

# EMERY AND RIMOIN'S PRINCIPLES AND PRACTICE OF MEDICAL GENETICS AND GENOMICS

SEVENTH EDITION

## Clinical Principles and Applications

Edited by
Reed E. Pyeritz, Bruce R. Korf, Wayne W. Grody

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# Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics

## Clinical Principles and Applications

SEVENTH EDITION

**Editors**


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# Table of Contents

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Title page

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List of Contributors

Preface to the Seventh Edition of Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics

Preface to Clinical Principles and Applications

1. A Clinical Approach to the Dysmorphic Child

    1.1. Introduction

    1.2. Prenatal Versus Postnatal Onset of Developmental Problems

    1.3. Prenatal-Onset Problems in Development

    1.4. Postnatal-Onset Problems in Development

    1.5. Conclusion

2. Clinical Teratology

2.1. Introduction

2.2. Evaluating the Patient and Her Exposure

2.3. Recognized Teratogenic Exposures

2.4. Paternal Exposures and Maternal Exposures Before or Shortly After Conception

2.5. Future Perspective

2.6. Conclusion

3. Neurodevelopmental Disabilities: Global Developmental Delay, Intellectual Disability, and Autism

3.1. Intellectual Disability and Global Developmental Delay

3.2. Global Developmental Delay

3.3. Definition of a Diagnosis

3.4. Whole Exome Sequencing

3.5. Whole Genome Sequencing

3.6. Phenotyping

3.7. Genetic Mechanisms of ID

3.8. Diagnostic Testing of Patients With ID of Unknown Cause

3.9. Cytogenomic Copy Number Abnormalities

3.10. X-Linked ID

3.11. Fragile X Syndrome

3.12. Autism Spectrum Disorders

3.13. Inborn Errors of Metabolism and ID

3.14. CNS Malformations, Intellectual Disability and Brain Imaging

3.15. Summary

4. Abnormal Body Size and Proportion

Glossary

4.1. Introduction

4.2. Approach to the Patient With Abnormal Stature

4.3. Mechanisms of Growth

4.4. Pathologic Short Stature

4.5. Pathologic Overgrowth

4.6. Conclusion

Chapter 5. Cytogenetic Analysis

5.1. Introduction

5.2. Milestones in Human Cytogenetics

5.3. Indications for Cytogenetic Analysis

5.4. Tissue Samples and Cell Culture

5.5. Chromosome Banding

5.6. Normal Human Karyotype

5.7. Chromosome Abnormalities

5.8. In Situ Hybridization

6. Diagnostic Molecular Genetics

6.1. Introduction

6.2. Indications for Molecular Genetic Testing

6.3. Technical Approaches to Molecular Genetic Testing

6.4. Molecular Genetic Diagnosis of Some Commonly Tested Diseases

6.5. Mitochondrial DNA Disorders

6.6. Other Targets of Molecular Genetic Screening

6.7. Pharmacogenetic Testing

6.8. Quality Assurance, Reimbursement, and Regulatory Issues

6.9. Internet Resources for Molecular Genetic Testing

6.10. Societal Impact of the New Genetic Technology

6.11. Future Directions

7. Therapies for Lysosomal Storage Diseases

7.1. Introduction

7.2. Enzyme Replacement Therapy

7.3. Bone Marrow Transplantation

7.4. Substrate Reduction Therapy

7.5. Pharmacologic Chaperone Therapy

7.6. Emerging Therapies: Gene Therapy and Genome Editing

8. Transplantation Genetics

8.1. The Major Histocompatibility Complex

8.2. Historical Iter Toward Histocompatibility Definition

8.3. Currently Most Used Methods for HLA Typing

8.4. Clinical Significance of HLA Molecular Typing

8.5. Stem Cells and Transplantation

8.6. Concluding Remarks

9. Genetic Evaluation for Common, Chronic Disorders of Adulthood

9.1. Background

9.2. Outcomes of Genetic Services

9.3. The Process of Genetic Consultation for Common, Chronic Diseases of Adulthood

9.4. Genetic Healthcare Models

Summary

10. Carrier Screening and Heterozygote Testing

Glossary

10.1. Introduction

10.2. Carrier Screening in Clinical Practice

10.3. Carrier Screening in Individuals of Defined Subpopulation Groups

10.4. Therapeutic Implications for Heterozygotes

10.5. Sensitivity and Specificity

10.6. Cost and Feasibility

10.7. Genetic Counseling and Informed Consent

10.8. Conclusions

11. Circadian Rhythms and Disease

11.1. Introduction

11.2. Molecular Mechanisms

11.3. Central and Peripheral Clocks

11.4. Circadian Diseases

11.5. Metabolic and Cardiovascular Disorders

11.6. Cancer

11.7. Psychologic and Neurologic Diseases and Circadian Rhythms

11.8. Chronotherapy

11.9. Concluding Remarks

12. The Genomic Health Record: Current Status and Vision for the Future

12.1. Introduction

12.2. Conclusion

13. Ethical and Social Issues in Clinical Genetics

13.1. Introduction

13.2. The Historical Context

13.3. Genetic Counseling, Testing, and Screening

13.4. Diagnostic Genetic Testing

13.5. Predictive Genetic Testing

13.6. Confidentiality

13.7. Genetic Testing in Childhood

13.8. Population Genetic Screening

13.9. Other Challenges in Genetic Counseling

14. Genetics and Genomics in Public Health

14.1. What Is Public Health Genetics/Genomics?

14.2. The Purposes of Public Health

14.3. The Public Health System Infrastructure

14.4. Evolution and Convergence of Two Fields of Science—Public Health and Genetics/Genomics

14.5. Future Direction for Public Health Genetics/Genomics

15. Implementation of Genomic Medicine: An International Perspective

15.1. Introduction

15.2. Large-Scale Genomic Medicine Initiatives

15.3. National Genomic Medicine Initiatives

15.4. Large-Scale Regional Genome Initiatives

15.5. Corporate Genomic Medicine Initiatives

15.6. Studying Founder Populations

15.7. Conclusions and Future Perspectives

15.8. Competing Interests

Index

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# Preface to the Seventh Edition of *Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics*

The first edition of *Emery and Rimoin's Principles and Practice of Medical Genetics* appeared in 1983. This was several years prior to the start of the Human Genome Project in the early days of molecular genetic testing, a time when linkage analysis was often performed for diagnostic purposes. Medical genetics was not yet a recognized medical specialty in the United States, or anywhere else in the world. Therapy was mostly limited to a number of biochemical genetic conditions, and the underlying pathophysiology of most genetic disorders was unknown. The first edition was nevertheless published in two volumes, reflecting the fact that genetics was relevant to all areas of medical practice.

Thirty-five years later we are publishing the seventh edition of *Principles and Practice of Medical Genetics and Genomics*. Adding "genomics" to the title recognizes the pivotal role of genomic approaches in medicine, with the human genome sequence now in hand and exome/genome-level diagnostic sequencing becoming increasingly commonplace. Thousands of genetic disorders have been matched with the underlying genes, often illuminating pathophysiological mechanisms and in some cases enabling targeted therapies. Genetic testing is becoming increasingly incorporated into specialty medical care, though applications of adequate family

history, genetic risk assessment, and pharmacogenetic testing are only gradually being integrated into routine medical practice. Sadly, this is the first edition of the book to be produced without the guidance of one of the founding coeditors, Dr David Rimoin, who passed away just as the previous edition went to press.

The seventh edition incorporates two major changes from previous editions. The first is publication of the text in 11 separate volumes. Over the years the book had grown from two to three massive volumes, until the electronic version was introduced in the previous edition. The decision to split the book into multiple smaller volumes represents an attempt to divide the content into smaller, more accessible units. Most of these are organized around a unifying theme, for the most part based on specific body systems. This may make the book more useful to specialists who are interested in the application of medical genetics to their area but do not wish to invest in a larger volume that covers all areas of medicine. It also reflects our recognition that genetic concepts and determinants now underpin all medical specialties and subspecialties. The second change might seem on the surface to be a regressive one in today's high-tech world—the publication of the 11 volumes in print rather than strictly electronic form. However, feedback from our readers, as well as the experience of the editors, indicated that access to the web version via a password-protected site was cumbersome, and printing a smaller volume with two-page summaries was not useful. We have therefore returned to a full print version, although an eBook is available for those who prefer an electronic version.

One might ask whether there is a need for a comprehensive text in an era of instantaneous internet searches for virtually any information, including authoritative open sources such as *Online Mendelian Inheritance in Man* and *GeneReviews*. We recognize the value of these and other online resources, but believe that there is still a place for the long-form prose approach of a textbook. Here the authors have the opportunity to tell the story of their area of medical genetics and genomics, including in-depth background about pathophysiology, as well as giving practical advice for medical practice. The willingness of

our authors to embrace this approach indicates that there is still enthusiasm for a textbook on medical genetics; we will appreciate feedback from our readers as well.

The realities of editing an 11-volume set have become obvious to the three of us as editors. We are grateful to our authors, many of whom have contributed to multiple past volumes, including some who have updated their contributions from the first or second editions. We are also indebted to staff from Elsevier, particularly Peter Linsley and Pat Gonzalez, who have worked patiently with us in the conception and production of this large project. Finally, we thank our families, who have indulged our occasional disappearances into writing and editing. As always, we look forward to feedback from our readers, as this has played a critical role in shaping the evolution of *Principles and Practice of Medical Genetics and Genomics* in the face of the exponential changes that have occurred in the landscape of our discipline.

# Preface to *Clinical Principles and Applications*

This volume of *Principles and Practice of Medical Genetics and Genomics* presents topics focused on fundamental principles that underlie clinical applications of genetics and genomics. Some of the authors (Robert J. Desnick, John M. Graham, Kenneth Lyons Jones, and Marilyn Jones) have composed and updated their chapters since the first or second editions of this treatise. Due to the recognition of the evolution of genomic, not just genetic, application, a number of new chapters have been added since the sixth edition. The knowledge and perspectives gained from the chapters in this volume provide the foundation for interpreting and applying the information contained in all subsequent volumes.

***Reed E. Pyeritz, MD, PhD***

1

# A Clinical Approach to the Dysmorphic Child

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## Abstract

This chapter outlines a clinical approach to the child with structural defects, defines many terms used in dysmorphology, and presents a parsimonious approach to genetic testing.

## Keywords

Malformation; Deformation; Disruption; DysplasiaSingle primary defect; Sequence; Multiple malformation syndrome; Etiology; Pathogenesis; Prognosis; Recurrence risk; Chromosome; Comparative genomic hybridization; Whole exome sequencing

## 1.1. Introduction

The purpose of this chapter is to present a clinical approach to the child with structural defects. The approach is predicated on the concept that the nature of the structural defects presents clues to the time of onset, mechanism of injury, and probable etiology of the problem, all of which determine the direction of the evaluation. It presumes that the dysmorphic child represents an experiment in human development, which, if interpreted correctly, can provide

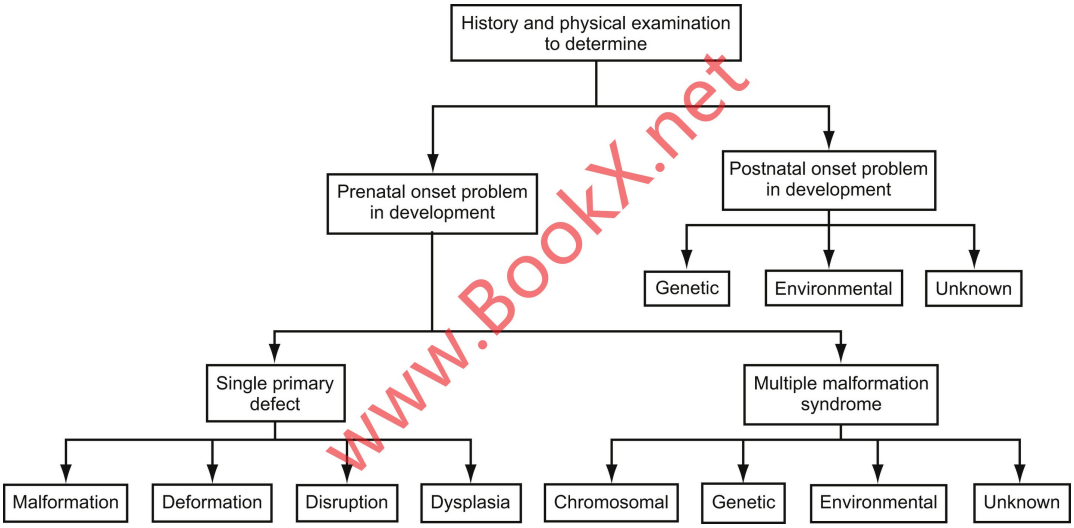
answers regarding the etiology of various structural defects, as well as permit insights into mechanisms of normal and abnormal morphogenesis. The method on which this approach is based has been most articulately set forth by Sir Arthur Conan Doyle's fictional character Sherlock Holmes, who showed "how much an observant man might learn by accurate and systematic examination of all that came within his way" [1]. This chapter adapts this method to the evaluation of the child with structural defects. By sharpening the faculties of observation, the clinician can narrow systematically the diagnostic possibilities so that the laboratory and the literature can be consulted in a rational fashion to arrive at an accurate diagnosis. The precise cause of many malformations and malformation syndromes is not known. However, careful clinical evaluation in combination with an expanded range of cytogenetic, cytogenomic, and molecular testing has allowed the elucidation of the mechanism underlying a growing list of clinical disorders. The separation between genetic and environmental factors as well as cytogenetic (copy number) and single gene abnormalities is somewhat arbitrary. However, the approach is intended to be practical and to facilitate detection and prevention of human malformations. *Gorlin's Syndromes of the Head and Neck* [2] and *Smith's Recognizable Patterns of Human Malformation* [3] are particularly useful. In recent years, computerized databases available online and on CD-ROM have become useful adjuncts to diagnosis (London Dysmorphology Database [Face2Gene]; Possum Web [4]; Online Mendelian Inheritance in Man [5]; and Decipher [6]).

## 1.2. Prenatal Versus Postnatal Onset of Developmental Problems

A method of approach to children with structural defects is set forth diagrammatically in Fig. 1.1. Although the lists of exceptions is growing, a history and physical examination usually make it possible to determine if the structural abnormality is of prenatal or postnatal onset. In this chapter, "prenatal onset" designates structural abnormalities that are present at birth, and "postnatal onset"

designates structures that have previously developed and differentiated normally. Whereas the genetic alteration responsible for many of the disorders included under postnatal-onset structural defects is present at the time of conception, the structural manifestations of that genetic alteration do not become obvious until postnatal life. On the basis of this distinction, a more rational approach to the problem can be developed, as this determination narrows considerably the diagnostic probabilities and, it is hoped, permits a more judicious selection of adjunctive laboratory tests.

Generally speaking, prenatal-onset problems in development are a consequence of genetic or chromosomal (copy number) alterations that cause programming problems in the development and/or differentiation of structure or are the result of factors unique to the pregnancy itself, such as environmental agents, abnormalities of placentation, or mechanical constraint. Although always evident at birth, most prenatal-onset problems remain static or improve postnatally without evidence of neurologic deterioration. By contrast, postnatal-onset problems in development usually result in deterioration in structure or function that has previously been normal. Deterioration may reflect postnatal accumulation of a toxic metabolic product (as in phenylketonuria), progressive storage of a metabolite (as in Hurler syndrome), deteriorating energy production (as in mitochondrial myopathies), or ongoing infection (as in deafness from cytomegalovirus). Children with postnatal problems usually appear to have thrived in utero. The structural and functional consequences of the problem manifest after the newborn period.



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**FIGURE 1.1** Approach to a child with structural defects.

Certain historical information can be particularly helpful in determining onset of the problem. Structural defects of prenatal onset are frequently associated with the following abnormalities noted by the mother during pregnancy and at the time of delivery, whereas, by contrast, with postnatal-onset structural defects, the pregnancy and delivery usually are normal.

Alterations of pregnancy associated with prenatal onset of developmental problems are as follows:

1. Alterations in gestational timing (prematurity or postmaturity). As discussed in several other chapters, the majority of

   conceptuses do not survive to be born at 40 weeks' gestation. Much of this loss occurs in the very early part of pregnancy and is the result of gross chromosomal abnormities and/or malformation. Numerous studies have documented an increased frequency of chromosomal and genetic abnormalities in losses from the second and third trimesters. Thus, premature delivery may reflect late fetal wastage rather than maternal disease. Postmaturity rarely occurs today because of improved fetal monitoring techniques. In years before the widespread use of ultrasound, anencephaly typically presented in pregnancies that continued well beyond

the due date because the fetal pituitary-adrenal axis is involved in the triggering of labor.

2. Alterations in onset of fetal activity, nature of fetal activity, or both. Although it is clear that fetal activity begins much earlier, it is usually not felt by the mother until about 18 weeks of gestation. Fetal activity increases in amount and intensity from that time, reaches a maximum between the 29th and 38th weeks, and then decreases somewhat until delivery. Discussion with mothers who have given birth to babies with structural defects suggests that certain structural defects are often associated with delayed onset and/or decreased intensity of fetal activity. Moreover, fetal movement may be localized to one particular quadrant of the abdomen, for example, when the defect represents deformation due to intrauterine compression in a previously normally formed structure. Other examples are defects in brain development and meningomyelocele, conditions in which the decreased fetal activity is secondary to neurologic impairment.
3. Abnormalities in amount of amniotic fluid, for example, polyhydramnios or oligohydramnios. During the latter part of gestation, amniotic fluid is maintained in equilibrium by fetal urination and fetal swallowing. Polyhydramnios occurs when the fetus has difficulty swallowing amniotic fluid; for example, early problems in central nervous system development or upper gastrointestinal obstruction. Oligohydramnios is usually present after chronic leakage of amniotic fluid or whenever fetal urinary excretion is decreased, such as renal agenesis, infantile polycystic kidney disease, or urethral obstruction.

Alterations noted at delivery associated with prenatal onset of developmental problems are as follows:

1. Increased incidence of breech presentation. Breech presentation

occurs in 3.1% of normal deliveries at 40 weeks' gestation. However, it occurs much more frequently in some disorders that adversely affect the form and/or function of the fetus. Defects of form include structural abnormalities such as hydrocephalus, which would be less compatible with the vertex position because of the large head, and joint dislocations, which may limit the capacity of the fetus to alter its position. Defects of function include some conditions associated with neuromuscular dysfunction, for example, the trisomy 18 syndrome and Smith–Lemli–Opitz syndrome associated with hypertonia and the Prader–Willi syndrome and Zellweger syndrome associated with hypotonia.

2. Prenatal onset growth deficiency. Drillen [7] studied the incidence of malformations, intellectual disability, and/or neurologic defects in 180 children who were 1 year old, whose birth weight was 2000 g or less, and who were small for gestational age (SGA). She documented an increased incidence of prenatal onset malformations as weight-for-gestational age decreased. In addition, she showed a marked increase in suspected mental and neurologic defects in those SGA children who had some structural anomaly. The association between both prematurity and SGA has more recently been confirmed in a population-based registry study in which birth defects were present in 17.2% of SGA infants as opposed to 7.8% of controls [8].
3. Difficulty with neonatal adaptation. Children with prenatal-onset structural defects frequently have problems with neonatal respiratory adaptation, probably secondary to malformations of brain structure. Therefore, one should always be cautious when attributing intellectual disability to a perinatal insult in a child who has associated prenatal-onset structural malformations. Intellectual disability in such

patients may well be related to a problem in brain development of prenatal onset.

Other historical information that will be useful in determining etiology includes:

1. Family history with attention to any health, developmental, or functional issues in first-, second-, or third-degree relatives as well as the presence or absence of consanguinity;
2. Past obstetrical history with attention to unexplained fetal losses;
3. Maternal health and exposure history in that mothers with diabetes, epilepsy, and certain immunological conditions may have a higher risk for adverse outcomes. Certain drugs, chemicals, and infections are known to increase risk.

The most helpful way to determine whether a structural defect is of prenatal or postnatal onset is a careful physical examination [9]. In the vast majority of situations, the nature of the problem will determine the direction the diagnostic evaluation should take. The physical examination should focus on delineating the pattern of major and particularly minor malformations. Major malformations are birth defects which have significant cosmetic and/or functional consequences to the individual concerned. About 15%–20% of stillborn babies and 2%–3% of all liveborns have a major malformation with the addition of an additional 2% as occult cardiac, renal and nervous system malformations become manifest by age 5 years. Major anomalies are often what brings an individual to clinical attention, but they rarely lead to an etiologic diagnosis.

Minor malformations, on the other hand, have no functional or cosmetic consequences for the affected individual. Minor malformations include such things as complete 2–3 syndactyly of the toes or a single transverse palmar crease. By definition, minor malformations occur in less than 4% of the general population. Although these anomalies themselves have few adverse implications,

the presence of two or more minor anomalies greatly increases the likelihood of a major anomaly. In addition, certain minor anomalies (such as a preauricular tag) may be associated with specific major anomalies (such as hearing loss). Moreover, most syndromes are diagnosed based upon the pattern of minor malformations rather than the major malformations with which they occur. For example, a diagnosis of Down syndrome may be suspected in an infant with upslanting palpebral fissures, epicanthal folds, small ears, a flat face, loose nuchal skin, a single transverse palmar crease, and wide spaces between toes 1 and 2 regardless of the presence of a cardiac defect. Last, minor malformations may provide clues as to the timing of the insult in development. For example, the interphalangeal flexion creases develop at about 9 weeks' gestation in response to movement across the joint. Hand contractures associated with absent palmar creases suggest that the fetal hand was not moving at 9 weeks' gestation, whereas contractures in the face of normal creases suggest that early hand movement was normal.

## Table 1.1

### Common Single Primary Defects in Development

| |
|---|
| Malformation: cleft lip ± cleft palate, cleft palate, cardiac septal defects, defects in neural tube closure |
| Deformation: congenital hip dislocation, talipes equinovarus |
| Dysplasia: pyloric stenosis |

In addition to structure, posture, tone, and behavior may all provide diagnostic clues as in the high pitched cry of the neonate with deletion 5p (cri du chat) syndrome, the overfriendliness and lack of stranger anxiety in the toddler with Williams syndrome, or the hand-wringing

behavior of the youngster with Rett syndrome.

## 1.3. Prenatal-Onset Problems in Development

Once a given problem has been determined to be of prenatal onset, a distinction should be made between those that are single primary defects in development and those that are multiple malformation syndromes. Although the concepts are not totally analogous, separation of prenatal problems into these two categories permits some practical generalizations that can be extremely helpful in counseling about recurrence risk.

Conceptually, "single primary defect in development" is an anatomic or morphogenetic designation. In most cases, the defect involves only a single structure, and the child is otherwise completely normal. Table 1.1 sets forth the seven most common single primary defects in development. For most of them, the specific etiology is unknown, making definitive recurrence risk counseling difficult. However, from a practical standpoint, most single primary defects are explained on the basis of multifactorial inheritance, which is thought to carry a recurrence risk for first-degree relatives of between 2% and 5%. Thus, recognition that a child's structural defect represents a single primary defect in development usually enables the clinician to suggest recurrence risk percentages between 2% and 5% for unaffected parents with one affected child.

The multifactorial model is a theoretical construct that was developed to explain the observed 2%–5% risk for first-degree relatives, the twin discordance, and gender inequality observed for many common single defects. The model stipulates that expression of a given characteristic represents the interaction between genetic susceptibility to a given biologic error and a threshold beyond which a given characteristic is expressed (later volumes will address such and such topic more fully). Susceptibility is depicted as a normal distribution in the population. The threshold may be manipulated by environmental factors, which may either increase or decrease the likelihood that a defect will be evident. The extent to which multifactorial inheritance contributes to the etiology of some of the

less common single defects in development is at present unclear. The fact that single primary defects are etiologically heterogeneous implies that some will have a clearly environmental etiology, whereas others will result from dominantly or recessively inherited single altered genes. Craniosynostosis secondary to in utero constraint is an example of the former, and postaxial polydactyly illustrates the latter. Before multifactorial risk figures are used for counseling, references Online Mendelian Inheritance in Man [5] or *Harper's Practical Genetic Counselling* [10] should be consulted to determine if other risk figures have been reported. Currently available genetic tests such as comparative genomic hybridization (CGH) array and whole exome sequencing (WES) rarely identify the etiology of most single defects in development.

In contrast to the anatomic concept of the single primary defect in development, the designation "multiple malformation syndrome" indicates that all the observed structural defects have the same cause. The defects themselves usually include a number of anatomically unrelated errors in morphogenesis. Multiple malformation syndromes are caused by gross chromosomal abnormalities, smaller copy number abnormalities resulting in microdeletions and duplications, teratogens, and single-gene defects usually inherited in Mendelian patterns. Recurrence risk depends on an accurate diagnosis and ranges from zero in cases that represent fresh gene mutations or are caused by one-time teratogen exposures to 100% for the unusual case of a child with the Down syndrome in which one parent is a balanced 21/21 translocation carrier. To review, recognition that a child has a prenatal-onset single primary defect in development suggests a 2%–5% risk; recognition that a child has a multiple malformation syndrome is not helpful with respect to recurrence risk counseling unless a specific diagnosis can be made.

## 1.3.1. Single Primary Defect in Development

Single primary defects can be subcategorized, as shown in Table 1.1, according to the nature of the error in morphogenesis that has produced the observed structural defect Thus, single primary defects

involve either malformation, deformation, disruption, or dysplasia of developing structure. A malformation implies a primary structural defect arising from a localized error in morphogenesis. A deformation should be thought of as an alteration, usually through compression, in shape and/or structure of a part that has differentiated normally; the term *disruption* is used for a structural defect resulting from destruction of a previously normally formed part. The term *dysplasia* refers to an abnormal organization of cells and the structural consequences. Dysplasias may be localized or generalized. Localized dysplasias (e.g., hemangiomas) are generally single primary defects in development. However, generalized dysplasias such as connective tissue disorders usually present as multiple malformation syndromes in that a variety of structures are involved because of the widespread distribution of the dysplastic tissue.

Table 1.1 sets forth the most common single primary defects in development. Four are malformations, the result of a localized error in morphogenesis. Two (congenital hip dislocation and talipes equinovarus) are the result of intrauterine molding and thus represent deformation of previously normally formed structures. One (pyloric stenosis) is a dysplasia resulting from abnormal muscular hypertrophy at the gastric outlet. Each of these seven anomalies occurs with a frequency of 0.5–1 per 1000 live-born infants. The major reason for separating single defects in development into malformation, deformation, disruption, and dysplasia is to gain information that can be helpful relative to prognosis. Of the deformations noted at birth, 90% will correct spontaneously; of those that do not, the vast majority can be corrected with early postural interventions, such as casting or bracing. Conversely, spontaneous correction of both malformations and disruptions almost never occurs, and, when correction is possible, surgery is virtually always necessary. Because dysplasias involve abnormal organization and localized deregulation of growth in affected structures, many change over time with involution occurring in some and malignant transformation taking place in others.

## 1.3.2. Malformations

Most children with a localized malformation in development, such as a cardiac septal defect or cleft lip and palate, are otherwise completely normal. After appropriate reconstruction, prognosis is excellent. In those cases in which Mendelian inheritance has not been previously documented, multifactorial recurrence risk figures (2%–5%) can be given to unaffected parents. If the malformation in development involves a structure that is not amenable to surgical correction, such as the brain, the long-term prognosis may be poor. The environmental factors that modify the threshold for expression of most malformations are for the most part unknown. For neural tube malformations, however, folic acid supplementation before conception substantially reduces the risk for recurrence [11].

## 1.3.3. Deformations

Most deformations involve the musculoskeletal system and are believed to be caused by intrauterine molding [12]. The pressure required to produce such molding may be intrinsic (e.g., neuromuscular imbalance within the fetus) or more likely be extrinsic (e.g., fetal crowding). In either case, the ability of the fetus to kick is impaired, resulting in decreased fetal movement, an important factor in the development of a normal musculoskeletal system. This is particularly true with respect to joint development because motion is essential for normal development of the joints. In addition, because of fetal plasticity, marked positional deformation of any body part can occur when the fetus is unable to change position and thus alter the direction along which potentially deforming extrinsic forces are being directed.

### 1.3.3.1. Intrinsically Derived Prenatal-Onset Deformations

Disorders involving muscle degeneration, such as the Steinert myotonic dystrophy, and disorders involving motor neurons, such as Werdnig–Hoffmann disease, are uncommon causes of positional

deformations. Early defects in development of the central nervous system are more common causes and should be seriously considered whenever a structural defect is thought to be an intrinsically derived prenatal-onset deformation.

### 1.3.3.2. Extrinsically Derived Prenatal-Onset Deformations

Fetal crowding, the common pathway in extrinsically derived postural deformations, is usually due to a decreased volume of amniotic fluid, a situation that occurs normally during the later weeks of gestation when the fetus undergoes extremely rapid growth. However, it also occurs abnormally when fetal urinary output is diminished and in cases of chronic leakage of amniotic fluid. Other extrinsic factors associated with the development of deformations include breech presentation and the shape of the amniotic cavity. When a fetus is held in the breech position, the legs may be trapped between the body and the uterine wall. In that position, the fetus is unable to kick optimally and therefore is immobilized and more susceptible to molding and deformation. Breech presentation is associated with a 10-fold increase in the incidence of deformations. The shape of the amniotic cavity, which has a profound influence on the shape of the fetus that lies within it, is influenced by many factors, among which are the following: uterine shape; volume of amniotic fluid; size and shape of the fetus; presence of more than one fetus; site of placental implantation; presence of uterine tumors; shape of the abdominal cavity, which is influenced by the pelvis, sacral promontory; and neighboring abdominal organs; and tightness of the abdominal musculature.

The various forms of talipes and congenital hip dislocation are the most frequently observed congenital postural deformities. Most children with these deformations are otherwise completely normal, and their prognosis is excellent. Correction usually occurs spontaneously. However, recognition that a structural defect represents a deformation does not always imply fetal crowding and should lead to careful consideration of other etiologic possibilities that

might have far greater importance to the child. For example, because decreased fetal movement can be secondary to serious neurologic abnormalities, multiple joint contractures should always alert the clinician to the possibility of a malformation in central nervous system development. Although the most common deformational single primary defects—congenital hip dislocation and talipes—have a 2%–5% recurrence risk, most deformations are the result of physiologic crowding and have virtually no recurrence risk. Deformations that are due to pathologic crowding (e.g., uterine tumors or malformation) have a much higher recurrence risk unless the factors leading to crowding are altered before subsequent pregnancies. Deformations that are the result of an underlying malformation (e.g., renal agenesis) have a recurrence risk similar to that for the underlying malformation.

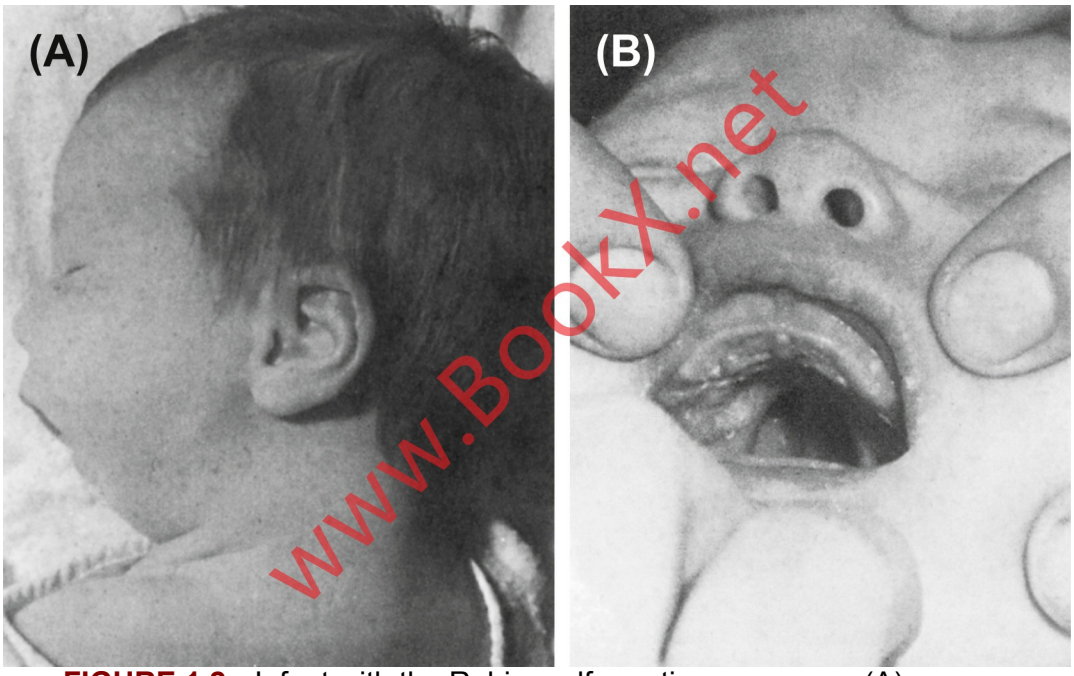
## 1.3.4. Disruptive Defects

Disruptive defects occur when there is destruction of a previously normally formed part. There are at least two basic mechanisms believed to produce disruption. One involves entanglement followed by renting, amputation, or both of a normally developed structure, usually a digit, arm, or leg, by strands of amnion floating within amniotic fluid ("amniotic bands") [13].

The second mechanism through which disruption occurs involves the interruption of blood supply to a developing part, leading to infarction, necrosis, and resorption of structures distal to the insult. If interruption of blood supply occurs early in gestation, the disruptive defect seen at term usually involves atresia or absence of a particular part. If the infarction occurs later, necrosis is more likely to be present. Examples of disruptive single primary defects for which infarctive mechanisms have been implicated include nonduodenal intestinal atresia [14], gastroschisis [15], porencephaly, and transverse limb reduction defects [16]. The extent to which disruption of developing structures plays a role in dysmorphogenesis is unknown [7].

Because disruptions typically do not involve programing errors intrinsic to the fetus, genetic factors appear to play a minor role in their pathogenesis. Thus, most disruptive defects are sporadic events

in otherwise normal families. Cocaine is an environmental agent whose mechanism of action is vascular disruption. Multiple disruptive defects have been seen in the offspring of women who abuse this agent in pregnancy [9]. The prognosis for a disruptive defect is determined entirely by the extent and location of the tissue loss. Thus, a child with a limb amputation has an excellent prognosis for normal function but a child with porencephaly does not.



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**FIGURE 1.2** Infant with the Robin malformation sequence. (A) Micrognathia. (B) U-shaped palatal cleft.

## 1.3.5. Dysplasia

The causes of many localized dysplasias have recently been elucidated and reflect somatic mutation in specific tissues. Mosaicism is consistent with the observation that empirical recurrence risks for localized dysplasias are low. Germline mutations in the genes responsible for many localized dysplasias are likely embryonic lethals. The process of dysplasia typically involves deregulation of growth; hence, most dysplasias change over time. Capillary hemangiomas

become involuted (the bathing trunk nevus illustrated in Fig. 1.5 carries a risk for malignant transformation). Knowledge of the natural history of a lesion is critical in the long-term follow-up of children with localized dysplasias.

## 1.3.6. Sequence

*Sequence* describes the pattern of multiple anomalies that occurs when a single primary defect in early morphogenesis produces multiple abnormalities through a cascading process of secondary and tertiary errors in morphogenesis [17]. When evaluating a child with multiple anomalies, it is extremely important from the standpoint of recurrence risk counseling to differentiate between multiple anomalies secondary to a single localized error in morphogenesis (a sequence) and a multiple malformation syndrome. In a sequence, recurrence risk counseling for the multiple anomalies depends entirely on the recurrence risk for the initiating, localized error. The words *malformation, deformation, disruption*, and *dysplasia sequence* are used if the nature of the initiating error in morphogenesis is known.

The patient depicted in Fig. 1.2 has mandibular hypoplasia, glossoptosis, and cleft palate, an example of multiple anomalies secondary to a single localized error in morphogenesis. The primary defect in this case is mandibular hypoplasia, a malformation. Because the tongue is relatively large for the oral cavity, it drops back (glossoptosis), blocking closure of the posterior palatal shelves, resulting in a U-shaped cleft palate. This condition has previously been referred to as the Pierre Robin syndrome. However, because both the glossoptosis and cleft palate are secondary to mandibular hypoplasia, the disorder is now understood as the Robin malformation sequence. Recognition that all of the observed defects are due to a single localized error in morphogenesis (mandibular hypoplasia) permits recurrence risk counseling based on the etiology of the single defect, which could be a localized malformation of the mandible or a consequence of a genetic abnormality as in Stickler syndrome [18]. The patient depicted in Fig. 1.3 has bathrocephaly, torticollis, facial asymmetry, a dislocated right hip, and valgus

anomalies of both feet. All of the structural defects are the result of compression of developing fetal parts. The pattern of abnormalities in this patient is referred to as the breech deformation sequence. Intrauterine crowding in this situation was the result of a large infant with a birth length of 54 cm, birth weight of 3.9 kg, and head circumference of 36 cm, delivered from a breech position to a small, primigravida mother. Recurrence risk is therefore negligible. Recognition of the deformational nature of the abnormalities is helpful with respect to prognosis. All of the problems should resolve spontaneously or with postural therapy.



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**FIGURE 1.3** Newborn infant with breech deformation sequence. Note the deformed cranial shape and positional deformities at the hips and feet.

The patient depicted in Fig. 1.4 has the amnion rupture sequence. All of the craniofacial and limb defects are secondary to multiple fibrous strands of amnion extending from the placental insertion of the umbilical cord to the surface of the amnion-denuded chorion or floating freely within the chorionic sac. These strands of amnion, which result from disruption of the normally formed membrane, cause secondary defects through any one or more of the following mechanisms. Malformations occur if a strand of amnion interferes with the normal sequence of embryologic development. For example, a strand of amnion could interrupt fusion of the facial processes so that a cleft lip would result. Disruptions, on the other hand, are secondary to tearing apart of structures that have previously developed normally. As such, an amniotic band might act to cleave areas in the developing craniofacies along a line not conforming to the normal planes of facial closure. Deformations due to fetal compression occur secondary to oligohydramnios, tethering of a fetal part, or both. The former situation may result from rupture of both amnion and chorion, leading to chronic leakage of amniotic fluid. Tethering occurs when the fetus or one of its parts becomes immobilized by the constraining effect of an amniotic band such that it is unable to change position and thus alters the direction along which potentially deforming forces are being directed.



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**FIGURE 1.4** Infant with amniotic band disruption sequence. Note the asymmetrical encephalocele, severe disruption of facial development, and digital anomalies.


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**FIGURE 1.5** Infant with neurocutaneous melanosis. Note the bathing trunk nevus. This infant also had seizures, presumably from melanocytic infiltration of the pia and arachnoid.

When used in conjunction with the word sequence, malformation, deformation, disruption, and dysplasia describe only the initiating error in morphogenesis of the sequence. The nature of the individual secondary defects that ensue from the initiating event depend on the manner in which the initiating error alters subsequent morphogenesis. In the case of the amnion rupture sequence, the initiating event, disruption, can lead to multiple structural defects through three of the mechanisms listed above.

The child depicted in Fig. 1.5 has a neurocutaneous melanosis sequence. In this dysplasia sequence, melanocytic hamartosis of the skin occurs in conjunction with similar changes in the pia and arachnoid. Affected individuals are at risk for malignant degeneration within the hamartoses and are also at risk for neurologic sequelae, including seizures and intellectual disability. The cause of this condition, in most cases, is somatic mosaicism for mutations in NRAS Proto-Oncogene, GTPase (*NRAS*) [19]. The patchy phenotype is a function of which tissues harbor the mutation.

Finally, a sequence, like any other single defect in development, can occur by itself in an otherwise normal individual or may be one feature in a multiple malformation syndrome. Stickler syndrome, cerebrocostomandibular syndrome, and spondyloepiphyseal dysplasia congenita are examples of multiple malformation syndromes in which the Robin malformation sequence represents one feature [18]. In this situation, recurrence risk counseling is based on the etiology of the overall condition.

## 1.3.7. Multiple Malformation Syndromes

The category of multiple malformation syndromes includes patients in whom a primary developmental anomaly of two or more systems has occurred, all of which are thought to be due to a common etiology. Other than Down syndrome, which has an incidence of 1:660, and XXY syndrome (1:500 males), few of these disorders occur more frequently than 1 in 3000 live births.

As shown in Fig. 1.1, multiple malformation syndromes can be categorized on the basis of etiology.

## 1.3.8. Chromosomal (Copy Number) Abnormalities

The ability to perform chromosomal studies and, more recently, CGH arrays has led to the recognition of a number of multiple malformation syndromes due to copy number abnormalities secondary to gross chromosomal rearrangements or more subtle deletions or duplications. Certain generalizations are important to consider when deciding whether a copy number abnormality should be suspected. First, because chromosomes are present in most cells of the body, a chromosome aberration may be expected to affect adversely many parts of the body. Consequently, a person with only an incurved fifth finger and a heart defect is very unlikely to have those features on the basis of a copy number abnormality. Second, some sex-chromosome disorders (e.g., XXX, XXY, and XYY) have few, if any, defects recognizable at birth and may present as postnatal problems in development associated with learning difficulties and behavioral challenges. In addition, as more experience is gained with copy number abnormalities involving very small deletions and duplications it has become increasingly clear that prenatal growth deficiency and intellectual disability should not be the only requirements for this type of testing.

The most common disorder associated with a chromosomal abnormality is Down syndrome (trisomy 21). The principal features of the disorder (flat facies with upward slant to the palpebral fissures, hypotonicity, and small ears) are usually present at birth, making a clinical diagnosis possible in the newborn period. Ear length is measured by the maximum distance from the superior aspect to the inferior aspect of the ear. Aase and colleagues [20] documented decreased ear length as a consistent feature in newborn infants with Down syndrome. In their series, no full-term white infant with Down syndrome had an ear length greater than 3.4 cm (mean 3 cm), and no normal full-term white infant had an ear length less than 3.2 cm

(mean 3.8 cm).

Until recently, the most commonly performed screening test to define the etiology of an unrecognized, prenatal-onset multiple malformation syndrome was a high-resolution chromosome analysis (>550-band resolution). Chromosome analysis as a first-tier test has effectively been replaced by array technologies combining CGH and single nucleotide polymorphism (SNP) platforms. Array-based CGH identifies copy number variation (either duplication or deletion) across the entire genome at high resolution. The most broadly available arrays use fluorescence techniques to compare DNA content in two differentially labeled genomes. Thousands of individual DNA sequences can be simultaneously interrogated, providing precise information about copy number at specific genomic locations. SNP arrays have the capacity to detect areas of homozygosity that could suggest unknown or undisclosed consanguinity or uniparental disomy. Arrays vary in resolution and coverage. Most currently provide less than 1-Mb resolution and dense coverage in areas of known clinical significance. A recent meta-analysis of diagnostic yield of array GCH in the evaluation of individuals with learning disabilities and congenital anomalies has suggested a 10% rate of causal copy number abnormalities. The down side of the technology is that it also detects copy number abnormalities that will eventually be determined to be noncausal (usually based on parental studies) at almost the same rate—7%, the false-positive rate [17]. Most practice guidelines have advocated that array CGH should be a first-line diagnostic test in the evaluation of a child with a multiple malformation syndrome given the ability of the technology to identify causal copy number changes that might not be specifically suspected on clinical grounds alone as well as all of the aneuploid anomalies identified on routine chromosome analysis and fluorescent in situ hybridization testing for specific microdeletion syndromes [21,22]. Arguments against the routine use of array CGH focus primarily on cost, the false-positive rate (which adds cost if parental studies are required), and the inability of the technology to detect balanced

rearrangements. When using array CGH as a first-line test, it must be borne in mind that the technology cannot detect balanced rearrangements such as translocations and inversions that may relate directly to the phenotype or represent a predisposing factor to rearrangement.

Recurrence risk counseling for copy number abnormalities depends on the nature of the cytogenetic abnormality identified. Risk is usually low in aneuploidy; however, the risk for certain trisomies (21, 18, XXX, and XXY) may increase with increasing maternal age. In case of an unbalanced translocation, parental karyotypes are warranted before specific risks are cited. In cases of de novo abnormalities, the risk is usually low except for the rare circumstance of gonadal mosaicism in a parent. Most microdeletion and microduplication syndromes are mediated by low copy repeats or inversions and carry a low risk for recurrence [23].

## 1.3.9. Disorders With Known Genetic Etiology

A single mutant allele or a pair of mutant alleles has been implicated as the cause of some recognizable multiple malformation syndromes of prenatal onset. Testing technology has dramatically altered the ability of the laboratory to assist the clinician when the pattern of malformation is not clinically recognized. A family history of a similarly, affected individual can be extremely helpful in suspecting that a single gene might be operative. However, many patients with multiple malformation syndromes of genetic etiology represent simplex events as a result of fresh gene mutation or the first presentation of an autosomal recessive disorder in a family. In such situations, family history will be noncontributory although older paternal age might suggest fresh gene mutation or parental consanguinity the effects of an autosomal recessive mutation. For many multiple malformation syndromes in which the abnormalities reflect perturbation of a specific system, such as the skeletal system in bone dysplasias, adjunctive testing might first involve radiographs or tissue histopathology. Increasingly, laboratories are offering platforms that include panels of genes, mutations in which produce overlapping

phenotypes such as for lethal skeletal dysplasias or aortopathies that resemble Marfan syndrome. For multiple malformation syndromes with no known copy number abnormality, WES or whole genome sequencing (WGS) may be a viable approach. Although these platforms sequence the whole exome or genome, interpretation reflects analysis of variants in genes known to relate to the phenotype in question. As the technology improves, WGS may replace array technology as a methodology that can identify both copy number abnormalities and single gene variants.

In addition to Mendelian patterns of inheritance, some multiple malformation syndromes may arise as a consequence of dosage imbalance of a gene. At some loci in the genome, only one copy of a gene is active even though two copies are normally present. Inactivation of one member of a gene pair involves the process of imprinting. Parent of origin effects are evident at imprinted sites where it is possible in a normal individual to document that either the maternally inherited copy of the gene or the paternally inherited copy of the gene is active. Prenatal-onset multiple malformation syndromes may occur if an abnormality in the imprinting process causes both the maternal and paternal genes to be active (or inactive) at a specific locus or if an affected individual inherits two copies of a gene from one parent and none from the other. The latter situation is termed uniparental disomy (UPD). Each of these mechanisms accounts for some cases of the Beckwith–Wiedemann syndrome. The extent to which imprinting and UPD play a role in the etiology of genetically determined multiple malformation syndromes is at present unknown.

## 1.3.10. Disorders Caused by Teratogens

Disorders caused by teratogens include multiple malformation syndromes due to the effect of specific infections and drugs or chemical agents with which the embryo or fetus has come into contact (later volumes will address such and such topic more fully). These disorders take on special importance because they represent the only group of dysmorphologic conditions in which prevention before conception may be feasible. This is particularly true in the case of

drugs and chemicals if the mother is aware that the agent in question can affect her baby. It is difficult, on the other hand, for a pregnant woman to avoid contact with all infectious agents. Immunization of at-risk individuals may assist in prevention of birth defects caused by specific viral infections (e.g., rubella or varicella-zoster virus).

A careful history of drug intake, chemical exposure, and, in some cases, travel should be obtained from the parents of all children with multiple malformation syndromes. This is particularly true when the etiology of the disorder is unknown. An excellent source of information regarding what is known about various exposures in pregnancy is MotherToBaby (https://mothertobaby.org/). In addition, several online databases are available through subscription, including Reprotox (https://reprotox.org) and TERIS (http://depts.washington.edu/terisdb/teriswerb/index.html).

Although a specific and easily distinguishable phenotype does not exist for each of the infectious agents that are commonly associated with altered fetal development, intrauterine infection can be suspected, based on the overall pattern of malformation. Any SGA patient with microcephaly or hydrocephalus, ocular findings including microphthalmia, chorioretinitis, cataracts, and/or glaucoma, or hepatosplenomegaly and thrombocytopenia, and who is developmentally delayed may be suspected of having had an intrauterine infection. It should be emphasized that each of these intrauterine infections has a wide spectrum, from fetal death, to the severely affected newborn infant with multiple malformations, to the child with no malformation disabilities. The latter situation is illustrated by a study by Hanshaw and coworkers [24] indicating a significant increase in school failure and deafness after clinically unapparent congenital cytomegalovirus infection (another exception to the prenatal–postnatal distinction set forth at the beginning of the chapter).

## 1.3.11. Recognized Patterns

Many multiple malformation syndromes are diagnosed based on the pattern of malformation in the affected individual, specifically the

pattern of minor malformations. As the molecular basis for many syndromes is elucidated, etiologic heterogeneity has become the rule rather than the exception. A case in point is Rubenstein–Taybi syndrome (RTS). This disorder is a well-recognized pattern of malformation associated with prenatal and postnatal growth deficiency, intellectual disability, typical craniofacial features that change over time, and distinctive limb anomalies. Most cases of RTS are produced by functional loss of one copy of the gene encoding the transcriptional coactivator CREB-binding protein (*CREBBP*) on chromosome 16p13.3 [25]. Microdeletions of this region, first thought to be causal in the majority of cases, actually account for only 10% of affected cases and produce a more severe phenotype than point mutations in the gene, which account for the majority of cases. Moreover, mutations in a second gene, *EP300* on chromosome 22q13, also produce a similar phenotype. Thus, RTS is both a chromosomal and a single gene disorder. RTS is typical of a growing number of recognized patterns of malformation in which different genetic mechanisms may produce the same phenotype. As a note of caution, array CGH testing will identify the deletion cases but not those due to point mutation in the gene. As the technology of WGS advances and the cost decreases, it may be possible for one test to detect both the copy number abnormality cases and those with a single gene mutation.

Array CGH and WES have both allowed delineation of the molecular basis of previously unrecognized patterns of malformation, such as the Potock–Lupski syndrome [26] and mandibulofacial dysostosis with microcephaly [27]. It is interesting that, in many cases, the distinguishing phenotype has not been delineated until after the molecular cause was known. In some newly described disorders, defined first in the laboratory, the phenotype appears to be so nonspecific as to be unrecognizable.

The molecular basis of many multiple malformation syndromes remains unknown although most will likely be determined to be a consequence of altered copy number, single gene mutation, epigenetic factors that alter expression or function of existing genes, or as yet

unrecognized environmental exposure.

## 1.4. Postnatal-Onset Problems in Development

Most children with postnatal-onset malformation problems are normal at birth, having appeared to thrive in utero. Neurologic problems frequently begin within the first week of life, and deterioration is often rapid. A specific pattern of malformation is typically not present at birth; structural abnormalities develop as the result of neurologic deterioration, storage of metabolites, or progressive loss of function in a specific tissue. In disorders with a known metabolic aberration, other manifestations of the metabolic defect such as cataracts, sparse hair, coarse facies, unusual skin pigmentation, and hepatosplenomegaly are frequently present. As set forth in Fig. 1.1, these disorders can be categorized on the basis of etiology.

### 1.4.1. Genetic

#### 1.4.1.1. Metabolic

Most metabolic conditions are the result of deficiency of a specific enzyme, transporter, or cofactor. Because of the possibility that early institution of dietary or enzyme replacement therapy may help to prevent intellectual disability, these disorders are of particular interest, and newborn screening is offered for an expanding list of these conditions throughout the United States. Because the placenta is able to compensate for the metabolic deficiency, affected infants are typically normal at birth (e.g., aminoacidurias and organic acidurias). Most of these conditions have an autosomal recessive mode of inheritance. Their incidence is extremely low. Phenylketonuria (1:14,000) is the most common and represented, in its untreated state, about 1% of most institutionalized populations surveyed before newborn screening programs were instituted. A second group of metabolic conditions produces a phenotype through abnormal accumulation or storage of material in various tissues in the body.

Although the placenta does not compensate for the enzymatic deficiency, the phenotype does not manifest until a period of time has passed during which accumulation of metabolites occurs (e.g., glycogen storage diseases and mucopolysaccharidoses). Consequently, there is usually a postnatal presentation.

#### 1.4.1.2. Central Nervous System Degenerative States

A genetic etiology is increasingly known for many of these disorders. The clinical diagnosis has previously relied on imaging and histopathology. However, genetic panel testing and WES have significantly changed the rate and ease of diagnosis in these conditions.

#### 1.4.1.3. Myopathies and Connective Tissue Disorders

Myopathies and connective tissue disorders represent a group of conditions in which structures deteriorate with wear and tear over time—hence, the typically postnatal presentation. A diagnosis may be suspected by the communality of the involved tissue (i.e., wound-healing problems and ligamentous laxity for connective tissue disorders or cardiomyopathy and weakness for muscle disorders). The ability to sequence mitochondrial and genomic DNA using panels or whole exome platforms has greatly facilitated diagnosis in all of these disorders.

### 1.4.2. Environmental Factors

Trauma, infection, hypoxia, and metabolic derangements can result in severe neurologic impairment. Progressive joint immobility, abnormal positioning, and paralysis secondary to central nervous system deterioration are the most frequent structural anomalies resulting from this type of injury. Deafness and cataract may also be seen.

## 1.5. Conclusion

A clinical approach to children with structural defects has been set

forth. It is based on the concept that the diagnostic evaluation should be directed by the nature of the structural defects. The ultimate goal of this approach is a specific overall diagnosis. The purpose of a diagnosis is to address the two questions facing all parents when a child with a birth defect is born: What does this condition mean to my child? What are my chances of having another child with a similar problem? An accurate overall diagnosis is critical to understanding the natural history of the disorder such that associated complications may be anticipated through screening, appropriate interventions offered and precise reproductive counseling be provided. The birth of a child with congenital anomalies usually places severe stress on a family eliciting all of the stages of a grief response. Understanding the cause and implications of the condition is often helpful in mitigating this stress. When an overall diagnosis is lacking, a better understanding of the nature and onset of the problem is commonly possible. That in itself can often be helpful to parents and to all others dealing with children who have structural defects. Last, reaching an overall diagnosis may take time as some characteristic developmental and behavioral findings may not manifest until an individual is older. Reevaluation is a critical component of this approach. Revisiting exomes and genomes as more is known about the function of human genes will also be an important adjunct to diagnosis.

## References


[1] Doyle A.C. *The complete Sherlock Holmes*. Garden City, NY: Doubleday; 1930:23.

[2] Hennekam R.C.M, Krantz I.D, Allanson J.E. *Gorlin's syndromes of the head and neck*. 5th ed. New York: Oxford University Press; 2010.

[3] Jones K.L, Jones M.C, Del Campo M. *Smith's recognizable patterns of human malformation*. 7th ed. Philadelphia: Elsevier-Saunders; 2013.

[4] POSSUM Web, 2017. http://www.possum.net.au/

(requires subscription).
[5] Online Mendelian Inheritance in Man. OMIM. 2017. http://www.ncbi.nlm.nih.gov/omi
[6] Decipher, 2017. https://decipher.sanger.ac.uk.
[7] Drillen C.M. The small-for-date infant: etiology and prognosis. *Pediatr Clin N Am*. 1970;17:9–24.
[8] Miguel-Verges F, Mosley B.S, Block A.S, Hobbs C.A. A spectrum project: preterm birth and small-for-gestational age among infants with birth defects. *J Perinatol*. 2015;35:198–203.
[9] Hoyme H.E, Jones K.L, Dixon S.D, et al. Prenatal cocaine exposure and fetal vascular disruption. *An Anales de Pediatía*. 1990;85:743–747.
[10] Clarke A. *Harper's practical genetic counselling*. 8th ed. Oxfordshire, England: Taylor and Francis, Ltd.; 2018.
[11] MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the medical research council vitamin study. *Lancet*. 1991;338:131–137.
[12] Graham J.M, Lara-Sanchez P. *Smith's recognizable patterns of human deformation*. 4th ed. Philadelphia: Elsevier; 2016.
[13] Higginbottom M.C, Jones K.L, Hall B.D, Smith D.W. T amniotic band disruption complex: timing of amniotic rupture and variable spectra of consequent defects. *J Pediatr*. 1979;95:544–549.
[14] Louw J.H. Jejunoileal atresia and stenosis. *J Pediatr Surg*. 1966;1:8–23.
[15] Hoyme H.E, Higginbottom M.C, Jones K.L. The

vascular pathogenesis of gastroschisis: intrauterine interruption of the omphalomesenteric artery. *J Pediatr*. 1981;98:228–231.
[16] Hoyme H.E, Jones K.L, Van Allen M.I, et al. Vascular pathogenesis of transverse limb reduction defects. *J Pediatr*. 1982;101:839–843.
[17] Spranger J, Benirschke K, Hall J.G, et al. Errors of morphogenesis: concepts and terms. *J Pediatr*. 1982;100:160–165.
[18] IzumiK K.L.L, Mitchell A.L, Jones M.C. Underlying genetic diagnosis of Pierre Robin sequence: a retrospective chart review at two children's hospitals and a systematic literature review. *J Pediatr*. 2012;160:645–650.
[19] Kinsler V.A, Thomas A.C, Ishida M, et al. Multiple congenital melanocytic nevi and neurocutaneous melanosis are caused by postzygotic mutations in codon 61 of NRAS. *J Invest Dermatol*. 2013;133:2229–2236.
[20] Aase J.M, Wilson A.C, Smith D.W. Small ears in Down's syndrome: a helpful diagnostic aid. *J Pediatr*. 1973;82:845–847.
[21] Edelmann L, Hirschhorn K. Clinical utility of array CGH for the detection of chromosomal imbalances associated with mental retardation and multiple congenital anomalies. *Ann NY Acad Sci*. 2009;1151:157–166.
[22] Manning M, Hudgins L, Professional Practice and Guidelines Committee. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal

abnormalities. *Genet Med*. 2010;12:742–745. .
[23] Shaw C.J, Lupski J.R. Implication of human genome architecture for rearrangement –based disorders: the genomic basis of disease. *Hum Mol Genet*. 2004;13:R57–R64.
[24] Hanshaw J.B, Scheiner A.P, Moxley A.W, et al. School failure and deafness after silent congenital cytomegalovirus infection. *N Engl J Med*. 1976;295:468–470.
[25] Sagoo G.S, Butterworth A.S, Sanderson S, Shaw-Smith C, Higgins J.P.T, Burton H. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects. *Gen Med*. 2009;11:139–146.
[26] Potocki L, Bi W, Treadwell-Deering D, Carvalho C.M, et al. Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a disage-sensitive critical interval that can convey an autism phenotype. *Am J Hum Genet*. 2007;80:633–649.
[27] Lines M.A, Huang L, Schwartzentruber J, et al. Haploinsufficiency of a splceisomal GTPase encoded by EFTUD2 causes mandibulofacial dysostosis with microcephaly. *Am J Hum Genet*. 2012;90:369–377.

2

# Clinical Teratology

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## Abstract

Clinical teratology involves recognizing and counseling patients and families about the effects of exposures that may cause birth defects. Such exposures include certain illnesses, infections, chemicals, and physical agents or drugs that may affect the developing embryo or fetus. Clinical geneticists and genetic counselors need to consider the possibility of teratogenic effects in three clinical settings: (1) during the evaluation of a patient whose clinical feature suggests the possibility of a teratogenic effect; (2) in a woman who is not currently pregnant but wishes to have children and is concerned about possible teratogenic effects of a current or possible future exposure; and (3) in a patient who is concerned about adverse effects of an exposure that has already occurred in her current pregnancy. An approach to these clinical problems is presented, and examples of some pregnancy exposures that can cause birth defects are discussed.

## Keywords

Adverse effects; Birth defects; Exposures; Pregnancy; Teratogenic

## 2.1. Introduction

## 2.1.1. Overview

Pregnant patients who are undergoing genetic evaluation or prenatal diagnosis often have concerns about possible adverse effects of nongenetic factors, such as exposures to drugs, infections, or occupational agents. Only about half of families seen in most genetics clinics have conditions that are principally genetic in origin, and the differential diagnosis often includes disorders that have a predominant nongenetic cause. Accordingly, it is incumbent on practitioners of clinical genetics to be aware of the noninheritable sources of human variability, their potential interactions with genetic factors, and their implications for human health, growth, and development. *Teratology* is the branch of medical science devoted to study of the causes of abnormal prenatal growth and development, and in this chapter our focus is on the nongenetic causes. Teratological effects include structural congenital anomalies, growth disturbances, and functional deficits such as behavioral and cognitive abnormalities that may not be apparent until sometime after birth.

The term "teratogen" has been used to denote an agent that can cause abnormalities of form, function, or both in an exposed embryo or fetus, but this use is somewhat misleading. It implies that any given agent either is or is not teratogenic and that clinical teratology consists merely of memorizing a list of "human teratogens." In reality, teratogenicity is a property of an *exposure*, which involves not only the physical and chemical nature of the agent but also the dose, route, and gestational timing involved. The occurrence of other, concurrent exposures and the biological susceptibility of the mother and embryo or fetus are also factors that can determine whether a given exposure produces damage in a particular instance.

Regulatory agencies have erected barriers against the introduction of teratogenic exposures into our environment. Furthermore, the past three decades have seen the development of teratogen information services in North America (www.mothertobaby.org) and around the world (https://www.entis-org.eu/). These services, supported by computer-based information resources such as *TERIS*

(http://depts.washington.edu/~terisweb/teris/index.html) and *REPROTOX* (http://www.reprotox.org), have improved the access of pregnant women and their health care providers to available information and have fostered a systematic approach to risk assessment and risk management. Unfortunately, significant public health barriers remain, and the goal of primary prevention through the avoidance of hazardous prenatal exposures remains substantially unrealized. Although progress is being made in our understanding normal and abnormal embryonic development, the mechanisms by which most teratogenic exposures produce their pathogenic effects are still unknown.

## 2.1.2. Mechanisms of Teratogenesis

James Wilson, in the magisterial four-volume *Handbook of Teratology* that he published with Clarke Fraser in 1977 [1], formulated six Principles of Teratology, the third of which is, "Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate abnormal embryogenesis." Wilson used the term "mechanisms" in a very precise manner to mean the earliest, if not the first, event in the pathogenic pathway between a teratogenic exposure and its effect. He explained that later events in the pathogenic process might be expressed as one or more higher-level effects, such as excessive or reduced cell death, abnormal cellular interactions, reduced biosynthesis of important products, aberrant morphogenic movement, or mechanical disruption of tissues, and that these, in turn, might result in a few final common pathways of abnormal development. Table 2.1 lists some of the mechanisms that we now know to be important in mammalian embryonic development. Of these, epigenetic control of gene expression has been very intensively studied [2–5]. Epigenetic mechanisms provide a possible link between the complex genetic program that underlies early development and teratogenic exposures. For the various mutagenic mechanisms listed in the table, it is important to point out that teratogenesis and mutagenesis are not the same thing [6]. Many teratogenic exposures, including those involving retinoids and thalidomide, are *not*

mutagenic, and many chemicals that have mutagenic potential do not appear to be teratogenic when administered to pregnant women in therapeutic doses.

**Table 2.1**

**Mechanisms of Teratogenesis**

- Alterations of the cytoskeleton
- Alterations of the integrity of intracellular organelles
- Altered energy sources[a]
- Altered membrane characteristics[a]
- Altered nucleic acid integrity or function[a]
- Chromosomal nondisjunction and breaks[a]
- Disturbances of intracellular or intercellular signalling
- Dysfunction of molecular chaperones
- Effects of mechanical forces on embryogenesis
- Effects of small regulatory RNAs
- Effects on the distribution of molecules into subcellular compartments
- Enzyme inhibitions[a]
- Epigenetic control of gene expression
- Genomic imbalance resulting from copy number changes
- Lack of precursors and substrates needed for biosynthesis[a]
- Mitotic interference[a]
- Mutation[a]
- Osmolar imbalance[a]
- Perturbations of the extracellular matrix

[a] Included among the mechanisms presented previously Ref. [1].

## 2.1.3. Genetic Susceptibility to Teratogenic Effects

It is no surprise that genetic factors influence susceptibility to teratogenic effects, given that maternal absorption, maternal and fetal metabolism, and maternal and fetal excretion of potentially teratogenic agents, as well as embryonic development itself, are under genetic control. Genetic susceptibility to teratogenic effects has been unequivocally demonstrated in experimental animals, but direct evidence in humans is more limited [7]. One striking example of the importance of the genetic susceptibility to teratogenesis is the higher rate of concordance for fetal alcohol syndrome among monozygotic than dizygotic twins of mothers who heavily abuse alcohol during pregnancy [8]. Clinical observers have also repeatedly noted that the risk for recurrence of other teratogenic syndromes (assuming the exposure continues) is much higher in the subsequent children of a woman who has had one affected child than in exposed pregnancies in general [9].

Genetic susceptibility to teratogenic effects has also been assessed through case–control studies of common congenital anomalies that focus on gene–environment interactions. For example, it appears that individuals with thalidomide embryopathy have a higher frequency of alleles associated with reduced expression of the endothelial nitric oxide synthase (*NOS3*) gene, indicating a possible genetic susceptibility to thalidomide embryopathy [10,11]. Similar studies have found associations between maternal cigarette smoking and candidate genes such as *TGFA* and *NOS3* in orofacial clefts, and with candidate genes like *MTHFR* and *MTRR* that are involved in folic acid metabolism in relationship to neural tube [7,12]. Although statistically significant gene–environment interactions have been demonstrated in some of these studies, no significant associations have been found in others.

## 2.1.4. Characterization of Teratogenic Exposures

Teratogenic exposures act via a relatively limited number of pathogenetic processes [13,14] that may produce cellular death, alter

tissue growth (hyperplasia, hypoplasia, or asynchronous growth) or interfere with cellular differentiation or other basic morphogenetic processes, including mechanical ones. Some agents may also act by destroying ("disrupting") normally developing structures.

Similar effects may be produced by different teratogenic exposures. For instance, many exposures may stunt growth, resulting in a neonate who is small for gestational age. Thus, certain characteristics are common to a wide range of teratogenic exposures and may be used as general indicators of potential teratogenicity. Such indicators that a teratogenic effect may have occurred include:

1. Infertility or fetal wastage
2. Prenatal-onset growth deficiency
3. Alterations of morphogenesis, i.e., congenital anomalies
4. Alterations of central nervous system (CNS) function

Exposures that have been reproducibly associated with one or more of these effects should be considered possibly teratogenic. Fetal wastage and prenatal-onset growth deficiency seem to be especially frequent indicators of teratogenicity.

These general indicators of teratogenic activity reflect disturbances of basic processes occurring in many tissues. Such processes affect critical events in growing cells and developing organisms, and commonly are manifest in more than one tissue or organ in the developing embryo or fetus at any one time. It is not surprising, therefore, to find that teratogenic exposures usually have the capability of producing abnormalities in more than one tissue or organ system and that teratogenic exposures tend to produce characteristic patterns of abnormal growth and morphogenesis. For this reason, while individual abnormalities of morphogenesis are not specific for a particular teratogenic exposure, certain patterns of abnormal growth and development may be distinctive.

Agents are teratogenic only under certain conditions of exposure. This is why classifying some agents as "teratogens" and others as "nonteratogens" is misleading [15]. One critical factor is developmental stage of the embryo at the time of exposure. The most

sensitive period for most teratogenic exposures appears to be from roughly 2 weeks after conception to the eighth week after conception (i.e., gestational weeks 4–10, counting from the beginning of the last menstrual period). Data from animal experiments suggest that earlier adverse exposures are usually either lethal to the embryo or produce no demonstrable effect on morphogenesis. Exposures occurring after the period of embryogenesis may produce problems of cell depletion or organ function and can, therefore, be related to such effects as growth retardation or renal failure. Exposures that lead to fetal constraint and consequent deformations are likely to have their most significant effect in the third trimester of pregnancy, during the phase of most rapid fetal growth. Exposures, such as those to infectious agents that produce cell death or tissue necrosis, may cause disruption at any stage of gestation.

Thalidomide and angiotensin-converting enzyme (ACE) inhibitors provide especially vivid illustrations of the importance of timing to teratogenesis. The pattern of limb reduction defects, facial hemangioma, microtia, ocular abnormalities, renal malformations, and congenital heart disease that characterizes the thalidomide embryopathy only occurs in children whose mothers are treated between 27 and 40 days of gestation [16]. In contrast, maternal treatment with ACE inhibitors like captopril or enalapril produces fetal renal failure and oligohydramnios only during the latter stages of pregnancy [17]. These effects appear to result from exquisite sensitivity of the fetus to the pharmacological hypotensive action of ACE inhibitors during the second and third trimesters of gestation [18].

Dose is a critical feature of any teratogenic exposure. Teratogenic effects occur only when the dose exceeds a certain threshold [19]. Exposures to agents that are generally considered to be safe may have adverse effects on the embryo or fetus if given in doses high enough to produce maternal toxicity. This is an especially important consideration in exposures associated with suicide attempts, drugs of

abuse (e.g., toluene inhalation), or agents encountered occupationally. Chronic exposure is usually of more concern than a single exposure, given similar doses.

The route of exposure is also of importance; there is unlikely to be a risk associated with any agent when the exposure occurs by a route that does not permit systemic absorption. This is the case with many dermal exposures. Exposure to methylene blue illustrates the importance of route of exposure to teratogenicity. Several studies have found a strong association between the occurrence of intestinal atresia and the instillation of methylene blue into the amniotic sac during midtrimester genetic amniocentesis in twin pregnancies [20]. The risk of intestinal atresia in an infant born after this procedure is about 20% [21]. Neither oral nor topical administration of methylene blue to the mother has been associated with a similar teratogenic risk.

Some agents, such as ionizing radiation, have direct access to the embryo, whereas others do not reach the embryo until after extensive metabolism by the mother. The teratogenicity of agents that are metabolized by the mother may depend on whether teratogenic metabolites reach the embryo or fetus in sufficient quantities to produce adverse effects. This, in turn, depends on a number of factors, including route of entry, physical properties of the agent, maternal dose, amount of systemic absorption, and maternal metabolic capacity.

Another factor that influences teratogenicity is the chemical and/or physical nature of the agent itself. Some agents are inherently more risky than others, and some pose a much greater risk to the embryo or fetus than to the mother. Maternal thalidomide treatment during embryogenesis is the classic example of an exposure that usually presents little direct risk to the mother but has strong developmental toxicity. There are only a few other exposures that exhibit such selective developmental toxicity.

Together, these factors probably account for most variation encountered among patients adversely affected by prenatal exposures. It must be recognized that the teratogenic potential of an exposure is commonly expressed over a wide spectrum when all exposed

individuals are considered. That is to say, when dealing with affected individuals, variability of effect is the rule, not the exception. These generalizations lead to the following conclusions:

1. Teratogenic exposures are most easily recognized (i.e., the effects are most specific) at the severe end of the spectrum where a clear-cut pattern of abnormalities of growth and development often emerges.
2. "Milder" or partial effects, including single major abnormalities of morphogenesis, may be more frequent among exposed individuals and less specific to individual teratogenic exposures. Such effects may sometimes be viewed as being "consistent with a prenatal teratogenic exposure" even though they are not specific.
3. Certain general features of teratogenic exposures may be sought in screening for potentially hazardous agents, and the use of agents displaying some of these characteristics would be most prudently avoided during pregnancy.
4. All of these features should be taken into account for complete clinical characterization of the effects of a teratogenic exposure and in order to interpret this information to patients in a meaningful way. A checklist of the types of information needed for the clinical characterization of a teratogenic exposure is presented in Table 2.2. Table 2.3 presents a list of features used to characterize effects that may occur in the child as the result of such exposures. Much more information on each of these points is needed for virtually every known or suspected teratogenic exposure in humans.

## Table 2.2

### Characterization of Teratogenic Exposures

| Agent |
|---|

- Nature of the chemical, physical, or infectious agent
- Inherent developmental toxicity
- Capacity to produce other kinds of toxicity in the mother

Dosage to embryo or fetus

- Single, repeated, or chronic exposure
- Duration of exposure
- Maternal dose
- Maternal route of exposure
- Maternal absorption
- Maternal metabolism and clearance
- Placental transfer
- Fetal metabolism and clearance

Time of exposure in pregnancy expressed in gestational weeks (or days)

- Between conception and onset of embryogenesis
- Embryogenesis
- Fetal period

Other factors

- Genetic susceptibility of mother
- Genetic susceptibility of the fetus
- Other concurrent exposures
- Maternal illness or other condition associated with exposure
- Availability of tests to quantify the magnitude of maternal exposure

## 2.1.5. Risk Assessment and Counseling for Teratogenic Effects

### 2.1.5.1. Clinical Settings

Clinical geneticists and genetic counselors need to consider the possibility of teratogenic effects in three clinical settings. The first is during the evaluation of a patient, usually a child, in whom the medical history and/or physical findings suggest the possibility of a teratogenic effect. Both a high index of suspicion and considerable

skepticism are required in such cases. Careful review of the prenatal history for the nature and circumstances of any potentially teratogenic exposure is essential, and the child should be examined for major and minor abnormalities known to be associated with such exposures. Except in the case of some infectious agents, laboratory confirmation of the diagnosis is not possible, and recognition of a teratogenic cause may require the skills of an expert dysmorphologist [22–24]. In many instances, a firm diagnosis can only be made by exclusion of alternative explanations for any abnormalities identified and long-term follow-up of the patient.

## Table 2.3

### Characterization of Teratogenic Effects for Counselling

General effects

- Alterations of morphogenesis
- Alterations of CNS function
- Other functional impairments
- Death of the conceptus, embryo, or fetus
- Prenatal-onset growth deficiency
- Carcinogenesis

Specific effects

- Recognizable syndrome
- Other distinctive features

Magnitude of risk

- Absolute
- Relative

Prenatal diagnosis

- Detailed ultrasound examination
- Amniocentesis or other invasive method

- Availability
- Reliability
- Utility

Concern is often voiced that informing couples that a child's birth defects have resulted from the adverse effects of a drug or other potential teratogenic exposure may create serious psychological problems, particularly in situations that involve maternal drug abuse or excessive consumption of ethanol. However, only through such a frank approach will there be an opportunity to provide optimal care for the affected child and the potential for prevention of similar problems in future children. It is clear, however, that the sharing of such information may indeed produce psychological problems. Under these circumstances long-term support for affected families through community and health care agencies is extremely important.

A second setting in which the geneticist or genetic counselor must consider a teratogenic effect is when a patient who is not currently pregnant but wishes to have children is concerned about possible teratogenic effects of a current or future medical treatment or occupational exposure, or for the management of a condition, such as diabetes. The most certain way to prevent birth defects from teratogenic exposures is exclusion of the exposure from the prenatal environment. The approach in this instance involves determining whether the exposure is of concern and, if so, whether it can be avoided, replaced by a safer alternative, completed before pregnancy, or deferred until the pregnancy is over. Although this is not always possible, increased attention should be paid by physicians to patient education to help avoid unnecessary exposures to potentially teratogenic agents during pregnancy, particularly during critical periods of embryonic or fetal development. When a woman of reproductive age requires drug therapy, the prescribing physician's discussion of risks and benefits should include the potential for teratogenic risks. Failure to inform a woman of reproductive age of teratogenic hazards relating to medical treatment or procedures may place the physician in legal jeopardy.

Sometimes it is impossible or unwise for a woman to avoid a

potentially teratogenic exposure, such as when failure to use a potentially teratogenic treatment poses a greater risk to the woman than the treatment does, even if she is pregnant. When it is not possible or prudent to avoid such treatment, it may still be possible to minimize the magnitude of the exposure or to avoid it during the most sensitive period of embryonic development. In the final analysis, decisions regarding the use of potentially teratogenic treatments during pregnancy are best left to an informed couple, supported through a comprehensive pregnancy risk assessment, risk communication, and management process by a sensitive and knowledgeable physician or counselor.

A third circumstance in which a geneticist or genetic counselor must consider a teratogenic effect is when a patient is concerned about the possible adverse effect of an exposure during her current pregnancy. For example, half of all pregnancies in the United States are unintended, and a patient may have abused alcohol or "recreational drugs" before she realized that she was pregnant. Alternatively, she may have become pregnant while taking a particular medication that she has now stopped. In these instances, any benefits of the exposure are no longer relevant, and the patient's concern may be whether she should terminate the pregnancy, consider prenatal diagnosis, or accept the additional risk (if any) and continue the pregnancy. Providing appropriate teratogen risk counseling in such situations requires careful evaluation of the patient, her fetus and the exposure, as well as a review of relevant scientific literature regarding the risk and nature of potential adverse outcomes, their ability to be diagnosed prenatally, and the effectiveness with which they can be treated if they do occur.

### 2.1.5.2. What Is the Risk?

Three kinds of risk are used in the medical literature to describe a teratogenic effect: absolute risk, relative risk (or odds ratio), and population-attributable risk. *Absolute risk* is the chance that a woman who has had a particular exposure during pregnancy will have an affected baby. Absolute risk is useful in counseling because it answers

the question that most pregnant women ask about an exposure: "What is the risk of birth defects in my baby?" In addition, absolute risk can be compared directly to other familiar risks such as the risk of miscarriage following prenatal diagnosis or the risk of nontreatment of the mother's disease.

Relative risk is a statement of how much more likely a woman who has had a particular exposure during pregnancy is to have an affected baby than a woman who has not had that exposure. Relative risks (or odds ratios, which are numerically equivalent for uncommon events) are usually reported in birth defects epidemiology studies because they are easy to calculate and easy to interpret. In counseling, it often is helpful to convert a relative risk (or odds ratio) to an absolute risk, but this requires knowledge of the incidence of the birth defect(s) of interest in the population that was studied.

Population-attributable risk is the proportion of adverse outcomes of a given type in the population as a whole that are caused by a particular treatment during pregnancy. Population-attributable risk is generally not relevant for counseling an individual patient, but it is useful for public health officials as an estimate of the amount by which the overall rate of a birth defect could be reduced by prevention of the teratogenic exposure.

The term "high risk" may mean two different things to patients. A teratogenic risk may be considered to be high if the severity of the effect is great, producing, for example, a severe brain malformation. Thus, the risk for Ebstein anomaly, a severe congenital heart defect associated with maternal lithium treatment during pregnancy, may be considered to be great even though the frequency of this malformation among the children of women treated with lithium during pregnancy is small [25–27]. Alternatively, a risk may be considered to be great if it is numerically large, even if the severity is mild. For example, maternal tetracycline treatment very frequently causes staining of the primary dentition in fetuses exposed during the second or third trimester of gestation, but this staining is only of cosmetic significance [28]. Maternal treatment with thalidomide or isotretinoin and maternal Zika virus (ZIKV) infection during critical

times of pregnancy are examples of teratogenic risks that are great in both severity and frequency.

### 2.1.5.3. Teratogen Risk Counseling

Providing teratogen risk counseling involves more than just identifying and estimating the magnitude of risk. The information must be communicated to the patient in a way that allows the patient to make informed decisions about the management of her pregnancy. The approach varies from patient to patient and depends on many factors, including the patient's cultural and social background, her understanding of the counselor's language, her level of general and scientific knowledge, and her commitment to the pregnancy. The uncertainty about possible effects that usually exists complicates teratogen counseling and requires that information be provided as risks, which are difficult for many people to understand. Counselors need to be aware of the information and misinformation patients bring with them to the counseling session and how it affects their perception of risk [29,30]. Finally, the importance most women place on having healthy children and the emotional circumstances that often surround teratogen risk counseling require that the counselor have considerable skill as well as a thorough knowledge of clinical teratology.

### 2.1.5.4. Dealing With Uncertainty

One of the most difficult aspects of counseling pregnant women about teratogenic risks associated with various exposures during pregnancy is the fact that there are very few exposures for which the available information is sufficient to estimate the magnitude and severity of risk with any confidence. Available data are insufficient to determine the teratogenic risk associated with conventional treatment of pregnant women with most prescription medications [31,32], and the same thing is true for treatment with many over-the-counter drugs or herbal remedies and occupational or environmental exposures. Nevertheless, geneticists, genetic counselors, and other health professionals who take care of pregnant women must advise them

about these risks.

It is important for health professionals to admit the limitations of their knowledge to themselves and to their patients. When counseling pregnant women, risks should be presented as best estimates and couched in appropriate uncertainty. Admitting the limitations of one's knowledge in this way may be unsatisfactory for some patients and is certainly unsatisfying for the counsellor, but it is better than assuming that a lack of information means a lack of risk or, alternatively, that any maternal exposure during pregnancy may pose a significant risk to the developing embryo.

## 2.2. Evaluating the Patient and Her Exposure

### 2.2.1. Evaluation of the Pregnant Patient for Teratogenic Exposures

To provide a context for counseling, the counselor should review the patient's general medical, obstetrical, and family histories. The purpose of teratogenic risk assessment is to determine whether a pregnant woman's exposure increases her risk of having a child with congenital anomalies above the risk that she would have if she were unexposed. The "background" risk of serious congenital anomalies usually quoted is 3%–5% for the general population, but the risk for a particular woman may be much greater because of her age, family history, medical conditions, or other exposures. It is common for the risk of congenital anomalies associated with these other factors to equal or exceed the risk associated with a particular exposure of concern to the patient, and it is important for any teratogenic risk to be presented in the context of these other risks for adverse pregnancy outcomes when they are present.

The dose, route, duration, and timing for each exposure of concern should be determined as precisely as possible. For example, one can usually obtain the name, amount, frequency, and length of time that a medication was taken. This is much more informative than just knowing what was prescribed. In some cases, blood levels of a drug or

occupational chemical to which the patient was exposed can be used to define the exposure very precisely. In other instances, measurements of a chemical or its metabolite in the urine or an occupational hygiene assessment can help to quantify an exposure of particular concern. One should always establish the reason the exposure occurred (e.g., medical treatment of a particular disease) and whether the woman experienced any toxic effects herself as a result of the exposure. If toxic effects did occur, they should be described as fully as possible. The evaluation should also include information about any relevant occupational exposures and the patient's use of other medications, alcohol, tobacco and other "recreational" drugs.

## 2.2.2. Assessing the Scientific Literature

Clinical assessment of human teratogenic risk requires careful interpretation of data obtained from several kinds of studies [33]. Fortunately, the problem of collecting and analyzing the available data has been greatly simplified by the advent of online clinical teratology knowledge bases (http://www.reprotox.org/; http://depts.washington.edu/terisweb/teris/). In addition, pharmaceutical labels developed in accordance with the revised US Food and Drug Administration Pregnancy and Lactation Labeling Rule [34] provide clinicians a narrative guide for communicating risk to their patients.

In some places, health professionals and patients can obtain information on teratogenic risks over the telephone (and sometimes through text messaging) from dedicated teratology information services (TIS) [35,36]. TIS are unique in combining multidisciplinary expertise representing teratology, dysmorphology, toxicology, pharmacology, epidemiology, clinical genetics, obstetric medicine, infectious disease, and occupational health in order to understand the potential for a specific agent to interfere with normal embryonic or fetal development [37].

### 2.2.2.1. Animal Studies

For many exposures, the only data available on the effects of exposure during pregnancy are the results of studies done in laboratory animals. Such studies are valuable because they provide a means of identifying exposures with teratogenic potential before humans have been harmed. Unfortunately, however, it is usually impossible to extrapolate findings directly from animal experiments to a clinical situation involving an individual pregnant woman. Comparisons between species are confounded by differences in placentation, pharmacodynamics, embryonic development, and other factors that may influence the likelihood of teratogenic effects. In addition, the consistent response seen in a controlled experiment in a genetically inbred strain of laboratory animals often contrasts with the highly variable response seen in the outbred human population, and the doses and routes of exposure used in animal teratology experiments often are not comparable to those that usually occur in humans.

#### 2.2.2.2. Case Reports

Anecdotal descriptions of individual cases in which a child with birth defects was born to a mother who was exposed to a particular drug or other chemical or physical agent during pregnancy are often reported in the medical literature. These associations are usually coincidental rather than causal because both birth defects and maternal exposures during pregnancy are relatively frequent. Case reports may be useful for suggesting particular outcomes that require further investigation and for raising causal hypotheses, but other kinds of studies are needed to determine whether these hypotheses are true.

#### 2.2.2.3. Case Series

Teratogenic exposures typically produce qualitatively distinct patterns of congenital anomalies in affected children. Most of the exposures that are currently known to be teratogenic in humans were initially identified in clinical series on the basis of such characteristic patterns of anomalies [38]. The evidence of a causal relationship can be compelling when a highly characteristic pattern of congenital anomalies is seen repeatedly in children whose mothers experienced

similar well-defined exposures at similar times in pregnancy, especially if both the pattern of anomalies and the exposure are otherwise rare. Fetal alcohol syndrome provides the best known example of this [39,40], but the embryopathies associated with maternal exposure to rubella virus [41], thalidomide [16,42], isotretinoin [43], and ZIKV [44,45] were also first recognized by astute clinicians in case series.

Because clinical series can include very thorough assessment of the circumstances of maternal exposure and the phenotype of each affected child, they are well suited to the recognition of such syndromes. Unfortunately, however, case series are subject to extremely biased ascertainment and multiple sources of confounding. Because they do not include controls, clinical series cannot be used to provide quantitative estimates of the strength or statistical significance of a teratogenic effect. Properly conducted epidemiological studies are needed to determine the magnitude of a teratogenic risk.

#### 2.2.2.4. Pregnancy Registries

Pregnancy registries are an increasingly popular method of collecting information on outcomes among women who have taken a particular drug or group of drugs during pregnancy. These registries usually depend on voluntary reporting of exposed pregnancies by the women themselves or by physicians who are treating them. Pregnancy registries are most useful if they identify women for inclusion "prospectively"—that is, during or after the exposure but before the outcome of the pregnancy is known, to avoid the bias toward reporting adverse outcomes that occurs when cases are voluntarily submitted following delivery. A major limitation of most pregnancy registries is the lack of an appropriate control group. Comparisons are often made to "expected" rates of congenital anomalies obtained from population-based birth defects registries with active ascertainment and rigorous standards for diagnosis and classification, usually the Metropolitan Atlanta Congenital Defects Program [46]. Such comparisons are inappropriate because data are collected in a very different way for pregnancy registries. Pregnancy registries are often

also limited by the quality of available data regarding both birth defect outcomes and exposures, the diagnostic methods used in the children, the reliability and consistency of the outcome assessments, and the length of follow-up. These limitations can be overcome if appropriate control groups are available and if the exposure and outcome data are collected in a rigorous fashion. If these conditions are met, pregnancy registries can be used to ascertain patients for high-quality exposure cohort studies [47].

### 2.2.2.5. Randomized Controlled Trials

Randomized controlled trials are generally considered to be the optimal epidemiological approach to assessing the effects of a treatment, but randomized controlled trials are rarely used in clinical teratology. It would be unethical to conduct a trial in humans to determine if a particular maternal treatment during pregnancy caused birth defects in the infants, so trial data on fetal outcomes usually are collected in the assessment of efficacy and adverse effects of treatments for maternal conditions like hypertension or premature labor. The treatment in these studies almost always occurs after the period of embryogenesis, so available clinical trial data usually provide little or no information on teratogenic risks associated with first-trimester exposure.

### 2.2.2.6. Cohort Studies

Cohort studies are used in clinical teratology research to compare the frequency of birth defects among children born to women treated with an agent during pregnancy to the frequency among children whose mothers were not so treated. There are two different ways that these studies may be performed: as population-based cohorts of all newborns (or all pregnancies) or as exposure cohort studies through teratogen information services.

Population-based cohort studies can obtain information on many different outcomes, including ones that do not become apparent until later in childhood, if appropriate follow-up data are available. However, population-based cohort studies must be very large to be

useful for clinical teratology studies because both the exposures of interest and the outcomes of interest are infrequent. As a consequence, population-based cohort studies are very expensive to perform, and only a few have been done to assess teratogenic risks.

Exposure cohort studies provide a more practical approach because data only need to be collected on the outcomes of pregnancies in women who were exposed to a particular agent and the outcomes of appropriate control pregnancies. Women with the exposures of interest may call a teratogen information service for counseling, so subjects can be ascertained for exposure cohort studies in the course of regular service provision. This strength is also a limitation because studies performed through teratogen information services are not representative of the population as whole but only of women who call these services.

Both population-based and exposure cohort studies can be used to estimate relative risk and statistical significance of associations that are observed between maternal teratogenic exposures and birth defects in the children. Either type of study may be subject to serious biases and confounding if not well designed and if the effects of covariates are not appropriately considered. Both depend greatly on the quality of the information regarding exposures as well as on the quality of the outcome data. Insufficient statistical power is frequently a concern with cohort studies, especially if the exposures, the outcomes being assessed, or both are rare.

### 2.2.2.7. Case–Control Studies

Case–control studies are used in clinical teratology research to compare the frequency of a maternal exposure, such as treatment with a particular drug, during pregnancy among children with or without birth defects. Case–control studies are often population-based, an important factor in avoiding many kinds of ascertainment bias. Because case–control studies focus on women who have given birth to a baby with birth defects, case–control studies are usually far more statistically powerful than population-based cohort studies of an equivalent size. A major limitation of case-control studies is that they

only provide information regarding the outcome or outcomes selected for study, so that an association with birth defects of an unanticipated kind, such as a previously-unrecognized pattern of minor anomalies, cannot be identified. In this regard, case definition and the system used to classify birth defects into the group(s) chosen for inclusion in the study may be of particular importance, and selection of a group of anomalies that are thought to have similar pathogenesis – for example, vascular disruption – may be more relevant than conventional anatomic classifications.

Case–control studies can be used to estimate the odds ratio and statistical significance of an association observed between birth defects in children and maternal teratogenic exposures. Like cohort studies, case–control studies depend on the quality of both the outcome data (e.g., case ascertainment) and the exposure data (e.g., exposure characterization and timing) and may be subject to serious biases and confounding. Another frequent concern with large population-based case–control studies is that many case groups involving different kinds of birth defects may be analyzed for associations with several different maternal exposures simultaneously, creating a "multiple comparisons" problem that may not be resolvable without additional investigations.

Sample size and consequent statistical power are, of course, important considerations in any epidemiological study, but the very high power of the case–control design when used with birth defect groups that are rare in the general population may raise a different issue: identification of a statistically significant risk that is real but clinically irrelevant. An exposure that doubles the risk of, say, sirenomelia to 2:100,000 from 1:100,000 in unexposed pregnancies but does not affect the risk of any other congenital anomaly might be of great interest in terms of pathogenesis but would be of little clinical consequence to an individual pregnant woman who seeks counseling.

### 2.2.2.8. Record Linkage Studies

Record linkage studies provide a means of performing cohort studies or case–control studies or both in a cost-effective manner. Record

linkage studies are done by connecting information on exposures during pregnancy in the mother to information on birth defect outcomes in the infant through existing electronic medical records or administrative databases. These data systems may be very large, providing excellent sample sizes. A major limitation of most record linkage studies is that the exposure and outcome data are collected for other purposes and may be of less than ideal quality for epidemiological research. In addition, information on important covariates is usually limited, so these factors cannot be managed in the statistical analysis.

### 2.2.2.9. Ecological Studies

Ecological studies differ from all other epidemiological studies used for clinical teratology research in that the unit of analysis is groups of people rather than individuals. Information on exposure and on outcomes is collected on the group as a whole, and exposure metrics for the group, which generally includes men, nonpregnant women, and children as well as pregnant women, are used to estimate the level of exposure during pregnancy among the mothers of children with birth defects. An advantage of ecological studies is that they can usually be performed with data that are collected for other purposes, making them much less expensive than population-based cohort or case–control studies.

Ecological studies are usually done to investigate the effects of environmental or occupational exposures to toxic chemicals or radiation. Statistical tests for association are performed between a summary measure of exposure (e.g., average concentration of a particular chemical in drinking water) and a summary measure of disease (e.g., the frequency of miscarriage) in a group. Associations observed in ecological studies must be interpreted with great caution because they are subject to the "ecological fallacy": attributing correlations observed in groups to individual members of those groups. Because the analyses in ecological studies are performed on populations rather than individuals, consideration of confounding factors is problematic at best and often impossible.

### 2.2.2.10. Data Synthesis

Meta-analysis provides a systematic approach to identifying, evaluating, synthesizing and combining the results of epidemiological studies. Meta-analysis is useful because it may permit quantitative conclusions to emerge from the joint assessment of several studies that cannot be drawn from the analysis of any individual study. Meta-analysis also provides a way to assess the effects of biases and the limitations of the individual studies. However, a conclusion reached by meta-analysis is no better than the original studies on which it is based and is also subject to limitations inherent in the joint analysis. Of particular concern is combining studies that use fundamentally different definitions of exposure or outcome. Such differences are often encountered, and ignoring them may confound rather than inform interpretation of the available data.

Publication bias – the greater likelihood for studies that show an effect, especially a large effect, to be published in comparison to those that do not show a significant effect – has been found to occur in clinical teratology studies [48,49]. This "file drawer problem" can be assessed in a meta-analysis if many studies are available but may not be detectable if there are only a few published studies.

An alternative way of interpreting multiple clinical teratology studies available on a particular exposure is through expert consensus. Expert consensus is a qualitative, rather than rigorously quantitative, approach that can provide a summary assessment of studies of widely varying types, sizes, and quality, including nonepidemiological studies such as clinical series. The quality and value of an expert consensus depend on the thoroughness and rigor of the assessment and, quite critically, on who is making it.

Determining whether an exposure is teratogenic in humans requires careful assessment of all relevant available information, and especially data obtained directly by study of the outcomes of human pregnancies. A statistically significant association in one or more epidemiological studies is not sufficient to establish causality without other evidence to support such a conclusion. It is critically important that observed associations make biological sense: an association

between the occurrence of birth defects in a child and an exposure during pregnancy in the mother that is not biologically plausible is almost certainly not indicative of a teratogenic effect. A chemical exposure cannot be teratogenic unless it is systemically absorbed by the mother and it or its metabolic products reach susceptible sites in the embryo or placenta. Exposures that produce congenital anomalies do so only during times in which the involved structures in the embryo or fetus exhibit appropriate sensitivity. In most cases, exposure to a greater quantity of the agent can be expected to increase the likelihood of abnormalities. The existence of a reasonable pathogenic mechanism for the observed effect in animal or in vitro experiments may provide further support for a causal inference.

## 2.3. Recognized Teratogenic Exposures

Teratogenic exposures may be conveniently grouped into four major categories on the basis of the kind of agent involved: infectious agents, physical agents, drug and chemical agents, and maternal metabolic factors. In this chapter we present only general summaries for several of the more important teratogenic exposures in each group. **The exposures discussed in this chapter are intended to be illustrative, and exclusion of a particular exposure from this discussion does *not* mean that the exposure is safe or has no known teratogenic risk.** The reader is cautioned that considerable disagreement still exists over the role of many of these exposures in the production of human birth defects. Thus, the following discussion should be used as a guide, and standard clinical teratology resources and the current literature should be consulted for a more thorough consideration of any particular exposure. ***One should not use the information included in this chapter for counseling pregnant patients regarding the teratogenic potential of particular exposures without consulting up-to-date information resources such as TERIS (http://depts.washington.edu/~terisweb/teris/index.html) or REPROTOX (http://www.reprotox.org/Default.aspx) that are designed for this purpose.***

## 2.3.1. Infectious Agents

For many years clinicians have been aware of infectious agents that can attack the fetus in utero. Recognized effects on the fetus include death, intrauterine growth retardation, congenital defects, and intellectual disability. The pathogenesis of these abnormalities can generally be ascribed to direct fetal infection, which may be associated with inflammation of fetal tissues and cell death. Many, if not all, of these defects represent the "disruption" pathogenetic category.

Certain signs and symptoms characterize prenatal infections of the fetus and can therefore be used as general indicators of a potential infectious etiology for a child's congenital abnormalities. Direct invasion of the nervous system may result in microcephaly, often associated with cerebral calcifications, intellectual disability, disorders of movement and muscle tone, seizures, and central auditory and visual deficits. Other general abnormalities associated with prenatal infections include prematurity, low birth weight for gestational age, and failure to thrive. Affected infants may exhibit direct evidence of widespread infection, chronic skin rashes and certain kinds of congenital heart disease [50]. In contrast, vertebral segmentation defects, major intercalary limb reduction malformations, polydactyly, and syndactyly are not typically associated with teratogenic infections, and neither are open neural tube defects or renal agenesis or duplication. Serological studies in the mother and infant may be helpful, but "TORCH" screening alone is usually insufficient in newborns in whom a congenital infection is suspected because not all transplacental infections are included in this test and its sensitivity and specificity are limited. Specialized serological studies, culture of the organism, polymerase chain reaction for the organism's DNA, or other more specific approaches are usually necessary to diagnose a congenital infection with certainty in an affected infant [50].

### 2.3.1.1. Viruses

#### 2.3.1.1.1. Rubella

Abnormalities associated with prenatal infection with rubella vary

substantially in frequency, severity, and type according to the month of gestation in which the infection occurred [51,52]. From 40% to 85% of infants born to women with serologically proven rubella infection during the first trimester of pregnancy exhibit associated clinical abnormalities in early infancy. The incidence of severely affected children drops off rapidly with maternal rubella infection after the first trimester, but later-appearing manifestations, such as hearing loss, delayed intellectual development, and diabetes, may be encountered in offspring of women infected later in pregnancy.

Infants who are born after first-trimester rubella virus infection may display a wide variety of birth defects and health problems, including intrauterine growth retardation, subsequent failure to thrive, and congenital anomalies (Fig. 2.1). Ocular defects such as cataracts, pigmentary retinopathy, microphthalmia, and glaucoma are often present. Various cardiovascular anomalies, including patent ductus arteriosus, valvular and peripheral pulmonary arterial stenosis, atrial and ventricular septal defects, and possibly other vascular stenotic lesions and tetralogy of Fallot, may occur. Myocardial damage, presumably stemming from myocarditis, has also been observed. Central nervous system abnormalities may include microcephaly, intellectual disability, hypotonia, and convulsions. Signs of acute meningoencephalitis or progressive panencephalitis may occur, and sensorineural deafness or other sensory or functional disturbances often are present. Signs and symptoms of wide-spread systemic infection are common and may include hepatosplenomegaly, jaundice, thrombocytopenia, anemia, irregularities of ossification of the long bones, and delayed ossification of the calvarium. Affected children may also exhibit pneumonitis, a chronic rubelliform rash, generalized adenopathy, chronic diarrhea, diabetes mellitus, or thyroid disease. A variety of immune defects, such as thymic hypoplasia and hypogammaglobulinemia, may occur, often in association with recurrent or persistent infections. Intrauterine rubella infection tends to be chronic, and hearing and other neurological deficits and endocrine disturbances that are not apparent in the neonatal period may develop after several months or years of age.


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**FIGURE 2.1** A child with rubella embryopathy. Note the "blueberry muffin" petechial rash.
Photograph courtesy of Susan Reef, MD, National Congenital Rubella Syndrome Registry, Centers for Disease Control and Prevention.

Intrauterine diagnosis of fetal rubella infection can be accomplished by immunological or molecular methods in the second trimester of pregnancy, but these methods cannot distinguish fetuses that will have rubella embryopathy from those that will have asymptomatic infections at birth [53,54]. Manifestations of rubella embryopathy such as cardiac defects or fetal growth retardation can sometimes, but not always, be identified prenatally by detailed ultrasound examination [55].

Prevention of congenital rubella syndrome is possible through routine immunization of children with rubella vaccine. Although use

of attenuated live rubella vaccines in pregnant women is contraindicated, inadvertent immunization of women with such vaccines early in pregnancy has not been associated with an increased risk to the fetus [51].

#### 2.3.1.1.2. Cytomegalovirus

Congenital cytomegalovirus (CMV) infection has assumed a position of increasing importance in recent years as a recognized cause of perinatal morbidity and mortality [56]. Congenital CMV infection is common, but most infected infants are asymptomatic. About 0.6%–0.7% of newborn infants are congenitally infected with CMV, but only about 10% of these infants exhibit serious manifestations at birth [56,57]. The risk of symptomatic involvement is highest in the children of women who had a primary CMV infection during the first 6 months of gestation. Previous maternal infection or the presence of antibodies in the mother's blood does not prevent fetal infection, although such antibodies appear to reduce the risk of symptomatic disease.

The classic picture of severe congenital CMV infection includes CNS manifestations such as microcephaly, typically associated with diffuse periventricular calcifications reflecting an extensive encephalitis [56,58]. Less frequently, hydrocephalus may develop secondary to obstruction of the flow of cerebrospinal fluid. These CNS abnormalities are associated with functional disturbances, including intellectual disability, spasticity, hypotonia, seizures, and strabismus. Ocular involvement is common with chorioretinitis, optic atrophy, microphthalmia, cataracts, retinal necrosis, calcifications, and anomalies of the anterior chamber and optic disc, all of which may produce severe visual impairment. Hepatitis with resultant hepatosplenomegaly and jaundice may occur, and bone marrow disturbances may result in thrombocytopenia, with a generalized petechial rash or hemolytic anemia. Non-CNS malformations do not appear to be unusually frequent among infants with congenital CMV infections.

Although most infants infected in utero with CMV do not display clinical symptoms in the neonatal period, 10%–15% of these children develop neurodevelopmental handicaps later in life as a result of their infection. Sensorineural hearing loss is most common. Other manifestations may include intellectual disability, movement and coordination disorders, behavioral disturbances, and chorioretinitis [56,59]. Regular audiological assessments are recommended for children with congenital CMV infection until at least 6 years of age to allow early intervention and rehabilitation, while regular neurological, developmental, and ophthalmology assessments should be offered to symptomatic infants [60,61].

Detailed ultrasound examination and fetal magnetic resonance imaging (MRI) can be used for prenatal diagnosis of fetal ventriculomegaly, cerebral calcification, and some other serious manifestations of fetal CMV infection in the second or third trimester of pregnancy [62–64], although neurological dysfunction may occur in infants with congenital CMV infection even if the prenatal imaging studies are normal. Invasive testing can be used to demonstrate fetal infection but does not distinguish symptomatic from asymptomatic involvement of the fetus. Invasive testing is most informative when there is evidence of fetal disease on ultrasound examination.

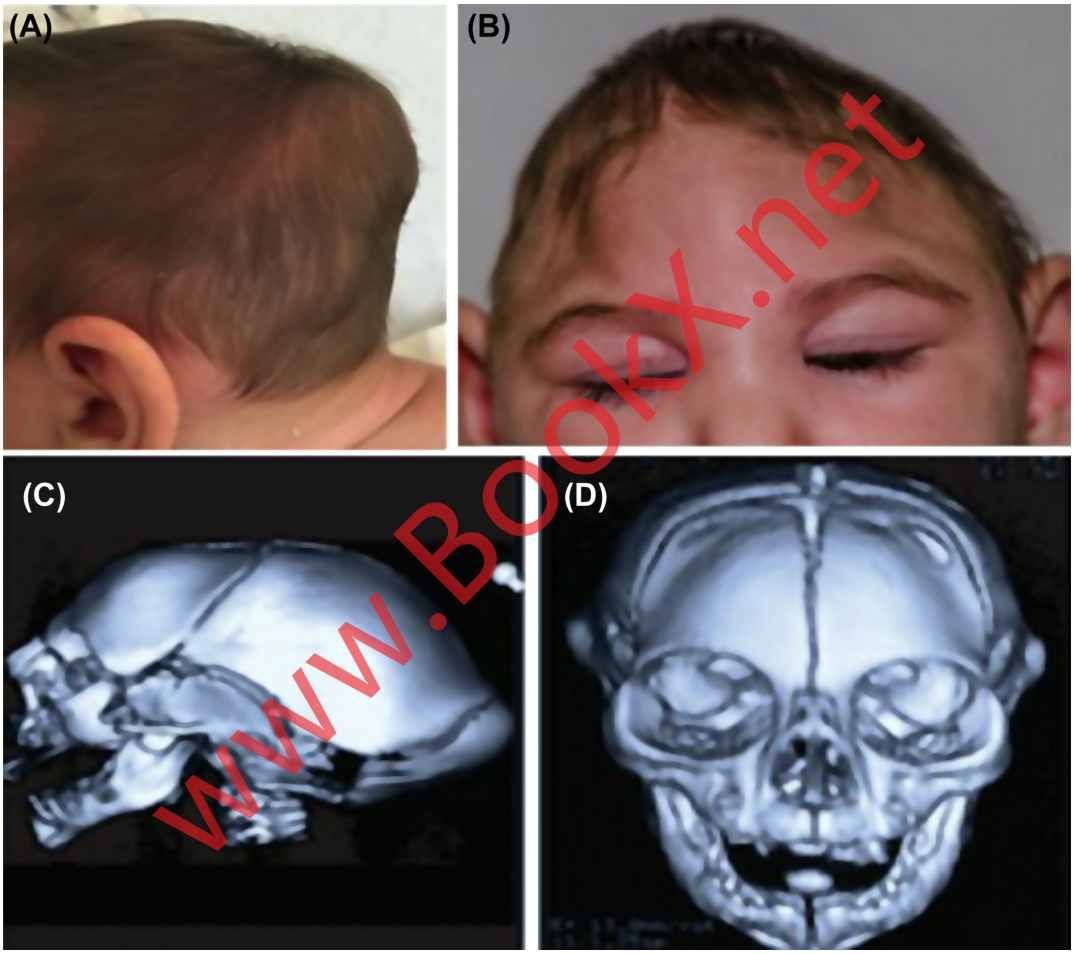
Vaccination of seronegative women may be useful in reducing the risk of fetal CMV infection associated with primary maternal infection during subsequent pregnancies [65]. Treatment of pregnant women with primary CMV infections and documented amniotic fluid involvement with specific hyperimmune globulin may reduce the frequency symptomatic CMV disease in the children [66,67].

#### 2.3.1.1.3. Zika virus

ZIKV is a flavivirus, primarily transmitted through infected *Aedes aegypti* mosquito bites, although transmission through sex or blood transfusion is also possible. A characteristic pattern of congenital anomalies has repeatedly been observed in case reports and clinical series of infants whose mothers had ZIKV infection during pregnancy

[44,45,68–71]. The embryopathy associated with congenital Zika syndrome (Figs. 2.2 and 2.3) is characterized by severe microcephaly, ocular abnormalities, and disruptive lesions of the brain on cranial imaging. Redundant scalp skin and congenital joint contractures may also occur. The most common ocular abnormalities are retinal pigment mottling, chorioretinal atrophy and optic nerve hypoplasia [72,73]. Brain abnormalities may include intracerebral calcifications; polymicrogyria, pachygyria, or other abnormalities of cortical development; ventriculomegaly; and dysgenesis of the corpus callosum, cerebellum, or brainstem [68,74]. Irritability, hypertonia or spasticity, and seizures have been reported, and stillbirth or death in infancy may occur. The functional consequences of ZIKV-associated microcephaly in childhood and later life are unknown, but normal neurodevelopment is unlikely in individuals with severe microcephaly and disruptive brain lesions.

The risk of microcephaly with maternal ZIKV infection in the first trimester of pregnancy is estimated to be 1%–13% [75] and appears to be smaller when ZIKV infection occurs in the second or third trimester of pregnancy. However, CNS abnormalities have been reported in fetuses infected as late as 27 weeks' gestation [76].




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**FIGURE 2.2** Congenital Zika syndrome. The occipital prominence can be pronounced and evident on external inspection (A) and represents the parietal-occipital junction with a very hypoplastic occipital bone on reconstructed CT image (C). A frontal midline bulge is apparent due to significant depression of the lateral aspects on the frontal bone (B), which appear more depressed than the parietal bones along with obvious supratemporal depression on CT (C and D). The vertical dimension of the cranium is reduced and the parietal vault is narrow (B and D).
From Del Campo, M, Feitosa, IM, Ribeiro, EM, Horovitz, DD, Pessoa, AL, Franca, GV, Garcia-Alix, A, Doriqui, MJ, Wanderley, HY, Sanseverino, MV, Neri, JI, Pina-Neto, JM, Santos, ES, Vercosa, I, Cernach, MC, Medeiros, PF, Kerbage, SC, Silva, AA, van der Linden, V, Martelli, CM, Cordeiro, MT, Dhalia, R, Vianna, FS, Victora, CG, Cavalcanti, DP, Schuler-Faccini, L. The phenotypic spectrum of congenital Zika syndrome. Am J Med Genet Part A 2017;173, 841–57; with permission.

Ultrasound examination or MRI in the second or third trimester of pregnancy may show microcephaly and other manifestations of fetal

ZIKV infection [77,78]. However, the fetal ultrasound examination may be normal in the first half of gestation in pregnancies in which the child is born with ZIKV-associated microcephaly and brain disruption [78,79]. RT-PCR can be used to identify IKV RNA in amniocentesis samples, but the presence of ZIKV RNA in amniotic fluid does not necessarily indicate that the fetus will be damaged. There are promising efforts to develop a vaccine in the near future, but some scientific challenges remain [80].

#### 2.3.1.1.4. HIV infection and AIDS

Rates of HIV transmission from mother to fetus range from 15% to 30% in studies from the United States and Europe in which maternal antiretroviral treatment was not given during pregnancy [81]. Maternal antiretroviral treatment late in pregnancy substantially decreases the rate of vertical transmission of HIV [82,83]. No evidence of a substantial teratogenic risk has been found in association with commonly prescribed antiretroviral treatments in pregnancy in a voluntary Antiretroviral Pregnancy Registry sponsored by pharmaceutical companies that manufacture these medications [84,85], however, a small but significant association with congenital heart defects has been reported [86].




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**FIGURE 2.3** Two infants with congenital Zika syndrome displaying abnormal neurological features. Hypertonic trunk extensor posture (A) and distal tremors and spasticity of lower limbs (B).
From Del Campo, M, Feitosa, IM, Ribeiro, EM, Horovitz, DD, Pessoa, AL, Franca, GV, Garcia-Alix, A, Doriqui, MJ, Wanderley, HY, Sanseverino, MV, Neri, JI, Pina-Neto, JM, Santos, ES, Vercosa, I, Cernach, MC, Medeiros, PF, Kerbage, SC, Silva, AA, van der Linden, V, Martelli, CM, Cordeiro, MT, Dhalia, R, Vianna, FS, Victora, CG, Cavalcanti, DP, Schuler-Faccini, L. The phenotypic spectrum of congenital Zika syndrome. Am J Med Genet Part A 2017;173, 841–57; with permission.

Congenital HIV infections usually follow one of two courses in untreated children [83]. Most have a slow progression that resembles AIDS in adults, but 10%–25% of congenitally infected infants become symptomatic in the first year of life, suffer rapid progression, and die very early. Affected infants may exhibit failure to thrive, interstitial

pneumonia, recurrent bacterial and other infections, chronic diarrhea, and generalized lymphadenopathy. Growth retardation is common, and microcephaly, developmental delay, progressive encephalopathy, and other neurological abnormalities are often seen. Malformations do not appear to be unusually frequent among the children of HIV-infected women [83,87].

#### 2.3.1.1.5. Parvovirus B19

Parvovirus infection causes fifth disease (erythema infectiosum) in children. Infections in adults may produce a rash or arthropathy but are often asymptomatic. Fetal infection with parvovirus B19 can cause severe anemia, hydrops, and death [88,89]. Many infected fetuses that develop hydrops die, but the hydrops may spontaneously resolve. Less-severely affected fetuses may have meconium peritonitis, isolated ascites, increased nuchal thickness, or pleural or pericardial effusions.

Transplacental parvovirus B19 infection occurs in 17%–33% of cases of maternal primary infection during pregnancy, but in most instances there is no apparent adverse effect on the fetus [90]. The excess fetal loss or hydrops attributable to parvovirus B19 infection during the first 20 weeks of gestation is estimated to be at 13%, while a lower risk (0.5%) is estimated with maternal infection later in pregnancy [90].

Persistent congenital anemia has been observed in infants born after intrauterine parvovirus B19 infection. Myocarditis, myositis, arthrogryposis, and ocular and CNS anomalies have also been reported after documented intrauterine parvovirus B19 infection [88], but such observations are rare. No measurable increase in the frequency of malformations or neurological abnormalities was found among the infants of women who had parvovirus B19 infections during pregnancy in most studies [91,92].

Testing for specific IgM antibody in serum is the standard means of diagnosing parvovirus B19 infection and of distinguishing primary and secondary infection in a pregnant woman. Prenatal diagnosis of

affected fetuses is often possible by ultrasonography and maternal serum alpha-fetoprotein and human chorionic gonadotropin screening. Measurement of IgM in amniotic fluid or fetal blood is not a reliable indicator of fetal infection, but detection of viral DNA in such specimens is useful. Intrauterine transfusion may be beneficial in some cases of fetal hydrops associated with parvovirus B19 infection [88–90,93,94].

#### 2.3.1.2. Bacteria

Although a host of bacterial agents may infect the fetus antenatally, only syphilis is thought to have a significant teratogenic potential. Nevertheless, many intra-amniotic bacterial infections can have devastating fetal consequences. Furthermore, as discussed later, high sustained fever associated with such infections may itself cause damage.

Transplacental transmission of *Borrelia burgdorferi*, the spirochete that causes Lyme disease, has been documented in humans, but whether this causes miscarriage or teratogenic damage to the embryo or fetus is controversial [95–97]. Maternal Lyme disease during pregnancy appears to be associated with very little, if any, increased risk of congenital anomalies in the infant, especially if the maternal illness is treated promptly with appropriate antibiotics.

##### 2.3.1.2.1. Syphilis

Congenital syphilis is certainly the oldest if not the most venerable of the known prenatal teratogenic infections, having been recognized for more than 500 years. The clinical manifestations of prenatal syphilis are related to both the time of gestation in which the fetal infection occurs and the duration of the untreated infection in the mother prior to pregnancy [98]. Although little direct information is available to confirm such a conclusion, it has been generally believed that infection of the fetus usually does not occur before the fourth month of pregnancy. The frequency of congenital syphilis in the child of a woman who has untreated primary or secondary syphilis during pregnancy is 40%–50%. Many such pregnancies are delivered

prematurely, and many of the infants are stillborn or die in the perinatal period. Syphilitic infections later in pregnancy result in lower risks to the fetus, with approximately 70% of late syphilitic infections resulting in the birth of normal healthy infants and only 10% showing signs of congenital syphilis. Fetal growth retardation may occur, and a wide variety of congenital problems may result.

The manifestations in the fetus may be overlooked in the newborn infant, and malformations are not frequent. Signs of syphilis in the child have been grouped into those that present within the first 2 years of life (early congenital syphilis) and those that appear later. Infants with early congenital syphilis are often hydropic, have a relatively large placenta and may display widespread evidence of hematogenous infection such as hepatosplenomegaly, jaundice, generalized lymphadenitis, anemia, thrombocytopenia, and leukemoid reactions. Rhinitis and various other cutaneous and mucosal rashes and lesions – including vesicular or bullous eruptions, maculopapular rashes that may desquamate, mucous patches, petechial lesions, edema, and condylomata lata – may occur in up to 60% of patients. Osteitis is frequent and may mimic an Erb's palsy or present as an irritable infant. Syphilitic nephrosis may appear during the second or third month of life, and other evidence of generalized infection such as bronchopneumonia, failure to thrive, or malabsorption may be encountered. Nervous system manifestations such as meningitis, cranial nerve palsies, intracerebral vascular lesions, and progressive hydrocephalus are frequent. Convulsions and hemiplegia may also occur. There may be a generalized chorioretinitis and uveitis, and optic atrophy, glaucoma, and chancres of the eyelid may be seen [98].

Later manifestations of congenital syphilis, commonly occurring beyond the age of 2 years, are also widespread. The most characteristic features are Hutchinson teeth (peg-shaped and notched permanent upper central incisors), mulberry molars, interstitial keratitis, and sensorineural deafness. Frontal bossing, poor maxillary

growth and saddle nose deformity are frequent craniofacial manifestations, and deep linear facial scars, particularly around body orifices (rhagades) are typical late cutaneous manifestations. Late neurological features include intellectual disability, hydrocephalus, convulsions, cranial nerve palsies, and optic atrophy [98,99].

Prompt treatment of mother and infant is extremely important in congenital syphilis, particularly if the infant is symptomatic. Penicillin is the drug of choice [100].

### 2.3.1.3. Parasites

Although several parasitic agents are known to cross the placenta and infect the fetus, only toxoplasmosis has been clearly shown to produce congenital anomalies. Maternal malarial infection during pregnancy has been associated with fetal growth retardation and premature delivery [101,102]. Fetal infection is usually asymptomatic, although intrauterine or neonatal death may occur [101].

#### 2.3.1.3.1. Toxoplasmosis

The major risk to the fetus arises from primary toxoplasmosis infections during pregnancy. The frequency and severity of fetal effects are strongly related to the stage of gestation in which the exposure occurs. The risk of transmission with primary maternal infection increases from about 1% around the time of conception to more than 90% near term. Most fetal infections do not cause permanent damage, but clinical manifestations of the disease are more likely and more severe with infections earlier in pregnancy. The highest risk for having a baby with severe manifestations is estimated to be 10%–40% with maternal infection between 10 and 24 weeks of gestation. Many more infants, especially those whose mothers acquire *Toxoplasma* infection in the second or third trimester of pregnancy, have mild or subclinical involvement, but these infants may develop chorioretinitis or neurological abnormalities later in childhood if not treated after birth [103–105].

As with other prenatal infections, congenital toxoplasmosis includes

a wide range of clinical manifestations. It is often overlooked in the newborn period because of the subtlety of its signs. Clinically apparent disease is commonly associated with CNS abnormalities. These may include microcephaly, hydrocephalus, intracranial calcification, intellectual disability and seizures. In addition, chorioretinitis, microphthalmia, glaucoma, and cataracts are frequent. Generalized infection may produce hepatitis, thrombocytopenia, pneumonitis, diarrhea, skin rash, or lymphadenopathy. Long-term follow-up has revealed a high percentage of intellectual disability, convulsions, spasticity, visual impairment, and deafness in children with serious toxoplasmosis infections. *Toxoplasma* infection during pregnancy can also cause miscarriage.

Diagnosis of primary maternal infection by *Toxoplasma* is usually based on serological studies. Prenatal diagnosis by amniocentesis can be used to demonstrate most, but not all, fetal infections in pregnant women with primary *Toxoplasma* infections. Ultrasound examination is useful in identifying hydrocephalus, hydrops, and other severe manifestations of fetal infection but is not a reliable means of determining whether transmission of *Toxoplasma* to the fetus has occurred. Prompt treatment of the infant is important when congenital toxoplasmosis is diagnosed at birth. Treatment of the mother is often recommended when maternal *Toxoplasma* infection is documented early in pregnancy. Screening of pregnant women remains controversial and is not generally recommend in North America. The only satisfactory preventive measure is avoidance of *Toxoplasma* infection during pregnancy by avoiding ingestion of infected foods and contact with the oocysts. Generally, this means avoiding handling or eating raw meat (especially red meat) and eggs and avoiding exposure to materials such as cat feces that may contain the oocysts [105,106].

## 2.3.2. Physical Agents

A wide variety of physical agents is potentially teratogenic for the fetus. Among these, the most important are ionizing radiation, mechanical factors, and possibly heat. Although public concern

continues, present evidence does not support any causal relationship between birth defects in humans and commonly encountered low-energy exposures to sound waves, ultrasound examination, magnetic resonance imaging, microwaves, or computer terminals [107–110].

### 2.3.2.1. Ionizing Radiation

Although most exposures to high-energy radiation during pregnancy are avoidable, valid medical indications exist for deliberate exposure of a pregnant woman to ionizing radiation in some cases. When exposure of a pregnant woman to ionizing radiation is necessary, the dosage to the fetus should be minimized.

Very high doses of high-energy radiation (>200 cGy) can produce prenatal-onset growth retardation, CNS damage including microcephaly, and ocular defects [111,112]. Both the rate and fractionation of the radiation dosage are extremely important considerations in determining the associated risk, as slow dosage rates or division of the dose over a period of days markedly reduces the effects of the total dosage. Under ordinary circumstances, only therapeutic levels of radiation delivered to the region of the developing embryo during the first months of pregnancy have a demonstrable risk of significant anomalies for the fetus [111,112].

Much lower doses of radiation, as might occur from gastrointestinal tract radiography, computed tomography scanning, or other diagnostic studies are, of course, far more frequent, but exposure of a pregnant woman to such low radiation doses at any time during gestation produces an extremely low or negligible risk for malformations in the offspring [111,112]. This is not to imply that radiographic exposures can be conducted with total impunity during pregnancy. Even an extremely low risk should be avoided unless there is an important benefit expected.

The mutagenic effect of high-energy radiation is well known. Present evidence suggests that there may be no threshold for this effect. Thus, even low-dose natural exposures or exposures to diagnostic radiation may have potential mutational implications for

an exposed fetus [111,112]. It is estimated that 1 cGy of radiation to a particular gene locus in a particular cell produces a risk of mutation on the order of $10^{-7}$. This would suggest that a germ cell exposed to 1 cGy of radiation may have on the order of 1:1000 chance to have undergone a mutation of some type. However, most such mutations are probably either without phenotypic effect or lethal, so the chance for fertilization of that cell to result in an individual with a genetic disorder is probably substantially less. One would not expect a radiation-exposed fetus to suffer from a genetic disorder produced in this fashion. Rather, potential offspring of that fetus in subsequent generations might manifest the disorder. By implication then, gonadal irradiation at any time before conception may have some small risk of producing a pathogenic mutation in the offspring. The load of such mutations presumably increases throughout a person's lifetime but in the aggregate is still very small in absolute terms under ordinary circumstances.

Concern over the carcinogenic potential of prenatal radiation may be slightly more realistic [113]. However, the magnitude of this risk appears to be less than the risk associated with similar exposures in early childhood [111]. Studies of leukemogenesis stemming from prenatal exposures to X-irradiation suggest that the relative risk from common low-dose exposures is no greater than 1.5 and may be substantially less. In other words, the absolute risk at worst may rise from 1:3000 to 1:2000 chance for such an exposed individual to develop leukemia.

#### 2.3.2.2. Heat

Possible sources of fetal hyperthermia include high fever of any cause and other factors that produce substantial elevation in the maternal body temperature, such as excessive use of a steam bath or hot tub. Studies in laboratory animals strongly suggest that hyperthermia may be teratogenic at critical stages of neural tube development [114,115]. The situation is less clear for humans, but most studies are limited by

poor documentation of the degree and duration of fever or other form of hyperthermia that occurred during pregnancy. In addition, fever usually accompanies serious infections, and such infections or their treatment may also have teratogenic potential in some instances. Nevertheless, an increased frequency of neural tube defects has been found in association with high fever during the period of neural tube closure in some studies [115,116]. A number of anecdotal cases of neuromigrational errors, Moebius syndrome, and neurogenic arthrogryposis have also been reported in the offspring of women who had hyperthermic events during the first or second trimester of pregnancy [115]. The risk associated with substantial hyperthermia during pregnancy has not been clearly defined and probably depends on the severity, duration, and gestational timing of the fever or other exposure. It would seem prudent to avoid extreme prolonged hyperthermic exposures whenever possible during pregnancy.

Serial detailed ultrasound examination is useful for prenatal diagnosis of some, but not all, kinds of severe CNS damage following such episodes, and maternal serum $\alpha$-fetoprotein screening for neural tube defects is indicated if the exposure occurred between 4 and 6 weeks after the last normal menstrual period.

## 2.3.2.3. Mechanical Factors

### 2.3.2.3.1. Constraint

Fetal growth or movement may be constrained by a variety of mechanical factors, including uterine malformations, large uterine myomas, oligohydramnios (whether of maternal or fetal origin), intra-amniotic fibrous bands, or multifetal gestation. It is easy to understand how fetal constraint could result in *deformations*. Such anomalies are the result of mechanical forces that entrap and physically compress structures of the developing fetus [117]. Included in this category are plagiocephaly and such positional limb deformities as clubfoot, dislocated hips, and perhaps arthrogryposis, particularly in predisposed fetuses. Such anomalies could

theoretically be produced at any time during prenatal life and would seem particularly likely to occur if the degree of constraint is severe or if the fetus is predisposed because of neurological impairment. All of these fetal anomalies are etiologically heterogenous, and similar defects may result from mechanisms other than constraint.

Recognition of factors that predispose to fetal constraint, such as uterine malformation, oligohydramnios, or multifetal gestation, should alert the physician to the possibility of adverse consequences to the fetus or newborn. These anomalies are often effectively treated by taking advantage of the relatively normal growth potential of the "deformed" tissues through casting or other essentially mechanical forms of therapy. By the same token, recognition of deformations in the newborn infant should alert the physician to the possibility of fetal constraint in utero. For instance, features of severe in utero compression are a common part of the so-called Potter sequence as a consequence of oligohydramnios, whether of fetal (renal hypoplasia) or nonfetal (premature rupture of the fetal membranes with amniotic fluid leakage) origin. The presence of deformations should alert the practitioner to the possibility of other disturbances of fetal growth that may commonly be associated, such as clubfoot and pulmonary hypoplasia with consequent respiratory distress resulting from oligohydramnios.

#### 2.3.2.3.2. Early invasive prenatal diagnosis

Evidence that the mechanical trauma associated with early chorionic villus sampling (CVS) or amniocentesis may occasionally cause fetal anomalies is of particular concern to medical geneticists. CVS prior to

10–11 weeks' gestation (i.e., during or shortly after formation of the limbs in the embryo) has been associated with an increased risk of limb reduction defects and oromandibular-limb hypogenesis syndrome [118,119]. The risk appears to be greater and the defects more severe with earlier procedures. It is unclear whether an increased risk for fetal limb defects exists with later CVS procedures; if so, the risk is very small [119,120]. There is also some evidence that

cavernous hemangiomas, intestinal atresia, gastroschisis, and clubfoot are more common than expected among children born to women who had CVS [121], but further study is needed to define these risks more clearly and to determine whether they also depend on the gestational age at which the procedure is done.

Two randomized controlled trials of early amniocentesis found a small but significantly increased frequency of talipes equinovarus among infants born to women who had undergone the procedure between 11 and 12 weeks 6 days of gestation [122,123]. The risk was much greater when amniotic fluid leakage occurred following early amniocentesis. Talipes equinovarus does not appear to be more frequent than expected among the children of women who have undergone amniocentesis after 15 weeks' gestation.

## 2.3.3. Drug and Chemical Agents

Health professionals who care for pregnant women often must deal with their concerns about possible teratogenic effects of drug and other chemical exposures. Far too little is known about the teratogenic potential of most drug and chemical exposures. Several major categories of agents are considered in this section: environmental chemicals, nonprescription drugs, and prescription drugs.

### 2.3.3.1. Environmental Agents

Recognition of the pollution of our environment by an ever-proliferating group of compounds has caused serious concern regarding the potential impact on the developing fetus. Relatively little is known about the teratogenic potential of many of these exposures in humans. Fortunately, however, efforts to limit exposures for the public at large and for occupationally exposed adults provide a measure of protection for the fetus as well. However, organic mercury compounds demonstrate the teratogenic potential of environmental exposures.

#### 2.3.3.1.1. Organic mercury compounds

Ingestion by a pregnant woman of food that is heavily contaminated with methylmercury can cause severe damage to the developing CNS of her fetus [124–126]. Clusters of affected infants were observed after maternal consumption of methylmercury-contaminated fish in Minamata, Japan, and of grain treated with methylmercury fungicides in Iraq. Affected children had severe CNS damage and microcephaly, resulting in a static encephalopathy. Maternal neurotoxicity often occurred in these cases as well, but the fetus appears to be more sensitive than the adult to this effect. Malformations were not unusually frequent in affected infants.

Subtle effects on fetal CNS development of much lower maternal exposures to organic mercury compounds, as may occur when the mother eats large amounts of swordfish or shark, have been reported in some studies but not others [125]. These studies remain controversial, but the US Food and Drug Administration has advised pregnant women to eat fish with lower rather than relatively high mercury contents (https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm397443.h

#### 2.3.3.1.2. Other environmental chemicals

Many natural and man-made chemicals have been shown to function as "endocrine disrupters" in experimental systems, and some adverse reproductive effects in wildlife have been linked to the presence of these chemicals in the environment [127–131]. Although similar adverse effects, including teratogenesis, have been suggested in humans, a measurable impact at the levels of exposure usually encountered seems unlikely [132].

There has been considerable concern about maternal exposures to other environmental contaminants, but there is no compelling evidence of teratogenic effects of such exposures in humans. Adequate studies are often difficult to perform because of uncertainty about the magnitude of individual exposures and the causal heterogeneity of birth defects. Avoiding toxic exposures during pregnancy as much as possible is a prudent precaution.

### 2.3.3.2. “Recreational” Drugs

Among the host of drugs that individuals in our society habitually use and abuse, alcohol and tobacco stand out as the most important public health problems for both adults and the developing embryo or fetus. Adverse effects on the offspring have also been associated with maternal abuse of cocaine and some other “recreational” drugs during pregnancy. According to the 2012 US Survey on Drug Use and Health, 5.9% of pregnant women use illicit drugs, 8.5% drink alcohol, and 15.9% smoke cigarettes [133].

#### 2.3.3.2.1. Ethyl alcohol

Maternal ethanol consumption during pregnancy can result in a wide spectrum of effects on the embryo and fetus. The frequency and severity of these anomalies appear to be dose related. They range from children with mild neurobehavioral problems to severely affected individuals with the fetal alcohol syndrome. Full fetal alcohol syndrome occurs among infants born to chronically alcoholic women, but there is considerable disagreement about the amount of ethanol necessary to cause milder degrees of fetal damage [134–138]. Some evidence suggests that consumption of as little as two drinks per day or periodic binge drinking (e.g., five or more drinks on a single occasion) in early pregnancy may be associated with recognizable (though milder) abnormalities in a substantial percentage of exposed newborns. Typical fetal alcohol syndrome occurs in about 6% of children of women who drink heavily during pregnancy, but the risk is much higher for alcoholic women who have already had an affected child. Less severe alcohol-related birth defects and neurocognitive deficits occur in a much larger proportion of these children, but the estimated frequency varies widely in different studies. The effects are less severe among the children of alcoholic women who stop drinking early in pregnancy.

The fetal alcohol syndrome is characterized by a distinctive facial appearance, prenatal-onset growth deficiency, intellectual disability, and an increased frequency of major congenital anomalies [135,139–142]. Children with this syndrome display a flat midface

with narrow palpebral fissures, a low nasal bridge, short upturned nose, and long smooth philtrum with a narrow vermilion border of the upper lip (Fig. 2.4). Presenting as small-for-gestational-age babies in the neonatal period, they continue to grow poorly and often are admitted to the hospital for evaluation of "failure to thrive." They may be described as jittery babies, a feature that often results in confusion with drug withdrawal symptoms. However, these neurological abnormalities persist, and, in addition to having developmental delay, such children are often poorly coordinated, tremulous, and sometimes hyperactive in later life. A wide variety of congenital anomalies has been associated with this syndrome, including cleft palate, cardiac malformations (especially atrial and ventricular septal defects), microphthalmia, joint anomalies, and a variety of dermal and skeletal abnormalities. Cranial MRI often shows abnormalities of neuronal migration, occasionally associated with microcephaly, hydrocephalus, absence of the corpus callosum, other midline anomalies, or cerebellar abnormalities [144,145].

Epidemiological studies suggest that prenatal damage from maternal alcohol abuse may be one of the most frequent recognizable causes of intellectual disability in the United States [146]. Associations with lower IQ scores, poor school performance, attention-deficit/hyperactivity disorder (ADHD), autism, impaired memory, and poor executive functioning have been reported [147,148]. Disabilities due to prenatal alcohol abuse are an important public health problem [146] – an observation that is particularly distressing because virtually all such exposures are avoidable. Pregnant women, or women who might become pregnant, should avoid drinking alcohol as much as possible – the safest amount is none at all. Continuing to work on developmental screening and intervention programs is a public health priority.

#### 2.3.3.2.2. Tobacco smoking

Maternal smoking during pregnancy interferes with fetal growth [149,150]. Birth weight and length and head circumference may be decreased in exposed babies. This growth deficiency is dose related

and reversible in early childhood, although obesity appears to be more frequent than expected among the offspring of women who smoked during pregnancy [151]. Women who smoke heavily during pregnancy also have higher than expected rates of spontaneous abortion, late fetal death, neonatal death, and prematurity [152].

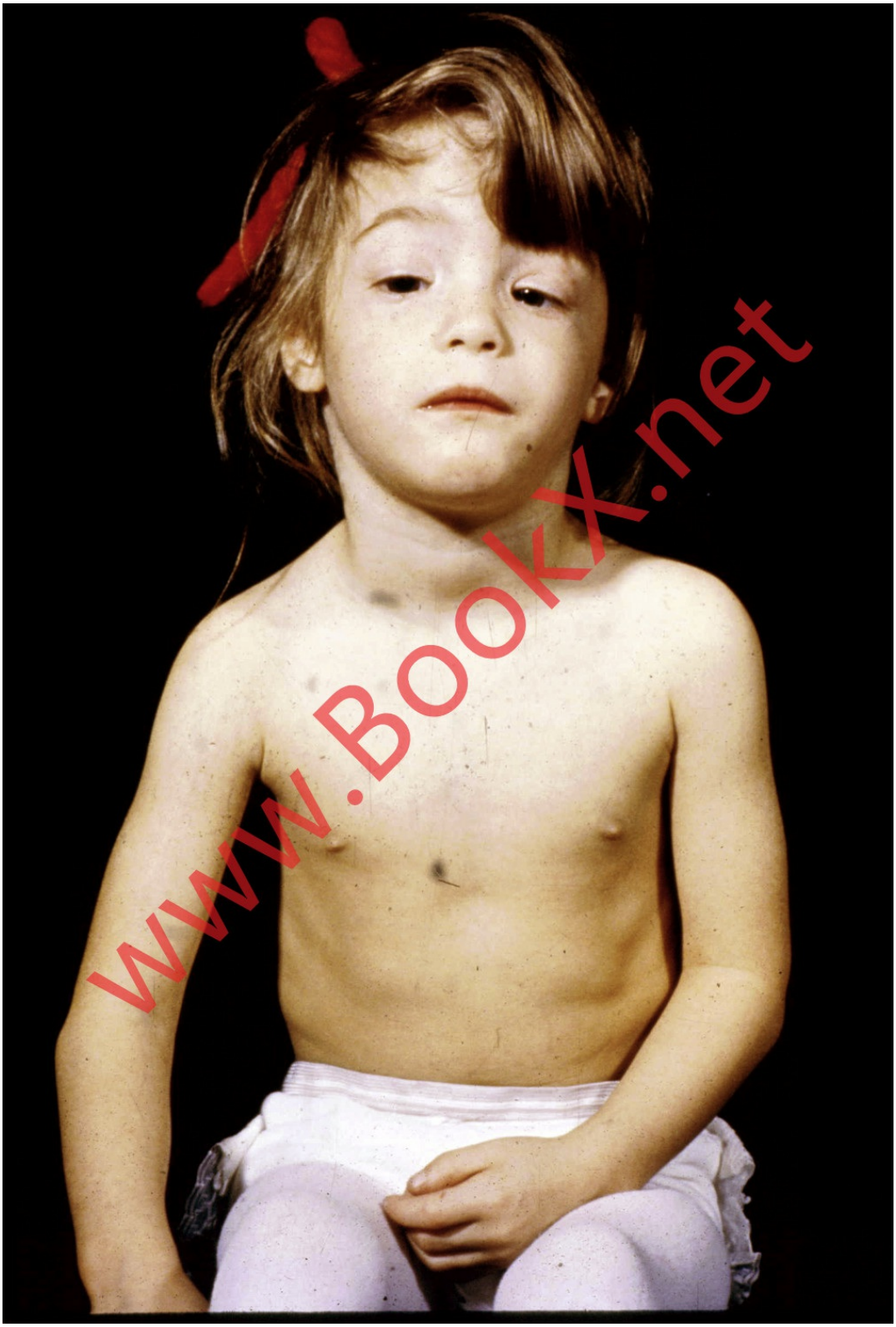

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**FIGURE 2.4** Fetal alcohol syndrome, note the short palpebral fissures; long, smooth philtrum; thin vermilion border; maxillary hypoplasia; and ptosis.

From Jones KL, Jones MC, Del Campo M. Smith's Recognizable Patterns of Human Malformation, Seventh Edition, with permission.

Maternal smoking during pregnancy has also been associated with a small increase in the frequencies of some birth defects, especially orofacial clefts [153]. The risk appears to be greater among a subset of infants who have genetic variants that predispose to orofacial clefting [154,155]. Slightly lower measured intelligence levels and increased frequencies of hyperactivity have also been reported in prenatally exposed children [156,157]. Pregnant women should be advised to avoid smoking. Smokers who are unable to stop should be advised to reduce their smoking as much as possible, as this appears to improve fetal outcome.

#### 2.3.3.2.3. Cocaine

The extensive medical literature on the effects of maternal cocaine use in pregnancy is difficult to interpret. Most studies are bedeviled by confounding factors, deficiencies in design, and poor documentation of the frequency, timing, and dosage of the mothers' use of cocaine, other illicit drugs, and alcohol. Moreover, there appears to be a systematic publication bias in favor of studies that show an association and against those that do not find an association between maternal cocaine use and untoward pregnancy outcomes [48,158].

Vascular disruptions occur with increased frequency in infants whose mothers abused cocaine during pregnancy, especially in the second or third trimester. Involvement of the CNS has been reported most often. Other congenital anomalies thought to result from vascular disruption – segmental intestinal atresia, gastroschisis, sirenomelia, limb-body wall complex, and limb reduction defects – have also been associated with maternal abuse of cocaine during pregnancy in some studies [159]. Associations with other kinds of congenital anomalies have been reported as well, but the findings are inconsistent. Cocaine's vasoconstrictive and hypertensive actions probably account for the increased frequency of placental abruption observed among pregnant women who abuse this drug [160]. Associations with preterm birth, low birth weight, and small for

gestational age infants have also been reported [161].

Prenatal growth retardation has consistently been noted among infants whose mothers abused cocaine during pregnancy, but the effect appears to be due at least in part to concomitant exposure to alcohol or cigarette smoking [136]. By school age, the growth of these children is indistinguishable from controls in most studies [162].

Many studies have demonstrated a subtle pattern of neonatal behavioral abnormalities among infants born to women who abused cocaine during pregnancy [159]. This pattern has been characterized as affecting "the four As of infancy": attention, arousal, affect, and action. Studies in older children have been inconsistent, and interpretation is confounded by other differences that distinguish mothers who abuse cocaine during pregnancy from other women [159,162–164]. Contingency management appears to be a promising intervention for pregnant women using cocaine, along with cognitive-behavioral therapy and motivational interviewing [165].

#### 2.3.3.2.4. Caffeine

While caffeine is teratogenic in high doses in some species, no convincing evidence linking this substance to human congenital anomalies has emerged [166,167]. Associations between maternal coffee drinking during pregnancy and miscarriage or poor fetal growth have been repeatedly observed in epidemiological studies, but these studies are often confounded by cigarette smoking and other factors [166,168]. It is unlikely that moderate coffee drinking by pregnant women adversely influences fetal growth or the rate of miscarriage, but it is sensible for pregnant women to avoid excessive caffeine intake.

### 2.3.3.3. Nonprescription Drugs

Over-the-counter preparations are widely used for the treatment of viral illnesses, allergies, headache, aches and pains, sleep disturbances, anxiety, and gastrointestinal discomfort. Little formal evaluation has been conducted of the potential reproductive toxicity of most over-the-counter drugs. In the absence of specific information,

it seems prudent to avoid these medications whenever possible, particularly in the earliest part of pregnancy.

#### 2.3.3.3.1. Salicylates and other anti-inflammatory drugs

Although aspirin and other salicylate compounds are believed to be among the safest and most effective drugs in the marketplace, excessive use during pregnancy may be associated with an increased risk of fetal hemorrhages, and limited evidence suggests that chronic aspirin consumption may interfere with fetal growth [169,170]. Malformations in general do not appear to be associated with the prenatal use of salicylates [169,171], but there is evidence that gastroschisis may be about 3 times more common among the infants of mothers who take aspirin early in pregnancy [169]. No consistent evidence of an effect on intelligence has been identified [172,173].

Epidemiological studies of children whose mothers took acetaminophen during the first trimester of pregnancy have found no consistent association with birth defects [174,175]. However, weak associations with hyperkinetic disorder or attention deficit hyperactivity disorder as well as psychomotor, behavioral, and temperament problems have been suggested in some studies [176–178].

The available data regarding maternal use of ibuprofen during pregnancy remain limited, but treatment during the first trimester seems unlikely to pose a major teratogenic risk [179,180]. A small increase in certain kinds of cardiac malformations, oral clefts, neural tube defects, or amniotic bands/limb body defects has been found in some studies but not others. Neonatal renal failure, oligohydramnios, and in utero closure of the ductus arteriosus have been associated with maternal ibuprofen treatment to arrest premature labor later in pregnancy [181].

### 2.3.3.4. Prescription Drugs

Half of all pregnant women take at least one prescription medication during the first trimester [182,183]. Treatment with some prescription medications is potentially teratogenic at conventional therapeutic

doses. Other drugs can be used safely during pregnancy, and in some instances such treatment is highly beneficial to both the mother and the fetus. Unfortunately, however, available data are insufficient to determine whether maternal treatment with most prescription drugs during pregnancy poses a substantial teratogenic risk. Postmarketing studies of prescription drugs for potential teratogenicity need to be greatly expanded to permit more adequate counseling of pregnant women. In the absence of specific information strongly supporting the safety of treatment with a particular agent during pregnancy, prudence dictates that such treatment be avoided whenever possible. All women of reproductive age who are given prescription drugs should be counseled regarding potential teratogenic effects, because many exposures occur when women become pregnant unintentionally or before they recognize that they are pregnant.

#### 2.3.3.4.1. Thalidomide

The most dramatic epidemic of drug-induced birth defects ever recognized occurred in the early 1960s when thalidomide was sold in several countries, but not the United States, as an over-the-counter sedative. More than 10,000 babies were damaged by this drug between 1958 and 1963. The association between maternal thalidomide treatment and birth defects was independently noted by Widukind Lenz [183a] and William McBride [183b]. Subsequent studies indicated that the susceptible period for the embryo was between 20 and 35 days after conception [16].

The thalidomide embryopathy includes a very unusual and characteristic pattern of congenital anomalies [42]. The manifestations depend primarily on the stage of embryonic development at which exposure occurred. Typical anomalies are phocomelia and other limb reduction malformations, anomalies of the external ear, ocular anomalies, and cardiovascular malformations ranging from septal defects to complex conotruncal defects. Involvement of the CNS and other organ systems may occur but is less common.

Discovery of thalidomide's immunomodulatory action has led to its

use to treat a variety of neoplasms and immunopathic disorders. Currently, it is being prescribed under strict controls designed to avoid use by pregnant women.

#### 2.3.3.4.2. Folic acid antagonists

##### 2.3.3.4.2.1. Aminopterin and methotrexate

An unusual and characteristic pattern of congenital anomalies has been reported in more than two dozen children whose mothers took aminopterin or methotrexate during pregnancy. In addition, to its use as an antineoplastic agent, methotrexate is used in the therapy of rheumatoid arthritis and other immunopathic diseases.

Children with aminopterin or methotrexate embryopathy have distinctive craniofacial anomalies with abnormal head shape and ocular hypertelorism, shallow orbits, mild midfacial hypoplasia, micrognathia, cleft palate, and facial asymmetry [184–186]. Malformations of the auricles, skin tags, and numerous skeletal anomalies, especially vertebral segmentation abnormalities with anomalous ribs, abnormalities of ossification of sacral structures, ectrodactyly, syndactyly, longitudinal limb reduction malformations, and positional limb deformities are common. CNS abnormalities may occur, and developmental delay is the rule in childhood, but affected adults may have normal or only mildly reduced intelligence. A different pattern of anomalies with tetralogy of Fallot and renal and limb malformations predominating may occur with earlier exposure in pregnancy [187,188]. Women who are treated with methotrexate or aminopterin in pregnancy have increased rates of miscarriage, early fetal growth retardation, stillbirth, and neonatal death.

The risk of a teratogenic effect after first-trimester maternal treatment with methotrexate is probably dose related, and much lower doses of the drug are used to treat immunopathic diseases than neoplasia. Most babies born to women treated with low-dose methotrexate during pregnancy appear normal at birth [189], but typical folic acid embryopathy has been reported even with low-dose treatment [190,191]. This may reflect a particular genetic susceptibility.

#### 2.3.3.4.2.2. Trimethoprim and other weak folic acid antagonists

An increased frequency of birth defects has been observed among the infants of women who were treated with trimethoprim, an antibiotic that acts as a weak dihydrofolate reductase inhibitor, during the first trimester of pregnancy. The risk among these infants appears to be greatest for neural tube defects, cardiovascular malformations, oral clefts, and urinary tract defects, which occur 2–5 times more often than expected [192–197]. Similar associations have been found among infants whose mothers were treated early in pregnancy with other weak folic acid antagonists such as triamterene, sulfasalazine, and anticonvulsant agents. These risks are reduced if the mother also takes a folic acid supplement early in pregnancy [194,198,199].

### 2.3.3.4.3. Anticancer agents

As the therapeutic efficacy of many antineoplastic agents is dependent on their ability to kill or suppress the growth of cells and many other agents block signaling pathways that are critical to normal embryonic development, treatment with any of these agents should be regarded as potentially teratogenic. The strong folic acid antagonists and several of the alkylating agents are of particular concern. Maternal treatment early in pregnancy with various cancer chemotherapeutic agents has been anecdotally associated with a variety of fetal anomalies [185,200]. The risk related to such exposures varies with the drug or (more often) combination of drugs used and with the dose, route, and gestational timing in a complex way that is unique for almost every woman. Early pregnancy appears to be a particularly hazardous time with respect to teratogenic risk, but providing a precise estimate of the risk to an individual patient is rarely possible.

Unlike most kinds of drug therapy, cancer chemotherapy is often given at the maximum dose tolerated by the mother (i.e., the doses are near or within the range of maternal toxicity). Under such conditions, treatment may carry a substantial risk of teratogenic effects. Nevertheless, apparently normal children have been born to women who underwent cancer chemotherapy during the first trimester or later in pregnancy [185,200–202]. In general, the risk of miscarriage

appears to be increased with many kinds of cancer chemotherapy during the first trimester of pregnancy, and the risks of premature delivery and of fetal death, growth retardation, and myelosuppression may be increased with treatment later in pregnancy [185,200–202]. Women who are undergoing cancer chemotherapy should be advised to avoid pregnancy during the period of treatment. Patients who require such treatment during pregnancy should receive appropriate counseling regarding the risk of congenital anomalies and other adverse effects in exposed offspring.

#### 2.3.3.4.4. Warfarin anticoagulants

A very uncommon and striking pattern of congenital anomalies has been reported repeatedly among children whose mothers were treated with coumarin derivatives (e.g., warfarin) during pregnancy (Fig. 2.5) [204,205]. Affected children typically have prenatal-onset growth deficiency, developmental delay, intellectual disability, and abnormal facial features with severe nasal hypoplasia. Microcephaly and optic atrophy have been repeatedly observed. Radiographic studies often reveal stippling of epiphyseal growth centers. Children with these abnormalities often have a poor outcome.


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**FIGURE 2.5** Fetal warfarin syndrome, note severe nasal hypoplasia. From Pauli, RM, Madden, JD, Kranzler, KJ, Culpepper, W, Port, R. Warfarin therapy initiated during pregnancy and phenotypic chondrodysplasia punctata. J Pediatr 1976;88, 506–508, with permission.

Children with similar defects whose mothers did not take coumarin

derivatives during pregnancy have been described who have a genetic disorder of vitamin K–dependent coagulation factors [206,207], suggesting that bleeding into developing tissues may be pathogenic in coumarin embryopathy.

The frequency of coumarin embryopathy among infants of women who are treated with warfarin throughout pregnancy has been estimated to be 3%–6% [208,209], with the risk increasing with treatment dose and duration [210]. Miscarriage appears to occur more often than expected when the mother is treated with coumarin derivatives early in pregnancy. Stillbirth and fetal, placental, and neonatal hemorrhage are substantially more frequent when the mother is treated with coumarin anticoagulants late in pregnancy [209].

#### 2.3.3.4.5. Antibiotics and other anti-infective agents

Available studies have generally not revealed an increased risk to the fetus with prenatal treatment with most anti-infective agents, including penicillins, sulfonamides, cephalosporins, or related agents [193,211,212]. Exceptions to this generalization are treatment with trimethaprim, a folic acid antagonist discussed earlier, or with tetracyclines, aminoglycosides, high-dose quinine, or high-dose fluconazole. Most newer antibacterial and antiviral agents have not been adequately studied with regard to their teratogenic potential in human pregnancy.

##### 2.3.3.4.5.1. Tetracyclines

Maternal treatment with tetracyclines during the second and third trimesters of pregnancy produces staining of the infant's primary teeth [213]. The risk of malformations does not appear to be increased among the children of women who were treated with tetracyclines during pregnancy in most studies, but a 7-fold increased risk of isolated cleft lip with or without cleft palate was observed in the infants of mothers treated with tetracycline or doxycycline, a closely related drug, in the second month of pregnancy in a Danish record-linkage study [214].

#### 2.3.3.4.5.2. Aminoglycosides

There is no indication that the risk of malformations in children of women treated with aminoglycosides, such as gentamicin, streptomycin, or tobramycin, during pregnancy is likely to be great [215]. However, several cases of sensorineural deafness, sometimes with accompanying vestibular dysfunction, have been reported in children whose mothers were treated during pregnancy with streptomycin [216], and asymptomatic abnormalities of auditory or vestibular function have been observed in up to 10% of such children [217].

#### 2.3.3.4.5.3. Quinine and related antimalarial agents

Maternal use of very high doses of quinine in an attempt to induce abortion hs been associated with deafness and optic nerve abnormalities in the offspring in case reports and clinical series [218]. A causal association seems possible because quinine treatment, especially in large doses, may cause auditory and visual damage in adults. Such abnormalities do not appear to be unusually common among the children of pregnant women who are given much lower doses of quinine to treat malaria or who take the usual prophylactic or therapeutic doses of chloroquine [219,220].

#### 2.3.3.4.5.4. Fluconazole

A few children have been described with a very unusual pattern of congenital anomalies whose mothers were treated for coccidioidomycosis meningitis during the first trimester of pregnancy with daily high-dose fluconazole [221]. The features in these infants include brachycephaly, abnormal calvarial development, cleft palate, arthrogryposis, and congenital heart disease. The pattern of anomalies resembles the Antley-Bixler syndrome, an autosomal recessive condition, but the occurrence of a similar rare pattern of congenital anomalies in children whose mothers received the same, very unusual treatment during pregnancy suggests a causal relationship. Epidemiological studies of maternal low-dose fluconazole treatment for vaginal candidiasis during pregnancy have been inconsistent, but

if there is an increased risk of malformations, it is likely to be minimal [222–224].

#### 2.3.3.4.6. Anticonvulsants

About 2% of women take anticonvulsant medications during pregnancy [225]. Treatment of pregnant women with a number of different anticonvulsant medications is associated with a risk for congenital abnormalities that is 2–3 times that of the normal population. The risk is higher in women who require treatment with multiple anticonvulsants, especially combinations that include valproic acid, than in those who are adequately treated with a single medication [226–231]. Some anticonvulsant agents are also used for treatment of bipolar disorder, neurogenic pain, and other conditions, but it is not known if the teratogenic potential of these drugs is different when they are used during pregnancy for indications other than control of seizures.

It seems prudent to suggest that all epileptic women be cautioned before beginning pregnancy about the potential adverse outcomes for their offspring. In some instances, a trial off drug therapy before conceiving may be warranted. In other cases, when it is deemed unsafe to take a woman off anticonvulsant therapy, she should be placed on the smallest number of agents and the lowest dosages compatible with adequate seizure control, and the potential hazards of anticonvulsant therapy to her offspring should be discussed in detail.

Maternal serum $\alpha$-fetoprotein measurement and detailed ultrasound examination are useful for prenatal diagnosis of fetal neural tube defects associated with maternal valproic acid or carbamazepine treatment during pregnancy [232]. Some other serious malformations have also been diagnosed prenatally in fetuses of women treated with anticonvulsant medications during pregnancy, but the subtle dysmorphic syndromes and functional deficits that occur more frequently in these children than major malformations cannot usually be identified before birth.

Many of the same major malformations and a similar pattern of

minor anomalies, sometimes called the "fetal anticonvulsant syndrome," have been reported among children of epileptic women treated with a variety of anticonvulsant medications [233]. The discussion that follows emphasizes these similarities as well as some major congenital anomalies that are only associated with maternal use of particular drugs during pregnancy. The influence of prenatal anticonvulsant treatment on behavioral and cognitive outcome in children is also of concern, especially with maternal valproic acid treatment, where there is clear evidence of detrimental effects on the child's IQ and neurodevelopment [234].

#### 2.3.3.4.6.1. Valproic acid

A characteristic pattern of craniofacial and other anomalies has been observed in up to half of children born to epileptic women treated with valproic acid during pregnancy [233,235,236]. Features of this "fetal valproate syndrome" include abnormalities of the skull with metopic ridging, trigonocephaly, narrow bifrontal diameter, relative deficiency of the outer orbital region, midfacial hypoplasia, short upturned nose with a broad flat bridge, and long flat philtrum with a thin vermilion border of the upper lip.

Major malformations occur in about 10% of infants whose mothers are treated with valproic acid for epilepsy during the first trimester of pregnancy, a rate that is higher than that associated with most other anticonvulsants [229,235,237,238]. The risk of spina bifida is about 2% among these children, but the risk for anencephaly does not appear to be increased. Beyond the risk for malformations, maternal valproic acid treatment during pregnancy has been associated with an increased risk of cognitive and behavioral deficits, including ADHD and autism spectrum disorders (ASDs) [234]. Inherited variations in maternal drug metabolism may increase the susceptibility of certain pregnancies to these adverse effects [235,239].

#### 2.3.3.4.6.2. Carbamazepine

A fetal anticonvulsant syndrome has also been observed among the children of epileptic women who were treated with carbamazepine

during pregnancy [233]. The features include minor craniofacial anomalies, fingernail hypoplasia, and delayed growth and development. The frequency of major malformations among the children of epileptic women treated with carbamazepine during pregnancy appears to be similar to that observed in children of women treated with other anticonvulsants except that spina bifida occurs in about 1% of children whose mothers took carbamazepine while they were pregnant [229,237,238]. Dietary supplementation with folic acid is generally recommended for women of reproductive age with epilepsy taking carbamazepine to reduce the risk of neural tube defects in their children. Studies of carbamazepine's effect on cognitive development have been inconsistent [234].

#### 2.3.3.4.6.3. Other anticonvulsant agents

Phenobarbital is not often used as an anticonvulsant in adults, but primidone, which is partially metabolized to phenobarbital, is sometimes used. In most studies, the frequency of congenital anomalies among children of epileptic women treated with these drugs during pregnancy is similar to that observed with other anticonvulsants and greater than that expected without such treatment [229,237,238,240]. The most prominent malformations associated with prenatal phenobarbital treatment are facial clefts and heart defects. A distinctive pattern of minor dysmorphic features and poor growth (i.e., a "fetal anticonvulsant syndrome") occurs in some children whose mothers were treated for epilepsy with either phenobarbital or primidone during pregnancy [233,241].

Insufficient data are available to determine the risk of congenital anomalies in the children of pregnant women treated with the succinimide anticonvulsants, ethosuximide, or methsuximide. Treatment of maternal epilepsy with lamotrigine during pregnancy appears to be associated with similar rates of congenital anomalies among the infants as treatment with older anticonvulsants [229,237,238]. Information available on the teratogenic potential of other more recently-developed anticonvulsants, including vigabatrin, felbamate, gabapentin, clobazam, and clonazepam, is limited, but

recent data on topiramate treatment in pregnancy suggests a slight teratogenic potential, particularly in increasing the risk of oral clefting [242,243]. Registries have been established in the United States (http://www.mgh.harvard.edu/aed/) and other countries to collect and disseminate information on the teratogenic potential of maternal treatment with anticonvulsant agents during pregnancy.

#### 2.3.3.4.7. Endocrine agents

A number of reports have suggested possible teratogenic effects of treatment with endocrine agents. These medications are often taken by women of reproductive age.

##### 2.3.3.4.7.1. Female sex hormones

**Diethylstibestrol**. Diethylstilbestrol (DES) was widely used in the 1950s as a treatment for threatened abortion and for estrogen replacement during pregnancy. The daughters of women who received such treatment during pregnancy have a greatly increased risk of gross structural anomalies of the uterus and vagina and of developing clear cell adenocarcinoma of the vagina or cervix [244,245]. At least one-third to one-half of women who were exposed in utero to DES have gross or histological abnormalities of the genital tract, but the absolute risk of developing vaginal malignancy is, fortunately, quite small. Ectopic pregnancy, miscarriage, and premature delivery also occur with increased frequency among "DES daughters." The sons of women who were treated with DES during pregnancy have higher than expected frequencies of epididymal cysts, hypoplastic testes, and cryptorchidism, but fertility and sexual function are usually not impaired [245].

**Other estrogens and progestins**. Other estrogens have *not* been clearly associated with similar risks.

Maternal treatment during pregnancy with high doses of androgenic progestins, especially norethindrone, is associated with masculinization of the external genitalia in female fetuses. The degree of masculinization depends upon the time of treatment and is unlikely to occur after the 12th week of gestation. The magnitude of this risk is

no more than 1% with high doses and is less with lower doses [246]. Despite reports associating maternal progestin use during pregnancy with various other malformations in the offspring, no consistent pattern of abnormalities has emerged, and most epidemiological studies have failed to confirm such associations.

Studies claiming a relationship between maternal use of combinations of estrogens and lower-dose progestins in the form of contraceptive agents and various congenital anomalies infants have been reported, but the results have generally not been reproducible [246,247]. A woman who has inadvertently become pregnant while taking an oral contraceptive can be reassured that her use of birth control pills is very unlikely to have harmed the fetus.

#### 2.3.3.4.7.2. Other endocrine-active agents

**Clomiphene**. Induction of ovulation with clomiphene has been associated with an increased risk of neural tube defects in some studies, but most investigations do not show such an association [248,249]. The effects of treatment with this agent are often confounded with the effects of underlying maternal disorders that led to the need for therapy.

From 5% to 10% of pregnancies that result from ovulation induced by clomiphene treatment are multifetal [250,251], and these twin, triplet, or higher multiple pregnancies are at increased risk for premature delivery and positional limb deformations.

**Male sex hormone agents**. Maternal treatment with androgens during pregnancy can cause masculinization of the external genitalia of female fetuses [246]. The effect appears to be dose related. Similar masculinization of female fetuses may occur after maternal treatment with large doses of anabolic steroids such as danazol that have androgenic and antiestrogenic activity [252].

**Corticosteroids**. Some epidemiological studies have found an association of maternal treatment with systemic corticosteroids early in pregnancy and orofacial clefts in the offspring [253–255]. Even if this association is real and causal, the absolute risk for orofacial clefts among children of women who are treated with systemic

corticosteroids during the first trimester would be less than 1% [256]. The risk for malformations of other kinds does not appear to be substantially increased in these children [257].

**Antithyroid agents**. Maternal treatment during pregnancy with antithyroid agents such as radioactive iodine ($^{131}I$), propylthiouracil, or methimazole is associated with an increased risk of hypothyroidism and consequent intellectual disability in the offspring [258]. In addition, it appears that maternal treatment with methimazole or the related thioamide, carbimazole, during pregnancy may occasionally cause cutis aplasia of the scalp or, rarely, a methimazole embryopathy in the fetus [259,260]. The features of this unusual pattern of anomalies include developmental delay, cloanal atresia, esophageal atresia, absent or hypoplastic nipples, and scalp defects. Congenital goiter, hypothyroidism, or both may also occur among the children of women treated with methimazole or carbimazole during pregnancy [261].

Iodides have the potential for producing neonatal goiter and hypothyroidism, particularly when taken by a pregnant woman after the first trimester [258]. Fetal goiter can also be produced by maternal treatment with other medications, such as amiodarone, that contain large amounts of iodine [262].

#### 2.3.3.4.8. Retinoids and vitamin A

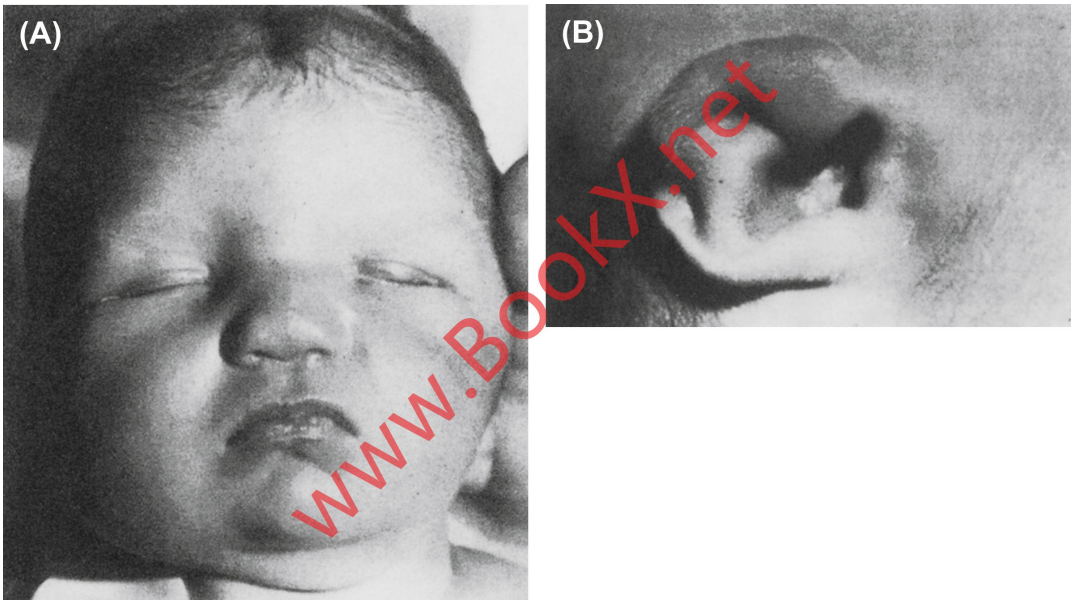
Preformed retinoids, including retinol, retinaldehyde, and retinoic acid, possess vitamin A activity directly. They are contained in substantial amounts in some foods, especially liver. Isotretinoin, etretinate, and acitretin are synthetic retinoid congeners that are used orally and topically to treat a variety of skin disorders. Absorption of the topical forms is usually quite limited. β-Carotene and related carotenoids, which are contained in many orange or dark green leafy vegetables, can be metabolized by the body to vitamin A.

A characteristic retinoid embryopathy may occur in children of women who are treated orally with isotretinoin during the first trimester of pregnancy [43,263]. Typical craniofacial anomalies include microcephaly, facial asymmetry with midfacial hypoplasia and facial

nerve palsy (Fig. 2.6A), microphthalmia, cleft palate, micrognathia, and microtia (Fig. 2.6B) or anotia. CNS abnormalities and cardiovascular malformations, especially conotruncal defects, are common. Thymic hypoplasia and genitourinary anomalies, including hypoplastic kidneys and hydroureter, are also frequent. Children with isotretinoin embryopathy who survive often have intellectual deficiency [266]. Clinical series and anecdotal evidence suggest that etretinate, acetretin and preformed vitamin A itself in very high doses may have similar teratogenic potential [263,267,268].

Dosage and timing are major factors, the highest risk being with maternal isotretinoin treatment between 2 and 5 weeks after conception. However, it should be noted that both etretinate and retinol have half-lives of weeks to months, implying longer risk periods after discontinuation of use and the possibility of substantial bioaccumulation [269]. The half-life of isotretinoin is much shorter – less than 1 day.

Women who are pregnant or may become pregnant should not be treated systemically with isotretinoin or related drugs. Despite a program designed by the manufacturer to prevent use of isotretinoin in pregnancy, substantial numbers of fetal exposures continue to occur [270–272]. Liver contains large amounts of retinol, and it has been recommended that pregnant women avoid eating excessive amounts of liver during pregnancy [273]. Ingestion of β-carotene, even in high doses, has not been associated with a teratogenic risk [268,274]. Vitamin supplements containing β-carotene are, therefore, preferable to those containing preformed vitamin A for women of reproductive age.



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**FIGURE 2.6** Isotretinoin embryopathy. (A) Dysmorphic facial features. (B) Microtia.
(A) From Lammer, EJ. Patterns of malformation among fetuses and infants exposed to retinoic acid (isotretinoin), Banbury report 26: developmental toxicology: mechanisms and action. Cold Spring Harbor Laboratory; 1987. p. 213–255, with permission; (B) From Fernhoff, PM, Lammer, EJ. Craniofacial features of isotretinoin embryopathy. J Pediatr 1984;105, 595–597, with permission.

### 2.3.3.4.9. Lithium carbonate

Children of women who are treated with lithium during pregnancy appear to have an increased risk of Ebstein anomaly and other cardiac malformations, although epidemiological studies indicate that this risk is likely to be small [25–27]. Fetal echocardiography should be offered to women who are treated with lithium during the first trimester of pregnancy.

Treatment of severe depression in pregnant women and those of reproductive age presents a clinical dilemma. The small but serious teratogenic risk associated with lithium use may need to be balanced against a substantial risk to the mother's health if treatment is stopped and with other possible maternal or fetal risks if alternative treatments are used [275,276].

### 2.3.3.4.10. Selective serotonin reuptake inhibitors

Selective serotonin reuptake inhibitors (SSRIs) are very widely used as antidepressants. These drugs are also given to treat obsessive compulsive disorder, panic disorders, and other psychiatric illnesses. Available literature suggests that the frequency of major malformations in general and or cardiac malformations in particular may be increased by a small amount over the background population risk following maternal treatment with citalopram/escitalopram, fluoxetine, paroxetine, or sertraline in early pregnancy. Maternal treatment with paroxetine also appears to be associated with a slightly higher risk for any birth defect, but particularly for heart defects, in comparison to treatment with other SSRIs [277–279].

Abnormalities of neonatal adaptation, as well as other neonatal complications, such as prematurity and persistent pulmonary hypertension of the newborn, may occur in infants whose mothers were treated with an SSRI near the time of delivery [277,280]. Furthermore, maternal SSRI treatment in late pregnancy has also been associated with neurodevelopmental disorders in childhood, specifically ASDs [277,281].

It is important to assess individual needs, provide comprehensive counseling and support, and discuss treatment options on a case-by-case basis for pregnant women with psychiatric illness. Fetal echocardiography should be offered to pregnant women who have taken an SSRI in early pregnancy, and detailed prenatal ultrasound examination should be offered to monitor for other fetal abnormalities.

#### 2.3.3.4.11. Codeine and other opioid analgesics

A small increase in the frequency of congenital heart defects has been observed repeatedly in case–control studies of children whose mothers were treated with codeine or another opioid analgesic early in pregnancy [282–285]. An increased frequency of spina bifida or cleft lip and palate has also been observed in some studies but not others [283,286–289]. Even if all of the observed associations are causal, the overall increase in the risk of congenital anomalies among infants of women who took opioid analgesics early in pregnancy is probably

less than 1%.

Narcotic withdrawal symptoms may occur in newborn infants whose mothers were treated chronically with opioid analgesics late in pregnancy [290]. Prenatal opioid exposure leads to neonatal abstinence syndrome (NAS) in 50%–80% of all opioid-exposed infants, which is characterized by transient gastrointestinal, respiratory, autonomic, and CNS disturbances [291].

#### 2.3.3.4.12. Misoprostol

Misoprostol is a prostaglandin analogue that is used in the prevention and treatment of peptic ulcer disease. The drug is also used in combination with mifepristone, a progesterone blocker, to induce abortion. Associations have been observed between unsuccessful maternal use of misoprostol to induce abortion in the first trimester of pregnancy and the occurrence of Moebius syndrome, terminal transverse limb reduction defects, arthrogryposis, and brain defects such as holoprosencephaly and hydrocephalus in the offspring [292–296]. Vascular disruption is a biologically plausible explanation for these associations [297]. The risks for severe anomalies among fetuses surviving attempted induced abortion with mifepristone and misoprostol should be explained to women contemplating such treatment. These risks contribute to the recommendation that pregnancies that continue despite attempted medical abortion be surgically terminated.

Oral doses of misoprostol similar to those used for pregnancy termination are taken chronically for prophylaxis or treatment of peptic ulcer disease. Induction of abortion and teratogenic risks would be expected to be at least as great when the drug is taken early in pregnancy for peptic ulcer disease as when it is taken to induce abortion, but little information is available on the outcome of such pregnancies.

#### 2.3.3.4.13. Mycophenolate mofetil

An unusual pattern of malformations has been repeatedly observed among children whose mothers were treated with mycophenolate

mofetil early in pregnancy [38,298–301]. Frequent features include microtia or anotia, auditory canal atresia, conductive deafness, cleft lip, cleft palate, dysmorphic facial features of the face, congenital heart defects, and short fifth finger.

The frequency of other adverse pregnancy outcomes, including stillbirths and premature delivery, also appear to be unusually high among pregnancies in women treated with mycophenolate mofetil [298,301]. All of these women have serious medical conditions, and most are treated with other immunosuppressive agents as well as mycophenolate mofetil, complicating interpretation of the relationship between mycophenolate mofetil and adverse pregnancy outcomes.

#### 2.3.3.4.14. Inhibitors of the renin-angiotensin system

##### 2.3.3.4.14.1. ACE inhibitors

Neonatal renal failure and hypotension as well as fetal anuria resulting in oligohydramnios, joint contractures, pulmonary hypoplasia and death, have been observed repeatedly after maternal treatment with ACE inhibitors like captopril, enalapril, or lisinopril during pregnancy [302]. Several cases of neonatal hypocalvaria and other skeletal anomalies have also been noted after maternal treatment during pregnancy with an ACE inhibitor. Accurate risk estimates are not available, but the effects appear to result from hypersensitivity of the fetus to the pharmacological action of ACE inhibitors during the second half of pregnancy [302]. There is some evidence suggesting that maternal ACE inhibitor therapy early in pregnancy increases the risk of cardiovascular and CNS malformations among the infants [303,304], but further study is necessary to determine whether the observed associations are causal. Women who conceive while taking an ACE inhibitor should be switched during the first trimester to an antihypertensive agent of a different class if treatment continues to be necessary.

##### 2.3.3.4.14.2. Angiotensin II receptor inhibitors

Losartan, candesartan, valsartan, and other antihypertensive drugs of

the "sartan" class block the activity of the renin-angiotensin system by a mechanism different from ACE inhibitors. Several case reports describe oligohydramnios, fetal growth retardation, pulmonary hypoplasia, limb contractures, and calvarial hypoplasia in various combinations in association with maternal sartan treatment during the second or third trimester of pregnancy [302,305]. Stillbirth or neonatal death is frequent in these reports, and surviving infants may exhibit renal damage. The fetal abnormalities, which are strikingly similar to those produced by maternal treatment with ACE inhibitors during pregnancy, are probably related to inhibition of the fetal renin-angiotensin system.

#### 2.3.3.4.15. Methylene blue

Up to 20% of twins born after genetic amniocentesis in which methylene blue was used as a marker develop small bowel atresia [20]. The affected twin was the one whose amniotic sac was injected with the dye, and the risk appears to be greater with higher doses. The risk of fetal death was also increased after injection of methylene blue during genetic amniocentesis [306]. It is important to note that these risks are only associated with intra-amniotic instillation of methylene blue, not with oral or topical use by the mother during pregnancy.

#### 2.3.3.4.16. Bendectin (Diclectin)

A fixed combination of pyridoxine (vitamin $B_6$) and doxylamine (an antihistamine), marketed in the past as Bendectin and more recently as Diclegis or Diclectin, is used in the treatment of nausea and vomiting of pregnancy. This medication was removed from the market by its manufacturer and was unavailable for many years in the United States because of excessive litigation alleging that Bendectin caused a variety of serious birth defects in children whose mothers took it early in pregnancy. However, extensive epidemiological studies provide no indication that maternal use of this medication during pregnancy increases the risk of congenital anomalies above the rate expected in the general population [307].

## 2.3.3.5. Maternal Metabolic Factors

Although not environmental in the strictest sense, factors that affect maternal metabolism may alter the intrauterine environment. Thus, an additional category of potential teratogenic effects is maternal metabolic factors. Of particular importance in this category is maternal diabetes mellitus, but maternal phenylketonuria and inadequate folic acid intake are also of concern.

### 2.3.3.5.1. Inadequate folic acid intake

The recognition that the risk of many malformations, including neural tube defects, can be substantially reduced by maternal folic acid supplementation has provided an unparalleled opportunity for the prevention of birth defects. Dietary supplementation with folic acid before and after conception may reduce the overall risk of birth defects by 20% or more [308]. Not all malformations are affected equally – neural tube defects may be reduced by as much as 85% [308–310], but the reductions in orofacial clefts, cardiac malformations, and renal anomalies are smaller [308,311]. The mechanism of prevention is not understood [312,313], although certain genetic variants (e.g., in folate metabolism) may put some patients at greater risk than others for birth defects when folic acid supplementation is not provided [313,314].

All women of childbearing age should take 0.4 mg of supplemental folic acid daily [315]. Larger doses of folic acid are sometimes recommended for women who are at increased risk to have a child with a neural tube defect. As compliance with recommendations for daily vitamin supplements is problematical, grain products are fortified with folic acid in some countries [316].

### 2.3.3.5.2. Diabetes mellitus

The principal maternal metabolic disorder that raises concern for the developing fetus is type 1 diabetes mellitus. The risk of congenital anomalies in infants of women who have insulin-dependent diabetes is 2–3 times greater than that in the general population [317,318]. A

variety of congenital anomalies is associated with maternal diabetes, but congenital heart defects and neural tube defects are most common [319,320]. Preconceptional and early postconceptional folic acid supplementation is, therefore, especially important for women with type 1 diabetes, and pregnant diabetic women should be offered prenatal diagnosis by detailed ultrasound examination, fetal echocardiography, and serum $\alpha$-fetoprotein measurement.

Caudal regression, focal femoral hypoplasia, and holoprosencephy are also more frequent than expected among the children of women with type 1 diabetes mellitus, but these malformations are, fortunately, quite rare. Diabetic pregnancies are also at increased risk for spontaneous abortion, abnormal fetal growth, neonatal hypoglycemia, and various obstetrical complications [321,322]. Risks for congenital anomalies and adverse neonatal outcomes can be minimized by very good control of maternal diabetes from the time of conception and throughout pregnancy [318,321–323].

Type 2 diabetes is occurring with increasing frequency in women of reproductive age, often in association with obesity. Although less well studied, infants of type 2 diabetic mothers appear to have increased risks of similar kinds of malformations and other complications of pregnancy as infants of type 1 diabetics [323–326].

There is controversy over whether women with gestational diabetes also are at increased risk to have children with malformations. When maternal diabetes does not develop until after the first trimester of pregnancy, an effect on embryogenesis would seem unlikely. However, some women with gestational diabetes have unusually high blood glucose levels at the time of diagnosis and may actually have preexisting undiagnosed abnormalities of glucose metabolism [327]. The infants of such women may be more likely to have malformations of the types seen in children of mothers with preexisting type 1 diabetes [328–330].

#### 2.3.3.5.3. Phenylketonuria

From 75% to 90% of children of women with phenylketonuria (PKU) who are not adequately treated during pregnancy are intellectually

disabled and microcephalic [331,332]. These children may also have prenatal growth retardation, congenital heart disease, and an appearance that is reminiscent of fetal alcohol syndrome. Children of women with PKU are usually heterozygous carriers and do not have PKU themselves. The children are damaged during gestation by exposure to very high levels of phenylalanine, phenylpyruvic acid, and other potentially toxic metabolites in the maternal blood [332]. The severity of malformations are dependent on the level of maternal serum phenylalanine [333].

Effective treatment of the mother beginning before conception and continuing throughout pregnancy reduces the risk substantially [332,334]. It is extremely important to ensure that all females with phenylketonuria receive this information before and during their reproductive years. Unfortunately, adult women with undiagnosed phenylketonuria have occasionally been identified after they have become pregnant [335,336]. A few of these women have near-normal intelligence; others present with major psychotic disorders or have milder degrees of intellectual deficit than is associated with classic phenylketonuria. Such women may come to attention only through the birth of an abnormal child. Thus, maternal screening for phenylketonuria subsequent to the birth of a child with the abnormalities described above is one way of avoiding the birth of additional affected children.

#### 2.3.3.5.4. Obesity

Obesity is usually defined in relationship to height as the body mass index [BMI = weight in kg/(height in m)$^2$]. A BMI of 25.0–29.9 is conventionally considered to indicate overweight, and a BMI of 30.0 greater, to indicate obesity. Maternal prepregnancy obesity is associated with an increased risk of neural tube defects [337,338] and cardiac malformations [339,340] among the infants. The risks of congenital anomalies in general [341] and of some other specific kinds of malformations may also be slightly increased [338].

### 2.3.3.6. Autoimmune and Isoimmune Disease

Rh hemolytic disease, which results from transplacental transfer of maternal antibodies reactive against fetal red blood cells, has been recognized as a cause of hydrops fetalis for more than 70 years. Isoimmunization of an $Rh^-$ woman usually occurs during pregnancy by fetomaternal hemorrhage; amniocentesis or CVS may be a predisposing factor. Sensitization of the mother, and thus Rh hemolytic disease in the fetus in subsequent pregnancies, can largely be prevented by appropriate administration of Rh immunoglobulin at appropriate times in pregnancy [342,343].

Fetal and neonatal disease may also be caused by transplacental transmission of various other maternal antibodies. This can occur in association with maternal Graves disease [344], idiopathic thrombocytopenia purpura [345,346], or systemic lupus erythematosus [347]. Although exposure to these maternal antibodies stops at the time of delivery, permanent damage to relevant organs or tissues may occur in the fetus. For example, maternal anti-Ro/La antibodies can cause permanent heart block in an infant [348,349], fetal isoimmune thrombocytopenia can produce cerebral infarction and consequent porencephaly [350], and maternal anti–acetylcholine receptor antibodies associated with myasthenia gravis can cause arthrogryposis in a child [351]. A particularly interesting but poorly understood association exists between maternal systemic lupus erythematosus and the occurrence of chondrodysplasia punctata in the infant [352]. Fortunately, all of these occurrences are rare.

## 2.4. Paternal Exposures and Maternal Exposures Before or Shortly After Conception

Concern is often voiced about the potential role of paternal exposure to toxic agents in the pathogenesis of birth defects [353]. However, it seems unlikely that such paternal exposures could produce birth defects in a subsequent child except by germ cell mutation or altering the pattern of imprinting [354–356]. Although these possibilities may exist for many agents to which fathers might be exposed, the associated increase in risk would apply to many different genetic loci, and one would not expect to see any consistent pattern of abnormalities among the offspring of an exposed male. Rather, an increase in the frequency of a variety of disorders resulting from new autosomal dominant or chromosomal mutations or from altered imprinting would be anticipated.

No paternal exposure to any chemical or physical agent, including a number of known mutagens, has been convincingly shown to increase the risk of birth defects in subsequently-conceived children [357]. Paternal mutagenic exposures may contribute to infertility or early miscarriage and may be of importance, from a population perspective, especially when considered over many generations. However, an individual couple who are concerned about exposure of the father to a potentially teratogenic or mutagenic agent can be reassured that such exposures probably present minimal risk to the fetus if they occur before conception and none at all if they occur postconceptionally.

Similar advice can be provided to women who were exposed to radiation or mutagenic chemicals, such as many cancer therapeutic agents, before conception. Available evidence indicates that the risk of congenital anomalies is not measurably increased among the children of women who have had such preconceptional exposures in comparison to unexposed women [108,358].

The first 2 weeks after conception – the time between creation of the zygote and formation of the third germ layer, which marks the

beginning of the embryonic period – is sometimes characterized as the "all-or-none period" with respect to teratogenic risk. Adverse environmental exposures that occur during this time usually either kill the conceptus, with loss of the pregnancy before it is recognized, or produce no permanent adverse effect because the multipotent cells that are present at this stage replace other cells that have been damaged or lost. Although many studies support the idea that embryogenesis proper is a time of greater susceptibility to teratogenic effects than the time around and immediately following conception, it is now clear that some congenital anomalies may be induced during the cleavage, blastocyst or bilaminar disc phases of development. The most compelling evidence for this comes from studies of infants who were conceived by in vitro fertilization. Alterations of imprinting are thought to be responsible for the increased risks of conditions such as Prader-Willi syndrome or Silver-Russell syndrome that have been associated with in vitro fertilization [359,360].

## 2.5. Future Perspective

The need for reliable information on potentially teratogenic exposures is likely to increase as a result of new technologies. One outcome of the Human Genome Project is a rapidly expanding capability to identify molecular targets for new therapeutic agents. However, in addition to mediating disease processes in adults, these targets may also be involved in morphogenesis or other critical functions in the embryo or fetus. Thus, in some cases the use of these agents in women who are pregnant or who become pregnant during therapy may pose specific and substantial risks for fetal development. The teratogenic potential of such agents will, therefore, be especially important considerations.

Nanotechnologies (molecular level devices or interventions) are being developed for both diagnostic and therapeutic purposes. Little is known about the risks of human exposures to such agents at present, and almost nothing is known about their possible effects on embryonic and fetal development.

Our recognition of epigenetic mechanisms (e.g., imprinting) that

may modify gene expression and may also create transgenerational consequences opens new possibilities that will need to be considered in terms of risk assessment. These concerns may be especially important in the context of in vitro fertilization, intracytoplasmic sperm injection, and other assisted reproductive technologies that may bypass normal epigenetic control mechanisms that operate around the time of conception.

## 2.6. Conclusion

A wide variety of potentially teratogenic exposures may be encountered during pregnancy. Insufficient evidence is available to characterize fully the teratogenic risks or safety of most such exposures, and further information in this area is badly needed. At present, emphasis should be placed on the avoidance of potentially hazardous exposures unless benefits to the mother or infant from the proposed exposure clearly outweigh the hazards to the fetus. Well-informed women, supported by knowledgeable and sensitive health care providers, form one of the strongest lines of defense for the embryo and fetus against potentially hazardous exposures.

## References


[1] Wilson J. Current status of teratology: general principles and mechanisms derived from animal studies. In: Wilson J, Fraser F, eds. *Handbook of teratalogy*. New York: Plenum Press; 1977:47–74.

[2] Corry G.N, Tanasijevic B, Barry E.R, Krueger W, Rasm regulatory mechanisms during preimplantation development. *Birth Defects Res C Embryo Today*. 2009;87:297–313.

[3] Geiman T.M, Muegge K. DNA methylation in early development. *Mol Reprod Dev*. 2010;77:105–113.

[4] Hemberger M, Dean W, Reik W. Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. *Nat Rev Mol Cell Biol*. 2009;10:526–537.
[5] Shi L, Wu J. Epigenetic regulation in mammalian preimplantation embryo development. *Reprod Biol Endocrinol*. 2009;7:59.
[6] Bishop J.B, Witt K.L, Sloane R.A. Genetic toxicities of human teratogens. *Mutat Res*. 1997;396:9–43.
[7] Zhu H, Kartiko S, Finnell R.H. Importance of gene-environment interactions in the etiology of selected birth defects. *Clin Genet*. 2009;75:409–423.
[8] Streissguth A.P, Dehaene P. Fetal alcohol syndrome in twins of alcoholic mothers: concordance of diagnosis and IQ. *Am J Med Genet*. 1993;47:857–861.
[9] Malm H, Kajantie E, Kivirikko S, Kaariainen H, Peipp embryopathy in three sets of siblings: further proof of hereditary susceptibility. *Neurology*. 2002;59:630–633.
[10] Kowalski T.W, Fraga L.R, Tovo-Rodrigues L, Sanseverino M.T, Hutz M.H, Schuler-Faccini L, Vianna F.S. New Findings in eNOS gene and Thalidomide Embryopathy Suggest pre-transcriptional effect variants as susceptibility factors. *Sci Rep*. 2016;6:23404.
[11] Vianna F.S, Fraga L.R, Tovo-Rodrigues L, Tagliani-Ribeiro A, Biondi F, Maximino C.M, Sanseverino M.T, Faccini L. Polymorphisms in the endothelial nitric oxide synthase gene in thalidomide embryopathy. *Nitric Oxide Biol ChemOff J Nitric Oxide Soc*. 2013;35:89–92.

[12] Shi M, Wehby G.L, Murray J.C. Review on genetic variants and maternal smoking in the etiology of oral clefts and other birth defects. *Birth Defects Res C Embryo Today*. 2008;84:16–29.
[13] Finnell R.H, Waes J.G, Eudy J.D, Rosenquist T.H. Mol basis of environmentally induced birth defects. *Annu Rev Pharmacol Toxicol*. 2002;42:181–208.
[14] Friedman J.M. The principles of teratology: are they still true? *Birth Defects Res Part A Clin Mol Teratol*. 2010;88:766–768.
[15] Scialli A.R. Identifying teratogens: the tyranny of lists. *Reprod Toxicol*. 1997;11:555–559.
[16] Lenz W, Knapp K. Thalidomide embryopathy. *Arch Environ Health*. 1962;5:100–105.
[17] Schaefer C. Angiotensin II-receptor-antagonists: further evidence of fetotoxicity but not teratogenicity. *Birth Defects Res Part A Clin Mol Teratol*. 2003;67:591–594.
[18] Barr Jr. M. Teratogen update: angiotensin-converting enzyme inhibitors. *Teratology*. 1994;50:399–409.
[19] Brent R.L. Environmental causes of human congenital malformations: the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. *Pediatrics*. 2004;113:957–968.
[20] Cragan J.D. Teratogen update: methylene blue. *Teratology*. 1999;60:42–48.
[21] van der Pol J.G, Wolf H, Boer K, Treffers P.E, Leschot N.J, Hey atresia related to the use of methylene blue in genetic

amniocentesis in twins. *Br J Obstet Gynaecol*. 1992;99:141–143.
[22] Graham Jr. J.M, Jones K.L, Brent R.L. Contribution of clinical teratologists and geneticists to the evaluation of the etiology of congenital malformations alleged to be caused by environmental agents: ionizing radiation, electromagnetic fields, microwaves, radionuclides, and ultrasound. *Teratology*. 1999;59:307–313.
[23] Jones K.L. *Smith's recognizable patterns of human malformations*. Philadelphia: Elsevier Saunders; 2006.
[24] Reardon W, Donnai D. Dysmorphology demystified. *Arch Dis Child Fetal Neonatal Ed*. 2007;92:F225–F229.
[25] Giles J.J, Bannigan J.G. Teratogenic and developmental effects of lithium. *Curr Pharm Des*. 2006;12:1531–1541.
[26] Grandjean E.M, Aubry J.M. Lithium: updated human knowledge using an evidence-based approach: part III: clinical safety. *CNS Drugs*. 2009;23:397–418.
[27] Yacobi S, Ornoy A. Is lithium a real teratogen? What can we conclude from the prospective versus retrospective studies? A review. *Isr J Psychiatry Relat Sci*. 2008;45:95–106.
[28] Billings R.J, Berkowitz R.J, Watson G. Teeth. *Pediatrics* 1127. .
[29] De Santis M, De Luca C, Quattrocchi T, Visconti D, Cesari E, Mappa I, of the Internet by women seeking information about

potentially teratogenic agents. *Eur J Obstet Gynecol Reprod Biol*. 2010;151:154–157.

[30] Koren G, Bologa M, Long D, Feldman Y, Shear N.H. P of teratogenic risk by pregnant women exposed to drugs and chemicals during the first trimester. *Am J Obstet Gynecol*. 1989;160:1190–1194.

[31] Adam M.P, Polifka J.E, Friedman J.M. Evolving knowledge of the teratogenicity of medications in human pregnancy. *Am J Med Genet Part C Semin Med Genet*. 2011;157:175–182.

[32] Lo W.Y, Friedman J.M. Teratogenicity of recently introduced medications in human pregnancy. *Obstet Gynecol*. 2002;100:465–473.

[33] Friedman J.M. How do we know if an exposure is actually teratogenic in humans? *Am J Med Genet C Semin Med Genet*. 2011;157:170–174.

[34] FDA. Content and format of labeling for human prescription drug and biological products; requirements for pregnancy and lactation labeling. Final rule. *Fed Regist*. 2014;79:72063–72103.

[35] Hancock R.L, Ungar W.J, Einarson A, Koren G. Intern practices in the provision of teratology information: a survey of international teratogen information programmes and comparisons with the North American model. *J Eval Clin Pract*. 2010;16:957–963.

[36] Schaefer C. Drug safety in pregnancy: utopia or achievable prospect? Risk information, risk research and advocacy in Teratology Information Services. *Congenit Anomalies*. 2011;51:6–11.

[37] Chambers C. The role of teratology information services in screening for teratogenic exposures: challenges and opportunities. *Am J Med Genet Part C Semin Med Genet*. 2011;157C:195–200.
[38] Carey J.C, Martinez L, Balken E, Leen-Mitchell M, Robertson J. Determination of human teratogenicity by the astute clinician method: review of illustrative agents and a proposal of guidelines. *Birth Defects Res Part A Clin Mol Teratol*. 2009;85:63–68.
[39] Jones K.L, Smith D.W. Recognition of the fetal alcohol syndrome in early infancy. *Lancet*. 1973;302:999–1001.
[40] Jones K.L, Smith D.W, Ulleland C.N, Streissguth P. Pa of malformation in offspring of chronic alcoholic mothers. *Lancet*. 1973;1:1267–1271.
[41] Gregg N.M. Congenital cataract following German measles in the mother. *Trans Ophthalmol Soc Aust*. 1941;3:35–46.
[42] Smithells R.W, Newman C.G. Recognition of thalidomide defects. *J Med Genet*. 1992;29:716–723.
[43] Lammer E.J, Chen D.T, Hoar R.M, Agnish N.D, Benke al. Retinoic acid embryopathy. *N Engl J Med*. 1985;313:837–841.
[44] Miranda-Filho Dde B, Martelli C.M, Ximenes R.A, Araujo T.V, Rocha Junior E.T, Rodrigues L.C. Initial description of the presumed congenital Zika syndrome. *Am J Public Health*. 2016;106:598–600.

[45] Petersen L.R, Jamieson D.J, Powers A.M, Honein M.A virus. *N Engl J Med*. 2016;374:1552–1563.
[46] Correa A, Cragan J.D, Kucik J.E, Alverson C.J, Gilboa Colarusso T, Siffel C, Gambrell D, Thompson D, Atkir birth defects surveillance data 1968-2003. *Birth Defects Res Part A Clin Mol Teratol*. 2007;79:65–186.
[47] Tomson T, Battino D, Craig J, Hernandez-Diaz S, Holmes L.B, Lindhout D, Morrow J, French J. I registries: differences, similarities, and possible harmonization. *Epilepsia*. 2010;51:909–915.
[48] Koren G, Graham K, Shear H, Einarson T. Bias against the null hypothesis: the reproductive hazards of cocaine. *Lancet*. 1989;2:1440–1442.
[49] Koren G, Nickel C. Perpetuating fears: bias against the null hypothesis in fetal safety of drugs as expressed in scientific citations. *J Popul Ther Clin Pharmacol*. 2011;18:e28–32.
[50] Maldonado Y.A, Nizet V, Klein J.O, Remington J.S, W concepts of infections of the fetus and newborn infant. In: Wilson C.B, Nizet V, Maldonado Y.A, Remi *diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016:3–23.
[51] Reef S.E, Plotkin S.A. Rubella. In: Wilson C.B, Nizet V *and Klein's infectious diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016:894–932.
[52] De

Santis M, Cavaliere A.F, Straface G, Caruso A. Rubella infection in pregnancy. *Reprod Toxicol*. 2006;21:390–398.

[53] Dontigny L, Arsenault M.Y, Martel M.J, Biringer A, C in pregnancy. *J Obstet Gynaecol Can*. 2008;30:152–168.

[54] Jin L, Thomas B. Application of molecular and serological assays to case based investigations of rubella and congenital rubella syndrome. *J Med Virol*. 2007;79:1017–1024.

[55] Cordier A.G, Vauloup-Fellous C, Grangeot-Keros L, Pinet C, Benachi A, Ayoubi J.M, Picone O. Pi in the diagnosis of congenital rubella syndrome in the first trimester of pregnancy. *Prenat Diagn*. 2012;32:496–497. .

[56] Britt W. Cytomegalovirus. In: Wilson C.B, Nizet V, Ma *Remington and Klein's infectious diseases of the fetus and newborn infant*. 7th ed. Philadelphia: Elsevier Saunders; 2016:724–781.

[57] Cannon M.J, Griffiths P.D, Aston V, Rawlinson W.D. U newborn screening for congenital CMV infection: what is the evidence of potential benefit? *Rev Med Virol*. 2014;24:291–307.

[58] Kylat R.I, Kelly E.N, Ford-Jones E.L. Clinical findings and adverse outcome in neonates with symptomatic congenital cytomegalovirus (SCCMV) infection. *Eur J Pediatr*. 2006;165:773–778.

[59] Goderis J, De Leenheer E, Smets K, Van Hoecke H, Keymeulen A, Dhooge I. Hearing loss and

congenital CMV infection: a systematic review. *Pediatrics*. 2014;134:972–982.
[60] Davis N.L, King C.C, Kourtis A.P. Cytomegalovirus infection in pregnancy. *Birth Defects Res*. 2017;109:336–346.
[61] Kadambari S, Williams E.J, Luck S, Griffiths P.D, Shar based management guidelines for the detection and treatment of congenital CMV. *Early Hum Dev*. 2011;87:723–728.
[62] Benoist G, Salomon L.J, Mohlo M, Suarez B, Jacquema related fetal brain lesions: comparison between targeted ultrasound examination and magnetic resonance imaging. *Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol*. 2008;32:900–905.
[63] Degani S. Sonographic findings in fetal viral infections: a systematic review. *Obstet Gynecol Surv*. 2006;61:329–336.
[64] Guerra B, Simonazzi G, Puccetti C, Lanari M, Farina A prediction of symptomatic congenital cytomegalovirus infection. *Am J Obstet Gynecol*. 2008;198(380):e381–387.
[65] Pass R.F, Zhang C, Evans A, Simpson T, Andrews W, prevention of maternal cytomegalovirus infection. *N Engl J Med*. 2009;360:1191–1199.
[66] Nigro G, Adler S.P, La Torre R, Best A.M. Passive immunization during pregnancy for congenital cytomegalovirus infection. *N Engl J*

*Med*. 2005;353:1350–1362.
[67] Yinon Y, Farine D, Yudin M.H. Screening, diagnosis, and management of cytomegalovirus infection in pregnancy. *Obstet Gynecol Surv*. 2010;65:736–743.
[68] Del
Campo M, Feitosa I.M, Ribeiro E.M, Horovitz D.D, Pe
Alix A, Doriqui M.J, Wanderley H.Y, Sanseverino M.V
Neto J.M, Santos E.S, Vercosa I, Cernach M.C, Medeir
der
Linden V, Martelli C.M, Cordeiro M.T, Dhalia R, Vian
Faccini L. The phenotypic spectrum of congenital Zika syndrome. *Am J Med Genet Part A*. 2017;173:841–857.
[69]
Karwowski M.P, Nelson J.M, Staples J.E, Fischer M, F
Dutra K.E, Villanueva J, Powers A.M, Mead P, Honein virus disease: a CDC update for pediatric health care providers. *Pediatrics*. 2016;137.
[70]
Rasmussen S.A, Jamieson D.J, Honein M.A, Petersen l
virus and birth defects–reviewing the evidence for causality. *N Engl J Med*. 2016;374:1981–1987.
[71] Schuler-
Faccini L, Ribeiro E.M, Feitosa I.M, Horovitz D.D, Cav
Husny A.S, Pone M.V, Serao C.L, Sanseverino M.T. Pc
association between Zika virus infection and microcephaly - Brazil, 2015. *MMWR. Morbi Mortal Weekly Rep*. 2016;65:59–62.
[72] de Paula Freitas B., de Oliveira
Dias J.R, Prazeres J., Sacramento G.A, Ko A.I, Maia M,
findings in infants with microcephaly associated with

presumed Zika virus congenital infection in Salvador, Brazil. *JAMA Ophthalmol*. 2016;134(5):529–535.

[73] Ventura C.V, Maia M, Travassos S.B, Martins T.T, Pat der Linden V, Ramos R.C, Rocha M.A, Silva P.S, Ventura factors associated with the ophthalmoscopic findings identified in infants with presumed Zika virus congenital infection. *JAMA Ophthalmol*. 2016;134:912–918.

[74] de Fatima Vasco Aragao M, van der Linden V, Brainer-Lima A.M, Coeli R.R, Rocha M.A, Sobral da Silva P, Durce Costa Gomes de Carvalho M, van der Linden A, Cesario de Holanda A, Valenca M.M. Clinical features and neuroimaging (CT and MRI) findings in presumed Zika virus related congenital infection and microcephaly: retrospective case series study. *BMJ*. 2016;353:i1901.

[75] Johansson M.A, Mier-y-Teran-Romero L, Reefhuis J, Gilboa S.M, Hills S.L. Zika and the risk of microcephaly. *N Engl J Med*. 2016;375:1–4.

[76] Brasil P, Pereira Jr. J.P, Moreira M.E, Ribeiro Nogueira R.M, Damasceno L, Wakimoto M, Rabello R Gabaglia C, Carvalho de Sequeira P, Pilotto J.H, Medialdea-Carrera R, Cotrim da Cunha D, Abreu de Carvalho L.M, Pone M, Machado Siqueira A, Calvet G.A, Rodrigues

Baiao A.E, Neves E.S, Nassar de Carvalho P.R, Hasue R.H, Marschik P.B, Einspieler C, de Filippis A.M, Nielsen-Saines K. Zika Virus Infection in Pregnant Women in Rio de Janeiro. *N Engl J Med*. 2016;375:2321–2334.

[77] Guillemette-Artur P, Besnard M, Eyrolle-Guignot D, Jouannic J.M, Garel C. Prenatal brain MRI of fetuses with Zika virus infection. *Pediatr Radiol*. 2016;46:1032–1039. .

[78] Meaney-Delman D, Rasmussen S.A, Staples J.E, Oduyebo T, El virus and pregnancy: what obstetric health care providers need to know. *Obstet Gynecol*. 2016;127:642–648.

[79] Driggers R.W, Ho C.Y, Korhonen E.M, Kuivanen S, Ja Plessis A, Vapalahti O. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. *N Engl J Med*. 2016;374:2142–2151.

[80] Marston H.D, Lurie N, Borio L.L, Fauci A.S. Consider for developing a Zika virus vaccine. *N Engl J Med*. 2016;375:1209–1212.

[81] Thorne C, Newell M.L. Safety of agents used to prevent mother-to-child transmission of HIV: is there any cause for concern? *Drug Saf*. 2007;30:203–213.

[82] Luzuriaga K, Mofenson L.M. Eliminating pediatric HIV-1 infection. *N Engl J Med*. 2016;375:193–194.

[83] Shetty A.K, Maldonado Y.A. Human immunodeficiency virus/Acquired immunodeficiency syndrome in the

infant. In: Wilson C.B, Nizet V, Maldonado Y.A, Remi
*and Klein's infectious diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016:619–674.
[84] Antiretroviral Pregnancy Registry Steering Committee. Antiretroviral pregnancy registry international interim report for 1 January 1989 through 31 July 2016. Registry coordinating center. December 2016., Wilmington, N.C. 2016 Available at. http://www.apregistry.com/forms/interim_report.p
[85] Mofenson L.M. Antiretroviral therapy and adverse pregnancy outcome: the elephant in the room? *J Infect Dis*. 2016;213:1051–1054.
[86] Sibiude J, Le Chenadec J, Bonnet D, Tubiana R, Faye A, Dollfus C, I utero exposure to zidovudine and heart anomalies in the ANRS French perinatal cohort and the nested PRIMEVA randomized trial. *Clin Infect Dis*. 2015;61:270–280.
[87] Brocklehurst P, French R. The association between maternal HIV infection and perinatal outcome: a systematic review of the literature and meta-analysis. *Br J Obstet Gynaecol*. 1998;105:836–848.
[88] Adler S.P, Koch W.C. Human parvovirus. In: Wilson C.B, Nizet V, Maldonado Y.A, *and Klein's infectious diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016:866–893.
[89] de Jong E.P, Walther F.J, Kroes A.C, Oepkes D. Parvoviru B19 infection in pregnancy: new insights and

management. *Prenat Diagn*. 2011;31:419–425.
[90] Crane J, Mundle W, Boucoiran I. Parvovirus B19 infection in pregnancy. *J Obstet Gynaecol Can*. 2014;36:1107–1116.
[91] Miller E, Fairley C.K, Cohen B.J, Seng C. Immediate and long term outcome of human parvovirus B19 infection in pregnancy. *Br J Obstet Gynaecol*. 1998;105:174–178.
[92] Rodis J.F, Rodner C, Hansen A.A, Borgida A.F, Deoliv Rosengren S. Long-term outcome of children following maternal human parvovirus B19 infection. *Obstet Gynecol*. 1998;91:125–128.
[93] Lamont R.F, Sobel J.D, Vaisbuch E, Kusanovic J.P, Ma Tovi S, Kim S.K, Uldbjerg N, Romero R. Parvovirus B19 infection in human pregnancy. *BJOG Int J Obstet Gynaecol*. 2011;118:175–186.
[94] Tolfvenstam T, Broliden K. Parvovirus B19 infection. *Semin Fetal Neonatal Med*. 2009;14:218–221.
[95] Feder H.M. Borrelia infections: Lyme disease and relapsing fever. In: Wilson C.B, Nizet V, Maldonado Y.A, Remir *and Klein's infectious diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016:544–557.
[96] Hercogova J, Vanousova D. Syphilis and borreliosis during pregnancy. *Dermatol Ther*. 2008;21:205–209.
[97] Lakos A, Solymosi N. Maternal Lyme borreliosis and pregnancy outcome. *Int J Infect Dis*. 2010;14:e494–498.
[98]

Kollmann T.R, Dobson R.M. Syphilis. In: Wilson C.B,
*and Klein's infectious diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016:512–543.

[99] Tsimis M.E, Sheffield J.S. Update on syphilis and pregnancy. *Birth Defects Res*. 2017;109:347–352.

[100] Chakraborty R, Luck S. Managing congenital syphilis again? The more things change. *Curr Opin Infect Dis*. 2007;20:247–252.

[101] Hartman T.K, Rogerson S.J, Fischer P.R. The impact of maternal malaria on newborns. *Ann Trop Paediatr*. 2010;30:271–282.

[102] Rogerson S.J. Malaria in pregnancy and the newborn. *Adv Exp Med Biol*. 2010;659:139–152.

[103] Dubey J.P, Jones J.L. Toxoplasma gondii infection in humans and animals in the United States. *Int J Parasitol*. 2008;38:1257–1278.

[104]
Peyron F, Wallon M, Kieffer F, Garweg J. Toxoplasmo
*and Kelin's infectious diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016:949–1042. .

[105] Rorman E, Zamir C.S, Rilkis I, Ben-David H. Congenital toxoplasmosis–prenatal aspects of Toxoplasma gondii infection. *Reprod Toxicol*. 2006;21:458–472.

[106] Montoya J.G, Remington J.S. Management of Toxoplasma gondii infection during pregnancy. *Clin Infect Dis*. 2008;47:554–566.

[107] Baysinger C.L. Imaging during pregnancy. *Anesth Analg*. 2010;110:863–867.

[108] Brent R.L. Utilization of developmental basic science principles in the evaluation of reproductive risks from pre- and postconception environmental radiation exposures. *Teratology*. 1999;59:182–204.
[109] Robert E. Intrauterine effects of electromagnetic fields–(low frequency, mid-frequency RF, and microwave): review of epidemiologic studies. *Teratology*. 1999;59:292–298.
[110] Ziskin M.C. Intrauterine effects of ultrasound: human epidemiology. *Teratology*. 1999;59:252–260.
[111] Brent R.L. Saving lives and changing family histories: appropriate counseling of pregnant women and men and women of reproductive age, concerning the risk of diagnostic radiation exposures during and before pregnancy. *Am J Obstet Gynecol*. 2009;200:4–24.
[112] De Santis M, Cesari E, Nobili E, Straface G, Cavaliere A.F effects on development. *Birth Defects Res C Embryo Today*. 2007;81:177–182.
[113] Boice Jr. J.D, Miller R.W. Childhood and adult cancer after intrauterine exposure to ionizing radiation. *Teratology*. 1999;59:227–233.
[114] Bennett G.D. Hyperthermia: malformations to chaperones. *Birth Defects Res B Dev Reprod Toxicol*. 2010;89:279–288.
[115] Edwards M.J. Review: hyperthermia and fever during pregnancy. *Birth Defects Res Part A Clin Mol Teratol*. 2006;76:507–516.
[116] Chambers C.D. Risks of hyperthermia associated with hot tub or spa use by pregnant women. *Birth Defects Res A Clin Mol Teratol*. 2006;76:569–573.

[117] Graham J.M.J, Sanchez-Lara P. *Smith's recognizable patterns of human deformation*. 4th ed. Philadelphia: Saunders, Elsevier; 2015.

[118] Botto L.D, Olney R.S, Mastroiacovo P, Khoury M.J, M villus sampling and transverse digital deficiencies: evidence for anatomic and gestational-age specificity of the digital deficiencies in two studies. *Am J Med Genet*. 1996;62:173–178.

[119] Firth H. Chorion villus sampling and limb deficiency–cause or coincidence? *Prenat Diagn*. 1997;17:1313–1330.

[120] Jenkins T.M, Wapner R.J. First trimester prenatal diagnosis: chorionic villus sampling. *Semin Perinatol*. 1999;23:403–413.

[121] Stoler J.M, McGuirk C.K, Lieberman E, Ryan L, Holm reported in chorionic villus sampling exposed children: a review and analytic synthesis of the literature. *Genet Med Off J Am Coll Med Genet*. 1999;1:315–322.

[122] Farrell S.A, Summers A.M, Dallaire L, Singer J, Johnsc foot, an adverse outcome of early amniocentesis: disruption or deformation? CEMAT. Canadian Early and Mid-Trimester Amniocentesis Trial. *J Med Genet*. 1999;36:843–846.

[123] Sundberg K, Bang J, Smidt-Jensen S, Brocks V, Lundsteen C, Parner J, Keiding N, study of risk of fetal loss related to early amniocentesis versus chorionic villus

sampling. *Lancet*. 1997;350:697–703.
[124] Ekino S, Susa M, Ninomiya T, Imamura K, Kitamura T disease revisited: an update on the acute and chronic manifestations of methyl mercury poisoning. *J Neurol Sci*. 2007;262:131–144.
[125] Grandjean P, Herz K.T. Methylmercury and brain development: imprecision and underestimation of developmental neurotoxicity in humans. *Mt Sinai J Med*. 2011;78:107–118.
[126] Kondo K. Congenital Minamata disease: warnings from Japan's experience. *J Child Neurol*. 2000;15:458–464.
[127] Bernanke J, Kohler H.R. The impact of environmental chemicals on wildlife vertebrates. *Rev Environ Contam Toxicol*. 2009;198:1–47.
[128] Choi S.M, Yoo S.D, Lee B.M. Toxicological characteristics of endocrine-disrupting chemicals: developmental toxicity, carcinogenicity, and mutagenicity. *J Toxicol Environ Health B Crit Rev*. 2004;7:1–24.
[129] Daston G.P, Cook J.C, Kavlock R.J. Uncertainties for endocrine disrupters: our view on progress. *Toxicol Sci*. 2003;74:245–252.
[130] Dickerson S.M, Gore A.C. Estrogenic environmental endocrine-disrupting chemical effects on reproductive neuroendocrine function and dysfunction across the life cycle. *Rev Endocr Metab Disord*. 2007;8:143–159.
[131] McLachlan J.A, Simpson E, Martin M. Endocrine disrupters and female reproductive health. *Best Pract*

*Res Clin Endocrinol Metab*. 2006;20:63–75.
[132] Sharpe R.M, Irvine D.S. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *BMJ*. 2004;328:447–451.
[133] Substance Abuse an Mental Health Services Administration. *Results from the 2013 national survey on drug use and health: summary of national findings*. 2014 NSDUH Series H-48, HHS Publication No. (SMA) 14–4863, Rockville, MD..
[134] Day N.L, Leech S.L, Richardson G.A, Cornelius M.D, alcohol exposure predicts continued deficits in offspring size at 14 years of age. *Alcohol Clin Exp Res*. 2002;26:1584–1591. .
[135] Jones K.L. The effects of alcohol on fetal development. *Birth Defects Res C Embryo Today*. 2011;93:3–11.
[136] Nordstrom-Klee B, Delaney-Black V, Covington C, Ager J, Sokol R. Growth from birth onwards of children prenatally exposed to drugs: a literature review. *Neurotoxicol Teratol*. 2002;24:481–488.
[137] Richardson G.A, Ryan C, Willford J, Day N.L, Goldscl alcohol and marijuana exposure: effects on neuropsychological outcomes at 10 years. *Neurotoxicol Teratol*. 2002;24:309–320.
[138] Testa M, Quigley B.M, Eiden R.D. The effects of prenatal alcohol exposure on infant mental development: a meta-analytical review. *Alcohol*

*Alcohol*. 2003;38:295–304.
[139]
Carter R.C, Jacobson J.L, Molteno C.D, Dodge N.C, M
alcohol growth restriction and cognitive
impairment. *Pediatrics*. 2016;138.
[140]
Hoyme H.E, Kalberg W.O, Elliott A.J, Blankenship J, E
Rahman O, Jewett T, Coles C.D, Chambers C, Jones K
clinical guidelines for diagnosing fetal alcohol
spectrum disorders. *Pediatrics*. 2016;138.
[141] Manning M.A, Eugene Hoyme H. Fetal alcohol
spectrum disorders: a practical clinical approach to
diagnosis. *Neurosci Biobehav Rev*. 2007;31:230–238.
[142]
Moore E.S, Ward R.E, Wetherill L.F, Rogers J.L, Autti-
Ramo I, Fagerlund A, Jacobson S.W, Robinson L.K, H
facial features distinguish fetal alcohol syndrome
patients and controls in diverse ethnic
populations. *Alcohol Clin Exp Res*. 2007;31:1707–1713.
[143] Hanson J.W, Jones K.L, Smith D.W. Fetal alcohol
syndrome. Experience with 41
patients. *JAMA*. 1976;235:1458–1460.
[144]
Norman A.L, Crocker N, Mattson S.N, Riley E.P. Neu
and fetal alcohol spectrum disorders. *Dev Disabil Res
Rev*. 2009;15:209–217.
[145]
Spadoni A.D, McGee C.L, Fryer S.L, Riley E.P. Neuroi
and fetal alcohol spectrum disorders. *Neurosci
Biobehav Rev*. 2007;31:239–245.
[146]

May P.A, Gossage J.P, Kalberg W.O, Robinson L.K, Bu and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Dev Disabil Res Rev*. 2009;15:176–192.

[147] Coles C.D, Platzman K.A, Lynch M.E, Freides D. Aud and visual sustained attention in adolescents prenatally exposed to alcohol. *Alcohol Clin Exp Res*. 2002;26:263–271.

[148] Kable J.A, Mukherjee R.A. Neurodevelopmental disorder associated with prenatal exposure to alcohol (ND-PAE): a proposed diagnostic method of capturing the neurocognitive phenotype of FASD. *Eur J Med Genet*. 2017;60:49–54.

[149] DiFranza J.R, Aligne C.A, Weitzman M. Prenatal and postnatal environmental tobacco smoke exposure and children's health. *Pediatrics*. 2004;113:1007–1015.

[150] Higgins S. Smoking in pregnancy. *Curr Opin Obstet Gynecol*. 2002;14:145–151.

[151] Oken E, Levitan E.B, Gillman M.W. Maternal smoking during pregnancy and child overweight: systematic review and meta-analysis. *Int J Obes (Lond)*. 2008;32:201–210.

[152] Salihu H.M, Wilson R.E. Epidemiology of prenatal smoking and perinatal outcomes. *Early Hum Dev*. 2007;83:713–720.

[153] Little J, Cardy A, Munger R.G. Tobacco smoking and oral clefts: a meta-analysis. *Bull World Health Organ*. 2004;82:213–218.

[154] Cummings A.M, Kavlock R.J. Gene-environment interactions: a review of effects on reproduction and development. *Crit Rev Toxicol*. 2004;34:461–485.
[155] Zeiger J.S, Beaty T.H, Liang K.Y. Oral clefts, maternal smoking, and TGFA: a meta-analysis of gene-environment interaction. *Cleft Palate Craniofac J*. 2005;42:58–63.
[156] Button T.M, Maughan B, McGuffin P. The relationship of maternal smoking to psychological problems in the offspring. *Early Hum Dev*. 2007;83:727–732.
[157] Huizink A.C, Mulder E.J. Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neurosci Biobehav Rev*. 2006;30:24–41.
[158] Coles C.D. Saying "goodbye" to the "crack baby". *Neurotoxicol Teratol*. 1993;15:290–292 discussion 311–292..
[159] Schardein J.L, Macina O.T. *Human developmental toxicants: aspects of toxicology and Chemistry*. Boca Raton, Fla: CRC Press; 2007.
[160] Addis A, Moretti M.E, Syed F.A, Einarson T.R, Koren effects of cocaine: an updated meta-analysis. *Reprod Toxicol*. 2001;15:341–369.
[161] Gouin K, Murphy K, Shah P.S. Effects of cocaine use during pregnancy on low birthweight and preterm birth: systematic review and metaanalyses. *Am J Obstet Gynecol*. 2011;204:340 e341–312..
[162] Ackerman J.P, Riggins T, Black M.M. A review of the effects of prenatal cocaine exposure among

school-aged children. *Pediatrics*. 2010;125:554–565.
[163] Dixon D.R, Kurtz P.F, Chin M.D. A systematic review of challenging behaviors in children exposed prenatally to substances of abuse. *Res Dev Disabil*. 2008;29:483–502. .
[164] Richardson G.A, Goldschmidt L, Larkby C, Day N.L. of prenatal cocaine exposure on adolescent development. *Neurotoxicol Teratol*. 2015;49:41–48.
[165] Terplan M, Ramanadhan S, Locke A, Longinaker N, L interventions for pregnant women in outpatient illicit drug treatment programs compared to other interventions. *Cochrane Database Syst Rev*. 2015:CD006037.
[166] Brent R.L, Christian M.S, Diener R.M. Evaluation of the reproductive and developmental risks of caffeine. *Birth Defects Res B Dev Reprod Toxicol*. 2011;92:152–187.
[167] Browne M.L. Maternal exposure to caffeine and risk of congenital anomalies: a systematic review. *Epidemiology*. 2006;17:324–331.
[168] Signorello L.B, McLaughlin J.K. Maternal caffeine consumption and spontaneous abortion: a review of the epidemiologic evidence. *Epidemiology*. 2004;15:229–239.
[169] James A.H, Brancazio L.R, Price T. Aspirin and reproductive outcomes. *Obstet Gynecol Surv*. 2008;63:49–57.
[170] Schardein J.L. *Agents used for pain, chemically induced birth defects*. 3rd ed. New York: Marcel

Dekker; 2000:139–166.
[171]
Kozer E, Nikfar S, Costei A, Boskovic R, Nulman I, Ko consumption during the first trimester of pregnancy and congenital anomalies: a meta-analysis. *Am J Obstet Gynecol*. 2002;187:1623–1630.
[172]
Marret S, Marchand L, Kaminski M, Larroque B, Arna low-dose aspirin and neurobehavioral outcomes of children born very preterm. *Pediatrics*. 2010;125:e29–34.
[173]
Parazzini F, Bortolus R, Chatenoud L, Restelli S, Bene up of children in the Italian study of aspirin in pregnancy. *Lancet*. 1994;343:1235.
[174]
Feldkamp M.L, Meyer R.E, Krikov S, Botto L.D. Aceta use in pregnancy and risk of birth defects: findings from the National Birth Defects Prevention Study. *Obstet Gynecol*. 2010;115:109–115.
[175] Rebordosa C, Kogevinas M, Horvath-Puho E, Norgard B, Morales M, Czeizel A.E, Vilstrup use during pregnancy: effects on risk for congenital abnormalities. *Am J Obstet Gynecol*. 2008;198(178):e171–177.
[176]
Brandlistuen R.E, Ystrom E, Nulman I, Koren G, Norc paracetamol exposure and child neurodevelopment: a sibling-controlled cohort study. *Int J Epidemiol*. 2013;42:1702–1713.
[177] Hoover R.M, Hayes V.A, Erramouspe J. Association

between prenatal acetaminophen exposure and future risk of attention deficit/hyperactivity disorder in children. *Ann Pharmacother*. 2015;49:1357–1361.

[178] Liew Z, Ritz B, Rebordosa C, Lee P.C, Olsen J. Acetam use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatr*. 2014;168:313–320.

[179] Ericson A, Kallen B.A. Nonsteroidal anti-inflammatory drugs in early pregnancy. *Reprod Toxicol*. 2001;15:371–375.

[180] Hernandez R.K, Werler M.M, Romitti P, Sun L, Ande antiinflammatory drug use among women and the risk of birth defects. *Am J Obstet Gynecol*. 2012;206(228):e221–228.

[181] Kaplan B.S, Restaino I, Raval D.S, Gottlieb R.P, Bernst failure in the neonate associated with in utero exposure to non-steroidal anti-inflammatory agents. *Pediatr Nephrol*. 1994;8:700–704.

[182] Gagne J.J, Maio V, Berghella V, Louis D.Z, Gonnella J. drug use during pregnancy: a population-based study in Regione Emilia-Romagna, Italy. *Eur J Clin Pharmacol*. 2008;64:1125–1132.

[183a]. Lenz W. Thalidomide and congenital anomalies. *Lancet*. 1962;1:45.

[183b]. McBride W.G. Thalidomide and congenital abnormalities. *Lancet*. 1961;2:1358.

[183]

Mitchell A.A, Gilboa S.M, Werler M.M, Kelley K.E, Lc Diaz S. Medication use during pregnancy, with particular focus on prescription drugs: 1976-2008. *Am J Obstet Gynecol*. 2011.

[184] Adam M.P, Manning M.A, Beck A.E, Kwan A, Enns G embryopathy: report of four cases resulting from failed medical abortion. *Am J Med Genet Part A*. 2003;123A:72–78.

[185] Schardein J.L. *Cancer chemotherapeutic agents, chemically induced birth defects*. 3rd ed. New York: Marcel Dekker; 2000:559–611.

[186] Warkany J. Aminopterin and methotrexate: folic acid deficiency. *Teratology*. 1978;17:353–357.

[187] Piggott K.D, Sorbello A, Riddle E, DeCampli W. Cong cardiac defects: a possible association of aminopterin syndrome and in utero methotrexate exposure? *Pediatr Cardiol*. 2011;32:518–520.

[188] Poggi S.H, Ghidini A. Importance of timing of gestational exposure to methotrexate for its teratogenic effects when used in setting of misdiagnosis of ectopic pregnancy. *Fertil Steril*. 2011;96:669–671.

[189] Lewden B, Vial T, Elefant E, Nelva A, Carlier P, Desco dose methotrexate in the first trimester of pregnancy: results of a French collaborative study. *J Rheumatol*. 2004;31:2360–2365.

[190] Buckley L.M, Bullaboy C.A, Leichtman L, Marquez M

congenital anomalies associated with weekly low-dose methotrexate treatment of the mother. *Arthritis Rheum*. 1997;40:971–973. .

[191] Wiebe V.J, Sipila P.E. Pharmacology of antineoplastic agents in pregnancy. *Crit Rev Oncol Hematol*. 1994;16:75–112.

[192] Andersen J.T, Petersen M, Jimenez-Solem E, Rasmussen J.N, Andersen N.L, Afzal S, Broe Pedersen C, Poulsen H.E. Trimethoprim use prior to pregnancy and the risk of congenital malformation: a register-based nationwide cohort study. *Obstet Gynecol Int*. 2013;2013:364526.

[193] Crider K.S, Cleves M.A, Reefhuis J, Berry R.J, Hobbs C medication use during pregnancy and risk of birth defects: National Birth Defects Prevention Study. *Arch Pediatr Adolesc Med*. 2009;163:978–985.

[194] Czeizel A.E, Rockenbauer M, Sorensen H.T, Olsen J. T teratogenic risk of trimethoprim-sulfonamides: a population based case-control study. *Reprod Toxicol*. 2001;15:637–646.

[195] Hansen C, Andrade S.E, Freiman H, Dublin S, Haffen sulfonamide use during the first trimester of pregnancy and the risk of congenital anomalies. *Pharmacoepidemiol Drug Saf*. 2016;25:170–178.

[196] Hernandez-Diaz S, Mitchell A.A. Folic acid antagonists during pregnancy and risk of birth defects Reply. *N Engl J Med*. 2001;344:934–935.

[197]
Matok I, Gorodischer R, Koren G, Landau D, Wiznitze to folic acid antagonists during the first trimester of pregnancy and the risk of major malformations. *Br J Clin Pharmacol*. 2009;68:956–962.
[198] Hernandez-
Diaz S, Werler M.M, Walker A.M, Mitchell A.A. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med*. 2000;343:1608–1614.
[199] Hernandez-
Diaz S, Werler M.M, Walker A.M, Mitchell A.A. Neur tube defects in relation to use of folic acid antagonists during pregnancy. *Am J Epidemiol*. 2001;153:961–968.
[200] Pereg D, Koren G, Lishner M. Cancer in pregnancy: gaps, challenges and solutions. *Cancer Treat Rev*. 2008;34:302–312.
[201] Azim Jr. H.A, Pavlidis N, Peccatori F.A. Treatment of the pregnant mother with cancer: a systematic review on the use of cytotoxic, endocrine, targeted agents and immunotherapy during pregnancy. Part II: hematological tumors. *Cancer Treat Rev*. 2010;36:110–121.
[202] Cardonick E, Iacobucci A. Use of chemotherapy during human pregnancy. *Lancet Oncol*. 2004;5:283–291.
[203]
Pauli R.M, Madden J.D, Kranzler K.J, Culpepper W, P therapy initiated during pregnancy and phenotypic chondrodysplasia punctata. *J Pediatr*. 1976;88:506–508.
[204] van Driel D, Wesseling J, Sauer P.J, Touwen B.C, van

der Veer E, Heymans H.S. Teratogen update: fetal effects after in utero exposure to coumarins overview of cases, follow-up findings, and pathogenesis. *Teratology*. 2002;66:127–140.
[205] Wainwright H, Beighton P. Warfarin embryopathy: fetal manifestations. *Virchows Arch*. 2010;457:735–739.
[206]
Menger H, Lin A.E, Toriello H.V, Bernert G, Spranger K deficiency embryopathy: a phenocopy of the warfarin embryopathy due to a disorder of embryonic vitamin K metabolism. *Am J Med Genet*. 1997;72:129–134.
[207]
Pauli R.M, Lian J.B, Mosher D.F, Suttie J.W. Associatic of congenital deficiency of multiple vitamin K-dependent coagulation factors and the phenotype of the warfarin embryopathy: clues to the mechanism of teratogenicity of coumarin derivatives. *Am J Hum Genet*. 1987;41:566–583.
[208]
Bates S.M, Greer I.A, Pabinger I, Sofaer S, Hirsh J. Ver thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy: American college of chest physicians evidence-based clinical practice guidelines (8th edition). *Chest*. 2008;133:844S–886S.
[209] Chan W.S, Anand S, Ginsberg J.S. Anticoagulation of pregnant women with mechanical heart valves: a systematic review of the literature. *Arch Intern Med*. 2000;160:191–196.
[210]
Schaefer C, Hannemann D, Meister R, Elefant E, Paul

Gnansia E, Arnon J, De Santis M, Clementi M, Rodriguez-Pinilla E, Dolivo A, Merlob P. Vitamin K antagonists and pregnancy outcome. A multi-centre prospective study. *Thromb Haemost*. 2006;95:949–957.

[211] Nahum G.G, Uhl K, Kennedy D.L. Antibiotic use in pregnancy and lactation: what is and is not known about teratogenic and toxic risks. *Obstet Gynecol*. 2006;107:1120–1138.

[212] Niebyl J.R. Antibiotics and other anti-infective agents in pregnancy and lactation. *Am J Perinatol*. 2003;20:405–414.

[213] Cohlan S.Q. Tetracycline staining of teeth. *Teratology*. 1977;15:127–129.

[214] Molgaard-Nielsen D, Hviid A. Maternal use of antibiotics and the risk of orofacial clefts: a nationwide cohort study. *Pharmacoepidemiol Drug Saf*. 2012;21:246–253.

[215] Czeizel A.E, Rockenbauer M, Olsen J, Sorensen H.T. A teratological study of aminoglycoside antibiotic treatment during pregnancy. *Scand J Infect Dis*. 2000;32:309–313.

[216] Warkany J. Antituberculous drugs. *Teratology*. 1979;20:133–137. .

[217] Donald P.R, Doherty E, Van Zyl F.J. Hearing loss in the child following streptomycin administration during pregnancy. *Cent Afr J Med*. 1991;37:268–271.

[218] Dannenberg A.L, Dorfman S.F, Johnson J. Use of quinine for self-induced abortion. *South Med J*. 1983;76:846–849.

[219]
McGready R, Lee S.J, Wiladphaingern J, Ashley E.A, I effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. *Lancet Infect Dis*. 2012;12:388–396.
[220]
Ward S.A, Sevene E.J, Hastings I.M, Nosten F, McGre drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. *Lancet Infect Dis*. 2007;7:136–144.
[221] Lopez-Rangel E, Van Allen M.I. Prenatal exposure to fluconazole: an identifiable dysmorphic phenotype. *Birth Defects Res Part A Clin Mol Teratol*. 2005;73:919–923.
[222]
Howley M.M, Carter T.C, Browne M.L, Romitti P.A, C use and birth defects in the national birth defects prevention study. *Am J Obstet Gynecol*. 2016;214:657 e651-659.
[223] Molgaard-Nielsen D, Pasternak B, Hviid A. Use of oral fluconazole during pregnancy and the risk of birth defects. *N Engl J Med*. 2013;369:830–839.
[224]
Norgaard M, Pedersen L, Gislum M, Erichsen R, Soga use of fluconazole and risk of congenital malformations: a Danish population-based cohort study. *J Antimicrob Chemother*. 2008;62:172–176.
[225]
Bobo W.V, Davis R.L, Toh S, Li D.K, Andrade S.E, Ch in the use of antiepileptic drugs among pregnant women in the US, 2001-2007: a medication exposure

in pregnancy risk evaluation program study. *Paediatr Perinat Epidemiol*. 2012;26:578–588.

[226] Campbell E, Kennedy F, Russell A, Smithson W.H, Pa risks of antiepileptic drug monotherapies in pregnancy: updated results from the UK and Ireland Epilepsy and Pregnancy Registers. *J Neurol Neurosurg Psychiatry*. 2014;85:1029–1034.

[227] Harden C.L, Meador K.J, Pennell P.B, Hauser W.A, G Guen C. Practice parameter update: management issues for women with epilepsy–focus on pregnancy (an evidence-based review): teratogenesis and perinatal outcomes: report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and American Epilepsy Society. *Neurology*. 2009;73:133–141.

[228] Hernandez-Diaz S, Smith C.R, Shen A, Mittendorf R, Hauser W.A safety of antiepileptic drugs during pregnancy. *Neurology*. 2012;78:1692–1699.

[229] Hill D.S, Wlodarczyk B.J, Palacios A.M, Finnell R.H. T effects of antiepileptic drugs. *Expert Rev Neurother*. 2010;10:943–959.

[230] Holmes L.B, Mittendorf R, Shen A, Smith C.R, Hernar Diaz S. Fetal effects of anticonvulsant polytherapies: different risks from different drug combinations. *Arch Neurol*. 2011;68:1275–1281.

[231] Vajda F.J, Hitchcock A.A, Graham J, O'Brien T.J, Land teratogenic risk of antiepileptic drug polytherapy. *Epilepsia*. 2010;51:805–810.
[232] Walker S.P, Permezel M, Berkovic S.F. The management of epilepsy in pregnancy. *BJOG Int J Obstet Gynaecol*. 2009;116:758–767.
[233] Kini U, Adab N, Vinten J, Fryer A, Clayton-Smith J. Dysmorphic features: an important clue to the diagnosis and severity of fetal anticonvulsant syndromes. *Arch Dis Child Fetal Neonatal Ed*. 2006;91:F90–F95.
[234] Gerard E.E, Meador K.J. An update on maternal use of antiepileptic medications in pregnancy and neurodevelopment outcomes. *J Pediatr Genet*. 2015;4:94–110.
[235] Duncan S. Teratogenesis of sodium valproate. *Curr Opin Neurol*. 2007;20:175–180.
[236] Ornoy A. Valproic acid in pregnancy: how much are we endangering the embryo and fetus? *Reprod Toxicol*. 2009;28:1–10.
[237] Meador K, Reynolds M.W, Crean S, Fahrbach K, Prob outcomes in women with epilepsy: a systematic review and meta-analysis of published pregnancy registries and cohorts. *Epilepsy Res*. 2008;81:1–13.
[238] Tomson T, Battino D. Teratogenic effects of antiepileptic medications. *Neurol Clin*. 2009;27:993–1002.
[239] Kini U, Lee R, Jones A, Smith S, Ramsden S, Fryer A, C

Smith J. Influence of the MTHFR genotype on the rate of malformations following exposure to antiepileptic drugs in utero. *Eur J Med Genet*. 2007;50:411–420.

[240] Ornoy A. Neuroteratogens in man: an overview with special emphasis on the teratogenicity of antiepileptic drugs in pregnancy. *Reprod Toxicol*. 2006;22:214–226.

[241] Holmes L.B, Harvey E.A, Coull B.A, Huntington K.B, teratogenicity of anticonvulsant drugs. *N Engl J Med*. 2001;344:1132–1138. .

[242] Margulis A.V, Mitchell A.A, Gilboa S.M, Werler M.M, Diaz S. Use of topiramate in pregnancy and risk of oral clefts. *Am J Obstet Gynecol*. 2012;207:405 e401-407..

[243] Mines D, Tennis P, Curkendall S.M, Li D.K, Peterson C use in pregnancy and the birth prevalence of oral clefts. *Pharmacoepidemiol Drug Saf*. 2014;23:1017–1025.

[244] Rubin M.M. Antenatal exposure to DES: lessons learned...future concerns. *Obstet Gynecol Surv*. 2007;62:548–555.

[245] Schrager S, Potter B.E. Diethylstilbestrol exposure. *Am Fam Physician*. 2004;69:2395–2400.

[246] Schardein J.L. *Hormones and hormonal antagonists, chemically induced birth defects*. 3rd ed. New York: Marcel Dekker; 2000:281–357.

[247] Brent R.L. Nongenital malformations following exposure to progestational drugs: the last chapter of

an erroneous allegation. *Birth Defects Res Part A Clin Mol Teratol*. 2005;73:906–918.
[248] Banhidy F, Acs N, Czeizel A.E. Ovarian cysts, clomiphene therapy, and the risk of neural tube defects. *Int J Gynaecol ObstetOff Organ Int Fed Gynaecol Obstet*. 2008;100:86–88.
[249] Elizur S.E, Tulandi T. Drugs in infertility and fetal safety. *Fertil Steril*. 2008;89:1595–1602.
[250] Lynch A, McDuffie R, Murphy J, Faber K, Leff M, Orl reproductive interventions and multiple birth. *Obstet Gynecol*. 2001;97:195–200.
[251] Sovino H, Sir-Petermann T, Devoto L. Clomiphene citrate and ovulation induction. *Reprod Biomed Online*. 2002;4:303–310.
[252] Brunskill P.J. The effects of fetal exposure to danazol. *Br J Obstet Gynaecol*. 1992;99:212–215.
[253] Carmichael S.L, Shaw G.M. Maternal corticosteroid use and risk of selected congenital anomalies. *Am J Med Genet*. 1999;86:242–244.
[254] Carmichael S.L, Shaw G.M, Ma C, Werler M.M, Rasm corticosteroid use and orofacial clefts. *Am J Obstet Gynecol*. 2007;197:585 e581-587; discussion 683-584, e581–587..
[255] Rodriguez-Pinilla E, Martinez-Frias M.L. Corticosteroids during pregnancy and oral clefts: a case-control study. *Teratology*. 1998;58:2–5.
[256] Shepard T.H, Brent R.L, Friedman J.M, Jones K.L, Mil on new developments in the study of human

teratogens. *Teratology*. 2002;65:153–161.
[257] Kallen B. The teratogenicity of antirheumatic drugs–what is the evidence? *Scand J Rheumatol Suppl*. 1998;107:119–124.
[258] Schardein J.L. *Thyroid-acting agents, chemically induced birth defects*. 3rd ed. New York: Marcel Dekker; 2000:463–473.
[259] Diav-Citrin O, Ornoy A. Teratogen update: antithyroid drugs-methimazole, carbimazole, and propylthiouracil. *Teratology*. 2002;65:38–44.
[260] Iwayama H, Hosono H, Yamamoto H, Oshiro M, Ued cutis congenita with skull defect in a monozygotic twin after exposure to methimazole in utero. *Birth Defects Res Part A Clin Mol Teratol*. 2007;79:680–684.
[261] Chattaway J.M, Klepser T.B. Propylthiouracil versus methimazole in treatment of Graves' disease during pregnancy. *Ann Pharmacother*. 2007;41:1018–1022.
[262] Bartalena L, Bogazzi F, Braverman L.E, Martino E. Eff of amiodarone administration during pregnancy on neonatal thyroid function and subsequent neurodevelopment. *J Endocrinol Invest*. 2001;24:116–130.
[263] Schardein J.L. *Foods, nutrients, and dietary factors, chemically induced birth defects*. 3rd ed. New York: Marcel Dekker; 2000:653–691.
[264] Lammer E.J. *Patterns of malformation among fetuses and infants exposed to retinoic acid (isotretinoin), Banbury report 26: developmental toxicology: mechanisms and action*. Cold Spring Harbor Laboratory; 1987:213–

255.
[265] Fernhoff P.M, Lammer E.J. Craniofacial features of isotretinoin embryopathy. *J Pediatr*. 1984;105:595–597.
[266] Adams J. The neurobehavioral teratology of retinoids: a 50-year history. *Birth Defects Res Part A Clin Mol Teratol*. 2010;88:895–905.
[267] Coberly S, Lammer E, Alashari M. Retinoic acid embryopathy: case report and review of literature. *Pediatr Pathol Lab Med*. 1996;16:823–836.
[268] Miller R.K, Hendrickx A.G, Mills J.L, Hummler H, Wi vitamin A use: how much is teratogenic? *Reprod Toxicol*. 1998;12:75–88.
[269] Lammer E.J. Embryopathy in infant conceived one year after termination of maternal etretinate. *Lancet*. 1988;2:1080–1081.
[270] Autret-Leca E, Kreft-Jais C, Elefant E, Cissoko H, Darrouzain F, Grimaldi-Bensouda L, Attia S, Jonville-Bera A.P. Isotretinoin exposure during pregnancy: assessment of spontaneous reports in France. *Drug Saf*. 2010;33:659–665.
[271] Crijns H.J, Straus S.M, Gispen-de Wied C, de Jong-van den Berg L.T. Compliance with pregnancy prevention programmes of isotretinoin in Europe: a systematic review. *Br J Dermatol*. 2011;164:238–244.
[272] Teichert M, Visser L.E, Dufour M, Rodenburg E, Strau Smet P.A, Stricker B.H. Isotretinoin use and compliance with the Dutch Pregnancy Prevention Programme: a retrospective cohort study in females

of reproductive age using pharmacy dispensing data. *Drug Saf*. 2010;33:315–326. .
[273] van Vliet T, Boelsma E, de Vries A.J, van den Berg H. Retinoic acid metabolites in plasma are higher after intake of liver paste compared with a vitamin A supplement in women. *J Nutr*. 2001;131:3197–3203.
[274] Polifka J.E, Dolan C.R, Donlan M.A, Friedman J.M. Cl teratology counseling and consultation report: high dose beta-carotene use during early pregnancy. *Teratology*. 1996;54:103–107.
[275] Pearlstein T. Perinatal depression: treatment options and dilemmas. *J Psychiatry Neurosci*. 2008;33:302–318.
[276] Viguera A.C, Whitfield T, Baldessarini R.J, Newport D of recurrence in women with bipolar disorder during pregnancy: prospective study of mood stabilizer discontinuation. *Am J Psychiatry*. 2007;164:1817–1824 quiz 1923..
[277] Alwan S, Friedman J.M, Chambers C. Safety of selective serotonin reuptake inhibitors in pregnancy: a review of current evidence. *CNS Drugs*. 2016;30:499–515.
[278] Kallen B, Borg N, Reis M. The use of central nervous system active drugs during pregnancy. *Pharmaceuticals*. 2013;6:1221–1286.
[279] Myles N, Newall H, Ward H, Large M. Systematic meta-analysis of individual selective serotonin reuptake inhibitor medications and congenital malformations. *Aust N Z J Psychiatr*. 2013;47:1002–

1012.
[280] Alwan S, Bandoli G, Chambers C.D. Maternal use of selective serotonin-reuptake inhibitors and risk of persistent pulmonary hypertension of the newborn. *Clin Pharmacol Ther*. 2016;100:34–41.
[281] Pedersen L.H. Prenatal antidepressant exposure and childhood autism spectrum disorders: cause for concern? *Paediatr Drugs*. 2015;17:443–448.
[282] Bracken M.B. Drug use in pregnancy and congenital heart disease in offspring. *N Engl J Med*. 1986;314:1120.
[283] Broussard C.S, Rasmussen S.A, Reefhuis J, Friedman J Colarusso T, Honein M.A. Maternal treatment with opioid analgesics and risk for birth defects. *Am J Obstet Gynecol*. 2011;204(314):e311–311.
[284] Rothman K.J, Fyler D.C, Goldblatt A, Kreidberg M.B. hormones and other drug exposures of children with congenital heart disease. *Am J Epidemiol*. 1979;109:433–439.
[285] Zierler S, Rothman K.J. Congenital heart disease in relation to maternal use of Bendectin and other drugs in early pregnancy. *N Engl J Med*. 1985;313:347–352.
[286] Saxen I. Associations between oral clefts and drugs taken during pregnancy. *Int J Epidemiol*. 1975;4:37–44.
[287] Saxen I. Epidemiology of cleft lip and palate. An attempt to rule out chance correlations. *Br J Prev Soc Med*. 1975;29:103–110.
[288] Shaw G.M, Todoroff K, Velie E.M, Lammer E.J. Mater

illness, including fever and medication use as risk factors for neural tube defects. *Teratology*. 1998;57:1–7.

[289] Yazdy M.M, Mitchell A.A, Tinker S.C, Parker S.E, We use of opioids and the risk of neural tube defects. *Obstet Gynecol*. 2013;122:838–844.

[290] Jansson L.M, Velez M, Harrow C. The opioid-exposed newborn: assessment and pharmacologic management. *J Opioid Manag*. 2009;5:47–55.

[291] Bagley S.M, Wachman E.M, Holland E, Brogly S.B. Re of the assessment and management of neonatal abstinence syndrome. *Addict Sci Clin Pract*. 2014;9:19.

[292] Barbero P, Liascovich R, Valdez R, Moresco A. [Misop teratogenicity: a prospective study in Argentina]. *Arch Argent Pediatr*. 2011;109:226–231.

[293] Dal Pizzol Tda S, Sanseverino M.T, Mengue S.S. Exposure to misoprostol and hormones during pregnancy and risk of congenital anomalies. *Cad Saude Publica*. 2008;24:1447–1453.

[294] Orioli I.M, Castilla E.E. Epidemiological assessment of misoprostol teratogenicity. *BJOG Int J Obstet Gynaecol*. 2000;107:519–523.

[295] Pastuszak A.L, Schuler L, Speck-Martins C.E, Coelho K.E, Cordello S.M, Vargas F, Bru of misoprostol during pregnancy and Mobius' syndrome in infants. *N Engl J Med*. 1998;338:1881–1885.

[296] Vargas F.R, Schuler-Faccini L, Brunoni D, Kim C, Meloni V.F, Sugayama S Bertollo E, Conte A, Koren G, Addis A. Prenatal exposure to misoprostol and vascular disruption defects: a case-control study. *Am J Med Genet*. 2000;95:302–306.

[297] Brent R.L. Congenital malformation case reports: the editor's and reviewer's dilemma. *Am J Med Genet*. 1993;47:872–874.

[298] Coscia L.A, Armenti D.P, King R.W, Sifontis N.M, Co on the teratogenicity of maternal mycophenolate mofetil. *J Pediatr Genet*. 2015;4:42–55.

[299] Jackson P, Paquette L, Watiker V, Randolph L, Raman exposure to mycophenolate mofetil and multiple congenital anomalies in a newborn: possible teratogenic effect. *Am J Med Genet Part A*. 2009;149A:1231–1236.

[300] Martin M.C, Cristiano E, Villanueva M, Bonora M.L, F atresia and prenatal exposure to mycophenolate. *Reprod Toxicol*. 2014;50:117–121. .

[301] NTPR. National transplantation pregnancy registry: 2015 annual report. Philadelphia, PA: Gift of Life Institute; 2016 Available at:. www.NTPRegistry.org.

[302] Quan A. Fetopathy associated with exposure to angiotensin converting enzyme inhibitors and angiotensin receptor antagonists. *Early Hum Dev*. 2006;82:23–28.

[303] Cooper W.O, Hernandez-

Diaz S, Arbogast P.G, Dudley J.A, Dyer S, Gideon P.S, congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med*. 2006;354:2443–2451.

[304] Fisher S.C, Van Zutphen A.R, Werler M.M, Lin A.E, Romitti P.A, Drus antihypertensive medication use and congenital heart defects: updated results from the national birth defects prevention study. *Hypertension*. 2017;69:798–805.

[305] Alwan S, Polifka J.E, Friedman J.M. Angiotensin II receptor antagonist treatment during pregnancy. *Birth Defects Res Part A Clin Mol Teratol*. 2005;73:123–130.

[306] Kidd S.A, Lancaster P.A, Anderson J.C, Boogert A, Fis death after exposure to methylene blue dye during mid-trimester amniocentesis in twin pregnancy. *Prenat Diagn*. 1996;16:39–47.

[307] Brent R. Bendectin and birth defects: hopefully, the final chapter. *Birth Defects Res Part A Clin Mol Teratol*. 2003;67:79–87.

[308] Goh Y.I, Bollano E, Einarson T.R, Koren G. Prenatal multivitamin supplementation and rates of congenital anomalies: a meta-analysis. *J Obstet Gynaecol Can*. 2006;28:680–689.

[309] De-Regil L.M, Fernandez-Gaxiola A.C, Dowswell T, Pena-Rosas J.P. Effects and safety of periconceptional folate supplementation for preventing birth defects. *Cochrane Database Syst Rev*. 2010:CD007950.

[310] Wolff T, Witkop C.T, Miller T, Syed S.B. Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2009;150:632–639.

[311] Bailey L.B, Berry R.J. Folic acid supplementation and the occurrence of congenital heart defects, orofacial clefts, multiple births, and miscarriage. *Am J clin Nutr*. 2005;81:1213S–1217S.

[312] Beaudin A.E, Stover P.J. Insights into metabolic mechanisms underlying folate-responsive neural tube defects: a minireview. *Birth Defects Res Part A Clin Mol Teratol*. 2009;85:274–284.

[313] Blom H.J. Folic acid, methylation and neural tube closure in humans. *Birth Defects Res Part A Clin Mol Teratol*. 2009;85:295–302.

[314] Molloy A.M, Brody L.C, Mills J.L, Scott J.M, Kirke P.N search for genetic polymorphisms in the homocysteine/folate pathway that contribute to the etiology of human neural tube defects. *Birth Defects Res Part A Clin Mol Teratol*. 2009;85:285–294.

[315] Bloom M.S, Houston A.S, Mills J.L, Molloy C.A, Hedig bone immaturity and 2D:4D ratio measurement error in the assessment of the hyperandrogenic hypothesis for the etiology of autism spectrum disorders. *Physiol Behav*. 2010;100:221–224.

[316] Bell K.N, Oakley Jr. G.P. Update on prevention of folic acid-preventable spina bifida and anencephaly. *Birth Defects Res Part A Clin Mol*

*Teratol*. 2009;85:102–107.
[317] Nold J.L, Georgieff M.K. Infants of diabetic mothers. *Pediatr Clin North Am*. 2004;51:619–637 viii..
[318] Reece E.A, Homko C.J. Prepregnancy care and the prevention of fetal malformations in the pregnancy complicated by diabetes. *Clin Obstet Gynecol*. 2007;50:990–997.
[319] Mitanchez D, Yzydorczyk C, Siddeek B, Boubred F, B offspring of the diabetic mother–short- and long-term implications. *Best Pract Res Clin Obstet Gynaecol*. 2015;29:256–269.
[320] Wren C, Birrell G, Hawthorne G. Cardiovascular malformations in infants of diabetic mothers. *Heart*. 2003;89:1217–1220.
[321] Kapoor N, Sankaran S, Hyer S, Shehata H. Diabetes in pregnancy: a review of current evidence. *Curr Opin Obstet Gynecol*. 2007;19:586–590.
[322] Walkinshaw S.A. Pregnancy in women with pre-existing diabetes: management issues. *Semin Fetal Neonatal Med*. 2005;10:307–315.
[323] Ali S, Dornhorst A. Diabetes in pregnancy: health risks and management. *Postgrad Med J*. 2011;87:417–427.
[324] Balsells M, Garcia-Patterson A, Gich I, Corcoy R. Maternal and fetal outcome in women with type 2 versus type 1 diabetes mellitus: a systematic review and metaanalysis. *J Clin Endocrinol Metab*. 2009;94:4284–4291.
[325] Boinpally T, Jovanovic L. Management of type 2

diabetes and gestational diabetes in pregnancy. *Mt Sinai J Med*. 2009;76:269–280.
[326] Temple R, Murphy H. Type 2 diabetes in pregnancy – an increasing problem. *Best Pract Res Clin Endocrinol Metab*. 2010;24:591–603.
[327] Parnell A.S, Correa A, Reece E.A. Pre-pregnancy obesity as a modifier of gestational diabetes and birth defects associations: a systematic review. *Matern Child Health J*. 2017;21:1105–1120.
[328] Allen V.M, Armson B.A, Wilson R.D, Blight C, Gagno Lindsay L, Morin V, Schneider C.E, Van Aerde J, Society of, O., Gynecologists of, C. Teratogenicity associated with pre-existing and gestational diabetes. *J Obstet Gynaecol Can*. 2007;29:927–944. .
[329] Clausen T.D, Mathiesen E, Ekbom P, Hellmuth E, Mar Poulsen T, Damm P. Poor pregnancy outcome in women with type 2 diabetes. *Diabetes Care*. 2005;28:323–328.
[330] Lapolla A, Dalfra M.G, Fedele D. Pregnancy complicated by type 2 diabetes: an emerging problem. *Diabetes Res Clin Pract*. 2008;80:2–7.
[331] Guttler F, Azen C, Guldberg P, Romstad A, Hanley W la Cruz F, Koch R. Impact of the phenylalanine hydroxylase gene on maternal phenylketonuria outcome. *Pediatrics*. 2003;112:1530–1533.
[332] Koch R, Hanley W, Levy H, Matalon K, Matalon R, Ro

la Cruz F. The maternal phenylketonuria international study: 1984-2002. *Pediatrics*. 2003;112:1523–1529.

[333] Prick B.W, Hop W.C, Duvekot J.J. Maternal phenylketonuria and hyperphenylalaninemia in pregnancy: pregnancy complications and neonatal sequelae in untreated and treated pregnancies. *Am J Clin Nutr*. 2012;95:374–382.

[334] Maillot F, Lilburn M, Baudin J, Morley D.W, Lee P.J. F influencing outcomes in the offspring of mothers with phenylketonuria during pregnancy: the importance of variation in maternal blood phenylalanine. *American J Clin Nutr*. 2008;88:700–705.

[335] Bouchlariotou S, Tsikouras P, Maroulis G. Undiagnos maternal phenylketonuria: own clinical experience and literature review. *J Matern Fetal Neonatal Med*. 2009;22:943–948.

[336] Hanley W.B. Finding the fertile woman with phenylketonuria. *Eur J Obstet Gynecol Reprod Biol*. 2008;137:131–135.

[337] Rasmussen S.A, Chu S.Y, Kim S.Y, Schmid C.H, Lau J obesity and risk of neural tube defects: a metaanalysis. *Am J Obstet Gynecol*. 2008;198:611–619.

[338] Stothard K.J, Tennant P.W, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *J Am Med Assoc*. 2009;301:636–650.

[339] Cai G.J, Sun X.X, Zhang L, Hong Q. Association between maternal body mass index and congenital heart defects in offspring: a systematic review. *Am J Obstet Gynecol*. 2014;211:91–117.

[340] Gilboa S.M, Correa A, Botto L.D, Rasmussen S.A, Wal Colarusso T.J. Association between prepregnancy body mass index and congenital heart defects. *Am J Obstet Gynecol*. 2010;202:51 e51-51 e10..

[341] Rankin J, Tennant P.W, Stothard K.J, Bythell M, Summ body mass index and congenital anomaly risk: a cohort study. *Int J Obes (Lond)*. 2010;34:1371–1380.

[342] Moise Jr. K.J. Management of rhesus alloimmunization in pregnancy. *Obstet Gynecol*. 2008;112:164–176.

[343] Pilgrim H, Lloyd-Jones M, Rees A. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. *Health Technol Assess*. 2009;13:1–103 iii, ix-xi.

[344] Dallas J.S. Autoimmune thyroid disease and pregnancy: relevance for the child. *Autoimmunity*. 2003;36:339–350.

[345] Skogen B, Killie M.K, Kjeldsen-Kragh J, Ahlen M.T, Tiller H, Stuge T.B, Husebekk A. fetal and neonatal alloimmune thrombocytopenia with a focus on screening and prevention. *Expert Rev Hematol*. 2010;3:559–566.

[346] Symington A, Paes B. Fetal and neonatal alloimmune thrombocytopenia: harvesting the evidence to develop a clinical approach to

management. *Am J Perinatol*. 2011;28:137–144.
[347] Lee L.A. The clinical spectrum of neonatal lupus. *Arch Dermatol Res*. 2009;301:107–110.
[348] Buyon J.P, Clancy R.M, Friedman D.M. Autoimmune associated congenital heart block: integration of clinical and research clues in the management of the maternal/foetal dyad at risk. *J Intern Med*. 2009;265:653–662.
[349] Salomonsson S, Strandberg L. Autoantibodies associated with congenital heart block. *Scand J Immunol*. 2010;72:185–188.
[350] Dale S.T, Coleman L.T. Neonatal alloimmune thrombocytopenia: antenatal and postnatal imaging findings in the pediatric brain. *AJNR Am J Neuroradiol*. 2002;23:1457–1465.
[351] Vincent A, McConville J, Farrugia M.E, Bowen J, Plest Davis J, Hoch W. Antibodies in myasthenia gravis and related disorders. *Ann N Y Acad Sci*. 2003;998:324–335.
[352] Shanske A.L, Bernstein L, Herzog R. Chondrodysplas punctata and maternal autoimmune disease: a new case and review of the literature. *Pediatrics*. 2007;120:e436–441.
[353] Robaire B, Hales B.F. *Advances in male mediated developmental toxicity*. New York: Kluwer Academic; 2003.
[354] Carone B.R, Fauquier L, Habib N, Shea J.M, Hart C.E,

induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell*. 2010;143:1084–1096. .
[355] Hirasawa R, Feil R. Genomic imprinting and human disease. *Essays Biochem*. 2010;48:187–200.
[356] John R.M, Lefebvre L. Developmental regulation of somatic imprints. *Differentiation*. 2011;81:270–280.
[357] Friedman J.M. Implications of research in male-mediated developmental toxicity to clinical counsellors, regulators, and occupational safety officers. *Adv Exp Med Biol*. 2003;518:219–226.
[358] Sankaranarayanan K. Estimation of the hereditary risks of exposure to ionizing radiation: history, current status, and emerging perspectives. *Health Phys*. 2001;80:363–369.
[359] Allen C, Reardon W. Assisted reproduction technology and defects of genomic imprinting. *BJOG Int J Obstet Gynaecol*. 2005;112:1589–1594.
[360] Manipalviratn S, DeCherney A, Segars J. Imprinting disorders and assisted reproductive technology. *Fertil Steril*. 2009;91:305–315.

## Further Reading

Briggs G.G, Freeman R.K, Towers C.V, Forinash A.B. *Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk*. 11th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2017.

Jones K.L, Jones M.C, del Campo M. *Smith's recognizable patterns of human malformations*. 7th ed. Philadelphia: Elsevier Saunders; 2013.

Schaefer C, Peters P, Miller R.K. *Drugs during preganancy and lactation: treatment options and risk assessment*. 3rd

ed. Amsterdam: Elsevier; 2014.

Wilson C.B, Nizet V, Maldonado Y.A, Remington J.S, Klein J.O. *Infectio diseases of the fetus and newborn infant*. 8th ed. Philadelphia: Elsevier Saunders; 2016.

3

# Neurodevelopmental Disabilities

## Global Developmental Delay, Intellectual Disability, and Autism

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## Abstract

This chapter addresses the genetic heterogeneity in intellectual disabilities and autism spectrum disorders. The author includes a review of the genomic deletions and duplications identified by chromosome microarray technology. Genes known to be involved in both intellectual disability and autism are discussed. Whole exome sequencing is now the best approach to such patients when the cause is not recognized. The chapter also reviews inborn errors of metabolism seen in patients with intellectual disabilities and the diagnostic approach to these metabolic disorders. It contains a review of the role of brain imaging and central nervous systemmalformations in the setting of intellectual disability. The promise of whole genome sequencing is discussed.

## Keywords

Autism; Developmental disability; Global developmental delay; Intellectual disability; Mental retardation

## 3.1. Intellectual Disability and Global Developmental Delay

Intellectual disability (ID) is a developmental disability presenting in infancy or the early childhood, though it cannot be diagnosed until the child is older than approximately 5 years, when standardized measures of developmental skills become reliable and valid. The American Association on Intellectual and Developmental Disability defines *intellectual disability* using measures of three domains: intelligence, adaptive behavior, and systems of supports afforded the individual [1]. Thus, one cannot rely solely on the measure of IQ to define intellectual disability. The term "intellectual disability" largely has replaced "mental retardation" [1]. Often, it is solely intelligence (typically measured by an intelligence quotient [IQ]) that is used to characterize intellectual function in the medical literature; however, standardized measures of "adaptive behavior" and of "social supports" should be considered to provide a more comprehensive and reliable data set for describing a person. Such measures may affect the medical genetic evaluation process and outcomes. The psychology profession requires completion of all measures before one can establish the diagnosis of intellectual disability in an individual. The gradations of disability are not different with this change in language: borderline, mild, moderate, severe, and profound.

For the purposes of this chapter, we define intellectual disability (ID) as a disability characterized by significant limitations both in intellectual functioning and in adaptive behavior as expressed in conceptual, social, and practical adaptive skills. The disability originates before age 18 years [1]. The prevalence of intellectual disability is estimated to be between 1% and 3% [2]. Costs (direct and indirect) to support individuals with ID are large, estimated to be as high as $1 million lifetime costs [3]. It has been estimated that the lifetime costs for medical care of US children born with an ID in 2000 will be $50 billion [4].

## 3.2. Global Developmental Delay

Global developmental delay (GDD) is defined as a significant delay in two or more developmental domains including gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living and is thought to predict a future diagnosis of intellectual [5]. Such delays require accurate documentation using norm-referenced and age-appropriate standardized measures of development administered by experienced developmental specialists. The term *global developmental delay* is usually reserved for younger children (typically less than 5 years of age). Children with GDDs are those who present with delays in the attainment of developmental milestones at the expected age; this implies deficits in learning and adaptation, which suggests that the delays are significant and predict later ID. However, delays in development, especially those that are mild, may be transient and lack predictive reliability for ID or other developmental disabilities. This requires the clinician to judge the significance of the presenting signs and symptoms and the likelihood that they imply a pathological problem and not a normal variation in development in the infant or very young child. This, in turn, will determine the optimal diagnostic evaluation pathway. The prevalence of GDD is estimated to be 1%–3% [6].

The purpose of identifying the cause is to address treatment options, discuss the expected clinical course, and offer information regarding risk of recurrences for the family. For parents or family members, the motivations to understand the cause of the disability vary. For example, Lenhard et al. [7] assessed the psychological benefit of a diagnosis by comparing the level of anxiety, guilt, and emotional burden among three groups: mothers of nondisabled children, mothers of children with Down syndrome, and mothers of children with idiopathic ID. They reported that mothers of children with Down syndrome and nondisabled children had similar scores for these measures, while mothers of children with idiopathic ID had "broad psychoemotional disadvantages." The authors offered the opinion that Down syndrome is so well known such that mothers of

affected children may have different experiences from mothers of children with ID of other causes.

Graungaard and Skov [8] interviewed parents of eight children aged 1–27 months who had recently been diagnosed with significant physical and mental disabilities and concluded that a diagnosis "could enable parents to regain predictability and control in their life" and provide them with active coping strategies.

Because "we do not know if the parents' ability to adapt to and cope with their child's ID is actually improved by knowing a specific genetic cause," Makela et al. [9] set out to address this question and interviewed families in depth in an effort to address the "value" of a specific etiological diagnosis in the child with an ID. Their qualitative research involving interviews of families of children with ID with and without an etiologic diagnosis and found these 20 families had specific stated needs and feelings about what a diagnosis offers:

1. Validation—A diagnosis established that the problem (ID) was credible, which empowered them to advocate for their child.
2. Information—A diagnosis was thought to help guide expectations and management immediately and provide hope for treatment or cure in the future.
3. Procuring services—The diagnosis assisted families in obtaining desired services, particularly in schools.
4. Support—Families expressed the need for emotional companionship that a specific diagnosis (or "similar challenges") assisted in accessing.
5. Need to know—Families widely differed in their "need to know" a specific diagnosis, ranging from strong to indifferent.
6. Prenatal testing—Families varied in their emotions, thoughts, and actions regarding prenatal genetic diagnosis.

Families differ on the importance of the specific etiology of their family member's disability and this may change with time [10]. The medical geneticist might be advised to explore the motivations with families as the evaluation process and treatment outcome are valued

differently by families.

## 3.3. Definition of a Diagnosis

There is not unanimity in the definition of a "genetic or genomic diagnosis." For many families and physicians, the diagnosis of the neurodevelopmental disability (e.g., "autism,""mild intellectual disability," etc.) is a sufficient clinical diagnosis and it does not always lead to counseling of the patient or family about the potential value of the etiological evaluation [6,9]. Furthermore, there are no shared definitions of "treatment" and "improved outcomes" resulting from a genetics evaluation, nor a body of literature demonstrating the value of a diagnosis in reference to the long-term outcome for those with neurodevelopmental disabilities. Nevertheless, expert opinions with support of many caseseries indicate that a comprehensive evaluation is required to plan health care for individuals with neurodevelopmental disorders [11,12].

Schaefer and Bodensteiner [13] wrote that a specific diagnosis "can be translated into useful clinical information for the family, including providing information about prognosis, recurrence risks, and preferred modes of available therapy." van Karnebeek et al. [14] defined *etiologic* diagnosis as "sufficient literature evidence … to make a causal relationship of the disorder with mental retardation likely, *and* if it met the Schaefer-Bodensteiner definition."

This chapter defines the "genetic or genomic diagnosis" as that in which there is sufficient literature to make a causal inference between the clinical and laboratory diagnostic information and the patient's phenotype, thus informing treatment options, recurrence risks, and patient outcomes.

**Table 3.1**

**Summary of Clinical Studies of Whole Exome Sequencing in Neurodevelo**

| Patients | Diagnosis | Candidate Genes | Total % of Cases | Total Number | C |
|---|---|---|---|---|---|

| | (%) | Identified | Diagnosed | in Study | |
|---|---|---|---|---|---|
| **Intellectual disability (ID)** | 91 (55) | 56 (24) | 79 | 232 | [1 |
| **ID and/or epileptic encephalopathy** | 14 (32.5) | Not reported (N.R.) | 32.5 | 43 | [1 |
| **ID, other neurological complications** | 44 (29.3) | 41 (27.3) | 56.6 | 150 | [1 |
| **"Dysmorphology syndromes"** | 28 (90) | N.R. | 90 | 31 | [1 |
| **"Neurodevelopmental phenotypes"** | 31 (31.6) | N.R. | 31.6 | 98 | [1 |
| **Neurodevelopmental disorders ("nonacute")** | 34[a](40) | N.R. | 40 | 85 | [2 |
| **ID** | 5 (29.4) | N. R. | 29.4 | 17 | [2 |
| **"Developmental disorders"** | 317 (28) | N.R. | 28 | 1133 | [2 |
| **Heterogeneous patient indications** | 504 (25.2) | N.R. | 25.2 | 2000 | [2 |
| **Developmental delay, patients <5 years of** | 45 (41) | N.R. | 45 | 109 | [2 |

| | | | | | |
|---|---|---|---|---|---|
| **age** | | | | | |
| **ID** | 24 (41.4) | N.R. | 41.4 | 58 | [2 |
| **ID, families with more than one affected member** | 3 (16) | 5 (26) | 42 | 19 | [2 |
| **Severe, nonsyndromic ID** | 16 (31.4) | 3 (6) | 37.4 | 51 | [2 |
| **Neurodevelopmental or dysmorphology** | 6 (50) | 1 (8) | 12 | 58 | [2 |
| **Severe ID** | 16 (16) | N.R. | 16 | 100 | [2 |

[a] 1 patient had staged WES then WGS; N.R., not reported.

## 3.4. Whole Exome Sequencing

The clinical utility and the potential cost savings to the diagnostic evaluation process of whole exome sequencing (WES) in the evaluation of the patient with neurodevelopmental disorders are now established. About 35%–40% of those patients with neurodevelopmental disorders and negative prior standard diagnostic evaluations will have a certain diagnosis with the use of WES (Table 3.1).

The question of when in the diagnostic process WES should be arranged also appears settled—early and before other (expensive) diagnostic testing. This question of timing relates to some extent to the costs associated with the counseling and testing as well as to the likely

outcome of such testing for the health and health care of the affected patient. There is some support in the literature for cost savings and for early application of diagnostic WES [16,17,19,21,30,31].

## 3.5. Whole Genome Sequencing

The role of whole genome sequencing (WGS) in the clinical evaluation of the patient with neurodevelopmental disabilities is not yet clear but promises to identify genetic causes in those who remain undiagnosed. For example, in one study of 50 patients (trios) with severe IDwho had been evaluated by expert clinicians with negative chromosome microarray studies and WES [32], WGS led to a conclusive diagnosis in 21 (42%) of the patients [32]. In a small study of eight patients with ID and central nervous system (CNS) anomalies [33], two patients were identified with known ID gene variants (*ARID1B*, *PHF6*), and in four additional patients, candidate genes for ID were noted [33].

## 3.6. Phenotyping

"Phenotyping" the patient to determine which test should be ordered should continue prior to any diagnostic testing. However, now phenotyping is also key in interpreting WES or WGS and applying focused phenotyping based on that sequence result [34–36]. Nevertheless, for the time being the clinical approach to the patient with neurodevelopmental disability will continue to be in the tradition of the profession to include these elements: the child medical history (including prenatal and birth histories); the family history which includes construction and analysis of a pedigree of three generations or more; the physical and neurologic examinations emphasizing the examination for minor anomalies (the "dysmorphology exam"); and, the examination for neurologic or behavioral signs that might suggest a specific recognizable syndrome or diagnosis. After the clinical genetic evaluation, judicious use of the laboratory tests, imaging, and other consultants based on best evidence are important in establishing the diagnosis (see: Table 3.2).

The medical geneticist experienced in the diagnostic evaluation and

treatment of patients with neurodevelopmental deficits remains the essential element in the evaluation process. This expert must be facile with the dysmorphology examination (and morphological pattern recognition) as well as the standard neurological examination. An international group of clinicians who are expert in the field of dysmorphology published a proposed nomenclature to be used to describe human morphology [37]. This effort improves the description of patient phenotype and facilitates comparison of findings among patients. It also serves to bring consistency and precision needed for communicating with colleagues in the research community. Pattern recognition of minor morphologic abnormalities (i.e., "dysmorphology" [38]) remains a pivotal skill of the medical geneticist in the evaluation of the patient with neurodevelopmental disability. Currently, the recognition of the pattern of dysmorphology, when present in the patient with neurodevelopmental disability, facilitates the efficient diagnostic evaluation process.

## Table 3.2

### MedicalGenetics Evaluation for Global Developmental Delays or Intellectual Delays

Medical genetics evaluation process:

1. Clinical history
2. Family history
3. Physical examination (especially for minor anomalies)
4. Neurological examination
5. Specific confirmatory genetic/genomic tests for suspected syndromes
6. Chromosome microarray if specific diagnosis not suspected
7. Fragile X molecular genetic testing if CMA is negative and phenotype is suggestive
8. Whole exome sequencing in all with significant disability
9. Metabolic screening in all
10. Targeted MRI brain imaging (microcephaly, macrocephaly, abnormal neurological examination)

Often patients present with nonspecific clinical findings, and because the etiology of ID is widely heterogeneous, it is advised that clinicians consider WES early in the diagnostic evaluation process and "phenotype" the patient with the variants identified by WES in mind [39]—as these authors state: *Diagnostic skills of medical specialists will shift from a pre-NGS [next generation sequencing]-test differential diagnostic mode to a post-NGS-test diagnostic assessment mode* [39].

Phenotyping support tools for clinicians and research have grown rapidly recently. Expert clinicians have published phenotypic nomenclature. An international group of clinicians working in the field of dysmorphology has initiated the standardization of terms used to describe human morphology. The goals are to standardize these terms and reach consensus regarding their definitions. Efforts to develop a standard set of computer readable terms to describe the phenotypic features have been published [40]. Others have developed Web-based tools designed to share information about other phenotypically similar patients with deleterious-appearing variants in the same candidate gene [41], (at www.phenomecentral.org), [41]. Others have developed "matchmaking" databases (www.genematcher.com) [42]. These matchmaking programs aim to connect clinicians/investigators based on interest in common genes and phenotypes. Face2Gene (www.face2gene.com, www.fdna.com) applies facial recognition tools to provide the expert clinician with a differential diagnosis for patients presenting with genetic syndromes [43]. Clinical geneticists should be facile with these tools in the roles of interpreting genetic and genomic testing results, providing an accurate diagnosis efficiently for patients, and to connect with researchers and with other affected individuals.

## 3.7. Genetic Mechanisms of ID

The number of genes identified that cause ID suggests that an ID phenotype can emerge as the final common pathway of many different types of abnormal cellular processing and that no one overriding mechanism is likely to be the cause of IS [44]. There are

more than2500 genes or phenotypes listed in OMIM with "mental retardation" as the search term, and it is estimated that one-third of the entire genome is expressed in the human brain. Mutations in more than 450 genes have been identified to cause IDs and related cognitive disorders. There are more than 200 X-linked genes for ID cloned or mapped. There are many inferred mechanisms by which pathogenic mutations affect CNS function (i.e., cause ID). It is emerging that ID can result from a wide range of protein abnormalities. The genes identified earliest were frequently signaling molecules in the RhoGTPase pathway (*GDI, PAK3, ARHGEF6*) or associated with chromatin remodeling (*RPS6KA3, ATRX*). Most recently, components of the synaptic vesicle or components necessary for its formation have been identified as defective (*SYN1, SLC6A8, NLGN4, DLG3*), and a number of novel transcription factors (*ZNF41, ZNF81, ZNF674*) have been found. Inlow and Restifo [45] identified the following categories of mechanism or dysfunction by an in silico review: enzymatic mediators of signal transduction, binding proteins, transporter proteins, cell adhesion molecules, structural molecules, motor proteins, tRNAs, apoptosis regulators, chaperones, and enzyme regulators. There are at least two curated online resources for X-linked genes causing ID: http://xlmr.interfree.it/home.htm; and http://www.ggc.org/xlmr_update.htm.

## 3.8. Diagnostic Testing of Patients With ID of Unknown Cause

Often, the expert medical geneticist or other specialist will recognize the likely or certain cause of the ID on completionof the history and examination [46]. More often, the cause of the disability is not certain and calls for a stepwise diagnostic evaluation with laboratory, imaging, and consultations with other expert clinicians. WES should be arranged early in this diagnostic process given the nonspecific phenotype in so many patients, the highly heterogenetic genetic and genomic etiologies, and the diagnostic power of WES. Cost savings is achieved only by early application of WES whereby other expensive

genetic testing can be omitted; if WES is done late in the diagnostic process, the costs are additive [17]. The costs for WES somewhat vary by location, as does insurance coverage for clinical WES testing. It is expected that with the existing literature demonstrating clinical utility and cost savings when done early in the diagnostic evaluation of patients with ID/NDD, early application of WES testing will be accepted as standard practice.

## 3.9. Cytogenomic Copy Number Abnormalities

Advances in molecular genetic technology have quickened the pace of the discovery of the genetic underpinnings of disorders. Chromosome SNP microarrays now enable the detection of deletions or duplications of candidate genes. This applies to those with ID and/or autism spectrum disorders (ASDs). The clinical application of the chromosomal microarray has replaced the standard karyotype, with certain clinical exceptions (e.g., in the use of the standard karyotype for confirming common syndromes such as Down syndrome). There are also recognizable syndromes associated with chromosomal abnormalities that are detectable by conventional karyotyping or by targeted fluorescent in situ hybridization (FISH) analysis based on suspicion of the specific syndrome, such as Prader-Willi and Angelman syndromes (15q11.2-q13), Williams-Beuren syndrome (7q11.23), Smith-Magenis syndrome (17p11.2), DiGeorge syndrome (22q11.2), and monosomy of 1p36.1. Some balanced chromosomal translocations, not detectable by microarray, may be causal of the ID viamechanisms such as position effects.

Nevertheless, the cytogenomic microarray is more sensitive than the standard karyotype at identifying genomic imbalance as the cause of ID or ASDs. The published diagnostic rates among study populations with ID range from 5% to 20%, about twice the rate by conventional karyotype [47]. Most of these cases are microdeletion/duplications with many recurrent patients with similar recognizable phenotypes (Table 3.3). The pathogenic mechanisms for these deletions or duplications are challenging to predict (i.e., whether a syndrome is due to a mutation in a single gene or the result of deletion,

duplication, or dysregulation of multiple genes). For example, application of microarray to known syndromes, like CHARGE syndrome, has allowed for the identification of haploinsufficiency of a single gene as cause for this known syndrome (Table 3.4). Many of the microdeletions/duplications are rare or unique. Consequently, there are several efforts to catalogue patient phenotypes associated with copy number abnormalities [48]. Table 3.4 provides a partial listing of prevalent dosage-sensitive genes by proposed mechanism. Curated comprehensive dosage-sensitive maps can be found online (e.g., https://www.clinicalgenome.org/data-sharing/clinvar/).

CMA diagnoses associated with specific medical recommendations are found in approximately 7% of all cases ($n$ = 28,526). Recommendations included pharmacological treatment, cancer-related screening or exclusion of screening, contraindications, and referrals for further evaluation [49,50].

More than700 genes have now been identified across studies of X-linked, autosomaldominant, and autosomalrecessive ID, which can be used for the molecular diagnosis of both isolated ID and ID-associated disorders [51].

## 3.10. X-Linked ID

A number of factors have contributed to the progress in understanding ID due to X-linked genes, particularly when the pace of discovery is compared withthat of autosomal genes. Some of these factors include longstanding awareness of the excess of males in many population studies dating to the 19th century,a large number of published clinical reports of large X-linked pedigrees dating back 70 years [52], the intense interest in fragile X syndrome (FXS) for some 40 years [53], and the relative ease of mapping and identifying X-linked genes compared with autosomal genes. Duringthe past 5or so years, advances in molecular genetic technology (WES, WGS) have quickened the pace of gene discovery [51]. And advances in genome

sequencing technology has made it possible for laboratories to sequence all X-linked genes in a single individual to identify pathogenic mutations.

Approximately 200 X-linked genes cause ID; about half have been identified and another 20% are regionally mapped. All genes responsible for X-linked ID (XLID) families reported before the rediscovery of FXS have now been identified,and, most, if not all, are the high-prevalence genes, that is, those of a prevalence of 1% or greater. Nevertheless, there remain many genes unmapped and mapped yet unidentified, and together they comprise a substantial proportion of XLID.

XLID often is subdivided into syndromic (S-XLID) and nonsyndromic (NS-XLID) forms, depending on whether further abnormalities are found on physical examination, laboratory investigation, and brain imaging [54,55]. Roughly two-thirds of X-linked MR (XLMR) cases are thought to be nonsyndromic; however, as the possibilities for classifying families through molecular studies improve and as patients are examined in more detail, it is likely that the proportion of syndromic cases that are diagnosed will increase, with a concomitant decrease in the nonsyndromic cases. This is illustrated by the FXS, which was initially described as nonsyndromic and is now considered to be the most frequent example of S-XLMR. Moreover, mutations in several XLID genes can give rise to both nonsyndromic and syndromic forms, which demonstrates the difficulties in such classification schema.

## Table 3.3

### Recurrent Interstitial CMA Deletions and Duplications in Intellectual Disability

| Name | Size (Mb)[a] | LCR | MIM | Clinical Features |
|---|---|---|---|---|
| **1q21.1 microdeletion** | 1.1 | + | 612474 | Mild-to-moderate MR, MC, cardiac abnormalities, |

| | | | | |
|---|---|---|---|---|
| | | | | cataracts, clear incomplete penetrance |
| **1q21.1 microduplication** | 1.1 | + | 612475 | Autism or autistic behaviours, mild to moderate MR, microcephaly, mild FD |
| **1q41q42 microdeletion** | 1.2 | − | − | MR, seizures, various dysmorphisms, cleft palate, diaphragmatic hernia |
| **2p15q16.1 microdeletion** | 3.9 | − | − | MR, MC, receding forehead, ptosis, telecanthus, short palpebral fissures, down-slanting palpebral fissures, broad/high nasal bridge, long/straight eyelashes, smooth and long philtrum, smooth upper vermillion border, everted lower lip, high narrow palate, hydronephrosis, optic nerve hypoplasia |
| **3q29 microdeletion** | 1.6 | + | 609425 | MR, mild FD, including high |

| | | | | |
|---|---|---|---|---|
| | | | | nasal bridge and short philtrum |
| **3q29 microduplication** | 1.6 | + | 611936 | Mild/moderate MR, MC, obesity |
| **7q11.23 microduplication** | 1.5 | + | 609757 | MR, speech and language delay, autism spectrum disorders, mild FD |
| **9q22.3 microdeletion** | 6.5 | − | – | MR, hyperactivity, overgrowth, trigonocephaly, macrocephaly, FD |
| **12q14 microdeletion** | 3.4 | − | – | Mild MR, failure to thrive, proportionate short stature and osteopoikilosis |
| **14q11.2 microdeletion** | 0.4 | − | – | MR, widely spaced eyes, short nose with flat nasal bridge, long philtrum, Cupid's bow of the upper lip, full lower lip, auricular anomalies |
| **15q13.3 microdeletion** | 1.5 | + | 612001 | MR, epilepsy, hypotonia, short stature, microcephaly and cardiac defects |
| **15q24 microdeletion** | 1.7 | + | – | MR, growth |

| | | | | |
|---|---|---|---|---|
| | | | | retardation, MC, digital abnormalities, genital abnormalities, hypospadias, loose connective tissue, high frontal hairline, broad medial eyebrows, down-slanted palpebral fissures, long philtrum |
| **16p11.2 microdeletion/duplication** | 0.6 | + | 611913 | Association with MR, autism, schizophrenia |
| **16p11.2p12.2 microdeletion** | 7.1 | + | – | MR, flat facies, down-slanting palpebral fissures, low-set and malformed ears, eye anomalies, orofacial clefting, heart defects, frequent ear infections, short stature, minor hand and foot anomalies, feeding difficulties, hypotonia |
| **16p13.1 microduplication** | 1.6 | + | – | Association with autism, significance |

| | | | | |
|---|---|---|---|---|
| | | | | uncertain |
| **16p13.1 microdeletion** | 1.6 | + | – | MR, MC, epilepsy, short stature, phenotypic variability |
| **17p11.2 microduplication**[b] | 3.7 | + | 610883 | MR, infantile hypotonia, failure to thrive, autistic features, sleep apnoea, and structural cardiovascular anomalies |
| **17q21.31 microdeletion** | 0.5 | + | 610443 | MR, hypotonia, long hypotonic face with ptosis, large and low set ears, tubular or pear shaped nose with bulbous nasal tip, long columella with hypoplastic alae nasi, broad chin |

Table Continued

| Name | Size (Mb)[a] | LCR | MIM | Clinical Features |
|---|---|---|---|---|
| **19q13.11 microdeletion** | 0.7 | – | – | MR, prenatal and postnatal growth retardation, primary microcephaly, hypospadias, ectodermal dysplasia including scalp |

| | | | | |
|---|---|---|---|---|
| | | | | aplasia, dysplastic nails and dry skin |
| **22q11.2 microduplication** | 3.7 | + | 608363 | Highly variable. MR, FD, for example widely spaced eyes and down-slanting palpebral fissures, velopharyngeal insufficiency, conotruncal heart disease |
| **22q11.2 distal microdeletion** | 1.4–2.1 | + | 611867 | MR, prematurity, prenatal/postnatal growth delay, mild skeletal abnormalities, arched eyebrows, deep set eyes, smooth philtrum, thin upper lip, hypoplastic alae nasi, small pointed chin |
| **Xq28 microduplication** | 0.4–0.8 | – | – | MR, severe hypotonia, progressive lower limb spasticity, absent or very limited speech |

*FD*, facial dysmorphisms; *LCR*, low copy repeat; *MC*, microcephaly; *MR*, mental retardation.

[a] Common region.

[b] Potockie-Lupski syndrome.

Vissers LE, de Vries BB, Veltman JA. Genomic microarrays in mental retardation: from copy number variation to gene, from research to diagnosis. J Med Genet 2010;47(5):289–297.

## Table 3.4

### Selected Dosage-Sensitive Genes Causing ID and Identified by Deletion

## and/or Duplication Strategies

| Syndrome | Chromosome | Size | Gene(s) involved | MIM |
|---|---|---|---|---|
| **Cystinuria with mitochondrial disease** | del(2)(p16) | 179 kb | SLC3A1, PPM1B, KIAA0436 | 606407 |
| **Adrenal hyperplasia with hypermobility** | del(6)(p21) | 33 kb | TNBX, CYP21A | – |
| **CHARGE syndrome** | del(8)(q12) | 2300 kb | CHD7 | 214800 |
| **Oto-facial-cercival syndrome** | del(8)(q13.3) | 316 kb | EYA1 | 166780 |
| **9q subtelomeric deletion syndrome** | del(9)(q34) | Diverse | EHMT1 | 610253 |
| **Potocki-Shaffer syndrome** | del(11)(p11.2) | 2100 kb | EXT2, ALX4 | 601224 |
| **Infantile hyperinsulinism enteropathy and deafness** | del(11) (p15p14) | 122 kb | USH1C, ABCC8, KCNJ11 | 606528 |
| **12q14 microdeletion syndrome** | del(12)(q14) | 3440 kb | LEMD3, HMGA2, GRIP1 | – |
| **Peters Plus syndrome** | del(13) (q12.3q13.1) | 1500 kb | B3GALTL | 261540 |
| **Tuberous sclerosis polycystic kidney disease** | del(16)(p13) | 87 kb | TSC2, PKD1 | 173900 |
| **PotockieLupski syndrome** | dup(17) (p11.2p11.2) | 3700 kb | RAI1 | 610883 |

| | | | | |
|---|---|---|---|---|
| **Alport leiomyomatosis** | del(X)(q22.3) | 133 kb | COL4A5, COL4A6 | 301050 |
| **MECP2 duplication syndrome** | dup(X)(q28) | Variable | MECP2 | – |

## 3.11. Fragile X Syndrome

In 1969, Lubs [56] identified a family with four male members diagnosed with ID, each of whom had an unusual chromosomal gap on his X chromosome long arm, a "marker X chromosome" later confirmed by Sutherland [57,58] to be related to certain cell culture conditions that render the chromosome "fragile." The *FMR1* gene was cloned in 1991 [59] and in almost all cases, the causative mutation is the expansion of a CGG repeat located in the 5′-untranslated region (UTR) of *FMR1*. The length of the CGG repeat in the healthy population is 6–54 repeats. When the number of repeats exceeds 200, the expansion is referred to as a full mutation allele and results in FXS. At the molecular level, the large number of CGG repeats in the full mutation leads to marked methylation of both the CGG repeats and the *FMR1* promoter, hypoacetylation of associated histones, and chromatin condensation; these epigenetic changes result in transcriptional silencing of *FMR1* and subsequent loss of its protein product, fragile X mental retardation protein (FMRP). Alleles with an intermediate number (55–200) of repeats are referred to as premutation alleles. Premutation alleles do not cause an FXS phenotype but are prone to large increases in repeat length during meiosis, especially female meiosis [60].

There are well-characterized clinical features in males with FXS that females, and rare males with a large expansion, may lack. Thus it is recommended that any child who presents with DD, borderline intellectual abilities, or ID or a diagnosis of autism without a specific etiology undergo molecular testing for FXS.

It is estimated that 2%–6% of such patients will be found to have an *FMR1* mutation.

## 3.12. Autism Spectrum Disorders

Autism is a pervasive developmental disorder involving deficits in threedomains: social skills, communication, and repetitive behaviors or restricted interests, all before the age of 36 months. Typically, the behavioral and developmental symptoms are noted shortly after age 1 year and before the second birthday. The ASDs include those with autism, pervasive developmental disorders-not otherwise specified, and Asperger syndrome.

There are several reports of a recent rise in prevalence of ASDs worldwide. The current estimates of prevalence for ASD is1:68 school-aged children.

ASDs are four times more common in males than in females [61]. There is a strong genetic basis to ASDs, as indicated by the recurrence risk in families, twin studies, and the co-occurrence with chromosomal disorders and rare genetic syndromes. More than 100 gene or genomic disorders are known to cause ASDs [62].

The clinical presentation of the child with an ASD overlaps that with GDD, particularly in the early years. For example, in the Childhood Autism Risks from Genetics and the Environment Study [63], which recruited preschool-aged children from the California administrative data system, 71% of those who meet criteria for autism on both the Autism Diagnostic Observation Schedule (ADOS) [64] and Autism Diagnostic Inventory-Revised (ADI-R) also met criteria for ID (Table 3.5). Many with ASDs will "outgrow" a diagnosis of GDD or ID in large part because the ability of the professionals to measure abilities improves as a child ages [65]. This overlap complicated the diagnostic decision-making by the medical specialist as well as the study of the genetic and metabolic cause of ASDs. For example, at least 103 specific genes and 44 genomic loci have been reported in patients with an ASD or autistic behavior. These genes and loci have all been causally implicated in ID, indicating that these two neurodevelopmental disorders share common genetic bases.

The genetic architecture of ASDs is highly heterogeneous. About

10%–20% of individuals with an ASD have an identified genetic etiology. Microscopically visible chromosomal alterations have been reported in about 5% of cases; the most frequent abnormalities are 15q11–q13 duplications and 2q37, 22q11.2, and 22q13.3 deletions. ASDs can also be due to mutations of single genes involved in autosomal dominant, autosomal recessive, and X-linked disorders. The most common single gene mutation in ASDs is FXS (*FMR1*), present in about 2% of cases (Table 3.6). Other monogenic disorders described in ASDs include tuberous sclerosis (*TSC1*, *TSC2*), neurofibromatosis (*NF1*), Angelman syndrome (*UBE3A*), Rett syndrome (*MECP2*), and *PTEN* mutations in patients with macrocephaly and autism. Rare mutations have been identified in synaptic genes, including *NLGN3*, *NLGN4X*, *SHANK3*,and *SHANK2*. Recent whole-genome microarray studies have revealed submicroscopic deletions and duplications, called copy number variation (CNV), affecting many loci and including de novo events in 5%–10% percent of ASD cases [66].

## Table 3.5

### Key Clinical Findings in ASDs

| Key Finding | Key Considerations |
|---|---|
| **X-linked pedigree** | • Fragile X testing<br>• Urine guanidinoacetate and creatine for disorders of creatine synthesis and/or transport<br>• X-linked intellectual disability genetic testing; X-chromosome sequencing |
| **Affected siblings** | None (see X-linked; nonspecific)<br>Rare autosomal recessive gene: e.g., *HOXA1* |
| **Female gender** | Rett syndrome genetic testing (*MECP2*, *CDKL5*) |
| **Macrocephaly** | • Sotos syndrome genetic testing<br>• *PTEN*-associated disorders (Riley-Ruvalcaba-Bannayan syndrome) particularly in males with |

| | |
|---|---|
| | genital freckling<br>• Brain MRI |
| **Microcephaly** | • Brain MRI<br>• 7-Dehydrocholestrolfor Smith-Lemli-Opitz syndrome |
| **Dysmorphology** | • Testing for specific suspected syndrome<br>• Microarray CGH |
| **Short stature** | • Testing for specific suspected syndrome<br>• Microarray CGH<br>• Other common metabolic tests |
| **Somatic overgrowth** | • Fragile X testing<br>• Sotos syndrome<br>• *PTEN*-associated disorders testing |
| **Dermatologic findings** | • Neurofibromatosis, type 1<br>• Tuberous sclerosis<br>• *PTEN*-associated conditions |
| **Motor delays, tone and coordination disorders** | • Metabolic screening<br>• Brain MRI, MRS<br>• Mitochondrial disorders |
| **Seizure disorder** | • Neurology consultation<br>• Landau-Kleffner syndrome (overnight sleep EEG)<br>• Metabolic testing |
| **Coarse features, organomegaly** | • Metabolic testing |

WES in patients with ASDs has been useful in identifying candidate genes involved in the phenotype. However, the literature is less clear as to the role in the assessment of patients with ASD in the clinical setting than it is in the setting of patients with ID. For example, the application of WES to more than 2500 patients from the Simons Simplex Collection (SSC) showed that 13% of de novo missense

mutations and 43% of de novo "likely gene-disrupting mutations" contribute to 12% and 9% of diagnoses, respectively [67]. Including CNVs, coding de novo mutations contribute to about 30% of all simplex and 45% of female diagnoses [68]. These findings did not establish "causality," however. Likewise, WES of 238 ASD simplex families from the SSC identified three with likely single gene causes (*SCN2A, CHD8, KATNAL2*) [69] and, in another study of 20 SSC patients, identified potentially causative de novo events in four, particularly among more severely affected individuals, in *FOXP1, GRIN2B, SCN1A*, and *LAMC3* [70].

## 3.13. Inborn Errors of Metabolism and ID

The inherited metabolic conditions that cause ID typically are associated with other neurological or systemic signs and symptoms. In several series of patients presenting with IDs, the incidence of diagnosed metabolic disorders ranges from 1% or less to about 5% [71]. While this incidence is lower than genomic or single gene conditions, the potential for response to treatment is relatively better [72]. In one systematic review of the literature, 81 inborn errors of metabolism (IEMs) that were responsive to treatment and associated with ID were identified; of these, 50 were diagnosed with routine metabolic screening tests: plasma amino acids, total serum homocysteine and acylcarnitine profile by tandem mass spectroscopy; urine organic acids, glycosaminoglycans, oligosaccharides, purines and pyrimidines, and creatine metabolites. Several of the 81 identified to be associated with ID are detectable by routine newborn screening currently in place in the United States and around the world. Of those IEMs not identified by the metabolic tests listed in Table 3.6, the identification is based on clinical judgment and a "test-by-test strategy" [73,74] or by WES. For 13 of these 81 IEMs that cause ID, WGS may be the best solution for diagnostic testing (Table 3.7) because of difficulties with current diagnostic: a biomarker is not available or is unreliable and/or the test requires invasive testing and/or the test is difficult to access. Screening for metabolic disorders in all patients with uncomplicated ("nonsyndromic") ASD is not

warranted; for example, in a review of about 8500 patients with IEMs, one was found to have a urea cycle disorder [75].

## Table 3.6

### X-Chromosome Genes Causing Both ID and ASD

| Gene | OMIM | Phenotype or Syndrome |
|---|---|---|
| *MID1* | 300502 | Opitz syndrome |
| *NLGN4X* | 300427 | |
| *AP1S2* | 300629 | Brain calcifications |
| *NHS* | 300457 | Nance-Horan s. |
| *CDKL5* | 300203 | Rett-like infantile spasms |
| *PTCHD1* | 300828 | |
| *ARX* | 300382 | XLAG |
| *DMD* | 300377 | Duchenne/Becker dystrophy |
| *OTC* | 300461 | Ornithine transcarbamylase deficiency |
| *CASK* | 300172 | Microcephaly, pontine, cerebellar hypoplasia |
| *IL1RAPL1* | 300206 | MRX 21 |
| *ZNF674* | 300573 | |
| *SYN1* | 313440 | Epilepsy |
| *ZNF81* | 314998 | |
| *FTSJ1* | 300499 | |
| *PQBP1* | 300463 | Renpenning s. |
| *NDP* | 300658 | Norrie disease |
| *CACNA1F* | 300110 | Severe congenital stationary night blindess |
| *SMC1A* | 300040 | Cornelia de Lange syndrome |
| | | |

| | | |
|---|---|---|
| ***PHF8*** | 300560 | Siderius-Hamel s. |
| ***JARID1C*** | 314690 | Microcephaly, spasticity, epilepsy |
| ***IQSEC2*** | 300522 | |
| ***FGD1*** | 300546 | Aarskog s |
| ***OPHN1*** | 300127 | Cerebellar Hypoplasia |
| ***MED12*** | 300188 | FG/Opitz-Kaveggia s. |
| ***NLGN3*** | 300336 | |
| ***KIAA2022*** | 300524 | Progressive quadriplegia |
| ***ATRX*** | 300032 | ATRX s. |
| ***PCDH19*** | 300460 | Female-limited epileptic encephalopathy |
| ***DCX*** | 300121 | Lissencephaly |
| ***ACSL4*** | 300157 | |
| ***AGTR2*** | 300034 | |
| ***UPF3B*** | 300298 | |
| ***LAMP2*** | 309060 | Danon disease |
| ***GRIA3*** | 305915 | |
| ***OCRL*** | 300535 | Lowe s. |
| ***PHF6*** | 300414 | Borjeson-Forssman-Lehmann s. |
| ***ARHGEF6*** | 300267 | |
| ***SLC9A6*** | 300231 | Christianson s. |
| ***FMR1*** | 309550 | Fragile X s. |
| ***AFF2*** | 300806 | |
| ***SLC6A8*** | 300036 | CreatineTransporter def. |
| ***MECP2*** | 300005 | Rett s. |
| ***L1CAM*** | 308840 | MASA s. |

| | | |
|---|---|---|
| *RAB39B* | 300774 | Epilepsy, Macrocephaly |

N.B. if Phenotype/syndrome not indicated, there are only “nonsyndromic” families noted to date.

## Table 3.7

### Inborn Errors of Metabolism (IEMs) for Which Molecular Genetic Testing Is Required

| IEMs | Gene | OMIM Numbers |
|---|---|---|
| **AGAT deficiency** | *AGAT* | 612718 |
| **Biotin responsive basal ganglia disease** | *SLC19A3* | 606152 |
| **Cerebral glucose transporter defect** | *SLC6A19* | 608893 |
| **Co Enzyme Q10 deficiency** | *COQ2, APTX, PDSS1, PDSS2, CABC1, COQ9* | 609825, 606350, 607429, 610564, 612837 |
| **CPS deficiency** | *CPS* | 117700 |
| **Creatine transporter deficiency** | *SLC6A8* | 300306 |
| **Hyperinsulinism-hyperammonia syndrome** | *GDH* | 138090 |
| **MELAS** | *MTTL1, MTTQ, MTTH, MTTK, MTTC, MTTS1, MTND1, MTND5, MTND6, MTTS2* | 590050, 590030, 590040, 590060, 590020, 590080, 516000, 516005, 516006, 590085 |
| **NAGS deficiency** | *NAGS* | 608300 |
| **Niemann-Pick** | *NPC1 & NPC2* | 607623, 601015 |

| | | |
|---|---|---|
| **disease type C** | | |
| **Serine Biosynthesis defects** | *PHGDH, PSAT, PSPH* | 606879, 610963, 172480 |
| **Sjögren-Larssen disease** | *FALDH* | 609523 |
| **Thiamine-responsive encephalopathy** | *SLC19A3* | 606152 |

## 3.14. CNS Malformations, Intellectual Disability and Brain Imaging

CNS malformations are a common finding in those with ID—abnormal findings on magnetic resonance imaging (MRI) are seen in approximately 30% of children with DD/ID. However, only in a fraction of these children does MRI lead to an etiologic or a syndromic diagnosis (Table 3.8).

Current recommendations range from performing brain images on all patients with DD/ID, to performing it only on those with indications on clinical examination, to being considered as a second-line investigation to be undertaken when features in addition to GDD are detected on either history or physical examination. The finding of a brain abnormality or anomaly on neuroimaging may lead to the recognition of a specific cause for an individual child's DD/ID in the same way that a dysmorphologic examination might lead to the inference of a particular clinical diagnosis. However, like other major or minor anomalies noted on physical examination, abnormalities on neuroimaging typically are not sufficient for determining the cause of the DD/ID; the underlying precise, and presumably frequently genetic in origin, cause of the brain anomaly is often left unknown. Thus, although a CNS anomaly (often also called a "CNS dysgenesis") is a useful finding, and indeed may be considered a useful "diagnosis,"it is, however, frequently not an etiologic or a syndromic diagnosis in

the usual sense of the term. This distinction is not always made in the literature on the utility of neuroimaging in the evaluation of children with DD/ID. The lack of a consistent use of this distinction has led to confusion regarding this particular issue (Table 3.9).

## Table 3.8

### Selected Syndromes With Agenesis of Corpus Callosum

| Syndrome | OMIM# | Gene(s) | Phenotype |
|---|---|---|---|
| **Chondrodysplasia punctata 2, XL type** | 302960 | *EBP* | Increased concentration of 8(9)-cholestenol, &8-dehydrocholesterol |
| **Donnai-Barrow s.** | 222448 | *LRP2* | Hypertelorism, ocular coloboma, diaphragmatic hernia |
| **Fumarate hydratase deficiency** | 136850 | *FH* | Isolated increased concentration of fumaric acid on urine organic acid analysis |
| **L1 syndrome** | 308840 | *L1CAM* | Hydrocephaly, spasticity, adducted thumbs |
| **Mowat-Wilson s.** | 235730 | *ZEB2* | Distinctive facial features, Hirschsprung disease |
| **Aicardi s.** | 304050 | *?* | Chrioretinal lacunae, infantile spasms |
| | | | |

| | | | |
|---|---|---|---|
| **Hereditary motor and sensory neuropathy with agenesis of corpus callosum** | 218000 | *SLC12A6* | Mild to severe ID |
| **Kallman s** | 300836, 136350, 607123, 607002, 605806, 600483 | *KAL1, FGFR1, PROKR2, PROK2, CHD7, and FGF8* | Anosmia, GnRH deficiency |
| **X-linked Opitz/BBB syndrome** | 300000 | *MID1* | Hypertelorism, hypospadias |
| **Tetra-Amelia s.** | 273395 | *WNT3* | Absence of all four limbs |
| **Oro-facial-digital syndrome, type 3** | | *OFD1* | Cleft palate, lobed tongue, polydactyly, syndactyly |

Shevell et al. [5] reported a range of findings in their review. For example, in three studies totaling 329 children with DD in which CT was used in almost all patients, and MRI was used in but a small sample, a specific cause was determined in 27%–32% of the children. In their systematic review of the literature, van Karnebeek et al. [14,76] reported on ninestudies on the use of MRI in children with ID. The mean rate of abnormalities found was 30%, with a range of 6.2%–48.7%. These investigators noted that more abnormalities were found in children with moderate to profound ID versus those with borderline to mild ID (mean yield of 30% and 21.2%, respectively). These authors also noted that none of the studies reported on the value of the absence of any neurologic abnormality for a diagnostic workup and concluded that "the value for finding abnormalities or the absence of abnormalities must be higher" than the 30% mean rate

implied.

If neuroimaging is performed in only selected cases, such as those children with an abnormal head circumference or an abnormal focal neurologic finding, the rate of abnormalities detected is increased further than when used on a screening basis in children with a normal neurologic examination except for than the documentation of DD.

## Table 3.9

### Microcephaly, Primary Autosomal Recessive

| Location | Phenotype | Inheritance | Phenotype Mapping Key | Phenotype MIM Number | Ge |
|---|---|---|---|---|---|
| **1p34.2** | Microcephaly 15, primary, autosomal recessive | Autosomal recessive | 3 | 616486 | *MI* |
| **1p33** | Microcephaly 7, primary, autosomal recessive | Autosomal recessive | 3 | 612703 | *ST* |
| **1p21.2** | ?Microcephaly 14, primary, autosomal recessive | Autosomal recessive | 3 | 616402 | *SA* |
| **1q31.3** | Microcephaly 5, primary, autosomal recessive | Autosomal recessive | 3 | 608716 | *AS* |
| **4q12** | Microcephaly 8, primary, autosomal recessive | Autosomal recessive | 3 | 614673 | *CE* |

| | | | | | |
|---|---|---|---|---|---|
| **4q21.23** | ?Microcephaly 18, primary, autosomal dominant | Autosomal dominant | 3 | 617520 | *WI* |
| **4q24** | ?Microcephaly 13, primary, autosomal recessive | Autosomal recessive | 3 | 616051 | *CE* |
| **7q21.2** | ?Microcephaly 12, primary, autosomal recessive | Autosomal recessive | 3 | 616080 | *CD* |
| **8p23.1** | Microcephaly 1, primary, autosomal recessive | Autosomal recessive | 3 | 251200 | *MC* |
| **9q33.2** | Microcephaly 3, primary, autosomal recessive | Autosomal recessive | 3 | 604804 | *CD* |
| **12p13.31** | ?Microcephaly 11, primary, autosomal recessive | Autosomal recessive | 3 | 615414 | *PH* |
| **12q24.23** | Microcephaly 17, primary, autosomal recessive | Autosomal recessive | 3 | 617090 | *CI* |
| **12q24.33** | ?Microcephaly 16, primary, autosomal recessive | Autosomal recessive | 3 | 616681 | *AN* |

| | | | | | |
|---|---|---|---|---|---|
| **13q12.12-q12.13** | Microcephaly 6, primary, autosomal recessive | Autosomal recessive | 3 | 608393 | *CE* |
| **15q15.1** | Microcephaly 4, primary, autosomal recessive | Autosomal recessive | 3 | 604321 | *KN* |
| | | | | | |

| Location | Phenotype | Inheritance | Phenotype Mapping Key | Phenotype MIM Number | Gene/ |
|---|---|---|---|---|---|
| **15q21.1** | Microcephaly 9, primary, autosomal recessive | Autosomal recessive | 3 | 614852 | *CEP15 KL MC SC* |
| **19q13.12** | Microcephaly 2, primary, autosomal recessive, with or without cortical malformations | Autosomal recessive | 3 | 604317 | *WDR6 C1. MC* |
| **20q13.12** | ?Microcephaly 10, primary, autosomal recessive | Autosomal recessive | 3 | 615095 | *ZNF33 NL NL MC* |

Phenotype Mapping Key: 1 - The disorder is placed on the map due to its association with a gene, but the underlying defect is not known. 2 - The disorder was placed on the map by statistical methods. 3 - The molecular basis of the disorder is known. 4 - A contiguous gene duplication or deletion syndrome in which multiple genes are involved.

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Brain imaging is often useful in guiding the etiological investigations based on the specific CNS abnormalities noted on imaging. To illustrate just two examples, findings such as microcephaly (Tables 3.2 and 3.9) andagenesis of the corpus callosum (Table 3.8) can often lead to a specific genetic diagnosis.

## 3.15. Summary

The DDs discussed in this chapter—ID and autism—are important public health problems. The accurate etiologic diagnosis provides the opportunity to improve health outcomes and quality of life as well as to save health and social services costs. These are extremely heterogeneous conditions with varied responses to treatment. The specialist is able to provide the proper diagnostic approach for individual patients and families. It is clear that early use of WES increases the diagnostic yield at lower costs and should be standard practice in the genetic evaluation of the child with neurodevelopmental disabilities [77]. The role of WGS technology in the clinical evaluation remains unclear at this time but promises to increase the diagnostic yield even beyond that of WES.

## References

[1] Schalock R.L, Luckasson R.A, Shogren K.A, et al. The renaming of mental retardation: understanding the change to the term intellectual disability. *Intellect Dev Disabil*. 2007;45(2):116–124.

[2] Leonard H, Wen X. The epidemiology of mental retardation: challenges and opportunities in the new millennium. *Ment Retard Dev Disabil Res Rev*. 2002;8(3):117–134.

[3] Genereaux D, van Karnebeek C.D, Birch P.H. Costs of caring for children with an intellectual developmental disorder. *Disabil Health J*. 2015;8(4):646–651.
[4] Patel D.R, Greydanus D.E, Calles Jr. J.L, Pratt H.D. De disabilities across the lifespan. *Dis Mon*. 2010;56(6):304–397.
[5] Shevell M, Ashwal S, Donley D, et al. Practice parameter: evaluation of the child with global developmental delay: report of the quality standards subcommittee of the American Academy of neurology and the practice cof the child neurology society. *Neurology*. 2003;60(3):367–380.
[6] Moeschler J.B, Shevell M. Clinical genetic evaluation of the child with mental retardation or developmental delays. *Pediatrics*. 2006;117(6):2304–2316. .
[7] Lenhard W, Breitenbach E, Ebert H, Schindelhauer-Deutscher H.J, Henn W. Psychological benefit of diagnostic certainty for mothers of children with disabilities: lessons from Down syndrome. *Am J Med Genet A*. 2005;133A(2):170–175.
[8] Graungaard A.H, Skov L. Why do we need a diagnosis? A qualitative study of parents' experiences, coping and needs, when the newborn child is severely disabled. *Child Care Health Dev*. 2007;33(3):296–307.
[9] Makela N.L, Birch P.H, Friedman J.M, Marra C.A. Par perceived value of a diagnosis for intellectual

disability (ID): a qualitative comparison of families with and without a diagnosis for their child's ID. *Am J Med Genet A*. 2009;149A(11):2393–2402.
[10] Rosenthal E.T, Biesecker L.G, Biesecker B.B. Parental attitudes toward a diagnosis in children with unidentified multiple congenital anomaly syndromes. *Am J Med Genet*. 2001;103(2):106–114.
[11] Curry C.J, Stevenson R.E, Aughton D, et al. Evaluation of mental retardation: recommendations of a consensus Conference: American College of medical genetics. *Am J Med Genet*. 1997;72(4):468–477.
[12] Moeschler J.B. Genetic evaluation of intellectual disabilities. *SeminPediatrNeurol*. 2008;15(1):2–9.
[13] Schaefer G.B, Bodensteiner J.B. Evaluation of the child with idiopathic mental retardation. *PediatrClin N Am*. 1992;39(4):929–943.
[14] van Karnebeek C.D, Jansweijer M.C, Leenders A.G, Offrin investigations in individuals with mental retardation: a systematic literature review of their usefulness. *Eur J Hum Genet*. 2005;13(1):6–25.
[15] Anazi S, Maddirevula S, Faqeih E, et al. Clinical genomics expands the morbid genome of intellectual disability and offers a high diagnostic yield. *Mol Psychiatry*. 2017;22(4):615–624.
[16] Thevenon J, Duffourd Y, Masurel-Paulet A, et al. Diagnostic odyssey in severe neurodevelopmental disorders: toward clinical whole-exome sequencing as a first-line diagnostic test. *Clin Genet*. 2016;89(6):700–707.

[17] Vissers L.E, van Nimwegen K.J, Schieving J.H, et al. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. *Genet Med*. 2017.
[18] Shaheen R, Patel N, Shamseldin H, et al. Accelerating matchmaking of novel dysmorphology syndromes through clinical and genomic characterization of a large cohort. *Genet Med*. 2016;18(7):686–695.
[19] Sawyer S.L, Hartley T, Dyment D.A, et al. Utility of whole-exome sequencing for those near the end of the diagnostic odyssey: time to address gaps in care. *Clin Genet*. 2016;89(3):275–284.
[20] Soden S.E, Saunders C.J, Willig L.K, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *SciTransl Med*. 2014;6(265):265ra168.
[21] Monroe G.R, Frederix G.W, Savelberg S.M, et al. Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. *Genet Med*. 2016;18(9):949–956.
[22] Large-scale discovery of novel genetic causes of developmental disorders. *Nature*. 2015;519(7542):223–228.
[23] Yang Y, Muzny D.M, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*. 2014;312(18):1870–1879.
[24] Lee H, Deignan J.L, Dorrani N, et al. Clinical exome sequencing for genetic identification of rare

Mendelian disorders. *JAMA*. 2014;312(18):1880–1887.
[25] Willemsen M.H, Kleefstra T. Making headway with genetic diagnostics of intellectual disabilities. *Clin Genet*. 2014;85(2):101–110.
[26] Schuurs-Hoeijmakers J.H, Vulto-van Silfhout A.T, Vissers L.E, et al. Identification of pathogenic gene variants in small families with intellectually disabled siblings by exome sequencing. *J Med Genet*. 2013;50(12):802–811.
[27] Rauch A, Wieczorek D, Graf E, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet*. 2012;380(9854):1674–1682.
[28] Need A.C, Shashi V, Hitomi Y, et al. Clinical application of exome sequencing in undiagnosed genetic conditions. *J Med Genet*. 2012;49(6):353–361.
[29] de Ligt J, Willemsen M.H, van Bon B.W, et al. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med*. 2012;367(20):1921–1929.
[30] Stark Z, Schofield D, Alam K, et al. Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. *Genet Med*. 2017.
[31] Biesecker L.G, Green R.C. Diagnostic clinical genome and exome sequencing. *N Engl J Med*. 2014;370(25):2418–2425.
[32] Gilissen C, Hehir-Kwa J.Y, Thung D.T, et al. Genome sequencing identifies major causes of severe intellectual disability. *Nature*. 2014;511(7509):344–347.

[33] Zahir F.R, Mwenifumbo J.C, Chun H.E, et al. Comprehensive whole genome sequence analyses yields novel genetic and structural insights for Intellectual Disability. *BMC Genom*. 2017;18(1):403.
[34] Ledbetter D.H. Cytogenetic technology–genotype and phenotype. *N Engl J Med*. 2008;359(16):1728–1730.
[35] Saul R.A, Moeschler J.B. How best to use CGH arrays in the clinical setting. *Genet Med*. 2009;11(5):371 author reply 371–372.
[36] Biesecker L.G. Editorial comment on "whole exome sequencing identifies compound heterozygous mutations in WDR62 in siblings with recurrent polymicrogyria. *Am J Med Genet A*. 2011;155(9):2069–2070.
[37] Allanson J.E, Biesecker L.G, Carey J.C, Hennekam R.C of morphology: introduction. *Am J Med Genet A*. 2009;149A(1):2–5.
[38] Smith D.W. Dysmorphology (teratology). *J Pediatr*. 1966;69(6):1150–1169.
[39] Hennekam R.C, Biesecker L.G. Next-generation sequencing demands next-generation phenotyping. *Hum Mutat*. 2012;33(5):884–886.
[40] Robinson P.N, Kohler S, Bauer S, Seelow D, Horn D, M Human Phenotype Ontology: a tool for annotating and analyzing human hereditary disease. *Am J Hum Genet*. 2008;83(5):610–615.
[41] Buske O.J, Girdea M, Dumitriu S, et al. PhenomeCentral: a portal for phenotypic and

gmatchmaking of patients with rare genetic diseases. *Hum Mutat*. 2015;36(10):931–940.

[42] Sobreira N, Schiettecatte F, Boehm C, Valle D, Hamosh tools for Mendelian disease gene identification: PhenoDB variant analysis module; and GeneMatcher, a web-based tool for linking investigators with an interest in the same gene. *Hum Mutat*. 2015;36(4):425–431.

[43] Gripp K.W, Baker L, Telegrafi A, Monaghan K.G. The role of objective facial analysis using FDNA in making diagnoses following whole exome analysis. Report of two patients with mutations in the BAF complex genes. *Am J Med Genet A*. 2016;170(7):1754–1762.

[44] Raymond F.L, Tarpey P. The genetics of mental retardation. *Hum Mol Genet*. 2006;15(Spec No 2):R110–R116.

[45] Inlow J.K, Restifo L.L. Molecular and comparative genetics of mental retardation. *Genetics*. 2004;166(2):835–881.

[46] Hunter A.G. Outcome of the routine assessment of patients with mental retardation in a genetics clinic. *Am J Med Genet*. 2000;90(1):60–68.

[47] Vissers L.E, de Vries B.B, Veltman J.A. Genomic microarrays in mental retardation: from copy number variation to gene, from research to diagnosis. *J Med Genet*. 2010;47(5):289–297.

[48] Miller D.T, Adam M.P, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier

clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*. 2010;86(5):749–764.
[49] Henderson L.B, Applegate C.D, Wohler E, Sheridan M Fong J, Batista D.A. The impact of chromosomal microarray on clinical management: a retrospective analysis. *Genet Med*. 2014;16(9):657–664.
[50] Manning M, Hudgins L. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genet Med*. 2010;12(11):742–745.
[51] Vissers L.E, Gilissen C, Veltman J.A. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet*. 2016;17(1):9–18.
[52] Neri G, Opitz J.M. Sixty years of X-linked mental retardation: a historical footnote. *Am J Med Genet*. 2000;97(3):228–233.
[53] Lubs H, Travers H, Lujan E, Carroll A. A large kindred with X-linked mental retardation, marker X and macroorchidism. *Am J Med Genet*. 1984;17(1):145–157.
[54] Stevenson R.E, Schwartz C.E. X-linked intellectual disability: unique vulnerability of the male genome. *Dev Disabil Res Rev*. 2009;15(4):361–368.
[55] Chiurazzi P, Schwartz C.E, Gecz J, Neri G. XLMR genes: update 2007. *Eur J Hum Genet*. 2008;16(4):422–434.
[56] Lubs H.A. A marker X chromosome. *Am J Hum Genet*. 1969;21(3):231–244.
[57] Sutherland G.R. Marker X chromosomes and mental

retardation. *N Engl J Med*. 1977;296(24):1415.
[58] Sutherland G.R. Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium. *Science*. 1977;197(4300):265–266.
[59] Verkerk A.J, Pieretti M, Sutcliffe J.S, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*. 1991;65(5):905–914.
[60] Fu Y.H, Kuhl D.P, Pizzuti A, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell*. 1991;67(6):1047–1058.
[61] Baird G, Simonoff E, Pickles A, et al. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the special needs and autism Project (SNAP). *Lancet*. 2006;368(9531):210–215.
[62] Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. *Brain Res*. 2011;1380:42–77.
[63] Hertz-Picciotto I, Croen L.A, Hansen R, Jones C.R, van de Water J, Pessah I.N. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect*. 2006;114(7):1119–1125.
[64] Lord C, Risi S, Lambrecht L, et al. The autism diagnostic observation schedule-generic: a standard

measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord*. 2000;30(3):205–223. .

[65] Hertz-Picciotto I, Delwiche L. The rise in autism and the role of age at diagnosis. *Epidemiology*. 2009;20(1):84–90.

[66] Pinto D, Pagnamenta A.T, Klei L, et al. Functional impact of global rare copy number variation in autism spectrum disorders. *Nature*. 2010;466(7304):368–372.

[67] Fischbach G.D, Lord C. The Simons Simplex Collection: a resource for identification of autism genetic risk factors. *Neuron*. 2010;68(2):192–195.

[68] Iossifov I, O'Roak B.J, Sanders S.J, et al. The contribution of de novo coding mutations to autism spectrum disorder. *Nature*. 2014;515(7526):216–221.

[69] Sanders S.J, Murtha M.T, Gupta A.R, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature*. 2012;485(7397):237–241.

[70] O'Roak B.J, Deriziotis P, Lee C, Vives L, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nature Genet*. 2011;43:585–589.

[71] Michelson D.J, Shevell M.I, Sherr E.H, Moeschler J.B, C report: genetic and metabolic testing on children with global developmental delay: report of the quality standards Subcommittee of the American Academy of neurology and the practice Committee of the child neurology

Society. *Neurology*. 2011;77(17):1629–1635.
[72] van Karnebeek C.D, Bowden K, Berry-Kravis E. Treatment of neurogeneticdevelopmental conditions: from 2016 into the future. *Pediatr Neurol*. 2016;65:1–13.
[73] van Karnebeek C.D, Stockler-Ipsiroglu S. Early identification of treatable inborn errors of metabolism in children with intellectual disability: the Treatable Intellectual Disability Endeavor protocol in British Columbia. *Paediatrics Child Health*. 2014;19(9):469–471.
[74] Tarailo-Graovac M, Shyr C, Ross C.J, et al. Exome sequencing and the management of neurometabolicdisorders. *N Engl J Med*. 2016;374(23):2246–2255.
[75] Campistol J, Diez-Juan M, Callejon L, et al. Inborn error metabolic screening in individuals with nonsyndromic autism spectrum disorders. *Dev Med Child Neurol*. 2016;58(8):842–847.
[76] van Karnebeek C.D, Scheper F.Y, Abeling N.G, et al. Etiology of mental retardation in children referred to a tertiary care center: a prospective study. *Am J Ment Retard*. 2005;110(4):253–267.
[77] Boycott K, Hartley T, Adam S, et al. The clinical application of genome-wide sequencing for monogenic diseases in Canada: position Statement of the Canadian College of Medical Geneticists. *J Med Genet*. 2015;52(7):431–437.

## Further Reading

Abrahams B.S, Geschwind D.H. Advances in autism genetics: on the

threshold of a new neurobiology. *Nat Rev Genet*. 2008;9(5):341–355.

Berkel S, Marshall C.R, Weiss B, et al. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nat Genet*. 2010;42(6):489–491.

Birnbaum R.Y.C.E, Everman D.B, Lomvardas S, Gurrieri F, Ahituv N, S as a result of disruption of limb enhancers contained within codingexons of DYNC1I1. In: David W, ed. *Smith Workshop on morphogenesis and malformations; September 9-14, 2011*. Lake Arrowhead, CA: UCLA Conference Center; 2011.

Chelly J, Khelfaoui M, Francis F, Cherif B, Bienvenu T. Genetics and pathophysiology of mental retardation. *Eur J Hum Genet*. 2006;14(6):701–713.

Christian S.L, Brune C.W, Sudi J, et al. Novel submicroscopic chromosomal abnormalities detected in autism spectrum disorder. *Biol Psychiatry*. 2008;63(12):1111–1117.

Coffee B, Zhang F, Ceman S, Warren S.T, Reines D. Histone modifications depict an aberrantly heterochromatinized FMR1 gene in fragile x syndrome. *Am J Hum Genet*. 2002;71(4):923–932.

Cohen Jr. M.M. ASD is an incorrect abbreviation for autism spectrum disorders. *Am J Med Genet A*. 2011;155(12):2906.

Coulter M.E, Miller D.T, Harris D.J, et al. Chromosomal microarray testing influences medical management. *Genet Med*. 2011;13(9):770–776.

Durand C.M, Betancur C, Boeckers T.M, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet*. 2007;39(1):25–27.

Engbers H.M, Berger R, van Hasselt P, et al. Yield of additional metabolic studies in neurodevelopmental disorders. *Ann Neurol*. 2008;64(2):212–217.

Fishburn J, Turner G, Daniel A, Brookwell R. The diagnosis and frequency of X-linked conditions in a cohort of moderately retarded males with affected brothers. *Am J Med Genet*. 1983;14(4):713–724.

Foster T, Regan-Smith M, Murray C, et al. Residency education,

preventive medicine, and population health care improvement: the Dartmouth-Hitchcock Leadership Preventive Medicine approach. *Acad Med*. 2008;83(4):390–398.

Fox J.W, Lamperti E.D, Eksioglu Y.Z, et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron*. 1998;21(6):1315–1325.

Hersh J.H, Saul R.A. Health supervision for children with fragile X syndrome. *Pediatrics*. 2011;127(5):994–1006. .

Hochstenbach R, van Binsbergen E, Engelen J, et al. Array analysis and karyotyping: workflow consequences based on a retrospective study of 36,325 patients with idiopathic developmental delay in The Netherlands. *Eur J Med Genet*. 2009;52(4):161–169.

Honeycutt A.D.L, Chen H, al Homsi G. Economic costs associated with mental retardation, cerebral palsy, hearing loss, and vision impairment – United States, 2003. *Natal Center Birth Defects Dev Disabil*. 2004;53:57–59 CDC.

*International classification of diseases. 10th revision*. 10th ed. Geneva, Switzerland: World Health Organization; 1994.

Jamain S, Quach H, Betancur C, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet*. 2003;34(1):27–29.

Majnemer A, Shevell M.I. Diagnostic yield of the neurologic assessment of the developmentally delayed child. *J Pediatr*. 1995;127(2):193–199.

Marshall C.R, Noor A, Vincent J.B, et al. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet*. 2008;82(2):477–488.

McGrew S.G, Peters B.R, Crittendon J.A, Veenstra-Vanderweele J. Diagnostic yield of chromosomal microarray analysis in anautism primary care practice: which guidelines to implement? *J Autism Dev Disord*. 2011.

Moeschler J.B. Medical genetics diagnostic evaluation of the child with global developmental delay or intellectual disability. *CurrOpin*

*Neurol*. 2008;21(2):117–122.

Riggs E, Wain K, Riethmaier D, et al. Chromosomal microarray impacts clinical management. *Clin Genet*. 2013.

Ropers H.H, Hamel B.C. X-linked mental retardation. *Nat Rev Genet*. 2005;6(1):46–57.

Sagoo G.S, Butterworth A.S, Sanderson S, Shaw-Smith C, Higgins J.P, Burton H. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects. *Genet Med*. 2009;11(3):139–146.

Schaefer G.B, Mendelsohn N.J. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders. *Genet Med*. 2008;10(4):301–305.

Sebat J, Lakshmi B, Malhotra D, et al. Strong association of de novo copy number mutations with autism. *Science*. 2007;316(5823):445–449.

Tarpey P.S, Smith R, Pleasance E, et al. A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. *Nat Genet*. 2009;41(5):535–543.

van Bokhoven H. Genetic and epigenetic networks inintellectual disabilities. *Annu Rev Genet*. 2010;45:81–104.

van Karnebeek C.D, Shevell M, Zschocke J, Moeschler J.B, Stockler S. The metabolic evaluation of the child with an intellectual developmental disorder: diagnostic algorithm for identification of treatable causes and new digital resource. *Mol Genet Metab*. 2014;111(4):428–438.

Whibley A.C, Plagnol V, Tarpey P.S, et al. Fine-scale survey of X chromosome copy number variants and indels underlying intellectual disability. *Am J Hum Genet*. 2010;87(2):173–188.

Yeargin-Allsopp M, Murphy C.C, Cordero J.F, Decoufle P, Hollowell J.G. Rep biomedical causes and associated medical conditions for mental retardation among 10-year-old children, metropolitan Atlanta, 1985

to 1987. *Dev Med Child Neurol*. 1997;39(3):142–149.

4

# Abnormal Body Size and Proportion

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## Abstract

Pathologic short and tall stature extends more than two standard deviations beyond the normal mean for age-appropriate human height (i.e., less than 3rd centile or greater than 97th centile). Identifying pathologic growth involves using growth curves appropriate for a person's ethnic and nutritional status. Allowances must also be made for parental height, the impact of chronic disease or medication, and the possibility of constitutional delay or acceleration of maturation. When it is established that a person is truly short or tall for age, considering all other relevant factors in the medical and social history, an accurate diagnosis of the cause is essential to providing good patient care: including prognosis, anticipating and treating complications, and providing accurate genetic counseling. The classification of the body habitus as proportionate or disproportionate, with the use of anthropometric measurements, and the recognition of the deficient or accelerated growth either

prenatally or postnatally can help identify the cause of the pathology. Next-generation sequencing strategies have the potential to identify causes for pathologic short or tall stature in 40%–50% of syndromic cases, especially when associated with intellectual disability. The authors describe algorithms to recognize pathologic overgrowth or short stature and discuss some common examples of abnormal body size and proportion.

## Keywords

Aarskog syndrome; Beckwith-Wiedemann syndrome; Bloom syndrome; Brachmann-de Lange syndrome; Coffin-Siris syndrome; Cohesinopathies; Donohue (leprechaunism) syndrome; Dubowitz syndrome; Elejalde syndrome; Fanconi pancytopenia syndrome; Floating Harbor syndrome; Hallermann-Streiff syndrome; Johanson-Blizzard syndrome; Marshall-Smith syndrome; Nevo syndrome; *NFIX* mutations; Noonan syndrome; Opitz syndrome; Overgrowth syndromes; Perlman syndrome; PIK3CA-related overgrowth; Proteus syndrome; PTEN hamartoma syndrome; Rasopathies; Robinow syndrome; Rubinstein-Taybi syndrome; Russell-Silver syndrome; Seckel syndrome; Segmental overgrowth; Short stature; SHORT syndrome; Simpson-Golabi Behmel syndrome; Smith-Lemli-Opitz syndrome; Sotos syndrome; Teratogenic exposures; Weaver syndrome; Williams syndrome

## Glossary

***Array comparative genomic hybridization* (*CGH*)** Molecular chromosomal analysis using a subtraction technique to compare a DNA sample from a patient to a control sample to find microscopic (visible with karyotype) or submicroscopic imbalances [deletions or duplications termed *copy number variants* (CNVs)] in the genome

***Genome-wide association studies* (*GWAS*) *or whole genome association study* (*WGAS*)** Examines a genome-wide set of

genetic variants in a cohort of individuals to investigate the association of a variant with a trait

***Linkage equilibrium* (*LE*)** Occurs when the genotype present at one locus is independent of the genotype at a second locus

***Linkage disequilibrium* (*LD*) or *gametic phase disequilibrium*** Nonrandom association of alleles at different loci

***Narrow-sense heritability*** Proportion of phenotypic variance due to additive genetic variance

***Quadriradial* (*Qr*)** Symmetrical, four-armed chromatid interchange configuration seen in Bloom syndrome; used as confirmatory testing

***Single nucleotide polymorphism* (*SNP*)** Variation in a single nucleotide at a specific location in the genome

***Chromosomal breakage study*** Examination of chromosomes using a clastogenic agent to determine if there is defective DNA repair that makes chromosomes more susceptible to chromosomal breakage as is seen in Fanconi pancytopenia syndrome

## 4.1. Introduction

Stature follows a normal distribution, varying between ethnic groups and within each ethnic group. *Short stature* and *tall stature* are therefore relative terms, influenced by a person's ethnic and familial background. Adult height is a classic polygenic trait with high narrow-sense heritability ($h^2 = 0.8$), influenced by genetic variants in at least 423 loci [1,2,3]. Allelic heterogeneity is a frequent feature of polygenic traits, and genome-wide association studies (GWAS) have discovered 180 single nucleotide polymorphisms (SNPs) that account for a fraction (16%) of the genetic variation in heights, with the remaining heritability variance explained by considering all SNPs simultaneously [4,5]. Each GWA locus typically comprises a set of contiguous genes, and among these loci are atleast 78 genes that are involved in growth plate function and regulation signaling pathways

[4]. Overall heritability estimates are around 80%, but nutritional deficits and other environmental factors common in developing countries can lead to a lower heritability and overall shorter stature [5]. The genetic basis of height is complex and likely caused by sophisticated interactions between multiple genetic loci, which may be influenced by growth factors not yet fully delineated. While there is demonstrated overlap between genetic loci associated with infant birth length and adult height, a genetic prediction score of 180 of the adult loci explained only 0.13% of the variance in infant length, suggesting there are different genetic factors influencing prenatal and adult growth [6]. While common variants/polymorphisms have been demonstrated to show a predicted effect across the spectrum of height, rare genetic nonadditive or rare nongenetic factors seem to play a more prominent role at the shorter end of the growth spectrum [7].

The optimum birth weight for the lowest perinatal mortality and morbidity is 3500–4000 g. In 2015 in the United States, 8% of newborns were considered low birth weight (<2500 g), including 1.4% very low birth weight (VLBW) infants (<1500 g) [8], while approximately 5%–8% of newborns exceeded 4000 g and 0.5%–1% exceeded 4500 g [9]. Though etiologically heterogeneous, abnormal size at birth may be associated with infant, childhood, and adult health implications [10]. Neonatal deaths account for 40% of deaths under the age of 5 years worldwide. Higher fetal mortality rates are associated with a birth weight of >4250 g in nondiabetic mothers and a birth weight of 4000 g in diabetic mothers [11]. On the other end of the spectrum, children born small (SGA) or very small for gestation

age are at high risk for postnatal complications and mortality; they remain at a significantly increased risk for poor school performance, and this risk increases with severity of SGA [12]. This risk diminishes after adequate catch-up growth [12].

## 4.2. Approach to the Patient With Abnormal Stature

A unified approach to a patient with abnormal stature is outlined in Table 4.1. As with any other genetic condition, the diagnostic evaluation begins with a comprehensive history and physical examination. Overall, the goal is to determine when the abnormal growth trajectory was initiated, that is, before or since birth. The history includes any antenatal maternal exposures [A summary of teratogens, including prenatal infections are included in Table 4.2]; chronic maternal conditions; infections; any complications of pregnancy, labor, and delivery (include any notable placental abnormalities); any anomalies of developmental milestones; and any physical or psychomotor anomalies noted by teachers or other health professionals. For example, dental maturity (calcification of premolars/molars) is associated with skeletal maturity and bone age [13], and an abnormal dental history may provide valuable insight. A history of similarly affected relatives helps contextualize growth aberrations. A complete physical examination includes attention to age-appropriate cognition, facial and axial symmetry, a thorough skin examination with attention to hair distribution and nail quality [14], and a thorough neurologic examination, including observation of gait. Any abnormality of proportion should be quantified through anthropometric measurements. Growth parameters and anthropometric measurements using appropriate growth curves help differentiate between pathologic short or tall stature and variants at each end of the normal range and quantify proportionality. Consideration must be given to parental height and ethic background as well as nutritional status and the impact of chronic disease or pharmacotherapy before an individual can be declared pathologically

short or tall. One must also rule out constitutional delay or acceleration of maturation by correlating bone age and height. This normal variation in the timing of growth accounts for a large proportion of children referred for variations in growth.

## TABLE 4.1

### Approach to the Patient With Abnormal Stature

**Clinical history**

Antenatal/maternal exposures/chronic conditions

Prenatal records/testing

Birth (labor/delivery complications) and developmental milestones, including any abnormalities of dentition

Family history (pedigree of other affected individuals)

Physical examination

Anthropometric measurements (use of appropriate growth charts), growth trajectory, head circumference

Facial dysmorphism

Intellectual ability

Neurological examination (including assessment of cranial nerves and gait)

Skin examination (distribution/texture or defects of hair and nails)

Form a clinical assessment based on gestalt/stature and historical aspects

**Confirmatory testing**

SNP chromosomal microarray, whole exome sequencing or single gene analysis

Metabolic screening

Management / appropriate surveillance recommendations guided by physical manifestations, known pathologic associations with uncovered cause of abnormal stature

## Table 4.2

### Common Teratogenic Causes of Abnormal Stature

| **Intrauterine Growth Restriction/Short Stature** | | |
|---|---|---|
| Exposure to Infectious Agents [108] | | |
| **To** | Toxoplasmosis (obligate intracellular protozoa) | Classic triad (rare): chorioretinitis (20% at birth, untreated up to 90% develop chorioretinitis by adulthood), hydrocephalus, and intracranial calcifications<br>Common: anemia, seizures, jaundice, splenomegaly, hepatomegaly, thrombocytopenia |
| | ***Treponema pallidum* (spirochete)** | Later manifestations: motor and cerebellar dysfunction, microcephaly, seizures, intellectual disability (mental retardation), sensorineural hearing loss<br>Syphilis: early findings may include hepatosplenomegaly, "snuffles" (nasal secretions), lymphadenopathy, mucous membrane lesions, pneumonia, osteochondritis and pseudoparalysis, maculopapular rash, edema, Coombs negative hemolytic anemia, and thrombocytopenia |
| **R** | Rubella | Classic: intrauterine growth restriction (IUGR) with deafness (66%), cataracts (78%) or other ocular findings: micro-ophthalmia, corneal opacity, glaucoma, cardiac anomalies (58%): patent ductus arteriosus, coarctation of aorta or pulmonary stenosis<br>Common findings: "blueberry muffin" rash, hepatosplenomegaly, thrombocytopenia |

| | | |
|---|---|---|
| | | Late sequelae include diabetes, hypertension, behavioral disorders, panencephalitis |
| **C** | CMV | Hepatosplenomegaly, cardiac lesions, petechiae/purpura/"blueberry muffin rash," microcephaly, periventricular calcifications, chorioretinitis, hearing deficits |
| **H** | Herpesvirus | HSV: Hepatosplenomegaly, myocarditis, petechia/purpura/"blueberry muffin" rash, microcephaly, chorioretinitis, hearing deficits |
| | **Hepatitis B virus** | Hepatitis B: generally asymptomatic (~90% of infants infected perinatally or in the first year of life develop chronic hepatitis B); mildly increased alanine aminotransferase concentrations, with detectable hepatitis B e-antigen and high hepatitis B virus DNA concentrations (>20,000 IU/mL) |
| | **Hepatitis C virus** | Hepatitis C: generally asymptomatic (80% develop chronic hepatitis C); short stature and osteodystrophy (increased serum osteocalcin) [246] |
| | **HIV** | HIV: generally asymptomatic HIV-exposed infant is generally considered to be HIV-1 negative if the HIV NAT is negative at up to 4 months of age |
| | **Varicella** | Varicella: Cicatricial skin defects, limb hypoplasia, mental deficiency, seizures |
| | **Parvovirus B19** | Parvovirus: *fetal infection may resolve spontaneously or lead to severe consequences such as nonimmune hydrops fetalis (secondary to cardiac failure) and miscarriage*<br>First 20 weeks: brain anomalies and neurodevelopmental insults, fetal loss; |

| | | |
|---|---|---|
| | | shortened half-life of erythrocytes—severe anemia, following fetal erythroid progenitor cells infection and apoptosis; severe anemia may cause high-output cardiac failure and nonimmune hydrops fetalis; directly, infection of myocardial cells produces myocarditis that further aggravates the cardiac failure |
| **Z** | Zika virus [247] | Microcephaly, fetal skull collapse, and redundancy of the scalp consistent with the fetal brain disruption sequence present in 70%; fetal<br><br>immobility, ranging from dimples (30.1%) to distal hand/finger contractures (20.5%) and feet malpositions (15.7%) to generalized arthrogryposis (9.6%), visual impairment regardless of retina and/or optic nerve abnormalities [16,17] |

Table Continued

| **Intrauterine Growth Restriction/Short Stature** | |
|---|---|
| Maternal Exposure to Chemicals/Chronic Conditions | |
| Intrauterine Growth Restriction | |
| **Fetal alcohol spectrum disorders** | Microcephaly, thin smooth philtrum, short palpebral fissures, growth deficiency, attention-deficit/hyperactivity disorder, neurobehavioral problems [18,19] |
| **Maternal nicotine use (including E-cigarettes)** | IUGR [20], cleft palate [21]<br>Abnormal fetal lung development: decreases in forced expiratory flows, decreased passive respiratory compliance, increased hospitalization for respiratory infections, increased prevalence of childhood wheeze |

| | |
|---|---|
| | and asthma [22,248] |
| **Maternal phenylketonuria** | Microcephaly, cardiac defects, progressive, severe mental deficiency, delayed motor development [23] |
| Large for Gestational Age | |
| **Maternal hyperglycemia (maternal diabetes mellitus)** | Phenotype variable, large for gestational age (may include small for gestational age), congenital heart defects [24] (reduced septal function at 1 month in the absence of septal hypertrophy, which is associated with altered maternal and infant lipid and glucose metabolism [25]), neural tube defects [26] |
| **Maternal obesity** | Adverse maternal and fetal outcomes, including preeclampsia, preterm birth, stillbirth, congenital anomalies, and macrosomia [27], placental dysfunction; risks of congenital malformations of the nervous system (highest risk of organ systems with increasing body mass index [BMI]), heart defects and limb defects also progressively increased with BMI from overweight to obesity class III (malformations of the genital and digestive systems were also increased in offspring of obese mothers) [28]<br>Increased incidence of metabolic and cardiovascular disease later in life in the mother and in the offspring [27] |

## 4.3. Mechanisms of Growth

Skeletal development proceeds through either endochondral or intramembranous ossification (see chapter on Skeletal Dysplasias), depending on bone type. Embryonic development and elongation of

long bones, tubular bones, and vertebrae depend on chondrocyte proliferation and differentiation at the growth plates through endochondral ossification, while other bones, primarily craniofacial bones, develop via intramembranous ossification [29]. Both pathways of ossification are also relevant in the repair of fractures: stabilized fractures heal via intramembranous ossification, while unstable fractures heal via endochondral ossification [30].

At the growth plate, histology demonstrates three distinct zones of chondrocytes: resting zone (adjacent to the epiphysis, consisting of progenitor chondrocytes); proliferative zone (the site of rapid cell proliferation); and hypertrophic zone (the site of hypertrophic chondrocytes where terminal differentiation occurs). As cells grow toward the metaphysis through chondrogenesis, there is growth of the cartilaginous growth plate osteoblasts and blood vessels invade the hypertrophic zone, remodeling newly formed cartilage to bone (endochondral ossification), lengthening the bone, and increasing overall height [31].

Chondrocyte proliferation and differentiation, ultimately resulting in linear bone growth, represent a highly regulated physiologic process that is influenced by nutrition, endocrine factors (including growth hormone [GH], estradiol [32], and leptin [33]), local environmental factors (including extracellular fluid constituency, pH, oxygenation, paracrines, and inflammatory cytokines), and extracellular matrix with complex interplay between these factors [34]. These complex mechanisms have been reviewed in the literature [29,33,34]. Long bone growth is rapid prenatally and in early postnatal life, with a decrease in chondrocyte proliferation associated with a clinical growth deceleration in adolescence as the growth plate enters senescence [35]. This capacity is gradually exhausted with cell division, and this proliferative exhaustion is followed by epiphyseal fusion where the cartilaginous growth plate is entirely replaced by bone [35]. Growth plates in the long bones function at a much greater pace than each of the individual growth plates in vertebrae, making them more susceptible to dysregulation [35]. The differential expression of genes in tubular versus vertebral growth plates is the

underlying precept behind dwarfism or disproportionate short stature.

Dysregulation at the growth plate can present as either idiopathic short stature or a skeletal dysplasia, depending on whether the mutation is heterozygous or homozygous/compound heterozygous with severely altered protein function. Common examples of monogenic causes of abnormally short stature can result from mutations in genes encoding proteins involved in regulating chondrogenesis such as transcription factors like *human signal transducer and activator of transcription (STAT)5b* ([36,37] *short stature homeobox SHOX* [38,39], C-type natriuretic peptide (*CNP*) and natriuretic peptide receptor-B, its principal receptor (encoded by *natriuretic peptide precursor-C* [*NPPC*] and *NPR2*) [40,41] or receptors such as *FGFR3* [43]. With the *SHOX* gene (and deletions of the pseudoautosomal region1 (PAR1) on chromosome X and Y), decreased copy number results in short stature (e.g., isolated or nonsyndromic short stature [44], short stature associated with Turner syndrome, and Leri-Weill dyschondrosteosis [OMIM 127300]) [45,46], while increased copy number is associated with syndromic tall stature (e.g., Kleinfelter syndrome) or tall stature at the upper level of normal *ACAN*[47]. Similarly, activating mutations in *NPR2* have been associated with tall stature [48]; homozygous inactivating mutations of *NPR2* cause a skeletal dysplasia with severe short stature (acromesomelic dysplasia [OMIM 602875], which is also linked to *GDF5* and *BMPR1B*) [49] and heterozygous *NPPC* mutations, leading to a defective/insufficient amount of CNP have been linked to autosomal dominant short stature [41]. Dominant *FGFR3* mutations have been linked to hypochondroplasia, achondroplasia, and thanatophoric dysplasia, while inactivating mutations have been related to tall stature [50]. Mutations in the *ACAN* gene, encoding extracellular matrix proteoglycan aggrecan, have been associated with proportionate short stature with an advanced bone age [51].

## 4.3.1. Genome Instability

Mutations resulting in genomic instability frequently result in short

stature and, while mechanisms are briefly discussed here, further elaboration is included in the short stature section.

#### 4.3.1.1. Defects of DNA Repair

Defects in DNA repair genes can present clinically with growth retardation, premature aging, skin abnormalities including abnormal pigmentation, telangiectasia, xerosis cutis, and pathological wound healing with a predisposition for certain types of cancers. RecQ proteins (DNA helicase) are essential to maintaining genomic stability, and defects thereof have been associated with Bloom (*RECQL3*) and Werner (*RECQL2*) syndromes. DNA helicase *RECQL4* interacts in an array of intracellular regulatory pathways from the initiation of DNA replication, through maintaining genomic stability (DNA repair) [52]. Fanconi pancytopenia syndrome (see section on DNA mismatch repair and genetics of hematologic disorders) is a clinically heterogeneous disorder can be caused by mutations in several different genes (Table 4.2), and it is characterized by bone marrow failure (aplastic anemia), developmental delay, and increased risk for malignancy [53]. Around 75% of patients present with a variable phenotype with short stature, skeletal malformations of the upper and lower limbs, microcephaly, ophthalmic and genitourinary tract anomalies, and abnormal skin pigmentation [53].

#### 4.3.1.2. Defects of Cell Replication

Disruption of DNA replication and chromosome segregation can result in chromosome dosage abnormalities, that is, aneuploidy/multiploidy. Cohesion, a multisubunit protein complex, plays a role in DNA repair and mediates chromatin organization and transcription regulation [54]. Mutations in cohesin, or its regulators, cause a spectrum of human developmental syndromes known as the "cohesinopathies," including Cornelia de Lange syndrome (CdLS), Roberts syndrome [55], Warsaw breakage syndrome, CAID (chronic atrial intestinal dysrhythmia) syndrome, CHOPS syndrome, prostate and colorectal cancer, and aneuploidy in neurons linked to Alzheimer disease) [55–57]. Five genes, encoding subunits of the cohesin complex

(*SMC1A, SMC3, RAD21*) and its regulators (*NIPBL, HDAC8*), account for at least 70% of patients with CdLS or CdLS-like phenotypes [57].

Mitotic spindle assembly and positioning are important for accurate and cell replication. Defects in spindle regulatory mechanisms might result in tissue overgrowth/tumorigenesis or growth insufficiency (such as that observed in the hereditary condition known as autosomal recessive primary microcephaly [MCPH, OMIM 251200] caused by a mutation in the *MCPH1* gene at 8p23.1 encoding microcephalin/BRIT1) [249]. MCPH and Seckel syndrome spectrum disorders are both characterized by microcephaly (Head Circumference (HC) <2 SDs below mean) with prenatal onset in the second trimester with slowed postnatal OFC growth and absence of visceral dysmorphisms [58]. There are 12 known MCPH loci: *Microcephalin, WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEP135, CEP152, ZNF335, PHC1,* and *CDK6.*

### 4.3.2. Signaling Pathways Involved in the Regulation of Growth

Numerous signaling pathways control osteogenic lineage commitment and progression, including *WNT, BMP,* and *FBG* [59]. These converge on bone-related transcription factors. Samsa et al. [60] review signaling pathways regulating cartilage growth plate development including the influence of circadian rhythm and COP9 signalosome.

Ubiquitin ligases are important proteins regulating bone turnover. E3 ubiquitin ligases c-Cbl and Cbl-b interact with RTKs and other molecules to control bone cell proliferation, differentiation, and survival. Inhibition of c-Cbl promotes osteoblast differentiation through decreased RTK degradation. Regulation of bone formation by E3 ubiquitin ligase was reviewed [61]. Dysregulation of the Ubiquitin Ligase pathway, including mutations in *CUL4*, *PHIP*, and *DDB1* have overlapping features of hypotonia, intellectual disability, obesity and dysmorphic features [64,65].

#### 4.3.2.1. Hedgehog Signaling

There are several prominent signaling pathways regulating bone development, homeostasis, (and tumorogenesis when disrupted). Endochondral and intramembraneous ossification are both regulated by Hedgehog (Hh) signaling [64]. In mammals, the Hh homologous proteins are: Sonic hedgehog (SHH), Indian hedgehog (IHH), and Desert hedgehog (DHH). Patch homologues 1 and 2 (Ptch1 and Ptch2) are transmembrane proteins that inhibit Hh signaling in the absence of Hh protein binding [65] via Smoothened (Smo), a transmembrane $G_i$-coupled receptor. Indian hedgehog (IHH) encoded by the *IHH* gene (chromosome 2q35)—produced by prehypertrophic chondrocytes (adjacent to the proliferation zone), hypertrophic chondrocytes, and osteoblasts—regulates both hypertrophic chondrocyte and osteoblast differentiation [66]. IHH stimulates production of parathyroid hormone–related protein (PTHrP) by perichondrial/periarticular cells and early chondrocytic cells, which diffuses through the growth plate, promoting chondrocytes proliferating in a less-differentiated state [67,68]. Chondrocytes then exit the cell cycle and undergo hypertrophy when the PTHrP level drops. IHH and PTHrP form a feedback loop that regulates the growth plate and ultimately the development of long bones, influenced by bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and mechanical loading [68].

Deficiency of *Ptch1* (mice and patients) has been noted to have increased bone mass, while absence of IHH was linked to reduced PTHrP expression (with accelerated hypertrophy of chondrocytes) and loss of endochondral ossification due to lack of osteoblast differentiation [68]. Homozygous mutation of the *IHH* gene is linked to acrocapitofemoral dysplasia (OMIM 6007778), while heterozygous mutation is linked to brachydactyly type A1 (Farabee-type) (OMIM 112500). Recently it was also demonstrated that *IHH* induces collagen type X expression through regulation of *Gli1* or *Gli2* or indirect interaction with Runx2/SMAD pathway [69]. *Runt-related transcription factor 2* or *RUNX2* (6p21.1) encodes transcription factor CBFA1 and is involved in the differentiation of osteoblasts and hypertrophy of

cartilage at the growth plate as well in cell migration and in vascular invasion of bone and is linked to vascular calcification of atherosclerotic lesions [70]. Heterozygous duplication (resulting in a gain-of-function mutation) results in metaphyseal dysplasia with maxillary hypoplasia with or without brachydactyly (OMIM 156510), while heterozygous loss-of-function mutations in *RUNX2* cause cleidocranial dysplasia (OMIM 119600). Mutations in *GLI3* can present with hypopituitarism as seen in children with Pallister-Hall syndrome (OMIM 146510: hypothalamic hamartoma or hamartoblastoma, polydactyly, anal or renal anomalies, and facial changes) and may present with hypopituitarism [71]. *GLI3* mutations are also associated with Greig cephalopolysyndactyly syndrome (GCPS, OMIM 175700) with macrocephaly; mild GCPS may have subtle craniofacial findings and polysyndactyly, while individuals with severe GCPS can have seizures, hydrocephalus, and intellectual disability [72].

#### 4.3.2.2. BMP Antagonists

Downstream of Hh signaling, endochondral bone development requires Wnt/β-catenin signaling (reviewed further in the chapter on Skeletal Dysplasias) to regulate osteoblast differentiation. BMP signaling also acts downstream of Hh pathway and regulates osteoblast cell differentiation from perichondral cells. *Noggin* (17q22) and *SOST/sclerostin* (17q21.31) are BMP antagonists that play a key role in bone homeostasis. Osteoblast hyperactivity can lead to increased bone mass, such as in *SOST*-related sclerosing bone dysplasias, Van Buchem disease (OMIM 239100), craniodiaphyseal dysplasia (autosomal dominant) (OMIM 122860), and sclerosteosis (OMIM 269500) [73]. Postnatally, *Noggin* is expressed in suture mesenchyme of patent cranial sutures and is suppressed by FGF2 and FGFR signaling [74]; mutations are associated with ankylosis of IP joints and carpal/tarsal bone fusion as seen in promixal symphalangiasm (SYM1A, OMIM 185800). Sclerostin–noggin interaction neutralized the ability of either protein to bind and inhibit BMP [75]. Recently, receptor activator of nuclear factor (NF)-κB ligand (RANKL), cathepsin K, and sclerostin have been identified as

important therapeutic targets in interventions related to manipulating bone mineral density [76]. RANKL and its receptor RANK activate SRC kinases and AKT1 (discussed later in relation to overgrowth disorders). *RANKL* is found to be deficient in osteopetrosis (OMIM 259710). Cathepsin K is a cysteine proteinase that regulates osteoclast function in bone remodeling and is dysregulated in pycnodysostosis (OMIM 265800). See chapters on Skeletal Dysplasia.

#### 4.3.2.3. Other Signaling Cascades

Beyond the growth plate, derangements of intracellular signaling pathways (such as the receptor tyrosine kinase [RTK]–phosphatidylinositol-3-kinase [PI3K]–the oncogene protein kinase B [AKT]–mechanistic target of rapamycin complex [mTOR] and rat sarcoma [RAS]–cyclic AMP/mitogen activated protein [MAP] kinase pathway) have been associated with growth abnormalities. Investigation of Cowden syndrome and tuberous sclerosis uncovered the significance of the RTK/PI3K/AKT/mTOR signaling cascade [77]. This pathway mediates cell growth, proliferation, and survival and has significant implications for somatic generalized, segmental, and localized overgrowth syndromes.

The RAS/MAPK signaling cascade, illustrated in Fig. 4.2, is a subsidiary pathway that is essential to normal growth and development, participating in regulation of the cell cycle, differentiation, growth, and cell senescence [78]. Disorders of the RAS/MAPK pathway are collectively called RASopathies and include developmental disorders associated with short stature and cardiovascular defects (Noonan syndrome (NS) and Noonan-related syndromes). These developmental disorders are caused by germline mutations in genes that encode protein components of the RAS/MAPK pathway. They result from germline mutations in genes that participate in the RAS/MAPK pathway. At least 15 genes linked to this pathway have been associated with specific RASopathies, and 11 have been found linked to NS or NS-like syndromes. *PTPN11* mutations cause ~50% of cases, and the other genes include *SOS1, CBL, BRAF, RASA1, RAF1, SHOC2, MAP2K1, RIT1, NRAS, KRAS,* and

*RRAS* [79]. The clinical features associated with mutations in these genes are discussed in Table 4.3.

PTEN is a tumor suppressor dual-specificity phosphatase that antagonizes the PI3K signaling pathway through its lipid phosphatase activity and negatively regulates the MAPK pathway through its protein phosphatase activity [79].

## 4.3.3. Epigenetic Contributions to Abnormal Growth

Gene expression is regulated by epigenetic processes that modify DNA and its associated proteins, controlling gene expression profiles for specific developmental stages of individual cell types. See chapter on Epigenetics. Small noncoding RNAs or microRNAs (miRNAs) are critical posttranscriptional regulators of gene expression by promoting mRNA degradation and translational inhibition [80]. miRNAs expressed in the placenta regulate trophoblast cell differentiation, proliferation, apoptosis, invasion/migration, and angiogenesis, and aberrant miRNAs expression has been linked to pregnancy complications, such as preeclampsia (in particular *miR-210* and *miR-193b-3p*) [90]. Placental insufficiency leads to slow fetal growth and leads to infants born who are SGA. Placental anomalies suggestive of uteroplacental malperfusion include single and multiple infarctions [91], as well as smaller and lighter placentas with thinner umbilical cords.

**Table 4.3**

**Prenatal-Onset Growth Deficiency Disorders**

| Disorder | Key Clinical Findings | Management Considerations/S |
|---|---|---|
| Teratogenic Disorders (see Table 4.2) | | |
| Chromosome Abnormalities | | |
| | | |

| | | | |
|---|---|---|---|
| **Trisomy 18** | Clenched hands, short sternum, low arch dermatoglyphic pattern | | |
| **Trisomy 13** | Scalp defects, polydactyly, holoprosencephaly facies | | |
| **Triploidy** | 3-4 Syndactyly, dysplastic calvaria, cystic placenta | | |
| **4p syndrome** | Ocular hypertelorism, hypospadias, preauricular pits | | |
| **5p syndrome** | Catlike cry, microcephaly, down-slanted palpebral fissures | | |
| **Williams Syndrome (194050)** | Prominent lips, periorbital fullness, supravalvular aortic stenosis (or other cardiovascular disease), mild MR, specific cognitive profile (overly friendly), endocrine abnormalities, hemizygous deletion 1.5- to 1.8-Mb Chr 7q11.23 including: *ELN, LIMK1, GTF2I, MAGI2, HIP1, YWHAG,* among others [81] | | |
| **13q syndrome** | Central nervous system defects, short thumbs, anal anomalies | | |
| **18p syndrome** | Ptosis, protruding ears, mental deficiency | | |
| **18q syndrome** | Prominent antihelix, midface hypoplasia, long palms | | |
| **45,X (Turner) syndrome** | Lymphedema of hands and feet, webbed neck, broad chest with wide-set nipples | | |
| Proportionate Syndromes | | | |
| **Disorder** | **Key Clinical** | **Inheritance** | **Gene (Chromoso** |

| (MIM) | Findings | | gene product |
|---|---|---|---|
| Epigenetic Changes | | | |
| **Russell-Silver (180860)** | IUGR with persistent postnatal growth deficiency, triangular face, normal-sized cranium, incurved (clinodactyly) fifth fingers, asymetric limb length (may lead to decreased growth on the affected side with hemi-hypertrophy), risk of motor and cognitive developmental delay with learning disabilities [13] | Sporadic heterogeneous | • Hypomethy 11p15.5 H19/ imprinting c region (*ICR1* 50%)<br>• Alterations c chromosome<br>• Maternal un disomy of Cl (mUPD7) in<br>• 7p11.2-11.3 r 1% |
| **3MC Syndrome (257920, 265050)** | hypertelorism, blepharophimosis, blepharoptosis, and highly arched eyebrows (75–90% cases)Cleft lip and palate, postnatal growth deficiency, cognitive impairment, and hearing loss (40–68% cases) | Autosomal recessive | Factors of the lect complement pathway3MC- (3q27.3) 3MC-2 *COLEC11* (2p2 3: *COLEC10* (8 [242] |

| | | | |
|---|---|---|---|
| | Craniosynostosis, radioulnar synostosis, and genital and vesicorenal anomalies (20–30% cases)Rare: anterior chamber defects, cardiac anomalies, caudal appendage, umbilical hernia (omphalocele), and diastasis recti [241] | | |

Table

| Disorder | Key Clinical Findings | | |
|---|---|---|---|
| **Dubowitz syndrome (223370)** | IUGR (69%) with postnatal growth deficiency (86%), microcephaly with a high or sloping forehead, sparse hair, and a broad and flat nasal bridge, flat or shallow supraorbital ridges, ptosis and blepharophimosis, with thin lateral eyebrows, soft high pitched voice, may have dental | Heterogeneous | |

| | | | |
|---|---|---|---|
| | anomalies or palate deformations eczema (~50% patients) 60% clears by age 2–4; immune deficiencies with increased infections<br>Clinodactyly of the fifth fingers, and cutaneous syndactyly/partial webbing of the second and third toes normal intelligence to mild to moderate MR, behavioral problems including ADHD. Genitourinary abnormalities may include hypospadias and cryptorchidism Malignancies: leukemia, lymphoma, aplastic anemia, neuroblastoma | | |
| **Cohesinopathies** – Intranuclear protein, Cohesin complex responsible for sist during mitosis (also, DNA damage repair, insulator function, gene regulat condensation) | | | |
| **Cornelia/Brachmann-de Lange**<br>• **CLDS1 (122470)**<br>• **CLDS2 (300590)** | Microcephaly, low anterior hair line, arched eyebrows, synophrys, thin downturned upper | Sporadic, autosomal dominant<br>X-linked | |

| | | | |
|---|---|---|---|
| • **CLDS3 (610759)**<br>• **CLDS4 (614701)** | lip (maxillary prognathism), small widely spaced teeth, micromelia, hirsutism, autistic tendencies, MR (IQ 30–102), occasionally with cardiac septal defects, hearing loss, myopia, gastrointestinal dysfunction, hypoplastic genitalia/cryptorchidism [Boyle] | | (fewer than 1% patients have an affected parent) |
| | | | |

| **Disorder** | **Key Clinical Findings** | | **Management Considerations/S** |
|---|---|---|---|
| **Roberts syndrome (268300)** | Slow intrauterine and postnatal growth, mild to severe intellectual impairment; shortened arm and leg bones (hypomelia or phocomelia), craniofacial abnormalities: hypertelorism, cleft lip/palate, microcephaly, heart, kidney, | Autosomal recessive | *ESCO2* (8p21.1) |

| | | | |
|---|---|---|---|
| | and genital abnormalities | | |
| Defects of Cell Replication | | | |
| **Meier-Gorlin syndrome Ear, patella short stature syndrome (224690)** | Severe intrauterine and postnatal growth retardation, bilateral microtia, microcephaly (variable) and aplasia or hypoplasia of the patellae (intellect usually normal) | Autosomal recessive Autosomal dominant | Mechanism: impa formation of t replication co (subunits code genes below) a subsequent di replication lic • MGORS1: *O* (1p32.3) (sho stature than MGORS mu • MGORS2: *O* (2q23.1) • MGORS3: *O* (16q11) • MGORS4: *C* (16q24) • MGORS5: *C* (17q21) • MGORS7: *C* (22q11) • MGORS6: *G* (6p22) |
| **IMAGe syndrome (614732)** | Intrauterine growth deficiency, metaphyseal dysplasia, adrenal hypoplasia, | Autosomal dominant | • *CDKN1C* (11 • Disrupted P binding (DN replication a protein at re fork) |

| | | | |
|---|---|---|---|
| | and genital anomalies: severe postnatal growth failure with severe, possibly life-threatening adrenal insufficiency, short adult height, hearing loss, and dysmorphic features (triangular face with frontal bossing, broad nasal bridge, low-set ears) | | |

Table

| Disorder | Key Clinical Findings | | Man Cons |
|---|---|---|---|
| **Seckel syndrome (201600, 606744, 613676,61382, 614728, 614850, 615807, 616777, 617253)** | IUGR and severe proportionate short stature (postnatal persistence) with Microcephaly, prominent nose, micrognathia<br>Phenotypic overlap | Autosomal recessive, genetically heterogeneous | • <br>• <br>• |

| | | | |
|---|---|---|---|
| | with MOPD2 (milder MR than SCKL, more severe growth retardation and radiologic abnormalities) | | • • • • • • |
| **MOPD II Microcephalic (Majewski) Osteodysplastic primordial dwarfism type (210720)** | Intrauterine growth retardation, severe persistent postnatal proportionate short stature, high forehead, pronounced, progressive microcephaly. radiological abnormalities (), and absent or mild mental retardation | Autosomal recessive | *PCN* c p P w th m se th |
| Defects of DNA Repair | | | |
| **Werner Syndrome (277700)** | Short stature, slender limbs with scleroderma-like texture to skin, stocky trunk, beaked nose. cataract, subcutaneous calcification, | Autosomal recessive | *REC* h |

| | | | |
|---|---|---|---|
| | premature arteriosclerosis, diabetes mellitus, and a wizened and prematurely aged facies, insulin resistance (defective signaling distal to insulin receptor) responsive to troglitazone [source] | | |
| | | | |

| Disorder | Key Clinical Findings | | Managen Consider |
|---|---|---|---|
| **Bloom syndrome (210900)** | Prenatal and postnatal growth deficiency, Microcephaly, sun sensitivity (hypo/hyperpigmented skin), malar erythema telangiectasia, malar hypoplasia, gastrointestinal reflux (possibly contributing to infections of lung [20%], middle ear and upper respiratory tract), sparse subcutaneous fat through infancy and early childhood, normal intelligence with poorly defined learning disability, early | Autosomal recessive | Homozyg heterc DNA 3 *REC* increa chrom (SCEs cultur dicent |

| | | | |
|---|---|---|---|
| | susceptibility to medical complications such as COPD, DM, and predisposition to malignancies (leukemia, lymphoma, adenocarcinoma, squamous cell carcinoma, and Wilms tumor (~44% by mean age 25) [31]<br>Male infertility/small testes; female fertile with premature menopause; | | |
| *S*ubset: RECQL4 Spectrum Diseases | | | |
| **Rothmund-Thomson syndrome (268400)** | Short stature, characteristic poikiloderma,may have skin atrophy, telangiectasia, hyper/hypopigmentation<br>RTSI: ectodermal dysplasia, juvenile cataracts<br>RTS II: congenital skeletal abnormalities especially radial defects (20%) (hypoplastic/absent thumbs), premature aging and increased risk of malignant disease (osteogenic sarcoma [32%] in childhood; skin cancer in later life) | Autosomal recessive | Genetic h<br>• RTS (8q2 encc<br>• RTS etiol |
| **Baller-** | Short stature, poikiloderma<br>Cardinal features: | Autosomal recessive | • *RECQL* |

| | | | |
|---|---|---|---|
| **Gerold syndrome (218600)** | craniosynostosis and radial aplasia some phenotypic overlap with Saethre-Chotzen syndrome | | |
| | | | |

| Disorder | Key Clinical Findings | |
|---|---|---|
| **RAPADILINO Radial and patellar aplasia, hypoplasia syndrome (266280)** | Short stature, no poikiloderma, radial aplasia or hypoplasia, absence of thumbs, absent or hypoplastic patellae, dislocations of joints, unusual face, cleft or highly arched palate, diarrhea in infancy, small stature, and normal intelligence | Autosomal recessive |
| **Trichothiodystrophy (601675, 616390, 616395, 234050, 300953, 616943)** | Hair: brittle, sulfur-deficient with alternating light and dark banding pattern, called "tiger tail banding," under polarizing microscopy (diagnostic), ichthyosis, intellectual/developmental disabilities, decreased fertility. Variable phenotype with cutaneous, neurologic, and ocular abnormalities (photosensitive and nonphotosensitive types) | Autosomal recessive |

| | | |
|---|---|---|
| | [84] | |
| **Nijmegen breakage syndrome** | Short stature, progressive and disproportionate microcephaly with normal intelligence (can see decline in intellectual intelligence with age), facial features: sloping forehead, upward slanted palpebral fissures, prominent nose, relatively large ears, and retrognathia immunodeficiency (recurrent upper respiratory (UR) infections), predisposition to cancer (by age 20: highest for T-cell [55%] and B-cell lymphomas [45%]; solid tumors: medulloblastoma, glioma, and rhabdomyosarcoma) (Clinically indistinguishable from Berlin breakage syndrome but differ in complementation studies) | Autosomal recessive |
| **Ataxia telangiectasia (208900)** | Progressive cerebellar ataxia beginning between ages one and | Autosomal Recessive |

| | | | |
|---|---|---|---|
| | 4 years, oculomotor apraxia, choreoathetosis, telangiectasias of the conjunctivae, immunodeficiency, frequent infections, and an increased risk for malignancy (leukemia and lymphoma); AT cells are abnormally sensitive to killing by ionizing radiation | | |
| | | | |

| Disorder | Key Clinical Findings | | Manage Consid |
|---|---|---|---|
| **3M syndrome (273750)** | Severe prenatal and postnatal growth retardation, and normal mental development, triangular face, full lips, hypoplastic midface, long philtrum, short thorax (transverse ribs, anterior inferior rib grooves) with sternal deformity, broad neck, square shoulders, prominent trapezius | Autosomal recessive | • *Cl* lig de pr ex inv res 77 • *O* (O CU • *C* |
| **Fanconi pancytopenia** | Prenatal and postnatal growth deficiencies, abnormal skin pigmentation microcephaly, | Autosomal recessive [19] Except *FANCB* | Biall i FAN p r |

| | | | | |
|---|---|---|---|---|
| | (hyperpigmentation)<br>Radial hypoplasia (malformations of upper or lower limbs), hypoplastic thumbs, pancytopenia (progressive bone marrow failure), increased risk of malignancy—acute myelogenous leukemia or myelodysplastic syndrome and solid tumors) [33]<br>May have malformations of the kidneys/urinary tract, heart, GI system, oral cavity, CNS, ears (including hearing loss), developmental delay<br>FANCB patients can present with VACTERL-H constellation of anomalies | mutations-<br>X-linked inheritance and | (<br>p<br>l<br>c<br>r<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>•<br>• |

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| Disorder | Key Clinical Findings | | Management Considerations/S |
|---|---|---|---|
| **De Sanctis-Cacchione syndrome (278800)** | Xeroderma pigmentosum, hypogonadism, microcephaly | Autosomal recessive | *ERCC6* (10q11.23) (excision repai complementin nucleotide exci repair pathwa |
| **Cockayne syndrome (216400, 133540)** | IUGR, postnatally, growth is slow, progeroid appearance, cutaneous photosensitivity, thin dry hair progressive pigmentary retinopathy, sensorineural hearing loss and characteristic "horse riding" stance secondary to knee contractures | Autosomal recessive | • CSA: *ERCC8* • CSB: *ERCC6* |
| **Johanson-Blizzard (243800)** | Aplastic or hypoplastic alae nasi, microcephaly, | Autosomal recessive | Homozygous or c heterozygous m of *UBR1* gene ( |

| | | | |
|---|---|---|---|
| | midline scalp defects, sensorineural deafness, exocrine pancreatic insufficiency, nasolacrimal system malformations, absent permanent teeth, hypothyroidism [36] | | *protein ligase E3 component N-re* (15q15.2) |
| **Hallermann-Streiff syndrome (243100)** | Characteristic skull: brachycephaly with frontal bossing, microphthalmia, cataracts, small pinched nose, hypotrichosis, dental anomalies, cutaneous atrophy | Sporadic | Not yet identified |
| **Smith-Lemli-Opitz syndrome (270400)** | Deficiency of enzyme 7-dehydrocholesterol reductase. Prenatal onset growth deficiency with postnatal persistence secondary to abnormal cholesterol metabolism | Autosomal recessive | Homozygous or c heterozygous r of *DHCR7*, ger encoding stero reductase/7-dehydrocholes reductase (11q confirmed by e of serum 7DH( |

| | | | |
|---|---|---|---|
| | Microcephaly, ptosis, cleft palate, syndactyly of second and third toes, postaxial polydactyly, hypospadias [40] | | |
| **Aarskog(-Scott) syndrome [faciogenital dysplasia] (305400)** | Ocular hypertelorism, optic nerve hypoplasia, retinal vessel tortuosity, deficient ocular elevation, hyperopia, and anisometropia [44] brachydactyly, shawl scrotum | X-linked recessive | *FGD1* (Xp11.2) |

Table

| **Disorder** | **Key Clinical Findings** | | **Mana Consi** |
|---|---|---|---|
| **Robinow syndrome (268310) (180700, 616331, 616894)** | Broad forehead with flat facial profile, short forearms, hypoplastic genitalia | Autosomal recessive Autosomal dominant | • I I I ( i a a • ] W • ] ( s a |

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| | | | t<br>• ] |
| **Opitz syndrome (300000, 145410)** | Ocular hypertelorism/telecanthus, swallowing difficulties secondary to abnormalities of the trachea/esophagus and larynx (laryngotracheoesophageal cleft; clefts of lip, palate, and uvula); also associated with cardiac anomalies, GU defects: hypospadias/splayed labia, imperforate anus, and developmental delay; anteverted nares and posterior pharyngeal cleft were seen only in the X-linked form [46] | X-linked recessive, autosomal dominant | • ]<br>(<br>• ]<br>(<br>T |
| **Coffin-Siris syndrome (135900, 614607-9, 616938)** | Hypoplastic fifth digits and nails, coarse facies, hirsutism with sparse scalp hair, psychomotor delay/MR [47] | Autosomal dominant | • A<br>g<br>c<br>S<br>c<br>r<br>• A<br>• S<br>• S<br>• S |
| Dysplasias—Also see chapter on Skeletal Dysplasias and Osteodystoses [240] | | | |
| **Myhre syndrome (139210)** | Prenatal and postnatal growth deficiency, microcephaly, midface hypoplasia, | Sporadic, autosomal dominant | *SMAI* |

| | | | |
|---|---|---|---|
| | prognathism, blepharophimosis, typical skeletal anomalies (short stature, square body shape, broad ribs, iliac hypoplasia, brachydactyly, flattened vertebrae), CV defects | | |
| | | | |

| Disorder | Key Clinical Findings | | M C |
|---|---|---|---|
| Selective Endocrine Causes—See chapter discussing Growth Hormone - IGF A | | | |
| **Donohue syndrome (leprechaunism) (246200)** | Severe IUGR Adipose deficiency, thick lips, islet cell hyperplasia results in hyperinsulinemia | Autosomal recessive | H |
| **Drayer syndrome (Chr 15q26-qter deletion syndrome) (612626)** | IUGR and postnatal growth deficiency, microcephaly, micrognathia, low-set ears, broad nasal bridge Can also have: renal anomalies, lung hypoplasia delayed growth/development | Sporadic | In |
| **Kowarski (262650)** | Short stature associated with bioinactive growth hormone, is characterized clinically by normal or slightly increased GH secretion, pathologically low IGF1, normal catch-up | Autosomal recessive | G |

| | | | |
|---|---|---|---|
| | growth on GH replacement therapy | | |
| ASXL Mutations | | | |
| **Bohring-Opitz syndrome (605039)** | IUGR, poor feeding, profound intellectual disability, trigonocephaly, prominent metopic suture, exophthalmos, facial nevus flammeus, flexion of the elbows/wrists with deviation of wrists/metacarpophalangeal joints [86,87] | Autosomal dominant | *A* |
| **Shashi-Pena syndrome (617190)** | Enlarged head circumference, delayed psychomotor development, variable intellectual disability, glabellar neus flameus, hypotonia, facial dysmorphism, deep palmar creases [88]. Variable: atrial septal defect, episodic hypoglycemia, changes in bone mineral density, and/or seizures | Autosomal dominant | *A* |
| **Bainbridge-Ropers syndrome (615485)** | Severe postnatal growth restriction, arched eyebrows, anteverted nares, feeding problems, severe psychomotor delay, ulnar deviation of hands [89] | De novo | *A* |

[a] A defect in repair of DNA double-strand break (DSB) likely due to defective nonhomologous end joining (NHEJ). DNA ligase IV mutation is solely responsible for the DNA repair defects.

At the growth plate, histone deacetylase (HDAC)3 controls temporal and spatial expression of tissue-remodeling genes and inflammatory responses in chondrocytes to ensure proper endochondral ossification during development. Carpio et al. [92] found that in HDAC3-deficient chondrocytes, there was (1) increased expression of cytokine and matrix-degrading genes (*IL-6, Mmp3, Mmp13*, and *Saa3*), (2) a reduced abundance of genes related to extracellular matrix production, bone development, and ossification (*Acan, Col2a1, Ihh,* and *Col10a*1), (3) increased acetylation and activation of NF-κB, and (4) increased cytokine signaling promoted autocrine activation of Janus kinase (JAK)-signal transducer and STAT and NF-κB pathways to suppress chondrocyte maturation, as well as paracrine activation of osteoclasts and bone resorption [92].

#### 4.3.3.1. Chromatin Remodeling by Nucleosome Complexes

Coffin-Siris syndrome (CSS, OMIM 135900) illustrated in Fig. 4.1, is characterized by coarse facial features, hypertrichosis, sparse scalp hair, and hypo-/aplastic digital nails and phalanges, typically of the fifth digits. Mutations in the neuron-specific Brg1/hBrm-associated factor (nBAF) complex [93] or SWItch/sucrose nonfermentable (SWI/SNF)-like chromatin remodeling complex subunits (*SMARCA4, SMARCE1, SMARCB1, SMARCA2, ARID1B*, and *ARID1A*) have been shown to cause CSS, Nicolaides-Baraitser syndrome (NCBRS, OMIM 601358), and *ARID1B*-intellectual disability syndrome (OMIM 614562) [94]. Mutations were associated with syndromic intellectual disability and severe–profound speech impairment, which was frequently accompanied by agenesis or hypoplasia of the corpus callosum [95]. *SMARCB1* mutations caused "classic" CSS with typical facial "coarseness" and significant digital/nail hypoplasia, while *SMARCA4* mutations caused CSS without typical facial coarseness and with significant nail hypoplasia. *SMARCA2* mutations caused NCBRS, typically with short stature, sparse hair, a thin vermillion of the upper lip, an everted lower lip, and prominent finger joints. A *SMARCE1* mutation caused CSS without typical facial coarseness and with

significant digital/nail hypoplasia. *ARID1A* mutations caused the most severe CSS with severe physical complications. *ARID1B* mutations caused CSS without typical facial coarseness (Fig. 4.1) and with mild digital/nail hypoplasia, or caused syndromic ID [95].


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**FIGURE 4.1** This 4-year-old child with Coffin-Siris Syndrome and an *ARID1B* mutation has growth deficiency, intellectual disability, microcephaly, coarse facial features including: small forehead, down

slanting palpebral fissures, thick eyebrows, thick lips.

#### 4.3.3.2. DNA Methylation and Imprinted Genes

Genomic imprinting is the epigenetic denotation of genes that allows for expression in a monoallelic parent-of-origin-specific manner (i.e., one of the two inherited alleles is expressed either from the paternal or maternal side). These *imprinted* genes are then arranged in clusters controlled by differentially methylated regions or imprinting control regions [96]. Critical periods of epigenetic processing takes place during gametogenesis and early preimplantation development. After fertilization, genome-wide demethylation is followed by de novo methylation, both processes regulated by imprinted loci [97].

DNA methylation at cytosine residues of CpG dinucleotides in gene promoters, transposons and imprinting control regions (ICRs) is one of the most widely studied epigenetic modifications [97]. Besides genomic imprinting, DNA methylation has been observed in cellular differentiation, embryogenesis, and X-chromosome inactivation [96]. DNA methylation dynamics are regulated by three processes: de novo DNA methylation, methylation maintenance, and DNA demethylation. Abnormal DNA methylation at the 11p15 imprinted region has been associated with two phenotypically contrasting syndromic disorders. The 11p15 imprinted region has two imprinting control regions (*ICR1* and*ICR2*) that are differentially methylated (*ICR1* is normally methylated on the paternal 11p15 and*ICR2* is normally methylated on the maternal 11p15 [98–100].

Loss of methylation (LOM) or hypomethylation of the paternal telomeric *ICR1* (regulating the expression of insulin-like growth factor [IGF]2 and *H19*) of chromosome 11p15.5 is identified in 35%–50% of individuals with Russell-Silver syndrome (RSS), an intrauterine and postnatal growth deficiency syndrome (*KCNQ10T1* Fig. 4.2A and B). About 10% of individuals with RSS have maternal uniparental disomy for chromosome 7 (UPD7) [100]. On the other hand, the overgrowth syndrome Beckwith-Wiedemann syndrome (BWS) (Fig. 4.3) is associated with gain of methylation in the telomeric *ICR1* or LOM in the centromeric *ICR2*-regulating the expression of *KCNQ1*, *CDKN1C*,

and *KCNQ10T1* [101].

Russo et al. [100] note that 20%–30% of clinically diagnosed RSS and BWS remain without molecular diagnosis, suggesting that the complex structure of the 11p15 region with variable CpG methylation and a low-rate mosaicism may account for some of these missed diagnoses.




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**FIGURE 4.2** (A) This 3-year-old male child with Russell-Silver syndrome had prenatal onset growth deficiency that persisted after

birth, triangular facial shape, fifth finger clinodactyly, mild skeletal asymmetry and reduced adipose tissue that placed this child at risk for fasting hypoglycemia. (B) This 4.5 year-old female child with Russell-Silver syndrome (left) with a normal skeletal survey, normal endocrine evaluation and biparental inheritance for of chromosome 7 with normal methylation of *DMR1*, upstream of *H19*, thus the basis of her disorder remains unknown. She was born premature with severe IUGR, and she is responding to growth hormone therapy with normal school

performance at age 6.5 years (right).

### 4.3.3.3. Gene Silencing

Polycomb-group proteins are transcriptional repressors that modulate chromatin structure. They reside in two complexes: polycomb repressive complex (PRC)1 and 2. PRC2 maintains gene silencing, which maintains embryonic stem cell pluripotency and plasticity [102,103]. The gene silencing activity of PRC2 depends on its ability to di- or trimethylate histone 3 lysine 27 by the catalytic SET domain of the methyltransferase enhancer of zeste (EZH2) subunit and at least two other subunits of the complex: embryonic ectoderm development (EED) and suppressor of zeste (SUZ) 12 [102,103]. Mutations in*EZH2* and embryonic stem cell pluripotency and plasticity EED have been associated with a Weaver syndrome–like (Fig. 4.16, discussed further in the overgrowth section of this chapter) phenotype [103,104].


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**FIGURE 4.3** This infant with Beckwith-Wiedemann syndrome was born macrosomic with macroglossia, omphalocele, glabellar nevus flammeous, infraorbital creases, and posterior auricular ear pits. These are typical features in this common overgrowth disorder.

## 4.4. Pathologic Short Stature

Of the myriad of causes for short stature, each of these have different prognoses, complications, and responses to treatments. Therefore, when it is established that a person is truly short for his or her genetic background (and constitutional delay or growth failure has been ruled out), the exact cause of the pathologic short stature must be delineated. The first step in the clinical evaluation of short stature is to determine whether the body habitus is proportionate or disproportionate (Fig. 4.4). Anthropometric measurements (such as sitting height, upper/lower segment ratio, and/or arm span) help qualify disproportionate short stature, which suggests a skeletal dysplasia or metabolic bone disease (see chapter on Skeletal Dysplasia), while proportionate short stature may have a more generalized disorder (i.e., malnutrition, chronic disease, psychosocial

dwarfism, endocrine disorder, genetic syndrome, or chromosomal or teratogenic disorder, as discussed (see chapters discussing Teratology)). Exceptions to this rule occur, and disproportionate dwarfism may occur in cases of severe cretinism, while proportionate shortening may occur in persons with osteogenesis imperfecta.

Once a person with short stature is found to be proportionate, it is helpful to determine whether the growth deficiency was of prenatal (congenital) or postnatal onset.

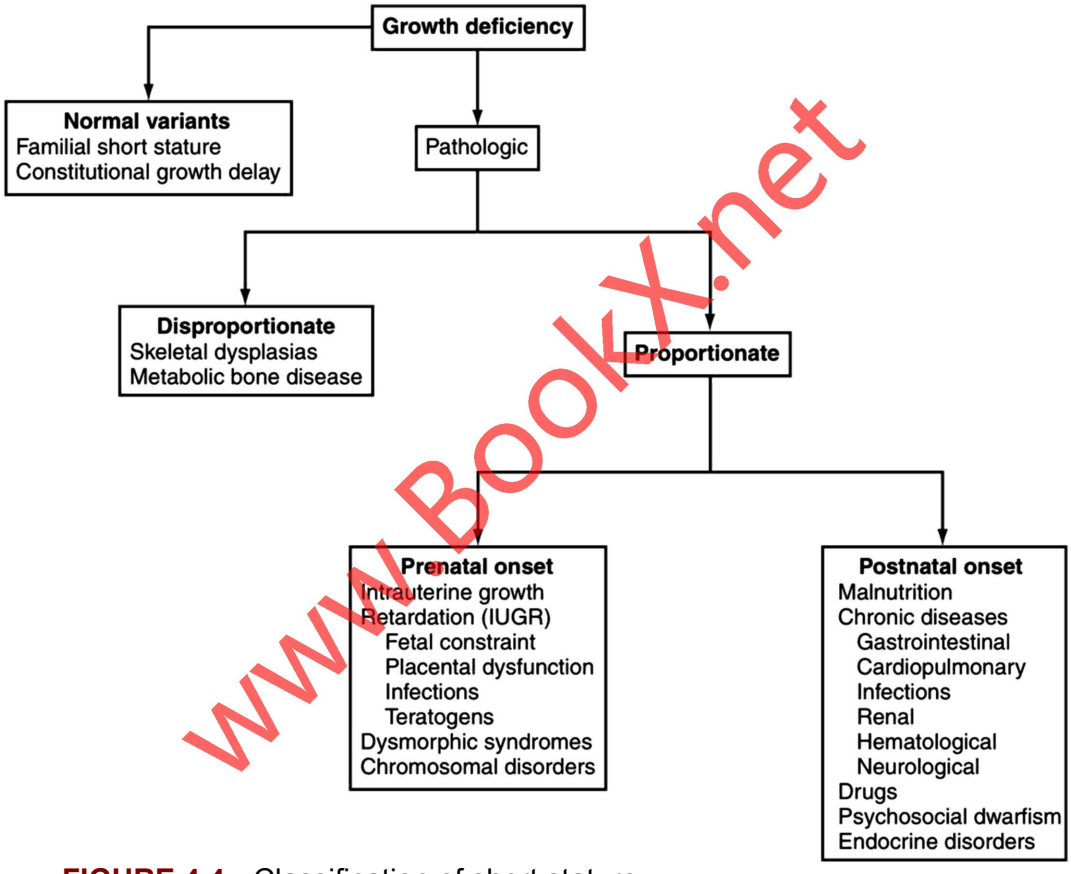
In general, prenatal-onset growth deficiency implicates a fetal environmental insult or a generalized cellular genetic defect that may limit cellular mitosis. Therefore, prenatal-growth restriction that persists after birth is generally assumed to be due to a genetic, syndromal, cytogenetic, or teratogenic disorder that results in generalized hypoplasia or disproportionate skeletal hypoplasia. Late fetal insults are more likely to demonstrate catch-up growth after birth compared with environmental factors, which have an early impact in fetal life [105]. Conversely, postnatal onset of proportionate growth deficiency usually implicates a postnatal environmental insult, such as infection, chronic disease, malnutrition, endocrine, psychological, or malabsorption disorder, and responds best to treatment of the primary disease state. Recurrence risk counseling must also be individualized based on the underlying diagnosis.

These generalizations hold true whether the cause of the short stature is a single-system disorder, such as GH deficiency or diabetes mellitus, or the short stature is associated with other abnormalities in a complex syndrome.

## 4.4.1. Proportionate Prenatal-Onset Short Stature

Declining fetal body growth in response to inadequate intrauterine supply, with relative preservation of fetal brain growth is viewed as an adaptive response to protect fetal brain development. Most SGA infants are identified on the basis of their birth weight for gestational age. Infants delivered preterm (as a group) are much smaller than

fetuses that remained in utero and delivered closer to term, and these data suggest an overlap between preterm birth and IUGR. When preterm IUGR infants and infants of multiple gestations are born after 33–34 weeks' gestation, they may have fewer complications of prematurity than expected for their gestational age, while infants born with IUGR before 34 weeks' gestation often have greater mortality and morbidity than preterm appropriate-for-gestational age infants of the same gestational age. These factors must be taken into account when evaluating growth parameters at birth, compared with subsequent growth and development.




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**FIGURE 4.4** Classification of short stature.

Prenatal growth deficiency (alternatively called fetal growth restriction [FGR] or IUGR) can be due to fetal, maternal, placental, or

environmental factors [106]. Mechanical late gestational constraint has been emphasized as the most common cause of prenatal growth deficiency. Most commonly, such growth-restricted babies are born to small mothers with fathers of normal size, and they readily catch up into the normal range within the first few months of postnatal life. A distinction is made between growth restriction mediated by the placenta versus non–placenta-mediated growth restriction [107]. Placenta-mediated causes include maternal conditions: preeclampsia, chronic or gestation hypertension, diabetes mellitus, vascular disease, renal disease, autoimmune diseases, or thrombophilia [106]. Non–placenta-mediated growth restriction includes congenital infections (TORCH infections; see Table 4.2 [108]), inborn errors of metabolism, and structural and chromosomal anomalies (see Table 4.3). Some of these disorders can be associated with a high degree of perinatal lethality, and these infants would not be expected to show catch-up growth.

Late fetal causes of growth deficiency, such as preeclampsia or placental insufficiency, usually show prompt resolution with postnatal catch-up growth. However, infants with disorders that adversely affect growth from early fetal life, such as chronic maternal hypertension/diabetes mellitus with vascular changes and heavy maternal smoking, may not show catch-up growth during the first 6 months of postnatal life. These latter conditions may be the result of diminished maternal blood flow to the placenta. IUGR is reviewed [109].

#### 4.4.1.1. Syndromic Short Stature

In many cases, a specific diagnosis of a recognizable syndrome can be made on the basis of associated characteristic clinical findings. Although maternal smoking may result in otherwise normal SGA babies, most other teratogens result in well-characterized, albeit clinically variable, teratogenic syndromes (Table 4.3; discussed in greater detail in chapter on Teratology), with the most frequent teratogenic cause of growth deficiency being fetal alcohol syndrome

(Fig. 4.6). Maternal disease [250] that can result in the accumulation of toxic substances that cross the placenta also can result in teratogenic effects on the fetus, as is evident in the maternal phenylketonuria syndrome [110]. Intrauterine infections with rubella, syphilis, toxoplasmosis, Zika virus, and cytomegalovirus (CMV) can produce prenatal growth retardation that results in postnatal proportionate short stature [108]. Affected neonates may show other systemic manifestations, including microcephaly, chorioretinitis, hepatosplenomegaly, petechiae, and seizures. The diagnosis should be suspected at birth, so that appropriate viral studies can be done to document the diagnosis and initiate therapy.

Other syndromic short stature with pathognomonic clinical features include Coffin-Siris syndrome (Fig. 4.1), Russell-Silver syndrome (Fig. 4.2A and B), CdLS (Fig. 4.5), Rubinstein-Taybi syndrome (RTS), or broad thumb-hallux syndrome (OMIM 180849 [86,87]) (Fig. 4.7A–D), Johanson-Blizzard syndrome (OMIM 243800) [114], Hallermann-Streiff syndrome (OMIM 243100) [115], Williams syndrome (OMIM 194050) (Fig. 4.8), Noonan syndrome (Fig. 4.9) [117], or Aarskog syndrome (OMIM 305400) (Fig. 4.10). In some cases, limb malformations may represent distinctive features of the syndrome (e.g., CdLS or RTS) (see Table 4.3). Thus, the clinical evaluation and analysis of the dysmorphic features present in each case often permit reaching a conclusive diagnosis, which can be confirmed through appropriate genetic or genomic testing.

In certain syndromes, diagnosis can be supported by specific laboratory studies, such as an increased rate of sister chromatid exchanges in Bloom syndrome, also known as Bloom-Torre-Machacek syndrome (confirmed by mutation analysis of the DNA helicase gene *BLM*) [118]; or analysis of chromosome breakage in the Fanconi pancytopenia syndrome, which can be caused by mutations in at least 15 different genes [53,119]; assessment of DNA repair genes in Cockayne syndrome (OMIM 216400, 133540) (Fig. 4.11A and B) [94,95], cerebro-oculo-facial-skeletal syndrome (OMIM 214150) [122] (Fig. 4.11C), and xeroderma pigmentosum [123]; use of microarray in Williams syndrome (Fig. 4.8) or velocardiofacial syndrome [124], and

insulin receptor mutation analysis in leprechaunism (caused by mutations in the insulin receptor gene *INSR* at 19p13.2) [125]. As more genes for various syndromes have become known, mutation analysis via clinical molecular testing and exome sequencing has become more available and useful, and routine use of chromosomal microarrays and exome sequencing has resulted in the molecular diagnosis of many syndromes. Array CGH has replaced chromosome analysis in most cases, and it can also be used to uncover the genomic basis for previously unknown causes of syndromic short stature, as well as to detect the deletion of a gene when mutation analysis of the causative gene demonstrates a normal sequence.

Some proportionate growth deficiency syndromes are inherited as autosomal recessive disorders, such as Bloom, Donohue, and microcephalic osteodysplastic primordial dwarfism, type II (MOPDII), whereas others usually occur sporadically, such as Hallermann-Streiff, RTS, RSS, CdLS, and Williams syndromes. Some disorders, like Cornelia-de Lange syndrome and Noonan syndrome, are genetically heterogeneous and may be due to a new mutation. Others may result from either a gene mutation or a submicroscopic chromosomal deletion, such as is seen in Rubinstein-Taybi syndrome (Fig. 4.6). In such cases, both mutation analysis and fluorescent in situ hybridization analysis may be necessary, because if there is a whole-gene deletion and mutation analysis is undertaken, only the remaining normal allele will be sequenced and found to have a normal coding sequence.

#### 4.4.1.2. Chromosomal Disorders

With the exception of sex chromosome aneuploidy, trisomy 21, and trisomy 8 mosaicism, most chromosomal disorders result in some degree of IUGR. In those syndromes compatible with survival beyond infancy, such as Turner syndrome and a variety of autosomal partial deletion or duplication syndromes, there is continued diminished growth throughout childhood and a blunted pubertal growth spurt as well. As the number of recognized chromosomal disorders increases due to the use of newer technologies, such as array CGH, it remains

important to make an accurate clinical diagnosis to select the appropriate laboratory techniques. A patient with IUGR, developmental delay, and dysmorphic features who does not manifest a recognizable syndrome may have an underlying genomic disorder, which could be detected by array CGH or exome sequencing. For example, deletion of 1q24q25 can result in primordial short stature with severe microcephaly due to deletion of the *CENPL* gene [126], which codes for a subunit of the CENPH-CENPI–associated centromeric complex and is required for kinetochore function and mitotic function. Similarly, Interstitial 2p15p16.1 microdeletion can result in short stature and microcephaly possibly secondary to the deletion of XPO1, which codes for exportin 1, nuclear protein essential for proliferation, and chromosome region maintenance [127].







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**FIGURE 4.5** This child with classic Cornelia–de Lange (Brachmann-de Lange) syndrome has not had any testing to determine the cause for her disorder. Note microcephaly with distinctive facial features, synophrys, highly arched eyebrows, long eyelashes, short nose with anteverted nares, small widely spaced teeth. This patient has distinctive micromelia (small hands), proximally placed thumbs, and nuanced fifth finger clinodactyly (Upper extremity deficiencies ranging from severe reduction defects with complete absence of the forearms to various forms of oligodactyly (missing digits) occur in approximately 30% [111].





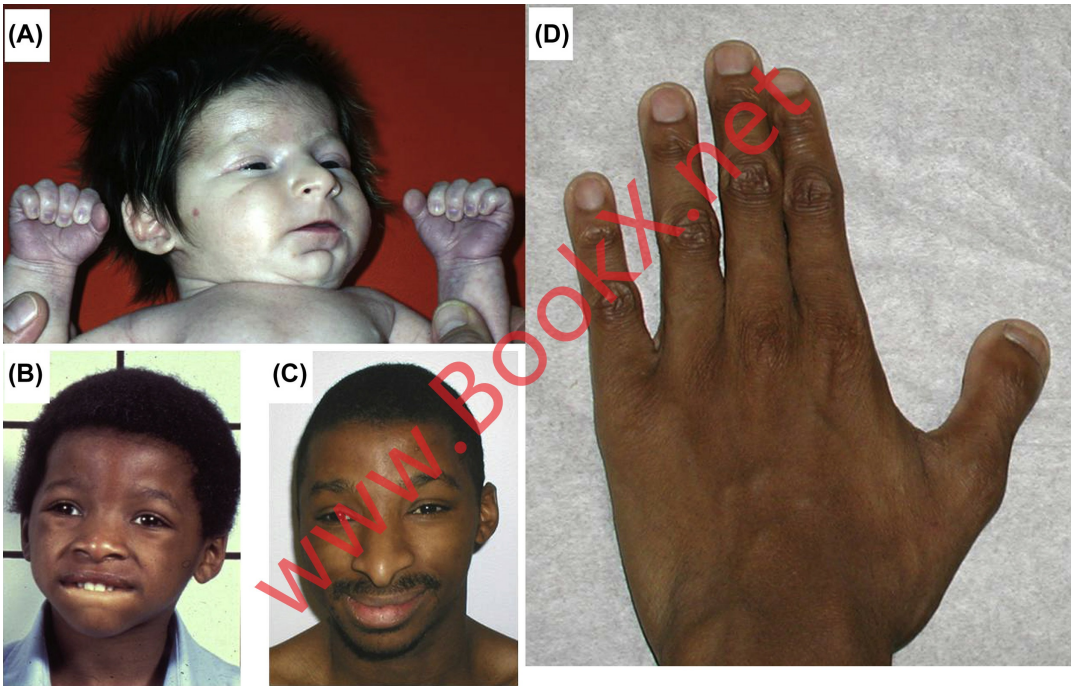
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**FIGURE 4.6** This 8-year-old girl with fetal alcohol syndrome has mild cognitive disability, ADHD, nproportionate short stature, microcephaly, short palpebral fissures, short nose, thin smooth upper lip, and camptodactyly with mild fingertip and nail hypoplasia.

In patients with a symptom complex suggestive of a specific chromosomal deletion syndrome (e.g., 18q syndrome Fig. 4.11 or 4p syndrome Fig. 4.12), it may be useful to begin the diagnostic evaluation with chromosomal microarray, particularly if previous chromosome studies were normal. Some chromosomal syndromes may be detectable only in skin fibroblast samples (e.g., diploid-triploid mixoploidy [128] or tetrasomy 12p [129]). It is also important to remember that some girls with Turner syndrome, especially those with mosaicism, express few phenotypic features prepubertally, except for short stature. Thus, chromosomal studies should be done on girls who appear to be short for their family background, with relatively normal body proportions, and for whom another diagnosis has not been made.

Several chromosomal deletion syndromes have also been found to be associated with GH insufficiency, indicating the locations of genes or chromosomal regions that result in short stature. Genetic defects in the lower end of the GH-IGF1 axis include the *GH1* gene on 17q22-q24, the GH receptor *GHR* gene on 5p13-12, the *IGF1* gene on 12q22q24.1, and the *IGF1R* receptor gene on 15q25q26 [130]. It is not surprising that mutations or deletions of any one of these genes will result in growth deficiency, often with an autosomal recessive basis. Donohue syndrome (leprechaunism) is due to mutations in the insulin receptor gene on 19p13.2, resulting in insulin resistance and IUGR, because insulin is a major fetal growth factor [131] (see sections in endocrinology chapters discussing Insulin, IGF2, and pituitary-hypothalamic axis). For some of the more common short stature and chromosome disorders, syndrome-specific growth curves are available through the Internet to aid in following these patients, and these curves are useful for detecting secondary hormonal problems that might be present and, likewise, treated. Growth deficiency in Turner syndrome has been attributed to haploinsufficiency of the *SHOX* gene at Xpter-p22.32 [132]. Altered imprinting on chromosome

7 or 11p15 has been identified in around half of patients with Russell-Silver syndrome [133].




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**FIGURE 4.7** The infant with Rubinstein-Taybi syndrome in the top left (A) shows the characteristic facial features, with broad and often angulated thumbs and great toes, short stature, and moderate to severe mental retardation. The characteristic craniofacial features include downslanting palpebral fissures with nasal columella extending below the nares. The individual on the bottom left (B) is shown at age

4 years and (C) age 30 years with similar features due to a

169.92 kb deletion on 16p13.3 which included *CREBBP*. (D) depicts the characteristic broad angulated thumbs and great toes.



**FIGURE 4.8** The child on the left with typical facial features for Williams syndrome and supravalvular aortic stenosis with peripheral

pulmonic stenosis has an atypical 1.26–1.31 MB deletion that includes *GTF2IRD1* but not *GTF2I*. She has typical visual-spatial construction defects without the usual increased eye contact and indiscriminant approach to strangers seen in children with the typical

1.55 MB deletion that includes *GTF2*I, illustrating the usefulness of chromosomal microarrays in understanding the clinical features of Williams syndrome. On the right (B) are shown children with the typical deletion at different ages.



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**FIGURE 4.9** These two children with Noonan syndrome show similar facial features (broad forehead, wide spaced down-slanted eyes, low-set posteriorly rotated ears) with proportionate short stature, wide set nipples, hypertrophic cardiomyopathy (left) and ASD with pulmonic stenosis (right). Both had feeding problems, with mildly delayed development later. Diagnostic criteria developed by van der Burgt in 1997 [116].


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**FIGURE 4.10** This child with Aarskog syndrome has proportionate short stature, ocular hypertelorism, anteverted nostrils, broad upper lip, with shawl scrotum, hyperextensible fingers, wide flat feet, ovoid periumbilical depression and ADHD.



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**FIGURE 4.11** This 12-year-old boy with Cockayne syndrome (top figures A and B) is the size of a 2 year old with typical features of Cockayne syndrome. These dizygotic twins with cerebro-oculo-facial-skeletal (COFS) syndrome (C) were from the Manitoba aboriginal family that was first reported with this syndrome. They were found to have a mutation in *CSB*, confirming that COFS syndrome and Cockayne syndrome are allelic
From Meira LB, Graham JM, Jr, et al. Am J Hum Genet 2000;66:1221–8.

#### 4.4.1.3. Defects of DNA Repair

Human physiology has evolved with highly conservative and effective mechanisms for DNA repair including base excision repair, nucleotide excision repair, double-strand break repair, mismatch repair, and DNA recombination (see chapter on DNA mismatch repair). Defects in DNA repair genes can present clinically with growth retardation, premature aging, skin abnormalities including abnormal pigmentation, telangiectasia, xerosis cutis, and pathological wound healing with a predisposition for certain types of cancers. RecQ proteins (DNA helicase) are essential to maintaining genomic stability and defects thereof have been associated with Bloom (*RECQL3*) and Werner (*RECQL2*) syndromes. DNA helicase *RECQL4* interacts in an array of intracellular regulatory pathways from the initiation of DNA replication, through maintaining genomic stability (DNA repair) [52], and mutations therein are associated with a spectrum of allelic disorders (Rothmund-Thomson syndrome, RAPADILINO syndrome, and Baller-Gerold syndrome) with skeletal developmental abnormalities including short stature with low bone mass, radial ray, and limb and craniofacial defects [134–136]. *RECQL4* has been shown to play a key role in DNA end resection, which is the initial and an essential step of homologous recombination (HR)-dependent DNA double-strand break repair (DSBR) [52,137]. Murine models demonstrate that *RECQL4* is critical for skeletal development by modulating p53 activity in vivo [138]. RTS patients have a highly increased incidence of osteosarcoma (OS). In a murine model by Ng et al., *RECQL4* deletion in vivo at the osteoblastic progenitor stage of differentiation resulted in mice with shorter bones and reduced bone volume, associated with an osteoblast intrinsic decrease in mineral apposition rate and bone formation rate in the *RECQL4*-deficient cohorts [50]. Deletion of *RECQL4* in mature osteoblasts/osteocytes in vivo, however, did not cause a detectable phenotype. Acute deletion of *RECQL4* in primary osteoblasts or shRNA knockdown in an osteoblastic cell line caused failed proliferation, accompanied by cell cycle arrest, induction of apoptosis, and impaired differentiation

[138]. Ng et al. [138] suggested that tumor suppression and osteosarcoma susceptibility are most likely a function of mutant, not null, alleles of *RECQL4* (Table 4.4).


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**FIGURE 4.12** This patient with 18q-syndrome has typical midface deficiency, conductive hearing loss, deep-set eyes, prominent antitragus and long palms with proximally-placed thumbs and long tapered fingers.

Fanconi pancytopenia, caused by mutations in a number of different genes (Table 4.2), is characterized by bone marrow failure (aplastic anemia), developmental delay, and increased risk for malignancy [53]. Around 75% of patients present with a variable phenotype with short stature, skeletal malformations of the upper and lower limbs, microcephaly, ophthalmic and genitourinary tract anomalies, and abnormal skin pigmentation [53].

### 4.4.1.4. Cohesionopathies

Stable transmission of genetic information from one generation of cells to the next is dependent on effective and orderly execution of DNA replication and chromosome segregation. Disruption of chromosome segregation can result in chromosome dosage abnormalities (i.e., aneuploidy/multiploidy). Cohesion mediates sister chromatid

cohesion during cell division and plays a role in DNA repair, chromatin organization, and transcription regulation [54]. The ring-shaped cohesin complex topologically entraps chromosomes [54,146]. Cohesin's core is composed of the structural maintenance of chromosome (SMC) proteins (Smc1–Smc3) ATPase [147], kleisin subunit sister chromatid cohesion 1 (Scc1) that links the two ATPase heads, and the Scc1-bound adaptor protein Scc3, while sister chromatid cohesion 2 and 4 (Scc2–Scc4) complex loads cohesin onto chromosomes [54,147].

SMC proteins are built from a globular hinge domain, a rod-shaped domain composed of long anti-parallel coiled-coil (CC), and a second globular ATPase domain called the head (the opening of the ring). DNA binding-mediated ATPase activity in Smc heads is regulated by the acetylation of several Lys residues located both in the Smc head and in the coiled coils. The function of CC is to transfer ATP binding/hydrolysis signals between the head and the hinge domains [148]. Marcos-Alcalde et al. used molecular dynamics to study cohesin ring opening and proposed that it is triggered by a sequential activation of ATP hydrolysis at the Smc1A site, which appeared to induce ATPase activity at the Smc3 site, opening of the head domains after the two ATP hydrolysis events [147].

The ring is opened either by separase, which cleaves Scc1 during anaphase, or by a releasing activity involving Wapl, Scc3, and Pds5, which bind to Scc1 and open its interface with Smc3 [149]. Binding to a kleisin subunit creates a closed tripartite ring, whose SMC arms act as barrier for DNA entrapment [149]. The interaction of cohesin with DNA is controlled by a number of additional regulatory proteins. The cohesin regulator Wapl promotes the release of cohesin from chromosomes during both interphase and mitosis [150]. Mutations in cohesin, or its regulators, cause a spectrum of human developmental syndromes known as the "cohesinopathies" including CdLS, Roberts syndrome [55], Warsaw breakage syndrome, CAID syndrome, CHOPS syndrome, prostate and colorectal cancer, and aneuploidy in neurons linked to Alzheimer disease) [55–57].

CdLS is characterized by facial dysmorphism (Fig. 4.5), growth

failure, intellectual (cognitive) disability (IQ ranges from below 30 to 102) with many individuals demonstrating autistic and self-destructive tendencies [111], limb defects (particularly upper extremity) malformations, and multiple organ involvement (cardiac septal defects, GI disturbances including reflux or malrotation, thrombocytopenia) [151]. CdLS is due to mutations in the genes for the structural and regulatory proteins that make up the cohesin complex and is considered a cohesinopathy disorder or, more recently, a transcriptomopathy [57]. Five genes, encoding subunits of the cohesin complex (*SMC1A, SMC3, RAD21*) and its regulators (*NIPBL, HDAC8*), account for at least 70% of patients with CdLS or CdLS-like phenotypes [57]. De novo *SMC3* mutations produce a dominant-negative effect with many SMC3-associated phenotypes characterized by postnatal microcephaly but with a less distinctive craniofacial appearance, a milder prenatal growth retardation that worsens in childhood, few congenital heart defects, and an absence of limb deficiencies [56,57]. Deardorff et al. [111] discuss management considerations including aggressive treatment of gastroesophageal reflux (sometimes necessitating fundoplication); physical, occupational, and speech therapy to optimize psychomotor development and communication skills; standard treatment for hearing loss, cardiac defects, seizures, vesicoureteral reflux, and cryptorchidism; and surveillance for monitoring heart and kidney abnormalities and preoperative evaluation for thrombocytopenia/cardiac disease with precautionary measures for malignant hyperthermia.

## Table 4.4

### Postnatal Growth Deficiency Syndromes

| Disorder (OMIM#) | Key Clinical Findings | Inheritance | Gene (Chromoson Loci), Gene Product |
|---|---|---|---|
| **Rubinstein-** | Normal prenatal growth | Sporadic/de novo | • RSTS1: |

| | | | |
|---|---|---|---|
| **Taybi syndrome (180849)** | with slowing of growth (height, weight and head circumference) in first few months of life, broad thumbs and halluces, down-slanted palpebral fissures, "beaked nose" with prominent nasal septum, high arched palate, grimacing smile and talon cusps, moderate to severe mental retardation (IQ from 25 to 79), childhood/adolescent obesity. Variable features: cataracts, colobomas, congenital renal or cardiac defects, cryptorchidism [10] | mutations, autosomal dominant | *CREBBI* (16p13.3 50%–70 • RSTS2: gene (22 |
| **Kabuki syndrome (147920, 300867)** | Postnatal growth deficiency, characteristic facies: long palpebral fissures with eversion of lateral third of lower eyelids, broad/depressed nasal tip, cleft or | • KS1: autosomal dominant<br>• KS2: X-linked dominant | • Kabuki *KMT2D* (12q13.1 • Kabuki *KDM6A* (Xp11.3 |

| | | | |
|---|---|---|---|
| | high arched palate, scoliosis, short fifth finger, radiographic anomalies, recurrent otitis media | | |
| Persistent Postnatal Growth Deficiency | | | |
| RASopathies/Noonan Spectrum Disorders (Fig. 4.14) | | | |
| **Noonan syndrome** | Broad forehead, hypertelorism, downward slanting palpebral fissures, high arched palate, webbed neck, skeletal chest/spine deformities: pectus excavatum, cardiac involvement in ~90% (most common: pulmonary stenosis, hypertrophic cardiomyopathy) motor delay | • Autosomal dominant, genetic heterogeneity<br>• NS2, autosomal recessive | • NS1: *PT* (12q24.1<br>• NS3: *KI* (12p12.1<br>• NS4: *SO* (2p22.1)<br>• NS5: *RA* (3p25.2)<br>• NS6: *NI* (1p13.2)<br>• NS7: *BI* (7q34)<br>• NS8: *RI* (1q22)<br>• NS9: *SO* (14q21.3<br>• NS10: *I* (22q11.2<br>• NS2: *SI* (10q25.2 |
| **Noonan syndrome with multiple lentigines (NSML; previously** | Short stature LEOPARD: lentigines, ECG anomalies, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, | Autosomal dominant, sporadic | • LPRD1 *PTPN11* (12q24.1<br>• LPRD-2 *RAF1* (3 (overlap NS5) |

| | | | |
|---|---|---|---|
| **known as LEOPARD) (151100, 611544, 613707)** | retardation of growth, sensorineural deafness<br>Mutations in CR2 domain of RAF1 have hypertrophic cardiomyopathy but patients with mutations in CR3 domain do not [139,140,141] | | • LPRD-3<br>*BRAF* (7 |

Table Con

| Disorder (OMIM#) | Key Clinical Findings | Inheritance |
|---|---|---|
| **Costello syndrome (218040)** | Short stature (congenital overgrowth/normal growth with postnatal growth failure) (due to constellation of features), distinct facial features: thick lips, macroglossia, and hand posture, redundant folds of skin or hyperkeratosis at neck as well as palms/soles (deep creases), fingers, papillomata/facial warts around mouth/nares (typical features may not be seen in preterm infants with decreased subcutaneous adipose tissue); also postnatal relative macrocephaly/posterior fossa | Autosomal dominant |

| | | |
|---|---|---|
| | crowding due to disproportionate brain growth [142]<br>Can present with distal arthrogryphosis/fetal akinesia due to congenital myopathy with neuromuscular spindle excess (p.Gly12Val) [143]<br>Can have mild to moderate developmental delay with pleasant disposition, pubertal delay, cardiac anomalies (extrasystoles/thick mitral valve, cardiac hypertrophy, rhythm anomaly), benign tumors or malignancies (bladder/rhabdomyosarcomas), benign breast disease/fibrosis | |
| **Cardio-facio-cutaneous (CFC) syndrome (115100, 615278-80)** | Postnatal growth deficiencies, hypotonia, cardiac anomalies (pulmonic stenosis, hypertrophic cardiomyopathy), ectodermal (hair and skin) anomalies: curly hair, hyperkeratosis, progressive nevi with age, characteristic facial features: developmental delay<br>*MAP2K*/*MEK* mutations linked to macrostomia, horizontal palpebral fissures (MEK mutations noted to more active in stimulating ERK phosphorylation) [144] | |
| **Neurofibromatosis-** | Short stature, facial features: | Autosomal |

| | | |
|---|---|---|
| **Noonan syndrome (601321)** | hypertelorism, epicanthal folds, downslanting palpebral features, low set ears, cafe au lait spots [145] | dominant |
| **Hutchinson-Gilford progeria (176670)** | Children typically look normal at birth and in early infancy, but then fail to gain weight, prominent eyes, a thin nose with a beaked tip, thin lips, small chin, protruding ears | Sporadic |

## 4.4.2. Proportionate Short Stature of Postnatal Onset

Persons with postnatal-onset proportionate short stature may have some kind of postnatal environmental insult, such as a chronic disease or an endocrine disorder, all of which may be associated with decreased IGF or peripheral unresponsiveness to its action (see discussion on Growth Hormone in Endocrine chapters on pituitary-hypothalamic axis). There may be direct suppression of hepatic IGF synthesis, deficient GH secretion with secondarily reduced IGF production, inability to respond to GH stimulation of IGF, circulating IGF inhibitors, or peripheral unresponsiveness to the actions of IGF.

These children usually have a bone age commensurate with, or even more severely retarded than, their height age, depending on the state of their gonadal and thyroid systems. An accurate diagnosis is especially important for persons with this group of disorders because many of them will respond to specific therapeutic measures. Key clinical findings of postnatal growth deficiencies are summarized in Table 4.5.

#### 4.4.2.1. Intrinsic Systemic Mechanisms of Proportionate Postnatal Short Stature: Biopsychosocial Features

A number of developmental anomalies or genetic syndromes affecting the hypothalamus and pituitary may result in GH deficiency with or without other tropic hormone deficiencies. The many genetic forms of GH deficiency or resistance are discussed in chapter on pituitary-hypothalamic axis. Dias et al. [152] suggested that decreased expression in the GHR on target cells may be an underlying cause in some cases of idiopathic short stature. Several genetic syndromes without known developmental malformations of the hypothalamus or pituitary have pituitary insufficiency as a common component (see chapter on Genetic disorders of the pituitary gland). In disorders such as histiocytosis X [153] and hemochromatosis, the hormonal deficiencies are due to a degenerative disease of the hypothalamus and pituitary. In other syndromes, however, the pathogenesis of the pituitary insufficiency is unknown. Both sickle cell anemia and thalassemia are associated with delayed growth and sexual development that result in adult short stature [154]. Interestingly, the *HFE* mutation linked to hereditary hemochromatosis p.Cys282Tyr has also been connected to increased height [155].

Underlying molecular defects causing GH deficiency (which are responsible for only a small fraction of GH-IGF axis disorders) that include *GH1* (which codes for GH), *GHRHR*, GH insensitivity or primary IGF-1 deficiency (*GHR, IGFI, IGFALS, STAT5B*), and IGF insensitivity (*IGFIR*) account for a minority of children with short stature. Savage et al. [29] reviewed the GH-IGF axis and specific defects causing short stature. Baron et al. [34] comment on this

paradigm shift from thinking of growth as dominated by the GH-IGF axis to the realization that this regulatory system is just one of many, shifting the overall understanding of linear growth in terms focusing on the cartilaginous growth plate in long bones.

Inflammation, associated with most infections and environmental enteric dysfunction, as well as endogenous inflammation associated with excess adiposity, inhibits endochondral ossification through the action of mediators including proinflammatory cytokines, the activin A-follistatin system, glucocorticoids, and FGF21. Chronic malnutrition retards growth and is associated with reduced synthesis of IGF. Chronic immune activation diverts nutrient resources toward fighting infection rather than growth, suppresses the GH-IGF axis, inhibits bone growth, and causes further damage to the intestinal mucosa, thereby exacerbating growth failure [166].

Many chronic childhood diseases are associated with growth failure. Chronic liver disease, renal disease, celiac disease, regional enteritis, infectious disease, diabetes mellitus, hemoglobinopathies, asthma, congenital heart disease, and many others fit into this category. Often, reduced growth may be the dominant clinical feature. Low IGF levels are usually found, and, in addition, many patients are in a state of negative nitrogen balance. After cure or control of the basic disease, there is usually significant catch-up growth, and some children may ultimately reach a near-normal final adult stature, depending on the duration and severity of the disease, the age at the onset of the disorder, and its therapy. For example, children with celiac disease and persistent short stature may reach normal stature when treated by GH. Teran et al. [167] review GH deficiency and treatment of patients with concurrent chronic disease, nutritional deficit, and SHOX deficiency.

## Table 4.5

### Prenatal-Onset Overgrowth Syndromes

| Proportionate Syndromes | |
|---|---|
| | Gene (Ch |

| Syndrome | Key Clinical Findings | Inheritance | Gene Proc |
|---|---|---|---|
| **Beckwith-Wiedemann (130650)** | Macroglossia, infraorbital creases, earlobe creases and pits, abdominal wall defects, neonatal hypoglycemia, visceromegaly, risk for abdominal neoplasms, hemihypertrophy, polyhydramnios, large placenta [106] | Sporadic, autosomal dominant due to mutation/deletion of imprinted genes | • *p57/K* (11p1 • *H19* ( Hyper varia • *H19/ contr* (11p1 impri of H1 [hypo same Russe syndr |
| **Sotos (117550, 614753)** | Macrocephaly, dolichocephaly, down-slanted palpebral fissures, hypertelorism, prognathism, high narrow palate, premature eruption of teeth, large hands and feet, kyphoscoliosis, mental deficiency [90] | Sporadic, autosomal dominant | • SOT( (5q35 • SOT( syndr haplo (19p1 • SOT( (19p1 |
| **Marshall-Smith (602535)** | Accelerated linear growth and skeletal maturation, postnatal failure to thrive, hypotonia, developmental | Autosomal dominant, sporadic | *NFIX* ( Activa |

| | | | |
|---|---|---|---|
| | delay, structural brain anomalies, respiratory tract anomalies, recurrent pneumonia, pulmonary hypertension, dolichocephaly, coarse eyebrows, shallow orbits, blue sclerae, upturned nose, low nasal bridge, small mandibular ramus, hypertrichosis, umbilical hernia, choanal atresia, omphalocele [135,136,146] | | |
| **Weaver (277590)** | Tall stature, developmental delay, hypertonia, hoarse voice, macrocephaly, round face, ocular hypertelorism, down-slanted palpebral fissures, long philtrum, large ears, micrognathia (retrognathia in children may resolve with age), camptodactyly, thin deep-set nails, | Sporadic, autosomal dominant | *Enhancer o*<br>(*Drosop*<br>*EZH2* (<br>(11q14.<br>(17q11. |

| | | | |
|---|---|---|---|
| | prominent fingertip pads [5]; neuromigration disorders for patients with EZH2-related overgrowth; also increased frequency of neuroblastoma | | |
| **Perlman (267000)** | Hypotonia, facial dysmorphic features: serration of upper alveolar ridge, deep-set eyes, prominent forehead, organomegaly: nephromegaly, nephroblastomatosis (precursor for Wilms), high (64%) incidence of Wilms tumor in patients surviving past infancy, high frequency of bilateral tumors(55%) frequent neurodevelopmental delay, high neonatal mortality | Autosomal recessive | • DIS 3 (*Sacc* *cerevi* Like 1 (2q37 • (DIS3 exon role i metal of cel divis pathv |
| | | | |

| **Proportionate Syndromes** |
|---|

| Syndrome | Key Clinical Findings | Inheritance |
|---|---|---|
| Selective Endocrine Causes | | |
| **Congenital nesidioblastosis/familial hyperinsulinism or persistent hyperinsulinemic hypoglycemia of infancy (PPHI) (256450, 601820, 602485, 609975, 609968, 606762, 610021)** | Prenatal macrosomnia/LGA, persistent hypoglycemia in infancy, nesidioblastosis (meaning neoformation of islets of Langerhans from pancreatic duct epithelium)<br>HHF2: focal islet cell adenomatous hyperplasia [161,162] | • Autosom dominar (1,3, 5, 6)<br>• Autosom recessive (2, 4) |
| **Cantu/hypertrichotic** | Congenital hypertrichosis, | Autosomal |

| | | |
|---|---|---|
| **osteochondrodysplasia syndrome; related disorders acromegaloid facial appearance (AFA) and hypertrichosis and acromegaloid facial features (HAFF) (239850)** [163] | macrosomia, distinctive facial features (broad nasal bridge, wide mouth with full lips and macroglossia); cardiac abnormalities (increased ventricular mass, enlarged chambers, and normal cardiac function; patent ductus arteriosus (50%), pericardial effusion (20%), increased vascular tortuosity or aortic aneurysm); skeletal abnormalities (thickening of the calvaria, broad ribs, scoliosis, and hypoplastic ischium and pubic bones, Erlenmeyer flask–like long bones with flaring of the metaphyses, narrow obturator foramen (may also have generalized osteopenia, delayed bone age, and craniosynostosis) | dominant, sporadic |
| PTEN Spectrum Syndromes | | |
| **Bannayan-Riley-Ruvalcaba (153480)** | Delayed gross motor development, | Autosomal dominant |

| | | |
|---|---|---|
| | hypotonia, speech delay, mental deficiency, macrocephaly, prominent Schwalbe rings, prominent corneal nerves, pseudopapilledema, mesodermal hamartomas, pigmented penile macules, lipid storage myopathy [119] | |

| **Proportionate Syndromes** | | | |
|---|---|---|---|
| **Syndrome** | **Key Clinical Findings** | **Inheritance** | **Gene (Ch Gene Pro** |
| **Cowden Syndrome-1 Lhermitte-Duclos (158350)** | Macrocephaly, trichilemmomas, and papillomatous papules<br>Hamartomatous polyps of the GI tract, benign and malignant tumors of the thyroid (usually follicular, rarely papillary, but never medullary thyroid cancer) is approximately | Autosomal dominant | • CWS (hete muta<br>• CWS<br>• CWS<br>• CWS<br>• CWS<br>• CWS<br>• CWS |

| | | | |
|---|---|---|---|
| | 35% lifetime risk), breast (85% lifetime risk), and endometrium (~28% lifetime risk) | | |
| ***PTEN*-related Proteus syndrome (PS)** | | | |
| **Simpson-Golabi-Behmel (300209) (312870)** | Macrocephaly, ocular hypertelorism, short broad nose, large mouth, macroglossia, variable mental retardation, hypotonia, postaxial polydactyly of hands, nail hypoplasia, partial cutaneous syndactyly, cryptorchidism, supernumerary nipples, cardiac defects, gastrointestinal defects, large cystic kidneys [135] | X-linked recessive | • SGB GPC<br>• SGB |
| **Elejalde (200995)** | Increased birth weight, globular body with thick skin, organomegaly, and fibrosis in multiple | Autosomal recessive | Acroceph dyspl |

| | | | |
|---|---|---|---|
| | tissues (including: pancreas, liver), apparently short limbs, postaxial polydactyly, craniosynostosis, cystic renal dysplasia, facial features: acrocephalic head, small and abnormally modeled ears, small and flat nose, big mouth, hypertelorism, epicanthal folds, short thick neck (Silhanova et al, ref below), pathology findings: perivascular proliferation of nerve fibers, excessive connective tissue [244] | | |
| **Nevo (601451) Ehlers-Danlos syndrome VI (kyphoscoliotic or ocular-scoliotic type)** | Intrauterine and postnatal overgrowth, large low-set malformed ears, cryptorchidism, accelerated osseous maturation, dolichocephaly, large extremities, | Autosomal recessive | *Procollage*<br>*oxoglu*<br>*PLOD*<br>or lysi<br>(LH1) |

| | | | |
|---|---|---|---|
| | clumsiness and retarded motor and speech development, generalized edema, hypotonia, contractures of the feet, wristdrop, clinodactyly [173], severe form with skeletal/dermal and ocular manifestations (lacking in hydroxylysine in skin collagen) | | |
| | | | |

| **Proportionate Syndromes** | | | |
|---|---|---|---|
| **Syndrome** | **Key Clinical Findings** | **Inheritance** | **Gene (L Loci), C** |
| **Tenorio syndrome (616260)** | Overgrowth, macrocephaly (mild hydrocephaly), intellectual disabilities/developmental delay, hypoglycemia, inflammatory disease (like Sjogren syndrome) [199] | Autosomal dominant | • R pr RN • [K pa th pa IP an sig |
| ***PP2A* deletion spectrum** | Increased height, increased head circumference/macrocephaly | Sporadic (de novo) | • Pr ph (P |

| | | | |
|---|---|---|---|
| | and intellectual disability/autistic spectrum; hypotonia | | he se ph (d ta if di PI gr sig [1 • P su (*P* (1 *PI* (g (1 *PI* [1 |
| **19q13.13 deletion (613638)** | Proportional overgrowth, macrocephaly, frontal bossing, down-slanting palpebral fissures with optic/ophthalmologic abnormalities like strabismus and/or optic nerve hypoplasia or atrophy, variable neurologic anomalies on brain MRI; abdominal pain, vomiting [134] | | |
| **Tatton-Brown-Rahman syndrome** | Tall stature (mean ± 3.0 SD), large head circumference | Autosomal dominant | *DNMT* cod de r |

| | | | |
|---|---|---|---|
| **(615879)** | (mean ± 2.5 SD), round face, heavy horizontal eyebrows, and narrow palpebral fissures. Mild to moderate intellectual disability | | met able unn hem DN |
| **Kosaki overgrowth syndrome (616592)** | Tall, elongated lower segment, hands and feet; facial features: prominent forehead, proptosis, down-slanting long palpebral fissures, wide nasal bridge, thin upper lip and pointed chin; hyperelastic and fragile skin, progressive neurologic deterioration (white matter lesions noted on brain MRI); scoliosis [165,245] | Autosomal dominant | *PDGFR* |
| **9q22.3 microdeletion** | Prenatal/postnatal developmental delay and/or intellectual disability, metopic craniosynostosis, obstructive hydrocephalus, prenatal and postnatal macrosomia, and seizures; increased risk of Wilms tumor | Autosomal dominant | (includ of gen mut syn *FAN* |

| **Proportionate Syndromes** |
|---|
| |

| Syndrome | Key Clinical Findings | Inheritance |
|---|---|---|
| **Distal 15q trisomy/tetrasomy** | Overgrowth and learning disabilities, long thin face, prominent nose and chin; renal anomalies: renal agenesis/horseshoe kidney and hydronephrosis | |
| Disproportionate Syndromes | | |
| **Proteus (176920)** | Regional overgrowth of hands and/or feet, asymmetry of limbs, plantar hyperplasia, hemangiomas, lipomas, lymphangiomas, varicosities, verrucous epidermal nevi, macrocephaly, cranial hyperostoses, long bone overgrowth, variable moderate mental deficiency [152,174]<br>Risk of DVT/PE | Sporadic |
| | | |

| | | |
|---|---|---|
| **Isolated lateralized overgrowth (previously isolated hemihypertrophy or hemihyperplasia)**<br>**Hemihyperplasia (including HHML and HIPO)** | Asymmetry present at birth with growth accompanying patient's growth. Can affect nearly any part of the body. Medullary sponge kidney is a frequent finding [5,175]; Wilms tumor is most frequent finding, followed by adrenal cortical carcinoma and hepatoblastoma [5] | Heterogenous causes |
| **Macrocephaly capillary malformation (polymicrogyria) syndrome (MCAP) (602501)** | Macrocephaly, prenatal overgrowth, thickened subcutaneous tissue; over half of patients have acquired cerebellar tonsillar herniation (vs. congenital Chiari I malformation) with dilated dural sinuses and ventriculomegaly secondary to rapid brain growth and progressive crowding of the posterior fossa [128–131] | |
| **CLOVES (congenital lipomatous asymmetric overgrowth of the trunk vascular malformations: lymphatic, capillary, venous,** | Progressive (sometimes complex) low- and high-flow vascular malformations, thoracic lipomatous hyperplasia, asymmetric growth, and visceral and neurological disorders | Somatic mosaicism or postzygotic activating mutations |

| | | |
|---|---|---|
| **and combined-type, epidermal nevi, skeletal and spinal anomalies: scoliosis)** | | |
| **Klippel-Trenaunay syndrome (149000)** | Cutaneous capillary malformations of a limb, venous varicosities/malformations, and accompanying segmental hypertrophy of soft tissue and/or bone [147] | Sporadic |

Thyroid hormone deficiency can result in either proportionate or disproportionate short stature, depending on the degree of secondary epiphyseal dysplasia. The prototype of endocrine short stature is GH deficiency, but growth can be unbalanced by several endocrine mechanisms. GH-related causes of short stature can result from many interruptions in the hypothalamic-pituitary-peripheral tissue axis. Cushing disease can lead to proportionate short stature similar to that seen with the exogenous administration of steroids. Excess androgen secretion, as in the adrenogenital syndrome, can lead to accelerated growth with advanced bone age in early childhood, but significant adult short stature resulting from premature closure of epiphyses (see chapter on Genetics of Adrenal disease). The various types of pituitary dwarfism can be classified on the basis of the level of the defect, whether genetic or acquired. The most common causes of acquired pituitary insufficiency are birth trauma, cranial irradiation for neoplasia, craniopharyngioma with the expanding tumor mass compromising pituitary function, and hemosiderosis following chronic transfusion therapy. In addition, trauma, surgical damage, infection, and sarcoidosis can lead to pituitary insufficiency.

Genetic syndromes and developmental anomalies associated with hypopituitarism are frequently associated with facial or optic

anomalies. In certain disorders, such as congenital absence of the pituitary, empty sella syndrome, transsphenoidal encephalocele, and two rare syndromes associated with an unusual or large sella turcica, lateral skull radiographs may be sufficient to suspect the intracranial anomaly. In other disorders, however, such as holoprosencephaly and septo-optic dysplasia, magnetic resonance imaging of the brain and hypothalamus may be necessary to detect the malformations. Therefore, any GH-deficient child with malformations of the face or eyes should have imaging of the brain and sella turcica to rule out a malformation affecting the hypothalamus or pituitary gland.

Psychosocial dwarfism, without a demonstrable organic cause, may be seen in children who suffer pronounced growth retardation secondary to emotional disturbances (affective deprivation [168]), insecure mother–child attachment due to any assortment of reasons for separation, marital discord, alcoholism, and social upheaval or personal traumatic experiences [169]. It must be noted that this phenomena can also be seen in well-to-do and seemingly well-adjusted families. Stress suppresses GH secretion [170], and these patients demonstrate a poor plasma GH response to GH stimulation tests, with low levels of circulating IGF. Bone age maturation is usually greatly delayed. When removed from their adverse environment, these children usually show a striking catch-up in their bone age, and GH and IGF levels return to normal.

#### 4.4.2.2. Extrinsic Mechanisms of Proportionate Postnatal Short Stature

In addition to various intrinsic causes of reduced human GH secretion, certain medications used in treating hyperactivity, such as methylphenidate hydrochloride, may alter the normal regulation of GH [171-173]. Children often show reduced growth rates during the first few years after treatment commences, but tolerance to the growth suppression may develop later. Similarly, glucocorticoids used in treating certain diseases, such as asthma, nephrotic syndrome, juvenile rheumatoid arthritis, and leukemia, may significantly retard growth. Steroids have anti-inflammatory effects involving ligand–

receptor interactions with other cellular signaling proteins (inhibition of NF-κB complexes) and transactivation effects mediated by ligand/glucocorticoid receptor (GR) translocation from the cytoplasm to the nucleus. Ligand/receptor dimers bind directly to glucocorticoid response elements (GREs) in the promoters of target genes (associated with deleterious side effects) [173]. The growth-suppressing effects of steroids may be seen with relatively small doses that are often thought to be harmless, such as prolonged use of topical ointments, nasal spray, or eye drops containing these compounds. The long-term effects of these and other drugs on final adult height have not been well established (Fig. 4.13).

#### 4.4.2.3. RASopathies

Dysregulation of the Ras/MAPK signaling pathway (Fig. 4.14) presents clinically as a set of disorders known as RASopathies and includes neurofibromatosis type I (discussed in chapter on Rasopathies), Legius syndrome, Noonan syndrome, Noonan syndrome with multiple lentigines (formerly called LEOPARD syndrome), Costello syndrome, cardiofaciocutaneous (CFC) syndrome, Noonan-like syndrome, hereditary gingival fibromatosis, and capillary malformation-arteriovenous malformation.




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**FIGURE 4.13** Two patients with 4p-syndrome (Wolf-Hirschhorn syndrome) with ocular hypertelorism, with cleft lip (A). Also present were hypospadius, preauricular pits, severe cognitive disability and intrauterine growth deficiency that persisted after birth.

Noonan syndrome is diagnosed on clinical features (Fig. 4.9) with associated short stature (normal birth length/weight), and congenital heart defects (the most common of which is pulmonic valve stenosis, often with dysplasia, ASD, or hypertrophic cardiomyopathy), variable developmental delay, broad or webbed neck, and widely set nipples with superior pectus carinatum with or without inferior pectus excavatum [116]. Patients may also have coagulation defects or lymphatic dysplasias of the lungs, intestines, or lower extremities. Affected individuals may have normal chromosome studies; 50% of patients have *PTPN11* pathogenic variant identified on molecular genetic testing, or *SOS1* (13%), *RAF1*/*RIT1* (each 5%) or *KRAS* (<5%) in <1% *NRAS, BRAF,* or *MAP2K1* might be affected. For patients, GH treatment increases growth velocity, and cardiovascular anomalies are treated as in the general population; surveillance includes monitoring of anomalies found in any system (particular cardiovascular) [116].

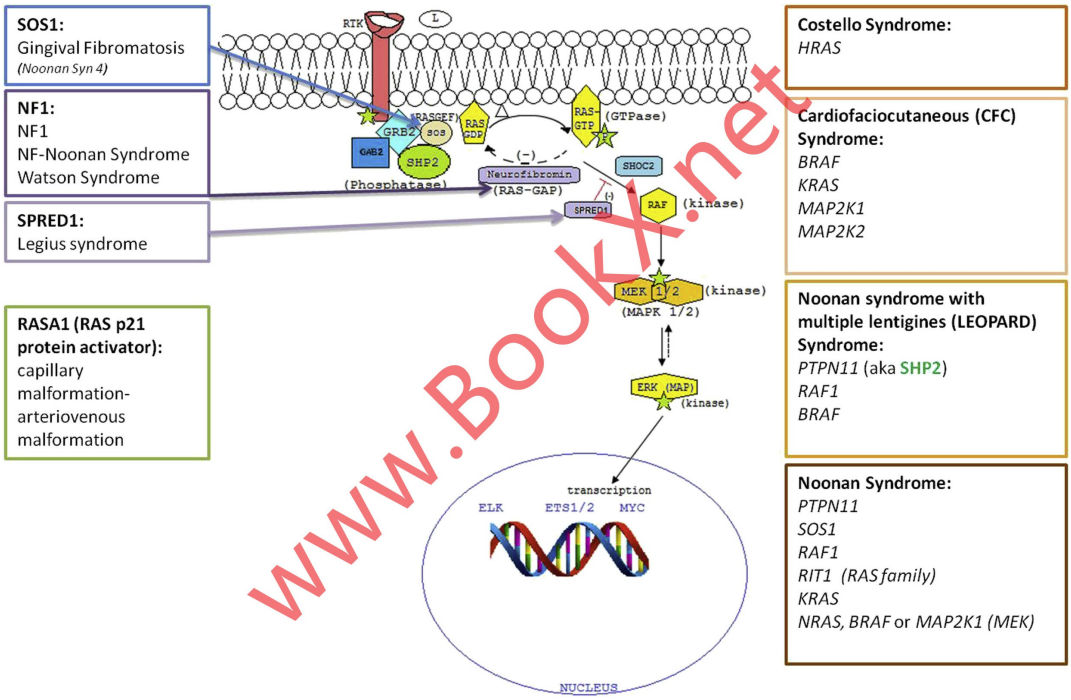
Clinical features of Noonan-spectrum disorders are further described in Table 4.3.

### 4.4.3. Disproportionate Short Stature

If a disproportionate body habitus is found on physical examination, the patient is likely to have a form of skeletal dysplasia. This is a heterogeneous group of heritable disorders affecting skeletal connective tissues in which the predominant clinical feature is dwarfism. The osteochondrodysplasias have been divided into 37 groups of disorders and three groups of dysostoses based on clinical and radiographic features [174]; each of these disorders is associated with a variety of skeletal and nonskeletal complications. These osteochondrodysplasias and dysostoses have also been classified using molecular, pathogenetic, and developmental approaches based on the structure and function of the causative genes and proteins; these classification systems are discussed in detail by Warman et al. [174]. Disproportionate short stature may require an entirely different diagnostic approach than what is used for proportionate short stature, and this is discussed in detail in chapter on skeletal dysplasias.

## 4.5. Pathologic Overgrowth

The array of pathologic conditions that can result in overgrowth is more restricted than the numerous conditions that have a negative impact on growth. Specific hormonal, molecular, and epigenetic pathways function at various times during development, impacting the rate of growth and tissues differentiation in either a generalized or a localized way. Evolving evidence demonstrates that catch-up growth is a local phenomenon at the growth plate related to senescence [175]. A disregulation of inhibition pathways of growth results in either localized or generalized effects on growth.




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**FIGURE 4.14** RAS/MAPK signaling cascade (Aka RAS-Raf-MEK-ERK pathway) is activated by a wide variety of receptors including receptor tyrosine kinases (RTKs), integrins, and ion channels. A set of adaptors (Shc, GRB2, PTPN11) link the receptor to a guanine nucleotide exchange factor (ex SOS) transducing the signal to small GTP-binding proteins (RAS, Rap1), which in turn activate the core unit of the cascade composed of a MAPKKK (Raf), a MAPKK (MEK1/2), and MAPK (Erk). An activated Erk dimer can regulate targets in the cytosol or translocate to the nucleus to phosphorylate a variety of transcription factors regulating gene expression. *Arrows* indicate

activating events, while *perpendicular lines with a minus sign* (−) indicate inhibitory events
Modified from Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta April 2010;1802(4):396–405; De Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N. The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: role in cancer pathogenesis and implications for therapeutic approaches. Expert Opin Ther Targets April 2012;16(Suppl. 2):S17–S27; Illustrated by Deepika Burkardt.

As with short stature, the pathologic determination of overgrowth should be made in relation to family and ethnic background. Once a careful assessment is made and pathologic overgrowth is apparent, anthropometric measurements help determine proportionality. Most prenatal overgrowth disorders persist after birth, and, in some cases, intellectual deficiency, risk for tumor development, or both are important associated features. The risk for tumor development usually involves embryonal neoplasms, perhaps related to overexpression of growth factors or lack of suppressing factors. A comprehensive review of overgrowth disorders has been presented [176]; a summary of key features of select overgrowth syndromes can be found in Table 4.5.

## 4.5.1. Generalized Overgrowth Disorders

Factors related to large fetal weight include genetic predispositions,maternal birthweight, pregravid BMI, excessive maternal weight gain, prior macrosomia and diabetes, postmaturity, and multiparity [177–180]. Interestingly, there has been noted to be a positive correlation between maternal birth weight (MBW) and offspring birth weight (OBW), suggesting intergenerational/vertical transmission of macrosomia from mother to child [180,181]. Although many macrosomic infants demonstrate proportionate overgrowth, infants of diabetic mothers are often disproportionately overgrown, with increased weight-to-length ratios. Infants born to diabetic and/or obese mothers also have an increased risk for certain malformations [182,183]. Some infants with anasarca are macrosomic primarily due to increased extracellular fluid volumes. True somatic overgrowth is

often accompanied by increased placental size, as is commonly seen in BWS [98].

At the cellular level, the onset of most prenatal overgrowth results from (1) excessive cellular proliferation (hyperplasia), (2) excessive cellular size (hypertrophy), (3) an increase in the interstitium (e.g., anasarca), or (4) a combination of these factors. Overgrowth conditions can be subdivided between normal variants, such as familial tall stature or familial rapid maturation, prenatal-onset growth excess, or postnatal-onset growth excess. However, primary growth excess results from intrinsic cellular hyperplasia, while secondary growth excess is due to humorally mediated factors outside the skeletal system. Among pathologic overgrowth disorders, most prenatal-onset growth excess is of the primary type, as is seen with BWS or Sotos syndrome. Secondary growth excess of prenatal onset is usually not syndromic. The best examples include diabetic macrosomia and congenital nesidioblastosis [184]. Postnatal-onset growth excess is usually secondary, involving overproduction of estrogens or androgens (precocious puberty), overproduction of GH (acromegaly), or overproduction of thyroid hormone. A few primary excess growth disorders manifest the majority of their overgrowth in the postnatal period (e.g., XYY syndrome, XXY syndrome, fragile X syndrome, and Marfan syndrome).

## 4.5.1.1. Prenatal-Onset Overgrowth

### 4.5.1.1.1. Beckwith-Wiedermann Spectrum

BWS is the most common congenital overgrowth syndrome, with a frequency of one case per 13,700 births [185]. Cardinal features (according to 2018 consensus guidelines) include macroglossia, exomphalos, lateralized overgrowth, mutifocal or bilateral Wilm's tumor or nephroblastomatosis, Hyperinsulinism lasting >1 week and pathology findings of adrenal cortex cytomegaly, placental mesenchymal dysplasia or pancreatic adenomatosis, while suggestive features include prenatal onset overgrowth (birthweight over 2 SDs above the mean), facial nevus simplex, polyhydramnios or

placentomegaly, ear creases or pits, transient hypoglycemia (<1 week), nephromegaly or hepatomegaly, umbilical hernia or diastasis recti, or BWS suggestive tumors including neuroblastoma, rhabdomyosarcoma, hepatoblastoma, unilateral Wilms tumor, adrenocortical carcinoma or pheochromocytoma [185] (Fig. 4.3). Large size at birth is associated with placental overgrowth, long umbilical cord, and polyhydramnios, which may result in premature delivery, contributing to an increased perinatal mortality [185]. The large size for age continues through early childhood and is associated with accelerated osseous maturation, but overgrowth is usually no longer evident by adulthood.

Because of the risk of tumor development in BWS Brioude et al. [185] provide evidence-based screening and management guidelines and note that AFP and Catecholamine screening is no longer recommended for patients with BWSp.

Sporadic cases account for 85% of cases. Familial cases (15% of cases) are inherited in an autosomal dominant fashion with incomplete penetrance, and most are born to female carriers. Approximately 80% of patients with BWSp have a defect in imprinted genes on the short arm of Chromosome 11 (11p15). The most common mechanism leading to BWS is a (complete or partial) loss of methylation at imprinting center 2 (IC2 LOM) on chromosome 11p15 (up to 50% of patients). Yu et al. [186] found an significant linear relationship between degree of methylation and clinical score calculated based on consensus guidelines by Brioude et al. Other mechanisms include gain of methylation at maternal IC1 (5–10% patients), mosaic, semental paternal uniparental isodisomy (upd (11) pat at 11p15.5, which accounts for 20% patients with BWSp) or an intragenic mutation of CDKN1C (5%). About half of the sporadic cases show biallelic expression of *IGF2*. Lateralized overgrowth is the most frequent feature in patients with segmental paternal UPD and are rare in patients with *CDKN1C* mutation (Fig. 4.14D) rarely occurs in familial cases, and lateralized overgrowth and neoplasms seem to occur more frequently in sporadic cases. Paternal trisomy or uniparental disomy for 11p15 could lead to an excess of expressed

paternal alleles, while relaxation of the maternal imprint for 11p15 might give rise to biallelic expression of *IGF2* and/or suppression of *H19*. Overexpression of *IGF2* appears to be the most common pathogenetic mechanism for the prenatal overgrowth.

#### 4.5.1.1.2. Simpson-Golabi-Behmel Syndrome (SGBS)

This distinctive X-linked syndrome with partial expression in female carriers was initially confused with BWS in earlier reports due to striking prenatal-onset overgrowth persisting into adulthood into adulthood. This disorder results from point mutations or rearrangements in the gene encoding cell surface heparan sulfate proteoglycan glipican gene *GPL3*, at Xq26. Glypican-3 appears to play a role in controlling the cellular growth and cell division of mesodermal tissues [187] (independent of IGF2). Murine models demonstrate that *GPC3*-null mouse embryos display normal levels of IGF-2 [188], but it was found that hedgehog signaling activity is increased in these *GPC3*-null mice [188,189].

Features of this syndrome, which can aid in prenatal diagnosis, include an increased alpha-fetoprotein level and increased nuchal translucency accompanied by body wall edema; at 30 weeks, macroglossia, polyhydramnios, nephromegaly, and hepatosplenomegaly occur, in addition to macrosomia, which may also aid with prenatal diagnosis [190]. Distinctive features include congenital macrocephaly, variable intelligence from normal to severe mental deficiency (language delay most characteristic), ocular hypertelorism with short broad nose, cleft palate, wide mouth that gives the face a square appearance, central groove of the lower lip, supernumerary nipples, pectus excavatum, rib and vertebral abnormalities, hypotonia, postaxial polydactyly of the hands, nail hypoplasia (especially involving the index fingers) [191], partial cutaneous syndactyly, cryptorchidism, heart defects (25% patients have structural defects, and 12% have arrhythmias noted on ECG), and visceromegaly (large cystic kidneys/nephromegaly, splenomegaly or hepatomegaly) [188,190]. Features in common with BWS include

prenatal overgrowth, macroglossia, earlobe creases and pits, umbilical hernia, intestinal malrotation, and hypoglycemia from excessive islets of Langerhans. Infant mortality is approximately 50%, occurring primarily from cardiorespiratory causes. The reported frequency of tumors is 10%, particularly embryonic tumors, including Wilms tumor, hepatoblastoma, adrenal neuroblastoma, gonadoblastoma [188,190], and hepatocellular carcinoma [192].

#### 4.5.1.1.3. Overgrowth syndromes linked to epigenetic mechanisms

Whole exome sequencing has been fruitful in the recognition of numerous epigenetic genes linked to overgrowth syndromes, including *NSD1, EZH2, DNMT3A, EED, HIST1H1E,* and *CHD8* [104]. *NSD1* and *EZH2* are SET-domain containing histone methyltransferases and regulate transcription through histone modification and chromatin modeling [193]. The *EZH2*-related overgrowth ranges from normal variant tall stature to Weaver syndrome. The PCR2 complex comprises 4 subunits: EZH1/2, Suz12, EED and RbAp46/48 and has 3 cofactors: AEBP2, Pcls and Jarid2. Germline mutations in the SUZ12, EZH2 and EED genes have been reported in Weaver syndrome (WS) or Weaver-like syndrome [194]. The *HIST1H1E* gene encodes histone H1.4 (a marker histone that helps mediate the formation of higher-order chromatin structure, regulating the accessiblity of regulatory proteins, chromatin remodeling factors, and histone-modifying enzymes) [176]. Chromodomain-helicase-DNA-binding 8 (*CHD8*) gene encodes duplin, a nuclear protein and ATP-dependent chromatin remodeling enzyme. While *CHD6* and *CHD7* both bind with high affinity to short linker DNA, *CHD8* binds longer DNA and slides nucleosomes into positions with more flanking linker DNA than *CHD7* [195].

##### 4.5.1.1.3.1. Cerebral gigantism (Sotos syndrome).

Is associated with prenatal-onset overgrowth and affects length more than weight. Rapid linear growth is more pronounced in the first year of life and is accompanied by variable advanced osseous maturation

and large hands and feet [196]. The head is usually large and dolichocephalic with frontoparietal balding. The facial features include a round face with a disproportionately prominent forehead seen in infancy elongates with age to a slim face (bitemporal narrowing) with a pointed chin seen in adolescence [196], flushed cheeks, and premature eruption of teeth [196] (Fig. 4.15). Neonatal complications include jaundice secondary to hyperbilirubinemia or hypoglycemia, often with hypotonia; difficulties with feedings secondary to trouble sucking or swallowing; and gastroesophageal reflux. Neurological features of Sotos syndrome include delayed developmental affecting both motor and speech functions, with associated clumsiness, and EEG abnormalities. There is a wide spectrum of psychosocial and behavioral problems with risks for learning difficulties, attention deficit disorder, hyperactivity disorder, and temper tantrums [196]. Although difficult to predict, final height tends to normalize after puberty with the most prominent feature seen in adults being macrocephaly [196]. Most cases are sporadic occurrences in otherwise normal families, with associated increased paternal age and fresh dominant mutations or whole-gene deletion of *NSD1* on 5q35. Roughly 93% of cases of Sotos syndrome result from a mutation of *NSD1* [196]. Recently mutations of *SETD2* and *DNMT3A* genes have occurred with some Sotos syndrome features [197]. *NSD1* and *SETD2* genes encode epigenetic "writer" proteins that catalyze methylation of histone 3 lysine 36 (H3K36me), and the *DNMT3A* gene encodes an epigenetic "reader" protein of the H3K36me chromatin mark [197]. Tlemsani et al. proposed that some Sotos-like cases result from disruption of apposition and reading of the epigenetic chromatin mark H3K36me [197]. Autosomal dominant inheritance has been demonstrated in some cases, with the affected parent showing mild mental retardation, long face, and large hands and feet but without tall stature [193]. Based on a review of the literature, the tumor frequency of patients with Sotos syndrome is <5% (ovarian fibromatosis, NBL, acute lymphoblastic leukemia, acute myelogenous leukemia, hepatoblastoma, sacrococcygeal teratomas, ganglioneuroma, small cell lung cancer, ganglioglioma, gastric

carcinoma, and testicular cancer), but there are no current recommendations for increased cancer surveillance [198].




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**FIGURE 4.15** Both these boys have Sotos syndrome with similar

facial features, but the boy on the left (A) has a NSD1 mutation with normal cognitive skills and the boy on the right (B) is deleted for NSD1 with cognitive disability, a tethered cord and sacral malformations. (C) This 3-year-old girl with Sotos syndrome demonstrates typical facial features (macrocephaly, dolichocephaly, frontoparietal balding, down-slanted palpebral fissures, flushed cheeks, prominently pointed chin, with premature eruption of teeth and accelerated osseous maturation.

## 4.5.1.1.3.2. *NFIX*-related Syndromes

### Malan Syndrome

Malan syndrome or Sotos syndrome 2 (OMIM 614753) is a clinical subset of patients with a "Sotos-like" syndrome caused by a mutation in the *NFIX* gene at 19q13.13 [199,200]. The *NFIX* gene encodes for NFI, X-type, a CCAAT-binding transcription factor. Marshall-Smith mutations are scattered through exons 6-10 of *NFIX* gene, while most point mutations causing Malan syndrome are clustered in exon 2 [201].

### Marshall-Smith Syndrome

Marshall-Smith syndrome is an overgrowth syndrome characterized by accelerated osseous maturation; failure to thrive with limited life expectancy, usually secondary to respiratory dysfunction; hypotonia, moderate to severe developmental delay, and craniofacial anomalies including a prominent forehead, shallow orbits resulting in prominent eyes, blue sclera, depressed nasal bridge, and micrognathia [202,203]. Diab et al. [204] underscored osseous fragility as an important aspect of this syndrome, and recent studies emphasize multiple nontraumatic fractures as significant clinical complication.

## 4.5.1.1.3.3. Weaver Spectrum and *PRC2* related Overgrowth Syndromes.

In Weaver syndrome, overgrowth is usually evident at birth, with most affected individuals at or above the 97th percentile for length and weight by age 1 year [5]. Overgrowth continues throughout life,

with adult height and weight above the 97th percentile, and markedly accelerated osseous maturation during childhood. Most affected individuals are mentally deficient. Their distinctive features include hypertonia, "doughy skin," hoarse low-pitched cry, macrocephaly, round face, ocular hypertelorism, down-slanting palpebral fissures/"almond-shaped eyes," long philtrum, large/fleshy ears, micrognathia, camptodactyly, clinodactyly, broad thumbs, prominent fingertip pads, and limited elbow and knee extension (Fig. 4.16**)** [193]. Younger children with Weaver syndrome have been known to have difficult intubations secondary to the relative micrognathia, anterior and cephalad positioning of the larynx and a relatively short neck; this difficulty seems to decrease with the growth of the mandible over time [193]. Somatic *EZH2* mutations, both activating and inactivating, have been identified in hematologic malignancies (including AML) and in solid tumors [198]. Increased clinical vigilance and work-up of potential cancer symptoms are prudent but as of yet, there are no recommendations for tumor surveillance.

#### 4.5.1.1.3.4. DNMT3A-related Overgrowth (Tatton Brown Rahman Syndrome TBRS OMIM#615879).

*DNMT3A* encodes a DNA methyltransferase essential for establishing methylation during embryogenesis (it is also commonly somatically mutated in acute myeloid leukemia) [205]. *DNMT3A* encodes a de novo methyltransferase, which can methylate unmethylated and hemimethylated DNA with equal efficiencies [206]. The DNMT3A-related Tatton Brown Rahman Syndrome [251] (OMIM 615879) is an overgrowth syndrome of tall stature, macrocephaly, facial dysmorphism, intellectual disability, round face, heavy horizontal eyebrows, and narrow palpebral fissures. Intellectual disability is moderate less common, and variable features include atrial septal defects, seizures, umbilical hernia, and scoliosis.

### 4.5.1.1.4. PP2A related overgrowth

Eukaryotic phosphatases can be divided into three superfamilies: the serine/threonine phosphatases (PSPs), the tyrosine phosphatases

(PTPs), and the dual specificity phosphatases (DSPs). There are two major Ser/Thr protein phosphatase families in the cell: protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A). PP2A forms about 100 heterotrimeric holoenzymes, and protein phosphatase 1 (PP1) forms about 400 heterodimeric holoenzymes. PP2A is a trimeric complex consisting of a catalytic subunit (C), a substrate binding regulatory subunit (B), and a scaffolding subunit (A) that links B and C [207,208]. PP2Ac is also found associated with $\alpha$4 protein and TOR signaling pathway regulator-like (TIPRL) [209].


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**FIGURE 4.16** Both these unrelated boys have Weaver syndrome at ages 18 and 11 months.

From Weaver DD, Graham CB, Thomas IT, Smith DW. A new overgrowth syndrome with accelerated skeletal maturation, unusual facies, and camptodactyly. J. Pediatr 1974;84:547–52.

*PP2A* is ubiquitously expressed and plays a critical role in the regulation of the cell cycle, in a multitude of signaling cascades that control cell proliferation, division, differentiation, apoptosis,

metabolism, adhesion, and migration and is thought to modulate the activity of over 30 different kinases and functions by dephosphorylating many critical cellular molecules like Akt, p53, c-Myc, and β-catenin [210]. PP2A has been shows to control the differentiation and function of mesenchymal descendants such as osteoblasts and adipocytes: reduction in PP2A promotes bone formation and osteoblast differentiation through the regulation of bone-related transcription factors such as Osterix. Downregulation of PP2A also stimulates adipocyte differentiation from undifferentiated mesenchymal cells under the appropriate adipogenic differentiation conditions and osteoblasts. PP2A is also involved in the ability to control osteoclastogenesis as well as in the proliferation and metastasis of osteosarcoma cells [211].

Mutations in the PP2A regulatory subunit B family genes *PPP2R5B, PPP2R5C,* and *PPP2R5D* cause human overgrowth. Loveday et al. [212] proposed mutations in PP2A genes affected substrate binding, perturbing the ability of PP2A to dephosphorylate particular protein substrates. They also identified that PP2A is a major negative regulator of v-akt murine thymoma viral oncogene homolog 1 (*AKT*) [213].

#### 4.5.1.1.5. Phosphoinositide-3 kinase–related overgrowth disorders

Phosphoinositide-3 kinase (PI3K)-related overgrowth spectrum (PROS) encompasses syndromes of overgrowth such as megalencephaly-capillary malformation (MCAP) syndrome, dysplatic megalencephaly (DMEG), congenital lipomatous asymmetric overgrowth of the trunk, lymphatic, capillary, venous, and combined-type vascular malformations, epidermal nevi, skeletal and spinal anomalies (CLOVES) syndrome [164], hemihyperplasia-multiple lipomatosis (HHML), fibroadipose overgrowth, and Klippel-Trenaunay syndrome (KTS) that are associated with somatic mosaic gain-of-function/activating mutations of *PIK3CA* gene, which in term lead to abnormal activation of the RTK/PI3K/AKT/mTOR signaling cascade illustrated in Fig. 4.17 [214]. Activated phosphatidylinositol 3-

kinase δ syndrome (APDS) or p110δ-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI) is a primary immunodeficiency disease linked to PI3K hyperactivity linked to *PIK3CD* and *PIK3R1* [215].

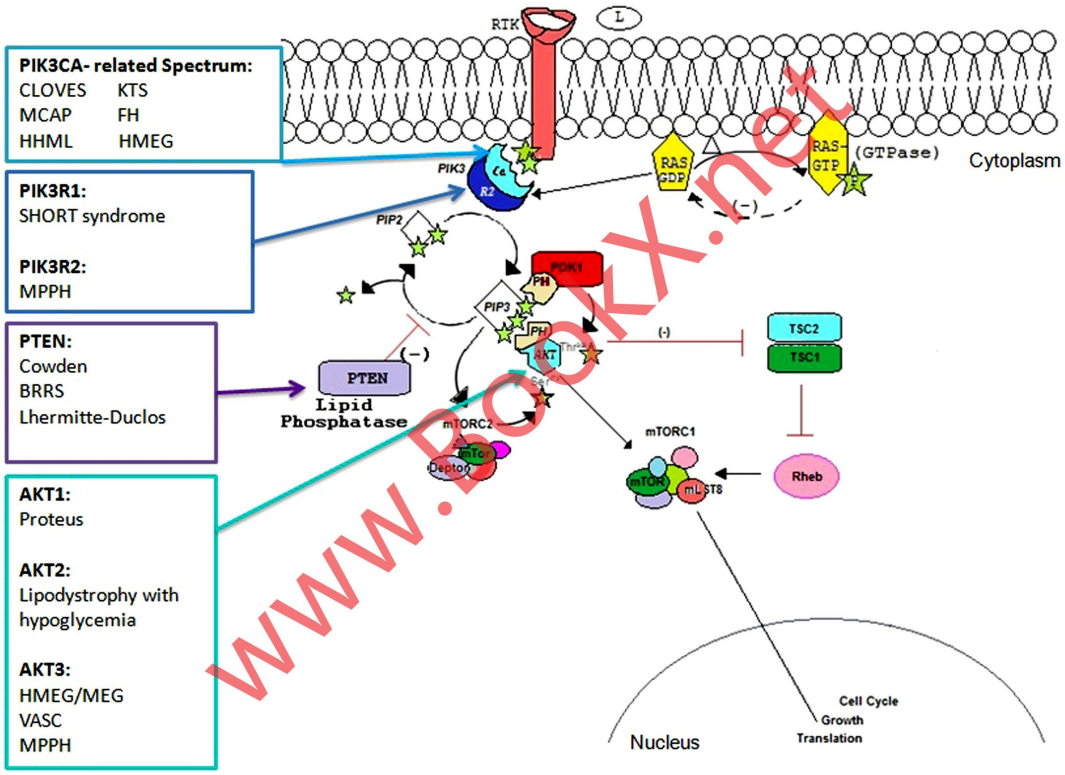



**FIGURE 4.17** RTK/PI3K/AKT/mTOR signaling: Ligand binding to the extracellular domain activates tyrosine kinase receptors (RTKs), phosphatidylinositol-3-kinases (PI3Ks) are a family of lipid kinases subdivided in three classes and eight isoforms. Class I PI3Ks act as dimers for p110α, p110β and p110δ, where the most common regulatory subunit is p85. main product of class I PI3Ks is PIP3 Activation of PI3K leads to phosphorylation of PIP2 (phosphatidylinositol 4,5-bisphosphate) to PIP3 (phosphatidylinositol-3,4,5-triphosphate) (reversed by PTEN), which in turn phosphorylates AKT (Oncogene Protein Kinase B). Activation of Akt and other PI-3K downstream kinases in the cytosol is mediated by phosphoinositide dependent kinase 1 (PDK1), a serine/threonine kinase. PDK1 phosphorylates Akt on $Thr^{308}$; full activation of AKT needs phosphorylation of Akt on $Ser^{473}$ by activated mTORC2 Active Akt inhibits TSC2 activity through direct phosphorylation. TSC2 is a GTP-ase activating protein (GAP) that functions in association with TSC1 to inactivate the small G protein Rheb. *Arrows* indicate activating events,

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while *perpendicular lines with a minus sign* (−) indicate inhibitory events.
Adapted from Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: an updated review. Ann Med September 2014;46(6):372–83. Illustrated by Deepika Burkardt.

Some disorders result in segmental or regional overgrowth (e.g., familial macrocephaly, congenital hemihyperplasia, KTS, Proteus syndrome, enchondromatosis syndromes, Maffucci syndrome or Ollier disease, Patterson-David syndrome). Parameter-specific overgrowth disorders contrast with generalized overgrowth disorders in that usually only a single growth parameter (e.g., weight) is affected. Examples of such disorders include those associated with obesity (e.g., Prader-Willi syndrome, Cohen syndrome, Börjeson-Forssman-Lehmann syndrome, Bardet-Biedle syndrome, and familial idiopathic obesity).

Klippel-Trenaunay syndrome (KTS, OMIM 149000) is a rare sporadic triad of congenital anomalies including cutaneous capillary malformations of a limb, venous malformations, and accompanying segmental hypertrophy of soft tissue and/or bone [216], of a limb (usually lower limb) [217], potentially involving the colon and related vessels [218], even a growing skull hemangioma [219]. Parkes-Weber syndrome (PWS) caused by a mutation in *RASA1* includes a clinically significant arteriovenous fistula [220]. Ultrasound (Doppler) is used to distinguish KTS from PWS since the prognosis and treatment are different with magnetic resonance angiography as the gold standard for diagnosis to confirm involvement of superficial or deep venous involvement [221,222]. Enoxaparin maintenance and recombinant coagulation factor VIIa have been successfully used to control potentially fatal consumptive coagulopathies resultant from the AVMs of PWS [217].

Proteus (OMIM 176920) is a variable syndrome of disproportionate or segmental overgrowth of limbs and macrocephaly associated with features including cranial hyperostoses, plantar hyperplasia, hemangiomas, lipomas or lymphangiomas, verrucous epidermal nevi, and variable psychomotor deficiency. Overgrowth and asymmetry are occasionally present at birth in <20% of cases [223,224]; but the

hallmark of disease is a random distribution of lesions throughout the body that develops during childhood [225]. Postnatal overgrowth of bone and soft tissue increases at a faster rate than the rest of the body [223], and this overgrowth tends to plateau at adolescence. Proteus seems to affect males more than females (1.9:1) and has serious complications including premature death, scoliosis due to megaspondyly, abnormalities of the CNS, ophthalmologic complications (especially strabismus), pulmonary, otolaryngologic and dental complications, and elongation of the long bones with thinning [223]. Most reports of Proteus suggest a sporadic disorder, and it was hypothesized [226] that the cause of the syndrome was a dominant lethal gene that survived by mosaicism. Reported cases of monozygotic twins that are discordant for Proteus syndrome support the idea of postzygotic mosaicism [227].

*C*ongenital *L*ipomatous *O*vergrowth, *V*ascular malformations, and *E*pidermal nevi (CLOVE syndrome, OMIM 612918) and hemihyperplasia lipomatosis syndrome (HHML)/isolated lateralized overgrowth (ILO) are segmental overgrowth syndromes that should be considered in the differential diagnosis for Proteus syndrome. ILO or HHML (Fig. 4.18) is characterized by subcutaneous lipomatosis, asymmetric overgrowth (hemihyperplasia), and occasional vascular malformations. The hemihyperplasia is not as severe and progressive as is seen in Proteus syndrome [228]. Findings that have been described in the literature as "isolated hemihypertrophy" and "isolated hemihyperplasia" are now termed "isolated lateralized overgrowth" (ILO); that is, lateralized overgrowth in the absence of a recognized pattern of malformations, dysplasia, or morphologic variants [229]. Unlike Proteus syndrome, lipomatosis is a main feature of HHML. Vascular malformations of HHML are capillary malformations without the involvement of the deep venous or lymphatic systems as seen in Proteus syndrome or KTS, and these are less susceptible to thrombosis [230]. CLOVE syndrome is an overgrowth syndrome characterized by congenital lipomatous overgrowth, vascular malformations, and epidermal nevi [212]. In 2015, the National Institutes of Health developed a consensus

document regarding diagnosis and treatment of patients with *PIK3CA*-associated somatic overgrowth disorders [231].

Megaencephaly is a clinical feature that accompanies constitutive activation of the PI3K-AKT-mTOR pathway for many patients. Negishi et al. [232] describe Western blot analysis to determine the expression levels of phosphorylated S6 ribosomal protein (phospho-S6 protein) in lymphoblastoid cell lines of patients with increased head circumference and neurological symptoms, Multiplex targeted sequencing analysis for 15 genes involved in the mTOR pathway was performed, and whole exome sequencing was performed to successfully identify mTOR pathway involvement in 70% of patients in the cohort (n = 13). Both megalencephaly-capillary malformation syndrome (MCAP, OMIM 602501) and megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH, OMIM 603387) have been shown to result from gain-of-function mutations in the PI3K-AKT-mTOR pathway (mTOR-pathway) (including AKT3, PIK3R2, and PIK3CA) [233]. MCAP is characterized clinically by prenatal onset overgrowth with variable features of cerebral and somatic asymmetry, macrocephaly, neonatal hypotonia, cutaneous vascular malformations/connective tissue dysplasia, syndactyly, an abnormal neurocognitive profile, and characteristic facial features (Fig. 4.19). MPPH is characterized by progressive megaencephaly, polymicrogyria, and polydactyly [232].



**FIGURE 4.18** This boy was born with hemihyperplasia of the right side of his body, including his tongue. These features are usually sporadic and noninheritable. They are typical of UPD and associated with the highest risk for hepatoblastoma and Wilms tumor.

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#### 4.5.1.1.6. *PTEN* Spectrum

*PTEN* hamartoma tumor syndrome is an umbrella term used to cover the continuum of autosomal dominant overgrowth conditions including Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome associated with multiple hamartomas and detectable mutations of the *PTEN* gene located on 10q23.3 [234]. Cowden syndrome (OMIM 158350) is a hamartomatous disorder with pathognomic features including cerebellar dysplastic gangliocytoma (adult Lhermitte-Duclos disease) and mucocutaneous lesions including facial trichilemmomas, acral keratoses, mucosal lesions, and papillomatous lesions and adult onset. Other major features of this syndrome include macrocephaly, thyroid (particularly follicular) and

endometrial carcinoma, and breast cancer. Occasional features include vascular malformations, lipomas, hamartomatous intestinal polyps, pigmented penile macules, Hashimoto thyroiditis (or other thyroid lesions), fibrocystic disease of the breast, uterine fibroids, genitourinary malformations, or tumors (especially renal cell carcinoma) [235].

Bannayan-Riley-Ruvalcaba syndrome (BRRS, OMIM 153480) is more rare and has a classic triad of macrocephaly, intestinal polyposis, and genital lentiginosis [236]. Birth length and weight are usually greater than the 97th percentile, although growth tends to normalize by mid-childhood, with the final height within the normal adult range. Bone age is usually consistent with chronologic age, and macrocephaly persists into adulthood with normal ventricular size. Delayed gross motor development may be associated with a lipid storage myopathy, which appears to respond to treatment with carnitine; 30% of patients have thyroid involvement [237]. A majority of patients with clinical features of Cowden syndrome and 65% of patients with BRRS had detectable *PTEN* gene mutations.



**FIGURE 4.19** One of di-zygotic twins with macrocephaly-capillary malformation syndrome at 12 (pictures on the left) and 40 (right) months showing typical macrocephaly with capillary malformations fading and postnatally decreased growth in comparison with his twin sister.

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## 4.5.1.1.7. Overgrowth Syndromes Diagnosed by Sequencing.

Array CGH has been found to be useful in the diagnosis of unknown overgrowth disorders. Among genetic causes of syndromic overgrowth, chromosomal deletions and duplications such as dup(4)(p16.3), dup(15)(q26qter), del(9)(q22.32q22.33), del(22)(q13), and del(5)(q35) have been identified, as well as a number of other rare CNVs, emphasizing the usefulness of this technique in unknown overgrowth disorders [199]. Tatton-Brown et al. [238] reported a series of five patients with trisomy or tetrasomy of 15q, all of whom shared clinical features of overgrowth and learning difficulties with characteristic

facial features including a long, thin face and prominent chin and nose. Three of these patients had renal anomalies, including renal agenesis, horseshoe kidney, and hydronephrosis [238]. Dolan et al. [239] reported a series of five patients with a Sotos-like syndrome and CNVs within 19p13.13. Four patients with a deletion shared a phenotype of overgrowth with macrocephaly, ophthalmologic anomalies, gastrointestinal abnormalities, and learning disabilities. One patient with a duplication within 19p13.13 had microcephaly and short stature with height affected more than weight [239]. Malan et al. [125] demonstrated that haploinsufficiency mutations in *NF1X* within 19p13.13 resulted in the same phenotype as Sotos-like syndrome and that patients with Marshall-Smith had activating mutations in *NF1X*.

## 4.6. Conclusion

Human growth is a sophisticated and highly regulated process. As our understanding of the mechanisms of growth and their temporal role in development evolves, the patient's features present an opportunity for the clinician to diagnose, prognosticate, and optimize patient care. From recognizing variants at either end of the spectrum (and potential underlying genetic causation) to evaluating pathologic short stature or overgrowth, this chapter presents a brief overview of mechanisms of growth at each level of physiology and clinical examples of syndromic and nonsyndromic genetic causes of abnormal stature. Advances in genetic diagnostics have boosted the clinician's ability to provide a molecular diagnosis for abnormal stature, but a meticulous history and thorough examination are central to an evaluation. An interdisciplinary approach helps with expectant management of the patient with abnormal stature. Periodic follow-up is necessary to monitor developmental progress and help coordinate care/anticipatory guidance. Learning/behavior/speech assessment and support may be benefit individuals with developmental delay and/or learning disability. For patients experiencing joint pain secondary to ligamentous laxity or joint contractures, physiotherapy or water therapy might provide relief. The appropriate specialist referral(s) should be made for other clinical issues.

## References


[1] Yang J, Benyamin B, McEvoy B.P, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet*. 2010;42:565–569.

[2] Durand C, Rappold G.A., Damsker J.M, et al. Height matters-from monogenic disorders to normal variationΔκ. *Nat Rev Endocrinol*. 2013;9(1):171–177.

[3] Lui JC, Nilsson O, Chan Y, Palmer CD, Andrade AC, genome-wide association studies and expression microarray reveals novel genes that act in the human growth plate to modulate height. *Hum Mol Genet.* 2012 Dec;21(23) 5193–201. https://doi:10.1093/hmg/dds347. Epub 2012 Aug 21. PubMed PMID: 22914739; PubMed Central PMCID: PMC3490510.

[4] Lango A.H, Estrada K, Lettre G, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature*. 2010;467:832–838.

[5] Lettre G. Recent progress in the study of the genetics of height. *Hum Genet*. May 2011;129(5):465–472.

[6] De Bruin C, Dauber A. Genomic insights into growth and its disorders: an update. *Curr Opin Endocrinol Diabetes Obes*. 2016;23(1):51–56.

[7] Chan Y, Holmen O.L, Dauber A, Vatten L, Havulinna variants show predicted polygenic effects on height in the tails of the distribution, except in extremely short individuals. *PLoS Genet*. 2011;7(12):e1002439.

[8] Martin J, Hamilton B.E, Osterman M, Driscoll A, Matł final data for 2015. *Natl Vital Stat Rep*. 2017;66(1):1–69.
[9] Najafian M, Cheraghi M. Occurrence of fetal macrosomia rate and its maternal and neonatal complications: a 5-year cohort study. *ISRN Obstet Gynecol*. 2012;2012:353791.
[10] Risnes K.R, Vatten L.J, Baker J.L, Jameson K, Sovio U, and mortality in adulthood: a systematic review and meta-analysis. *Int J Epidemiol*. 2011;40(3):647–661.
[11] Mondestin M.A, Ananth C.V, Smulian J.C, Vintzileos weight and fetal death in the United States: the effect of maternal diabetes during pregnancy. *Am J Obstet Gynecol*. October 2002;187(4):922–926.
[12] Lindström L., Wikström A.K, Bergman E, Lundgren M small for gestational age and poor school performance - how small is too small? *Horm Res Paediatr*. 2017;301(6):636–650 [Epub ahead of print].
[13] Bagherpour A, Pousti M, Adelianfar E. Hand skeletal maturity and its correlation with mandibular dental development. J Clin Exp Dent 2014 Jul. 1;6(3):e275–9. 10.4317/jced.51433. eCollection 2014 Jul. PubMed PMID:25136430; PubMed Central PMCID: PMC4134858
[14] Pavone P, Praticò AD, Falsaperla R, Ruggieri M, Zollino M, Corsello G, Neri G. Congenital generalized hypertrichosis: the skin as a clue to complex malformationsyndromes. Ital J Pediatr 2015

Aug 5;41:55. 10.1186/s13052-015-0161-3. Review. PubMed PMID: 26242548; PubMed Central PMCID: PMC4526284

[15] Neu N, Duchon J, Zachariah P. TORCH infections. *Clin Perinatol*. 2015 Mar;42(1):77–103. doi: 10.1016/j.clp.2014.11.001 viii. Epub 2014 Dec 20. Review.

[16] Moore C.A, Staples J.E, Dobyns W.B, Pessoa A, Ventu the Pattern of Anomalies in Congenital Zika Syndrome for Pediatric Clinicians. *JAMA Pediatr*. 2017;171(3):288–295. doi: 10.1001/jamapediatrics.2016.3982. .

[17] Ventura L.O, Ventura C.V, Lawrence L, van der Linden V, van der Linden A, Gois A.L, Cavalcanti M.M, Barros E.A, Dias impairment in children with congenital Zika syndrome. *J AAPOS*. 2017 Apr 24.

[18] Suttie M, Foroud T, Wetherill L, Jacobson J.L, Moltenc dysmorphism across the fetal alcohol spectrum. *Pediatrics*. 2013 Mar;131(3):e779–e788. doi: 10.1542/peds.2012-1371.

[19] Suttie M, Wetherill L, Jacobson S.W, Jacobson J.L, Hoy curvature detects and explicates ethnic differences in effects of prenatal alcohol exposure. *Alcohol Clin Exp Res*. 2017 Jun 13 doi: 10.1111/acer.13429.

[20] de Brito M.L, Nunes M, Bernardi J.R, Bosa V.L, Goldani M Silva C.H. Somatic growth in the first six months of

life of infants exposed to maternal smoking in pregnancy. *BMC Pediatr*. 2017 Mar 9;17(1):67. doi: 10.1186/s12887-017-0819-7.

[21] Wehby G.L, Uribe L.M, Wilcox A.J, Christensen K, Ro between smoking and body mass index and risk of oral clefts. *Ann Epidemiol*. 2017 Feb;27(2):103–107. doi: 10.1016/j.annepidem.2016.11.009 e2.

[22] McEvoy C.T, Spindel E.R. Pulmonary effects of maternal smoking on the fetus and child: effects on lung development, respiratory morbidities, and life long lung health. *Paediatr Respir Rev*. 2017 Jan;21:27–33. doi: 10.1016/j.prrv.2016.08.005.

[23] Murphy E. Pregnancy in women with inherited metabolic disease. *Obstet Med*. 2015 Jun;8(2):61–67. doi: 10.1177/1753495X15576442 Epub 2015 Mar 29. Review.

[24] Hoang T.T, Marengo L.K, Mitchell L.E, Canfield M.A, findings and updated meta-analysis for the association between maternal diabetes and risk for congenital heart disease phenotypes. *Am J Epidemiol*. 2017 May 13:1–11.

[25] Cade W.T, Levy P.T, Tinius R.A, Patel M.D, Choudhry of maternal and infant metabolism are associated with ventricular dysfunction in infants of obese Women with type 2 diabetes. *Pediatr Res*. 2017 Jun 12.

[26] Dong D, Reece E.A, Lin X, Wu Y, AriasVillela N, Yang development of the yolk sac theory in diabetic

embryopathy: molecular mechanism and link to structural birth defects. *Am J Obstet Gynecol*. 2016 Feb;214(2):192–202.

[27] Myatt L, Maloyan A. Obesity and placental function. *Semin Reprod Med*. 2016 Jan;34(1):42–49. doi: 10.1055/s-0035-1570027 Epub 2016 Jan 6. Review.

[28] Persson M, Cnattingius S, Villamor E, Söderling J, Pas of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singletons. *BMJ*. 2017 Jun 14;357 doi: 10.1136/bmj.j2563.

[29] Savage M.O, Hwa V, David A, Rosenfeld R.G, Mether Defects in the Growth Hormone-IGF-I Axis Causing Growth Hormone Insensitivity and Impaired Linear Growth. *Front Endocrinol (Lausanne)*. 2011 Dec 12;72(6):721–728 eCollection 2011. PubMed PMID: 22654835; PubMed Central PMCID: PMC3356141.

[30] Berendsen AD, Olsen BR. Bone development. Bone. 2015 Nov;80:14-18. 10.1016/j.bone.2015.04.035. Review. PubMed PMID: 26453494; PubMed Central PMCID:PMC4602167

[31] Jee Y.H, Baron J. The biology of stature. *J Pediatr*. June 2016;173:32–38.

[32] Zeng K, Zhang H.Q, Chen Y, Gao Q. Estradiol via estrogen receptor beta inhibits chondrogenesis of mouse vertebral growth plate in vitro. *Childs Nerv Syst*. 2016;32(3):461–465.

[33] Yu B, Jiang K, Chen B, Wang H, Li X, Liu Z. Leptin

differentially regulates chondrogenesis in mouse vertebral and tibial growth plates. *BMC Musculoskelet Disord*. 2017;18(1):235.

[34] Baron J, Sävendahl L, De Luca F, Dauber A, Phillip M, Wit J.M, Nilsson O. Shor and tall stature: a new paradigm emerges. *Nat Rev Endocrinol*. 2015;11(12):735–746.

[35] Lui J.C, Nilsson O, Baron J. Growth plate senescence and catch-up growth. *Endocr Dev*. 2011;21:23–29. doi: 10.1159/000328117 Epub 2011 Aug 22. Review. PubMed PMID: 21865751; PubMed Central PMCID: PMC3420820.

[36] Varco-Merth B., Feigerlová E, Shinde U, Rosenfeld R.G, Hwa growth deficiency is associated with STAT5b mutations that disrupt protein folding and activity. *Mol Endocrinol*. 2013;27(1):150–161.

[37] Scalco R.C, Hwa V, Domené H.M, et al. *STAT5B* mutations in heterozygous state have negative impact on height: another clue in human stature heritability. *Eur J Endocrinol*. 2015;173(3):291–296. .

[38] Benabbad I, Rosilio M, Child C.J, Carel J.C, Ross J.L, D outcomes and near-adult height gain of growth hormone-treated children with SHOX deficiency: data from an observational study and a clinical trial. *Horm Res Paediatr*. 2017;87(1):42–50.

[39] Marchini A, Ogata T, Rappold G.A. A track record on SHOX: from basic research to complex models and therapy,. *Endocr Rev*. 2016;37(4):417–448.

[40]

Wang S.R, Jacobsen C.M, Carmichael H, Edmund A.B mutations in natriuretic peptide receptor-B (NPR2) gene as a cause of short stature. *Hum Mutat*. 2015;36(4):474–481.

[41] Hisado-Oliva A, Ruzafa-Martin A, Sentchordi L, Funari M.F.A, Bezanilla-López C, Alonso-Bernáldez M, Barraza-García J, Rodriguez-Zabala M, Lerario A.M, Benito-Sanz S, Aza-Carmona M, Campos-Barros A, Jorge A.A.L, Heath K.E. Mutations in C-natriuretic peptide (NPPC): a novel cause of autosomal dominant short stature. *Genet Med*. June 29, 2017;20 [Epub ahead of print].

[42] Nilsson O., Guo M.H, Dunbar N., Popovic J, Flynn D, stature, accelerated bone maturation, and early growth cessation due to heterozygous aggrecan mutations. *J Clin Endocrinol Metab*. 2014;99(8):E1510–E1518.

[43] Decker E, Durand C, Bender S, Rödelsperger C, Glase is a target of the homeobox transcription factor SHOX in limb development. *Hum Mol Genet*. 2011;20(8):1524–1535.

[44] Fukami M, Seki A, Ogata T.. *SHOX* haploinsufficiency as a cause of syndromic and nonsyndromic short stature. *Mol Syndromol*. 2016;7(1):3–112.

[45] Valetto A, Bertini V, Michelucci A, Toschi B, Dati E, B stature in isodicentric Y chromosome and three

copies of the SHOX gene: clinical report and review of literature. *Mol Syndromol*. 2016;7(1):19–25.

[46] Bunyan D.J, Baffico M, Capone L, Vannelli S, Iughetti upstream and downstream of SHOX identified as novel causes of Leri-Weill dyschondrosteosis or idiopathic short stature. *Am J Med Genet*. 2016;170A(4):949–957.

[47] Upners E.N, Jensen R.B, Rajpert-De Meyts E, Dunø M, Aksglaede L, Juul A. Short stature homeobox-containing gene duplications in 3.7% of girls with tall stature and normal karyotypes. *Acta Paediatr*. July 1, 2017;106 [Epup ahead of print].

[48] Miura K, Kim O.H, Lee H.R, et al. Overgrowth syndrome associated with a gain-of-function mutation of the natriuretic peptide receptor 2 (*NPR2*) gene. *Am J Med Genet*. 2014;164A(1):156–163.

[49] Khan S, Basit S, Khan M.A, Muhammad N, Ahmad W of human isolated acromesomelic dysplasia. *Eur J Med Genet*. 2016;59(4):198–203.

[50] Makrythanasis P, Temtamy S, Aglan M.S, Otaify G.A, novel homozygous mutation in FGFR3 causes tall stature, severe lateral tibial deviation, scoliosis, hearing impairment, camptodactyly, and arachnodactyly. *Hum Mutat*. 2014;35(8):959–963.

[51] Quintos J.B, Guo M.H, Dauber A. Idiopathic short stature due to novel heterozygous mutation of the aggrecan gene. *J Pediatr Endocrinol Metab*. 2015;28(7–8):927–932.

[52] Lu L, Harutyunyan K, Jin W, Wu J, Yang T, Chen Y, Jo Regulates p53 Function In Vivo During Skeletogenesis. *J Bone Miner Res*. 2015 Jun;30(6):1077–1089. doi: 10.1002/jbmr.2436 PubMed PMID: 25556649.

[53] Mehta P.A, Tolar J. Fanconi anemia. In: Pagon R.A, Adam M.P, Ardinger H.H, et al., eds. *GeneReviews® [Internet]*. Seattle (WA): University of Washington; February 23, 2017:1993–2017 Available from:. https://www.ncbi.nlm.nih.gov/books/NBK1401

[54] Ouyang Z, Yu H. Releasing the cohesin ring: a rigid scaffold model for opening the DNA exit gate by Pds5 and Wapl. *Bioessays*. 2017;39(4) [submitted for publication].

[55] Banerji R, Skibbens R.V, Iovine M.K. How many roads lead to cohesinopathies? *Dev Dyn*. 2017 [submitted for publication].

[56] Gil-Rodríguez M.C, Deardorff M.A, Ansari M, Tan C.A, P Montoya C, Ousager L.B, Puisac B, Hernández-Marcos M, Teresa-Rodrigo M.E, Marcos-Alcalde I, Wesselink J.J, Lusa-Bernal S, Bijlsma E.K, Braunholz D, Bueno-Martinez I, Clark D, Cooper N.S, Curry C.J, Fisher R, I Kaesbach G, Guo Y, Hakonarson H, Hopkin R.J, Kaur Á P.J, Queralt E, Richer J, Roeder E, Sinha A, Scott R.F Puertas P, Casale C.H, Ström L, Selicorni A, Ramos F. novo heterozygous mutations in SMC3 cause a range of Cornelia de Lange syndrome-overlapping

phenotypes. *Hum Mutat*. 2015;36(4):454–462. .

[57] Kline A.D, Krantz I.D, Deardorff M.A, Shirahige K., D Snell E, Kerr L.M, Katz O, Calof A.L, Musio A, Egense de Lange syndrome and molecular implications of the cohesin complex: Abstracts from the 7th biennial scientific and educational symposium 2016. *Am J Med Genet*. 2017;173(5):1172–1185.

[58] Verloes A, Drunat S, Gressens P, Passemard S. Primar autosomal recessive microcephalies and Seckel syndrome spectrum disorders. In: Pagon R.A, Adam M.P, Ardinger H.H, e *[Internet]*. Seattle (WA): University of Washington; 2013:1993–2017.

[59] Khani F, Thaler R, Paradise C.R, Deyle D.R, Kruijthof-de Julio M, Galindo M, Gordon J.A, Stein G.S, Dudakovi Wijnen A.J. Histone H4 methyltransferase Suv420h2 maintains fidelity of osteoblast differentiation. *J Cell Biochem*. 2017;118(5):1262–1272.

[60] Samsa W.E, Zhou X, Zhou G. Signaling pathways regulating cartilage growth plate formation and activity. *Semin Cell Dev Biol*. 2017;62:3–15 [submitted for publication].

[61] Guo Y.C, Zhang S.W, Yuan Q. Deubiquitinating enzymes and bone remodeling. *Stem Cells Int*. 2018 Jul 8:3712083. doi: 10.1155/2018/3712083 eCollection 2018. Review. PubMed PMID: 30123285; PubMed Central PMCID: PMC6079350.

[62] Webster E, Cho M.T, Alexander N, Desai S, Naidu S, I A, Retterer K, Juusola J, Chung WK. De novo PHIP-predicted deleterious variants areassociated with developmental delay, intellectual disability, obesity, and dysmorphic features. *Cold Spring Harb Mol Case Stud*. 2016 Nov;2(6) a001172.PubMed PMID: 27900362; PubMed Central PMCID: PMC5111011.

[63] Jansen S, Hoischen A, Coe B.P, Carvill G.L, Van Esch H, Bosch D.G.M, Andersen U.A, Baker C, Bauter Bon B.W, Claahsen-van der Grinten H.L, Gecz J, Gilissen C, Grillo L, Hackett A, K Hoeijmakers J.H, Pfundt R, Steehouwer M, Stevens S. de Vorst M, Vries P, Witherspoon K, Veltman J.A, Brunn Vries B.B.A. A genotype-first approach identifies an intellectual disability-overweight syndrome caused by PHIP haploinsufficiency. *Eur J Hum Genet*. 2018 Jan;26(1):54–63. doi: 10.1038/s41431-017-0039-5 Epub 2017 Dec 5. PubMed PMID: 29209020; PubMed Central PMCID: PMC5839042.

[64] Yang J, Andre P, Ye L, Yang Y.-Z. The Hedgehog signalling pathway in bone formation. *Int J Oral Sci*. 2015;7(2):73–79.

[65] Polizio A.H, Chinchilla P, Chen X, Manning D.R, Riob Hedgehog activates the GTPases Rac1 and RhoA in a Gli-independent manner through coupling of Smoothened to Gi proteins. *Sci Signal*. November 22, 2011;4(200):pt.7 [Review].

[66] St-

Jacques B, Hammerschmidt M, McMahon A.P. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev*. 1999;13(16):2072–2086.
[67] Kronenberg H.M, Chung U. The parathyroid hormone-related protein and Indian hedgehog feedback loop in the growth plate. *Novartis Found Symp*. 2001;232:144–152 [discussion 152-7. Review].
[68] Kronenberg H.M. Developmental regulation of the growth plate. *Nature*. 2003;423(6937):332–336.
[69] Amano K, Densmore M, Nishimura R, et al. Indian hedgehog signaling regulates transcription and expression of collagen type X via Runx2/Smads interactions. *J Biol Chem*. 2014;289(36):24898–24910.
[70] Cohen Jr. M.M. Perspectives on RUNX genes: an update. *Am J Med Genet*. 2009;149A(12):2629–2646.
[71] Biesecker L.G. Pallister-Hall syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, al., eds. *GeneReviews [Internet]*. Seattle (WA): University of Washington; 2017:1993–2017.
[72] Biesecker L.G. Greig cephalopolysyndactyly syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, *[Internet]*. Seattle (WA): University of Washington; 2014:1993–2017.
[73] Beighton P.H, Hamersma H, Brunkow M.E. *SOST*-related sclerosing bone dysplasias, 2013. In: Pagon R.A, Adam M.P, Ardinger H.H, et al., eds. *GeneReviews [Internet]*. Seattle (WA): University of Washington; 2013:1993–2017.
[74] Warren S.M, Brunet L.J, Harland R.M, Economides A.

BMP antagonist noggin regulates cranial suture fusion. *Nature*. 2003;422:625–629.

[75] Winkler D.G, Yu C, Geoghegan J.C, Ojala E.W, Skonie and sclerostin bone morphogenetic protein antagonists form a mutually inhibitory complex. *J Biol Chem*. 2004;279:36293–36298.

[76] Boudin E, Fijalkowski I., Hendrickx G., Van Hul W. Genetic control of bone mass. *Mol Cell Endocrinol*. 2016;432(6):3–13199. .

[77] Barker K.T, Houlston R.S, Tchah H. Overgrowth syndromes: is dysfunctional PI3-kinase signalling a unifying mechanism? *Eur J Hum Genet*. 2003;11(1):665–667.

[78] Cao H, Alrejaye N, Klein O.D, Goodwin A.F, Oberoi S review of craniofacial and dental findings of the RASopathies. *Orthod Craniofac Res*. 2017;20(Suppl. 1):32–38.

[79] Pezzolesi M.G, Zbuk K.M, Waite K.A, Eng C. Compar genomic and functional analyses reveal a novel cis-acting PTEN regulatory element as a highly conserved functional E-box motif deleted in Cowden syndrome. *Hum Molec Genet*. 2007;16:1058–1071.

[80] Fu G, Brkić J, Hayder H, Peng C. MicroRNAs in human placental development and pregnancy complications. *Int J Mol Sci*. 2013;14(3):5519–5544.

[81] Pober B.R. Williams-Beuren syndrome. *N Engl J Med*. January 21, 2010;362(3):239–252 Review. Erratum in: N Engl J Med 2010 Jun 3;

362(22):2142. https://doi.org/10.1056/NEJMra0903074.
[82] Larizza L, Roversi G, Volpi L. Rothmund-Thomson syndrome. *Orphanet J Rare Dis*. Jan 2010;5:2. https://doi.org/10.1186/1750-1172-5-2.
[83] Van Maldergem L, Siitonen H.A, Jalkh N, Chouery E, De Roy M, Delague V, Muenke M, Jabs E.W, Cai J, Wang the craniosynostosis-radial ray hypoplasia association: Baller-Gerold syndrome caused by mutations in the RECQL4 gene. *J Med Genet*. February 2006;43(2):148–152.
[84] Faghri S, Tamura D, Kraemer K.H, DiGiovanna J.J. Tr a systematic review of 112 published cases characterises a wide spectrum of clinical manifestations. *J Med Genet*. 2008;45:609–621.
[85] Gatti R, Perlman S. Ataxia-Telangiectasia. In: Pagon R.A, Adam M.P, Ardinger H al., eds. *GeneReviews [Internet]*. Seattle (WA): University of Washington, Seattle; March 19, 1999:1993–2017 Available from. https://www.ncbi.nlm.nih.gov/books/NBK2646
[86] Hoischen A, van Bon B.W, Rodríguez-Santiago B, Gilissen C, Vissers L.E, de VriesP Janssen I, van Lier B, Hastings R, Smithson S.F, Newbury-Ecob R, Kjaergaard S,Goodship J, McGowan R, Bartholdi D, Rauch A, Pei D,Gillessen-Kaesbach G, Veltman J.A, Brunner H.G, de Vries B.B. De novo nonsensemutations in ASXL1 cause Bohring-Opitz syndrome. *Nat Genet*. 2011

Jun26;43(8):729–731. doi: 10.1038/ng.868 PubMed PMID: 21706002.
[87] Russell B, Tan W.H, Graham Jr. J.M. Bohring-Opitz Syndrome. In: AdamMP, Ardinger H.H, Pagon R.A, V *[Internet]*. Seattle (WA): University of Washington, Seattle; 2018 Feb 15:1993–2018 Available from. http://www.ncbi.nlm.nih.gov/books/NBK48183 29446906.
[88] Shashi V, Pena L.D.M, Kim K, Burton B, Hempel M, S novo truncating variants in ASXL2 are associated with a unique and recognizable clinical phenotype. *Am J Hum Genet*. 2016;99:991–999.
[89] Bainbridge M.N, Hu H, Muzny D.M, Musante L, Lups novo truncating mutations in ASXL3 are associated with a novel clinical phenotype with similarities to Bohring-Opitz syndrome. *Genome Med*. 2013;5:11.
[90] Zhou X, Li Q, Xu J, Zhang X, Zhang H, Xiang Y, Fang aberrantly expressed miR-193b-3p contributes to preeclampsia through regulating transforming growth factor-β signaling. *Sci Rep*. 2016;6:19910.
[91] Chisholm K.M, Folkins A.K. Placental and clinical characteristics of term small-for-gestational-age neonates: a case-control study. *Pediatr Dev Pathol*. 2016;19(1):37–46.
[92] Carpio L.R, Bradley E.W, McGee-Lawrence M.E, Weivoda M.M, Poston D.D, Dudakovi Wijnen A.J, Oursler M.J, Westendorf J.J. Histone deacetylase 3 supports endochondral bone formation

by controlling cytokine signaling and matrix remodeling. *Sci Signal*. 2016;9(440):ra79.
[93] López A.J, Wood M.A. Role of nucleosome remodeling in neurodevelopmental and intellectual disability disorders. *Front Behav Neurosci*. 2015;9:100.
[94] Zarate Y.A, Bhoj E, Kaylor J, Li D, Tsurusaki Y, Miyak Vergano S.A. SMARCE1, a rare cause of Coffin-Siris Syndrome: clinical description of three additional cases. *Am J Med Genet*. 2016;170(8):1967–1973.
[95] Kosho T, Okamoto N, Ohashi H, Tsurusaki Y, Imai Y, Ko Y, Kawame H, Homma T, Tanabe S, Kato M, Hiral correlations of mutations affecting six components of the SWI/SNF complex: detailed description of 21 patients and a review of the literature. *Am J Med Genet*. 2013;161A(6):1221–1237.
[96] Elhamamsy A.R. Role of DNA methylation in imprinting disorders: an updated review. *J Assist Reprod Genet*. 2017;34(5):549–562. .
[97] Le Bouc Y, Rossignol S, Azzi S, Steunou V, Netchine I, Gi genomic imprinting and assisted reproductive technology. *Ann Endocrinol*. 2010;71(3):237–238.
[98] Choufani S, Shuman C, Weksberg R. Beckwith–Wiedemann syndrome. *Am J Med Genet Part C*. 2010;154C:343–354.
[99] Õunap K. Silver-russell syndrome and Beckwith-Wiedemann syndrome: opposite phenotypes with heterogeneous molecular etiology. *Mol Syndromol*. July 2016;7(3):110–121.

[100]
Russo S, Calzari L, Mussa A, Mainini E, Cassina M, D
to: a multi-method approach to the molecular diagnosis of overt and borderline 11p15.5 defects underlying Silver-Russell and Beckwith-Wiedemann syndromes. *Clin Epigenet*. 2016;8:40.
[101] Berdasco M, Esteller M. Genetic syndromes caused by mutations in epigenetic genes. *Hum Genet*. 2013;132(4):359–383.
[102] Gall Trošelj K, Novak
Kujundzic R, Ugarkovic D. Polycomb repressive complex's evolutionary conserved function: the role of EZH2 status and cellular background. *Clin Epigenetics*. 2016;8:55.
[103]
Imagawa E, Higashimoto K, Sakai Y, Numakura C, O
in genes encoding polycomb repressive complex 2 subunits cause Weaver syndrome. *Hum Mutat*. June 2017;38(6):637–648.
[104] Cohen A.S, Gibson W.T. EED-associated overgrowth in a second male patient. *J Hum Genet*. 2016;61(9):831–834.
[105] Proos L, Gustafsson J. Is early puberty triggered by catch-up growth following undernutrition? *Int J Environ Res Public Health*. 2012;9(5):1791–1809.
[106]
Dall'Asta A, Brunelli V, Prefumo F, Frusca T, Lees C.C
onset fetal growth restriction. *Matern Health Neonatol Perinatol*. 2017;3:2.
[107]
Villar J, Altman D.G, Purwar M, Noble J.A, Knight H.

Ismail L, Barros F.C, Lambert A, Papageorghiou A.T, International Fetal and Newborn Growth Consortium for the 21st century (INTERGROWTH-21ST) the objectives, design and implementation of the INTERGROWTH-21ST project. *BJOG*. 2013;120(Suppl. 2):9–26.

[108] Neu N, Duchon J, Zachariah P. TORCH infections. *Clin Perinatol*. 2015;42(1):77–103. doi: 10.1016/j.clp.2014.11.001 viii. [Epub 2014 Dec 20. Review].

[109] Sharma D, Farahbakhsh N, Shastri S, Sharma P. Intrau growth restriction - part 2. *J Matern Fetal Neonatal Med*. 2016;29(24):4037–4048.

[110] Waisbren S.E, Rohr F, Anastasoaie V.M, Brown D, Ha Ozonoff A, Petrides S, Wessel A, Levy H.L. Maternal phenylketonuria: long-term outcomes in offspring and post-pregnancy maternal characteristics. *JIMD Rep*. 2015;21:23–33. doi: 10.1007/8904_2014_365.

[111] Deardorff M.A, Noon S.E, Krantz I.D. Cornelia de Lange Syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, al., eds. *GeneReviews® [Internet]*. Seattle (WA): University of Washington; 2016:1993–2017 Available from:. https://www.ncbi.nlm.nih.gov/books/NBK1104

[112] Stevens C.A. Rubinstein-taybi syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, al., eds. *GeneReviews [Internet]*. Seattle (WA): University of Washington; 2014:1993–2017.

correlation with mandibular dental development. *J Clin Exp Dent*. 2014 Jul 1;6(3):e275–e279. doi: 10.4317/jced.51433 eCollection 2014 Jul. PubMed PMID: 25136430; PubMed Central PMCID: PMC4134858.

[114] Sukalo M, Fiedler A, Guzmán C, Spranger S, Addor M Benavent M, Cobben J.M, Gillis L.A, Shealy A.G, Desł Karnebeek C.D, Bergmann C, Liu Z, Düker G, Rezaei Vega C.A, Verbeek N.E, Richmond E.J, Kirbiyik O, Ra in the human UBR1 gene and the associated phenotypic spectrum. *Hum Mutat*. 2014;35(5):521–531.

[115] Epée E, Beleho D, Bitang A.T, Njami V.A, Bengondo C Mvogo C. A familial study of Hallermann-Streiff-François syndrome. *Int Med Case Rep J*. 2017;10:193–201.

[116] Allanson J.E, Roberts A.E. Noonan syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, al., eds. *GeneReviews [Internet]*. Seattle (WA): University of Washington; 2016:1993–2017.

[117] Aoki Y, Niihori T, Inoue S, Matsubara Y. Recent advances in RASopathies. *J Hum Genet*. 2016;61(1):33–39.

[118] Sanz M.M, German J, Cunniff C. Bloom's syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, *[Internet]*. Seattle (WA): University of Washington; 2016:1993–2017 Available from:. https://www.ncbi.nlm.nih.gov/books/NBK1398

[119] Palovcak A, Liu W, Yuan F, Zhang Y. Maintenance of genome stability by Fanconi anemia proteins. *Cell Biosci*. 2017;7:8.

[120] Laugel V. Cockayne syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, *[Internet]*. Seattle (WA): University of Washington; 2012:1993–2017 Available from:. https://www.ncbi.nlm.nih.gov/books/NBK1342
[121] Wang Y, Chakravarty P, Ranes M, Kelly G, Brooks P.J of gene expression as a cause of Cockayne syndrome neurological disease. *Proc Natl Acad Sci U S A*. 2014;111(40):14454–14459.
[122] Faridounnia M, Wienk H, Kovačič L, Folkers G.E, Jasp Cerebro-oculo-facio-skeletal Syndrome Point Mutation F231L in the ERCC1 DNA Repair Protein Causes Dissociation of the ERCC1-XPF Complex. *J Biol Chem*. 2015 Aug 14;290(33):20541–20555. doi: 10.1074/jbc.M114.635169 Epub 2015 Jun 17. PubMed PMID: 26085086; PubMed Central PMCID: PMC4536458.
[123] Kramer K.H, DiGiovanna J.J. Xeroderma pigmentosum. In: Pagon R.A, Adam M.P, Ardinger H *[Internet]*. Seattle (WA): University of Washington; 2016:1993–2017.
[124] McDonald-McGinn D.M, Sullivan K.E, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Primer*. 2015;1:15071.
[125] Nijim Y, Awni Y, Adawi A, Bowirrat A. Classic case report of Donohue syndrome (leprechaunism; OMIM *246200): the impact of consanguineous mating. *Medicine*. 2016;95(6):e2710.
[126]

Burkardt D.D, Rosenfeld J.A, Helgeson M, et al. Distir phenotype in 9 patients with deletion of chromosome 1q24-q25. *Am J Med Genet*. 2011;155A:1336–1351.

[127] Lévy J, Coussement A, Dupont C, Guimiot F, Bauman and clinical delineation of 2p15p16.1 microdeletion syndrome. *Am J Med Genet*. 2017;173(8):2081–2087.

[128] Ono M, Maeda T, Ihara K. First mixoploid infant with full triploidy in blood cells. *Pediatr Int*. 2016;58(12):1354–1355.

[129] Blyth M, Maloney V, Beal S, Collinson M, Huang S, C Killian syndrome: a study of 22 British patients. *J Med Genet*. 2015;52(7):454–464.

[130] Domené H.M, Fierro-Carrión G. Genetic disorders of GH action pathway. *Growth Horm IGF Res*. 2018 Feb;38:19–23. doi: 10.1016/j.ghir.2017.12.004 Epub 2017 Dec 12. Review. PubMed PMID: 29249625.

[131] Kostopoulou E, Shah P, Ahmad N, Semple R, Hussair dysmotility and pancreatic insufficiency in 2 siblings with Donohue syndrome. *Pediatr Diabetes*. 2016 [Epub ahead of print].

[132] Fiot E, Zenaty D, Boizeau P, Haigneré J, Dos Santos S, Léger J, French Turner Syndrome Study Group. X-chromosome gene dosage as a determinant of impaired pre and postnatal growth and adult height in Turner syndrome. *Eur J Endocrinol*. 2016;174(3):281–288.

[133] Binder G, Eggermann T, Weber K, Ferrand N, Schwei

diagnostic value of IGF-2 and the IGF/IGFBP-3 system in Silver-Russell syndrome. *Horm Res Paediatr*. 2017;88 [Epub ahead of print].

[134]
Croteau D.L, Rossi M.L, Ross J, Dawut L, Dunn C, Ku RECQL4 mutant protein lacks helicase and ATPase activity. *Biochim Biophys Acta*. 2012;1822(11):1727–1734.

[135]
Fu W, Ligabue A, Rogers K.J, Akey J.M, Monnat Jr. R. RECQ helicase pathogenic variants, population variation and "missing" diseases. *Hum Mutat*. February 2017;38(2):193–203.

[136]
Siitonen H.A, Sotkasiira J, Biervliet M, Benmansour A Daire V, Crandall B, Hannula-Jouppi K, Hennekam R, Herzog D, Keymolen K, Lipsa Nyman M, Miny P, E Plon S, Riedl S, Sarkar A, R Vargas F, Verloes A, L Wang L, Kääriäinen H, Kestilä M. The mutation spectrum in RECQL4 diseases. *Eur J Human Genet*. 2009;17(2):151–158. doi: 10.1038/ejhg.2008.154.

[137]
Singh D.K, Ghosh A.K, Croteau D.L, Bohr V.A. RecQ helicases in DNA double strand break repair and telomere maintenance. *Mutat Res*. 2012;736(1–2):15–24.

[138]
Ng A.J, Walia M.K, Smeets M.F, Mutsaers A.J, Sims N DNA helicase recql4 is required for normal osteoblast expansion and osteosarcoma formation. *PLoS*

*Genet*. 2015 Apr 10;11(4):e1005160. doi: 10.1371/journal.pgen.1005160 e 2015 Apr. PubMed PMID: 25859855; PubMed Central PMCID: PMC4393104.

[139] Kobayashi T, Aoki Y, Niihori T, Cavé H, Verloes A, O Kaesbach G, Wieczorek D, Kurosawa K, Mizuno S, Ol *and clinical analysis of RAF1 in Noonan syndrome and related disorders:dephosphorylation of serine 259 as the essential mechanism for mutant activation. Hum Mutat*. 2010 Mar;31(3):284–294. doi: 10.1002/humu.21187 PubMed PMID: 20052757.

[140] Cançado F.H, Silva L.C, Taitson P.F, Andrade A.C, Pi you know this syndrome? Leopard syndrome. *An Bras Dermatol*. 2017 Jan-Feb;92(1):127–129. doi: 10.1590/abd1806-4841.20174505 PubMed PMID: 28225973; PubMed Central PMCID: PMC5312195.

[141] Kalev I, Muru K, Teek R, Zordania R, Reimand T, Köl syndrome with recurrent PTPN11 mutation Y279C and different cutaneousmanifestations: two case reports and a review of the literature. *Eur J Pediatr*. 2010 Apr;169(4):469–473. doi: 10.1007/s00431-009-1058-1 Epub 2009 Sep 20. Review. PubMed PMID: 19768645.

[142] Bertola D, Buscarilli M, Stabley D.L, Baker L, Doyle D Church K, Gripp K.W. Phenotypic spectrum of

Costello syndrome individualsharboring the rare HRAS mutation p.Gly13Asp. *Am J Med Genet A*. 2017 May;173(5):1309–1318. doi: 10.1002/ajmg.a.38178 Epub 2017 Apr 3. PubMed PMID:28371260; PubMed Central PMCID: PMC5397353.

[143] Quélin C, Loget P, Rozel C, D'Hervé D, Fradin M, Der costello syndrome with neuromuscular spindles excess and p.Gly12Val HRAS mutation. *Eur J Med Genet*. 2017 Jul;60(7):395–398. doi: 10.1016/j.ejmg.2017.03.014 Epub 2017 Apr 25. PubMed PMID: 28455154.

[144] Niemeyer C.M. RAS diseases in children. *Haematologica*. 2014 Nov;99(11):1653–1662. doi: 10.3324/haematol.2014.114595 Review. PubMed PMID: 25420281; PubMed CentralPMCID: PMC4222471.

[145] Ekvall S, Sjörs K, Jonzon A, Vihinen M, Annerén G, Bo association of neurofibromatosis type 1-causing mutations in families withneurofibromatosis-Noonan syndrome. *Am J Med Genet A*. 2014 Mar;164A(3):579–587. doi: 10.1002/ajmg.a.36313 Epub 2013 Dec 19. PubMed PMID: 24357598.

[146] Kikuchi S, Borek D.M, Otwinowski Z, Tomchick D.R, structure of the cohesin loader Scc2 and insight into cohesinopathy. *Proc Natl Acad Sci U S A*. 2016;113(44):12444–12449.

[147] Marcos-Alcalde Í, Mendieta-

Moreno J.I, Puisac B, Gil-Rodríguez M.C, Hernández-Marcos M, Soler-
Polo D, Ramos F.J, Ortega J, Pié J, Mendieta J, Gómez-Puertas P. Two-step ATP-driven opening of cohesin head. *Sci Rep*. 2017;7(1):3266.

[148] Orgil O, Mor H, Matityahu A, Onn I. Identification of a region in the coiled-coil domain of Smc3 that is essential for cohesin activity. *Nucleic Acids Res*. 2016;44(13):6309–6317.

[149]
Lee B.G, Roig M.B, Jansma M, Petela N, Metson J, Nas structure of the cohesin Gatekeeper Pds5 and in complex with kleisin Scc1. *Cell Rep*. 2016;14(9):2108–2115.

[150] Bürmann F, Basfeld A, Vazquez Nunez R, Diebold-Durand M.L, Wilhelm L, Gruber S. Tuned SMC arms drive chromosomal loading of prokaryotic condensin. *Mol Cell*. 2017;65(5):861–872 e9.

[151]
Barisic I, Tokic V, Loane M, Bianchi F, Calzolari E, Ga Working Group. Descriptive epidemiology of Cornelia de Lange syndrome in Europe. *Am J Med Genet Part A*. 2007;146A:51–59.

[152]
Dias C, Giordano M, Frechette R, Bellone S, Polychron variations at the human growth hormone receptor (GHR) gene locus are associated with idiopathic short stature. *J Cell Mol Med*. May 29, 2017;21.

[153] Xu C, Zhang X, Dong L, Zhu B, Xin T. MRI features of growth hormone deficiency in children with short stature caused by pituitary lesions. *Exp Ther*

*Med*. 2017;13(6):3474–3478.
[154] Soliman A.T, De Sanctis V, Yassin M, Adel A. Growth and growth hormone -insulin like growth factor -I (GH-IGF-I) Axis in chronic anemias. *Acta Biomed*. 2017;88(1):101–111.
[155] Cippà P.E, Krayenbuehl P.A. Increased height in HFE hemochromatosis. *N Engl J Med*. 2013;369(8):785–786.
[156] Aggarwal A, Nguyen J, Rivera-Davila M, Rodriguez-Buritica D. Marshall-Smithsyndrome: Novel pathogenic variant and previously unreported associations withprecocious puberty and aortic root dilatation. *Eur J Med Genet*. 2017 Jul;60(7):391–394. doi: 10.1016/j.ejmg.2017.04.012 Epub 2017 Apr 24. PubMedPMID: 28442439.
[157] Tatton-Brown K, Rahman N. EZH2-Related Overgrowth [updated 2018 Aug 2]. In: Adam M.P, Ardinger H.H, Pagon R.A, Wallace (WA): University of Washington, Seattle; 1993-2018; 2013 Jul 18 Available from. http://www.ncbi.nlm.nih.gov/books/NBK14882( PMID: 23865096.
[158] Cohen A.S.A, Gibson W.T. EED-associated overgrowth in a second male patient. *J. Hum. Genet*. 2016;61:831–834 [PubMed: https://www.ncbi.nlm.nih.gov/pubmed/271932202719
[159] Imagawa E, Higashimoto K, Sakai Y, Numakura C, O in genes encoding polycomb repressive complex 2

subunits cause Weaver syndrome. *Hum. Mutat.* 2017;38:637–648 [PubMed: https://www.ncbi.nlm.nih.gov/pubmed/282295142822

[160] Bharathavikru R, Hastie N.D. Overgrowth syndromes and pediatric cancers: howmany roads lead to IGF2? *Genes Dev*. 2018 Aug 1;32(15-16):993–995. doi: 10.1101/gad.317792.118 PubMed PMID: 30068702; PubMed Central PMCID: PMC6075144.

[161] Fournet J.C, Mayaud C, de Lonlay P, Gross-Morand M.S, Verkarre V, Castanet M, Devillers M, Ra Fekete C, Saudubray J.M, Junien C. Unbalanced expression of 11p15 imprinted genes in focal forms of congenital hyperinsulinism: association with a reduction to homozygosity of a mutation in ABCC8 or KCNJ11. *Am J Pathol*. 2001;158:2177–2184.

[162] Taneja T.K, Mankouri J, Karnik R, Kannan S, Smith A. GTPase-dependent ER exit of K(ATP) channels revealed by a mutation causing congenital hyperinsulinism. *Hum Molec Genet*. 2009;18:2400–2413.

[163] McClenaghan C, Hanson A, Sala-Rabanal M, Roessler H.I, Josifova D, Grange D.K, van Haaften G, Nichols C.G. Cantu syndrome-associated SUR2 (ABCC9) mutations in distinct structural domains result in K(ATP) channel gain-of-function by differential mechanisms. *J Biol Chem*. 2018 Feb 9;293(6):2041–2052. doi: 10.1074/jbc.RA117.000351 Epub 2017 Dec 22. PubMed PMID: 29275331; PubMed Central PMCID: PMC5808765.

[164] Shang L, Henderson L.B, Cho M.T, Petrey D.S, Fong C novo missense variants in PPP2R5D are associated with intellectual disability, macrocephaly, hypotonia, and autism. *Neurogenetics*. 2016;17(1):43–49.
[165] Minatogawa M, Takenouchi T, Tsuyusaki Y, Iwasaki I of the phenotype of Kosaki overgrowth syndrome. *Am J Med Genet A*. 2017 Sep;173(9):2422–2427. doi: 10.1002/ajmg.a.38310 Epub 2017Jun 22. PubMed PMID: 28639748.
[166] Millward D.J. Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutr Res Rev*. June 2017;30(1):50–72.
[167] Teran E, Chesner J, Rapaport R. Growth and growth hormone: an overview. *Growth Horm IGF Res*. 2016;28:3–5.
[168] Muñoz-Hoyos A, Molina-Carballo A, Augustin-Morales M, Contreras-Chova F, Naranjo-Gómez A, Justicia-Martínez F, Uberos J. Psychosocial dwarfism: psychopathological aspects and putative neuroendocrine markers. *Psychiatry Res*. June 30, 2011;188(1):96–101.
[169] Wattchow N, Lee H.E, Brock P. Psychosocial short stature with psychosis: a case report. *Australas Psychiatry*. February 2015;23(1):63–65.
[170] Deltondo J, Por I, Hu W, Merchenthaler I, Semeniken between the human growth hormone-releasing

hormone- and neuropeptide-Y-immunoreactive systems in the human diencephalon: a possible morphological substrate of the impact of stress on growth. *Neuroscience*. 2008;153(4):1146–1152.

[171] Faraone S.V, Biederman J, Morley C.P, et al. Effect of stimulants on height and weight: a review of the literature. *J Am Acad Child Adolesc Psychiatry*. 2008;47:994–1009.

[172] Phadke S.R, Aggarwal S, Kumari N. The expanding spectrum of Elejalde syndrome: overlap with other disorders of overgrowth. *Clin. Dysmorph.* 2011;20:98–101 PubMed: 21164339. https://doi.org/10.1136/bmjopen-2015-010433.

[173] Baudy A.R, Reeves E.K.M, Damsker J.M, et al. Δ-9,11 modification of glucocorticoids dissociates nuclear factor-κB inhibitory efficacy from glucocorticoid response element-associated side effects. *J Pharmacol Exp Therapeut*. 2012;343(1):225–232.

[174] Warman M.L, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2010 revision. *Am J Med Genet Part A*. 2011;155:943–968.

[175] Finkielstain G, Lui J, Baron J. Catch-up growth: cellular and molecular mechanisms. *World Rev Nutr Diet*. 2013;106:100–104.

[176] Tatton-Brown K, Loveday C, Yost S, Clarke M, Ramsay E, Za Overgrowth Collaboration, Mahamdallie S, Seal S, Ruark E, Rahma in epigenetic regulation genes are a major cause of

overgrowth with intellectual disability. *Am J Hum Genet*. 2017;100:725–736.

[177] Ehrenberg H.M, Mercer B.M, Catalano P.M. The influence of obesity and diabetes on the prevalence of macrosomia. *Am J Obstet Gynecol*. September 2004;191(3):964–968.

[178] Ouzounian J.G, Hernandez G.D, Korst L.M, Montoro pregnancy weight and excess weight gain are risk factors for macrosomia in women with gestational diabetes. *J Perinatol*. March 3, 2011. .

[179] Mohammadbeigi A, Farhadifar F, Soufi zadeh N, Mohammadsalehi N, Rezaiee M, Aghaei M. macrosomia: risk factors, maternal, and perinatal outcome. *Ann Med Health Sci Res*. 2013;3(4):546–550. doi: 10.4103/2141-9248.122098.

[180] Ogonowski J, Miazgowski T. Intergenerational transmission of macrosomia in women with gestational diabetes and normal glucose tolerance. *Eur J Obstet Gynecol Reprod Biol*. December 2015;195:113–116.

[181] Tyrrell J, Richmond R.C, Palmer T.M, Feenstra B, Ran Silva N.M, Wood A.R, Horikoshi M, Geller F, Myhre I Møller E, Huikari V, Painter J.N, Hottenga J.J, Allard Geus E.J, Martin N.G, Spector T.D, Power C, Järvelin Smith G, Hivert M.F, Felix J.F, Hyppönen E, Lowe Jr. growth genetics (EGG) Consortium. Genetic evidence for causal relationships between maternal obesity-related traits and birth weight. *J Am Med Assoc*. March 15, 2016;315(11):1129–1140 [Erratum in:

JAMA April 19 2016;315(15):1661].

[182]
Correa A, Gilboa S.M, Besser L.M, Botto L.D, Moore C Colarusso T.J, Waller D.K, Reece E.A. Diabetes mellitus and birth defects. *Am J Obstet Gynecol*. September 2008;199(3):237 e1-9. [Epub 2008 Jul 31].

[183]
Stothard K.J, Tennant P.W, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *J Am Med Assoc*. February 11, 2009;301(6):636–650 [submitted for publication].

[184]
Yu R, Nissen N.N, Dhall D, Heaney A.P. Nesidioblast and hyperplasia of alpha cells, microglucagonoma, and nonfunctioning islet cell tumor of the pancreas: review of the literature. *Pancreas*. 2008;36(4):428–431.

[185]
Brioude F, Kalish J.M, Mussa A, Foster A.C, Bliek J, Fe Pellegrin M, Hussain K, Ibrahim A, Kilby M.D, Krajev Walasek M, Kratz C.P, Ladusans E.J, Lapunzina P, Le Bouc Y, Maas S.M, Macdonald F, Õunap K, Peruzzi L, Brown K, Tenorio J, Tortora C, Grønskov K, Netchine consensus document: Clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. *Nat Rev Endocrinol*. 2018 Apr;14(4):229–249. doi: 10.1038/nrendo.2017.166 Epub 2018 Jan 29. Review. PubMed PMID: 29377879; PubMed Central PMCID: PMC6022848.

[186] Yu A, Duffy K, Hathaway E, Kelly J, Richards-Yutz J, Deardoff MA, Ganguly A, Kalish JM. The phenotypic spectrum of Beckwith Wiedemann Syndrome due to loss of methylation at Imprinting center 2. 39th David W. Smith Workshop on Malformations and Morphogenesis, August 24-29, 2018, Banff, Alberta, CA, American Journal of Medical Genetics, 39th Annual David W. Smith Workshop on Malformations and Morphogenesis: Abstracts of the 2018 Annual Meeting, in press March 2019.

[187] Vuillaume M.L, Moizard M.P, Rossignol S, Cottereau update for the GPC3 gene involved in Simpson-Golabi-Behmel syndrome and review of the literature. *Hum Mutat*. 2018 Jun;39(6):790–805. doi: 10.1002/humu.23428 Epub 2018 Apr 24. PubMed PMID: 29637653.

[188] Tenorio J, Arias P, Martínez-Glez V, Santos F, García-Miñaur S, Nevado J,Lapunzina P. Simpson-Golabi-Behmel syndrome types I and II. *Orphanet J Rare Dis*. 2014;20(9):138.

[189] Capurro M.I, Xu P, Shi W, Li F, Jia A, Filmus J. Glypic 3 inhibits Hedgehog signaling during development by competing with patched for Hedgehog binding. *Dev Cell*. 2008;14(5):700–711.

[190] Golabi M, Leung A, Lopez C. Simpson-golabi-behmel syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, al., eds. *GeneReviews [Internet]*. Seattle

(WA): University of Washington; 2011:1993–2017.
[191] Shi W, Filmus J. A patient with the Simpson-Golabi-Behmel syndrome displays a loss-of-function point mutation in GPC3 that inhibits the attachment of this proteoglycan to the cell surface. *Am J Med Genet*. 2009;149A:552–554.
[192] Suzuki M, Sugimoto K, Tanaka J, et al. Up-regulation of glypican-3 in human hepatocellular carcinoma. *Anticancer Res*. 2010;30(12):5055–5061. .
[193] Tatton-Brown K, Rahman N. EZH2-related overgrowth. In: Pagon R.A, Adam M.P, Ardinger H.H *[Internet]*. Seattle (WA): University of Washington; July 18, 2013:1993–2017 Available from:. http://www.ncbi.nlm.nih.gov/books/NBK14882
[194]
Imagawa E, Albuquerque E.V.A, Isidor B, Mitsuhashi SUZ12 mutations in Weaver-like syndrome. *Clin Genet*. 2018 Nov;94(5):461–466. doi: 10.1111/cge.13415 Epub 2018 Aug 6. PubMed PMID: 30019515.
[195] Manning B.J, Yusufzai T. The ATP-dependent chromatin remodeling enzymes CHD6, CHD7, and CHD8 exhibit distinct nucleosome binding and remodeling activities. *J Biol Chem*. 2017;292(28):11927–11936.
[196] Tatton-Brown K, Cole T.R.P, Rahman N. Sotos syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, al., eds. *GeneReviews® [Internet]*. Seattle (WA): University of Washington; December 17, 2004:1993–2017 Available from:. https://www.ncbi.nlm.nih.gov/books/NBK1479

[197] Tlemsani C, Luscan A, Leulliot N, Bieth E, Afenjar A, Fenzy M, Goldenberg A, Lacombe D, Lambert L, Ode Poirsier C, Briand-Suleau A, Laurendeau I, Chin M, Saugier-Veber P, Vidaud D, Cormier-Daire V, Vidaud M, Pasmant E, Burglen L. SETD2 and DNMT3A screen in the Sotos-like syndrome French cohort. *J Med Genet*. June 17, 2016.

[198] Villani A, Greer M.C, Kalish J.M, Nakagawara A, Nat for cancer surveillance in individuals with RASopathies and other rare genetic conditions with increased cancer risk. *Clin Cancer Res*. June 15, 2017;23(12):e83–e90.

[199] Tenorio J, Mansilla A, Valencia M, Martínez-Glez V, Romanelli V, Arias P, Castrejón N, Poletta F, C Navarro E, Gordo G, Mansilla E, García-Santiago F, González-Casado I, Vallespín E, Palomares M, Mori M.A, Santos Simarro F, García-Miñaur S, Fernández L, Mena R, Benito-Sanz S, del Pozo Á, Silla J.C, Ibañez K, López-Granados E, Martín-Trujillo A, Montaner D, , SOGRI Consortium, , Heath K.E, Campos-Barros Á, Dopazo J, Nevado J, Monk D, Ruiz-Pérez V.L, Lapunzina P. A new overgrowth syndrome is due to mutations in RNF125. *Hum Mutat*. 2014 Dec;35(12):1436–1441. doi: 10.1002/humu.22689 PubMed PMID: 25196541.

[200] Dong H.Y, Zeng H, Hu Y.Q, Xie L, Wang J, Wang X.Y Microdeletion including NFIX associated with overgrowth and intellectual disability suggestive of Malan syndrome. *Mol Cytogenet*. 2016;9:71.

[201] Martinez F, Marín-Reina P, Sanchis-Calvo A, Perez-Aytés A, Oltra S, Roselló M, Mayo S, Monfort S, Pantc mutations of NFIX gene causing Marshall-Smith syndrome or Sotos-like syndrome: one gene, two phenotypes. *Pediatr Res*. 2015 Nov;78(5):533–539. doi: 10.1038/pr.2015.135 Epub 2015 Jul 22. PubMed PMID: 26200704.

[202] Summers D.A, Cooper H.A, Butler M.G. Marshall-Smith syndrome: case report of a newborn male and review of the literature. *Clin Dysmorphol*. 1999;8(3):207–210.

[203] Salter M, Kalmat C, Kroll H, Kim D. Treatment of refractory hip pain with sodium hyaluronate (Hyalgan©) in a patient with the Marshall-Smith syndrome: a case report. *J Orthop Surg Res*. 2010;5:61.

[204] Diab M, Raff M, Gunther D.F. Osseous fragility in Marshall-Smith syndrome. *Am J Med Genet*. 2003;119A:218–222.

[205] Tatton-Brown K, Seal S, Ruark E, Harmer J, Ramsay E, Del Vecchio Duarte S, Zachariou A, Hanks S, O'Brien E, Aksglaede Maldergem L, Yachelevich N, Childhood Overgrowth Consortium, van Montfort R, Rahman N. Mutations in the DNA methyltransferase gene DNMT3A cause an

overgrowth syndrome with intellectual disability. *Nat Genet*. 2014 Apr;46(4):385–388. doi: 10.1038/ng.2917 Epub 2014 Mar 9. Erratum in: Nat Genet 2014 Jun;46(6):657. PubMed PMID: 24614070; PubMed Central PMCID: PMC3981653.

[206] Yanagisawa Y, Ito E, Yuasa Y, Maruyama K. The human DNA methyltransferases DNMT3A and DNMT3B have two types of promoters with different CpG contents. *Biochim Biophys Acta*. 2002;1577:457–465.

[207] Houge G, Haesen D, Vissers L.E, Mehta S, Parker M.J, Gassen K.L, Zonneveld-Huijssoon E, de Bie C.I, Hoischen A, Eichler E.E, Holdhus R, Steen V.M related protein phosphatase 2A dysfunction identified in patients with intellectual disability. *J Clin Invest*. 2015;125(8):3051–3062. .

[208] Nematullah M, Hoda M.N, Khan F. Protein phosphatase 2A: a double-faced phosphatase of cellular system and its role in neurodegenerative disorders. *Mol Neurobiol*. February 21, 2017 [Epub ahead of print, review].

[209] Nakashima A, Tanimura-Ito K, Oshiro N, et al. A positive role of mammalian Tip41-like protein, TIPRL, in the amino-acid dependent mTORC1-signaling pathway through interaction with PP2A. *FEBS Lett*. 2013;587:2924–2929.

[210] Yoon J.T, Ahn H.K, Pai H.S. The subfamily II catalytic subunits of protein phosphatase 2A (PP2A) are involved in cortical microtubule organization. *Planta*. 2018 Sep 6 doi: 10.1007/s00425-

018-3000-0 [Epub ahead of print] PubMed PMID: 30191298.

[211] Okamura H, Yoshida K, Morimoto H, Teramachi J, O of protein phosphatase 2A in osteoblast differentiation and function. *J Clin Med*. 2017;6(3).

[212] Martinez-Lopez A, Blasco-Morente G, Perez-Lopez I, Herrera-Garcia J.D, Luque-Valenzuela M, Sanchez-Cano D, Lopez-Gutierrez J.C, Ruiz-Villaverde R, Tercedor-Sanchez J. CLOVES syndrome: review of a PIK3CA-related overgrowth spectrum (PROS). *Clin Genet*. 2017 Jan;91(1):14–21. doi: 10.1111/cge.12832 Epub 2016 Aug 3. Review. PubMed PMID: 27426476.

[213] Loveday C, Tatton-Brown K, Clarke M, Westwood I, Renwick A, Ramsay Overgrowth Collaboration. Mutations in the PP2A regulatory subunit B family genes PPP2R5B, PPP2R5C and PPP2R5D cause human overgrowth. *Hum Mol Genet*. 2015;24(17):4775–4779.

[214] Venot Q, Canaud G. [PIK3CA-related overgrowth syndrome (PROS)]. *Nephrol Ther*. 2017;13(Suppl. 1):S155–S156 [French].

[215] Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh T.W, Mitsuiki N, Asano T, O and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase δ syndrome-like immunodeficiency. *J Allergy Clin Immunol*. 2016;138(6):1672–1680 e10.

[216] Pereira C, Espí Rito Santo R, Saldanha J. Klippel-

Trenaunay syndrome: diagnosis in a neonate. *BMJ Case Rep*. July 2017;24.

[217] Beier U.H, Schmidt M.L, Hast H, Kecskes S, Valentinc of disseminated intravascular coagulation in Klippel-Trenaunay-Weber syndrome using enoxaparin and recombinant activated factor VIIa: a case report. *J Med Case Rep*. 2010;19(4):92.

[218] Kaplan S.L, Zhuang H. Diffuse elevated abdominal activity on 99mTc-labeled red blood cell imaging in a pediatric patient with Klippel Trenaunay syndrome. *Clin Nucl Med*. 2016;41(11):899–901.

[219] van der Loo L.E, Beckervordersandforth J, Colon A.J, Schijns C skull hemangioma: first and unique description in a patient with Klippel-Trénaunay-Weber syndrome. *Acta Neurochir*. 2017;159(2):397–400.

[220] Banzic I, Brankovic M, Maksimović Ž, Davidović L, M Weber syndrome-Diagnostic and management paradigms: a systematic review. *Phlebology*. 2017;32(6):371–383.

[221] Ziyeh S, Spreer J, Rössler J, Strecker R, Hochmuth A, S Weber or Klippel-Trenaunay syndrome? Non-invasive diagnosis with MR projection angiography. *Eur Radiol*. 2004;14(11):2025–2029.

[222] Sung H.M, Chung H.Y, Lee S.J, Lee J.M, Huh S, Lee J.Y experience of the Klippel-Trenaunay syndrome. *Arch Plast Surg*. 2015;42(5):552–558.

[223] Biesecker L.G, Peters K.F, Darling T.N, et al. Clinical differentiation between Proteus syndrome and hemihyperplasia: description of a distinct form of hemihyperplasia. *Am J Med Genet*. 1998;79:311–318.
[224] Keppler-Noreuil K.M, Sapp J.C, Lindhurst M.J, et al. Clinical delineation and natural history of the *PIK3CA*-related overgrowth spectrum. *Am J Med Genet*. 2014;164(7):1713–1733.
[225] Lacerda L. da S, Alves Ú.D, Zanier J.F.C, Machado D.C, Camilo G.B, diagnoses of overgrowth syndromes: the most important clinical and radiological disease manifestations. *Radiol Res Pract*. 2014;2014:947451.
[226] Happle R. The categories of cutaneous mosaicism: a proposed classification. *Am J Med Genet*. 2016;170A(2):452–459.
[227] Biesecker L.G. Myths and misdiagnoses of proteus syndrome. *Asian J Anesthesiol*. 2018 Mar;56(1):14–22. doi: 10.6859/aja.201803.56(1):0006 PubMed PMID: 29847972.
[228] Mirzaa G, Conway R, Graham Jr. J.M, Dobyns W.B. P related segmental overgrowth. In: Pagon R.A, Adam M.P, Ardinger H.H *[Internet]*. Seattle (WA): University of Washington; August 15, 2013:1993–2017 Available from:. http://www.ncbi.nlm.nih.gov/books/NBK15372
[229] Kalish J.M, Biesecker L.G, Brioude F, Deardorff M.A, I Cesare-

Merlone A, Druley T, Ferrero G.B, Lapunzina P, Lariz
Agosto J.A, Mussa A, Robinson P, Russo S, Selicorni A
and definition in asymmetric regional body
overgrowth. *Am J Med Genet*. May 5 2017 [Epub
ahead of print].

[230]
Schulte T.L, Liljenqvist U, Görgens H, Hackenberg L,
multiple lipomatosis syndrome (HHML): a challenge
in spinal care. *Acta Orthop Belg*. 2008;74(5):714–719.

[231] Keppler-
Noreuil K.M, Rios J.J, Parker V.E, Semple R.K, Lindhu
related overgrowth spectrum (PROS): diagnostic and
testing eligibility criteria, differential diagnosis, and
evaluation. *Am J Med Genet*. 2015;167A(2):287–295.

[232]
Negishi Y, Miya F, Hattori A, Johmura Y, Nakagawa M
T,Aoyama K, Ohashi K, Fukumura S, Mizuno S, Umer
combination of genetic and biochemical analyses for
the diagnosis of PI3K-AKT-mTOR pathway-
associated megalencephaly. *BMC Med
Genet*. 2017;18(1):4.

[233]
Rivière J.B, Mirzaa G.M, O'Roak B.J, Beddaoui M, Alc
Onge J, Schwartzentruber J.A, Gripp K.W, Nikkel S.M
Sagie T, Uyanik G, Weksberg R, Zirn B, Beaulieu C.L,
of rare disease genes (FORGE) Canada
Consortium, Majewski J, Bulman D.E, O'Driscoll M, S
novo germline and postzygotic mutations in AKT3,
PIK3R2 and PIK3CA cause a spectrum of related
megalencephaly syndromes. *Nat Genet*. June 24,
2012;44(8):934–940.

[234] Ngeow J, Sesock K, Eng C. Clinical implications for germline PTEN spectrum disorders. *Endocrinol Metab Clin North Am*. 2017;46(2):503–517.
[235] Shuch B, Ricketts C.J, Vocke C.D, et al. Germline PTEN mutation Cowden syndrome: an under-appreciated form of hereditary kidney cancer. *J Urol*. 2013;190(6):1990–1998.
[236] Lee S.H, Ryoo E, Tchah H. Bannayan-riley-ruvalcaba syndrome in a patient with a PTEN mutation identified by chromosomal microar analysis: a case report. *Pediatr Gastroenterol Hepatol Nutr*. 2017;20(1):65–70.
[237] Peiretti V, Mussa A, Feyles F, et al. Thyroid involvement in two patients with Bannayan-Riley-Ruvalcaba syndrome. *J Clin Res Pediatr Endocrinol*. 2013;5(4):261–265.
[238] Tatton-
Brown K, Pilz D.T, Orstavik K.H, Patton M, Barber J.C overgrowth syndrome: a newly recognized phenotype associated with overgrowth, learning difficulties, characteristic facial appearance, renal anomalies and increased dosage of distal chromosome 15q. *Am J Med Genet Part A*. 2008;149A:147–154.
[239]
Dolan M, Mendelsohn N.J, Pierpont M.E, Schimmenti novel microdeletion/microduplication syndrome of 19p13.13. *Genet Med*. 2010;12(8):503–511.
[240]
Caputo V, Bocchinfuso G, Castori M, Traversa A, Pizz SMAD4 mutation causing Myhre syndrome. *Am. J.*

*Med. Genet. A*. 2014;164A:1835–1840. doi: 10.1002/ajmg.a.36544.

[241] Rooryck C, Diaz-Font A, Osborn D.P.S, Chabchoub E, Hernandez-Hernandez V, Shamseldin H, Kenny J, Waters A, Jenk Kaissi A, Leal G.F, Dallapiccola B, 9 others. Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. *Nature Genet*. 2011;43:197–203.

[242] Graul-Neumann L.M, Mensah M.A, Klopocki E, Uebe S, Eki intragenic deletion in MASP1 in an adult female with 3MC syndrome. *Eur J Med Genet*. 2018 Jul;61(7):363–368. doi: 10.1016/j.ejmg.2018.01.016 Epub 2018 Jan 31. PubMed PMID: 29407414.

[243] McClenaghan C, Hanson A, Sala-Rabanal M, Roessler H.I, Josifova D, Grange D.K, van Haaften G, Nichols C.G. Cantu syndrome-associated SUR2 (ABCC9) mutations in distinct structural domains result in K(ATP) channel gain-of-function by differential mechanisms. *J Biol Chem*. 2018 Feb 9;293(6):2041–2052. doi: 10.1074/jbc.RA117.000351 Epub 2017 Dec 22. PubMed PMID: 29275331; PubMed Central PMCID: PMC5808765.

[244] Silhanova E, Plevova P, Curik R, Kaspercik I, Krepelo syndrome--a case report. *Am. J. Med. Genet.* 2006;140A:2223–2226.

[245] Gawliński P, Pelc M, Ciara E, Jhangiani S, Jurkiewicz

Świątkowska A, Dawidziuk M, Coban-Akdemir Z.H, Guilbride D.L, Muzny D, Lupski J.R, K Walasek M. Phenotype expansion and development in Kosaki overgrowth syndrome. *Clin Genet*. 2018 Apr;93(4):919–924. doi: 10.1111/cge.13192 PubMed PMID: 29226947.

[246] Maccabruni A, Zaramella M, Pedrotti L, Lucanto S, Q disorders in children and adolescents with chronic HCV infection. *Clin Cases Miner Bone Metab*. 2014 May;11(2):99–36104 Review. PubMed PMID: 25285135; PubMed Central PMCID: PMC4172190.

[247] Shapiro-Mendoza CK, Rice ME, Galang RR, Fulton AC, VanMaldeghem K, Prado MV, Ellis E, Anesi MS, Simeone RM, Petersen EE, Ellington SR, Jones AM, Williams T, Reagan-Steiner S, Perez-Padilla J, Deseda CC, Beron A, Tufa AJ, Rosinger A, Roth NM, Green C, Martin S, Lopez CD, deWilde L, Goodwin M, Pagano HP, Mai CT, Gould C, Zaki S, Ferrer LN, Davis MS, Lathrop E, Polen K, Cragan JD, Reynolds M, Newsome KB, Huertas MM, Bhatangar J, Quiñones AM, Nahabedian JF, Adams L, Sharp TM, Hancock WT, Rasmussen SA, Moore CA, Jamieson DJ, Munoz-Jordan JL, Garstang H, Kambui A, Masao C, Honein MA, Meaney-Delman D. Zika Pregnancy and Infant Registries Working Group. Pregnancy Outcomes After Maternal Zika Virus Infection During Pregnancy - U.S. Territories, January 1, 2016-April 25, 2017. MMWR Morb Mortal Wkly Rep 2017 Jun 16;66(23):615–621.

[248] Environmental Tobacco exposure and childhood

respiratory function, Vanker A, Gie R.P, Zar H.J. The association between environmental tobacco smoke exposure and childhood respiratory disease: a review. *Expert Rev Respir Med*. 2017 Jun 14:1–13.

[249] Alderton G.K, Galbiati L, Griffith E, Surinya K.H, Nei of mitotic entry by microcephalin and its overlap with ATR signalling. *Nat Cell Biol*. 2006;8(7):725–733.

[250] Chetty S, Norton M.E. Obstetric care in women with genetic disorders. *Best Pract Res Clin Obstet Gynaecol*. 2017 Mar 18 doi: 10.1016/j.bpobgyn.2017.03.006 pii: S1521–6934(17)30053-6 Review.

[251] Tatton-Brown K, Zachariou A, Loveday C, Renwick A, Maha Schaapveld D, Blyth M, Bouma M, Breckpot J, Crabb I Daire V, Fauth C, Fisher R, Gener B, Goudie D, Homfi Borri C, Koolen D, Kumar A, Labilloy A, Lees M, Mar Ecob R, Pilz D.T, Posmyk R, Prada C, Ramsey K, Ranc Maldergem L, Varghese V, Veenstra-Knol H.E, Yachelevich N, Yates L, Clinical Assessment of the Utility of Sequencing and Evaluation as a Service (CAUSES) Research Study, Deciphering Developmental Disorders (DDD) Study, Rahman N. The Tatton-Brown-Rahman Syndrome: A clinical study of 55 individuals with de novo constitutive DNMT3A variants. *Wellcome Open Res*. 2018 Apr 23;3:46. doi: 10.12688/wellcomeopenres.14430.1.eColle PMID: 29900417; PubMed Central PMCID: PMC5964628.

## Further Reading


Balzeau J, Menezes M.R, Cao S, Hagan J.P. The LIN28/let-7 pathway in cancer. *Front Genet*. March 28, 2017;8(31). https://doi.org/10.3389/fgene.2017.00.031 eCollection 2017. Review.

Giuliano S, Iadarola P, Leva V, Montecucco A, Camerini S, Crescenzi M insight into the abundant proteome of 46BR.1G1 fibroblasts deficient of DNA ligase I. *Electrophoresis*. January 2012;33(2):307–315 PubMed PMID: 22,222,975. https://doi.org/10.1002/elps.201.100.332.

Huber C, Dias-Santagata D, Glaser A, O'Sullivan J, Brauner R, Wu K, Xu X, Pearce F Furga A, Dos Santos H, Mégarbané A, Morin G, Gillessen-Kaesbach G, Hennekam R, Van der Burgt I, Black G.C, Clayton P.E, Read A, Le Merrer M, Scambler P.J, Munnich A, Pan Z.Q, Winter R, Cormier-Daire V. Identification of mutations in CUL7 in 3-M syndrome. *Nat Genet*. October 2005;37(10):1119–1124 PI3K/AKT/mTOR pathway.

Liang Y, Gao H, Lin S.Y, Goss J.A, Du C, Li K. Mcph1/Brit1 deficiency promotes genomic instability and tumor formation in a mouse model. *Oncogene*. 2015;34(33):4368–4378.

Malan V, Chevallier S, Soler G, et al. Array-based comparative genomic hybridization identifies a high frequency of copy number variations in patients with syndromic overgrowth. *Eur J Hum Genet*. 2010;18(2):227–232. doi: 10.1038/ejhg.2009.162.

Nathan N, Keppler-Noreuil K.M, Biesecker L.G, Moss J, Darling T.N. Mosaic disorders of the PI3K/PTEN/AKT/TSC/mTORC1 signaling pathway. *Dermatol Clin*. January 2017;35(1):51–60. https://doi.org/10.1016/j.det.2016.07.001 Review.

Palovcak A, Liu W, Yuan F, Zhang Y. Maintenance of genome stability by Fanconi anemia proteins. *Cell Biosci*. 2017;8:7. https://doi.org/10.1186/s13578-016-0134-2. .

Proteins
Review1 Fanconi, Palovcak A, Liu W, Yuan F, Zhang Y. Maintenance of genome stability by Fanconi anemia proteins. *Cell Biosci*. 2017:7–8. https://doi.org/10.1186/s13578-016-0134-2.

Zheng G, Ouyang Z, Yu H. Biochemical and functional assays of human cohesin-releasing factor Wapl. *Methods Mol Biol*. 2017;1515:37–53.

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CHAPTER 5

# Cytogenetic Analysis

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## Abstract

The field of human cytogenetics has had steady growth since the discovery of the correct chromosome number in humans (46) in 1956. Since that time, our understanding of the importance of chromosome abnormalities to human health and disease has grown steadily, almost exponentially over the past 60 years. We review the historical trajectory of cytogenetics and introduce chromosome analysis methods, including chromosome banding, fluorescence in situ hybridization, chromosome microarray analysis, and, most recently, exome and genome sequencing. Recent advances are serving to blur the line between molecular and cytogenetic testing, with the result that the known contribution of abnormalities of the chromosomes to human disease is steadily increasing. The body of knowledge uncovered through the use of these techniques is reviewed, as an introduction to the more specific abnormalities and syndromes discussed throughout the volumes of this text.

## Keywords

Chromosome banding; Chromosome microarray analysis (CMA); Chromosomes; Copy number variation; Cytogenetics; Fluorescence in situ hybridization (FISH); Next-generation sequencing

## 5.1. Introduction

"Cytogenetics" traditionally refers to the study of chromosomes with the use of microscopy following the application of banding techniques, permitting the identification of abnormalities of chromosome number, loss or gain of chromosomal material, or positional changes. However, advances during the past 30 years have resulted in an increased reliance on molecular techniques such that the current field is a hybrid of microscopic and molecular technologies. Because chromosomes carry the genetic material, an understanding of their behavior, structure, and function is essential to an understanding of genetics. Visible or submicroscopic changes in the number and structure of chromosomes have been associated with a broad spectrum of disease, and the analysis of chromosomes has been an important tool in medical genetics for a number of decades. Several key technical advances in chromosome analysis include fluorescence in situ hybridization (FISH), first introduced in the mid 1980s, and then array-based genomic analysis or chromosomal microarray analysis (CMA), introduced in the 1990s, and brought into clinical practice beginning around 2003 [1,2,22]. This chapter describes the most useful techniques available for chromosome analysis. In recent years, array-based techniques have become the first line of investigation for analysis of chromosome abnormalities, with cytogenetic and FISH tools used to help interpret the results of imbalances diagnosed by array. However, array-based techniques generally identify only imbalances resulting in deletions or duplications and not copy neutral structural variation such as balanced translocations, inversions, or ring chromosomes. Structural variants are most easily identified by standard chromosome analysis,

but techniques for the identification of these alterations using exome or genome sequencing data have been emerging and are coming into more common use [3].

## 5.2. Milestones in Human Cytogenetics

Human chromosomes were probably first observed by Arnold in 1879 in dividing tumor cells. Hansemann in 1891 and Flemming in 1898 attempted to count the number of chromosomes in serial sections of dividing cells and obtained crude estimates of about 24. Quite different results were reported by de Winiwarter in 1912. He seems to have been the first to study sections of gonadal tissue, and he found counts of 47 in testes and 48 in ovary. He interpreted these results as indicating that, like some invertebrates, males had a single X chromosome and females had two. In 1921, Painter demonstrated in testis sections the presence of an additional small Y chromosome that was overlooked by de Winiwarter. Although he assumed that 48 was the correct chromosome number in both sexes, it is interesting to note that in his 1921 report he states that he could count only 46 chromosomes in the clearest mitotic figures. In a report published in 1923, Painter predicted the existence of individuals with unusual combinations of sex chromosomes, in particular intersexes with an XXY sex chromosome complement. No one seems to have tested this idea until 1942, when Severinghaus described an XY sex chromosome constitution in XY DSD. Largely as a result of the unfavorable material available for studying human chromosomes, there were few reports on this subject until the early 1950s. An important exception was a report by Koller (1937) [4] in which the X–Y pairing segment in human meiosis is described for the first time and the possibility of partial sex linkage is raised. The possibility that pathologic disorders might be due to abnormalities of chromosome number and structure seems to have been suggested first by Boveri. He described his theory on the origin of cancer from chromosomal aberrations in a classic monograph published in 1914. Then, 46 years later, the first specific chromosome abnormality associated with malignancy was described, namely, the

Philadelphia chromosome in chronic myeloid leukemia [5]. With regard to constitutional chromosome abnormalities, Waardenburg in 1932 was one of the first to suggest that Down syndrome might be due to a numeric chromosome aberration resulting from nondisjunction.

Human cytogenetics became a practical proposition with the discovery by Tjio and Levan [6] that the correct chromosome number was 46 and not 48 [6]. Both authors were experienced cytologists who exploited the availability in their laboratory of fetal tissue culture from abortus material. Levan had earlier introduced into plant cytogenetics the use of colchicine to arrest and accumulate mitoses at metaphase, and he knew of the effect of hypotonic solutions to separate individual chromosomes from one another by pretreatment before fixation. The hypotonic technique had been discovered independently by four scientists: Hsu (1952) in the United States, Makino and Nishimura (1952) in Japan, and Hughes (1952) in England. Apparently, both Hsu and Makino made the discovery fortuitously, after mistakenly adding hypotonic instead of isotonic salt solution during the washing stage before fixation. After fixation, the technique current at that time was to squash the dividing cells between the slide and coverslip so that the chromosomes could be spread in one optical plane. A variety of nuclear stains, including aceto-orcein, Giemsa, and Feulgen, were used to stain the chromosome preparations. All these methods, especially tissue culture, contributed to the rapid developments in human cytogenetics in the following years.

The chromosome number of 46 was soon confirmed in testis material by Ford and Hamerton (1956) [7]. Two years later, Ford and colleagues (1958) [8] introduced a method for chromosome analysis using bone marrow samples that was suitable for clinical studies in that it exploited the presence of actively dividing cells in that material. At about the same time, Lejeune and his colleagues were reinvestigating the chromosomes in Down syndrome from fibroblast cultures [9]. Their discovery of trisomy for one of the smallest autosomes was announced in 1958, although the description in nine patients was not published until early in 1959. Simultaneously, interest in the chromosomes of the Turner and Klinefelter syndromes

was prompted by the paradoxical nuclear sex chromatin (Barr body) findings in 1954 suggesting that amenorrheic women with Turner syndrome might be genetic males and that infertile men with Klinefelter syndrome might be genetic females. The latter seemed to be confirmed by Ford and colleagues (1958) [8], who found one Klinefelter patient to have an apparently normal female chromosome constitution on bone marrow preparations [7]. However, Ford reported in 1959 that Turner syndrome was usually associated with a 45, X chromosome complement and Jacobs and Strong found that Klinefelter syndrome had a 47, XXY complement [8]. The important conclusion from these studies was that human sex differentiation was determined by the Y chromosome and not by the number of X chromosomes.

It came as a surprise to clinicians that such gross numeric chromosome abnormalities could be associated with viability. There followed intense activity worldwide to determine whether other dysmorphic conditions were due to chromosomal abnormalities visible under the microscope. Trisomies 13 and 18 were quickly identified, followed by several instances of sex chromosomal mosaicism, translocation Down syndrome, and the deletion of the short arm of chromosome 5, which causes the cri du chat syndrome [9].

The search for new examples of conditions that are due to chromosome aberrations was accompanied by strenuous attempts to simplify techniques and improve the identification of individual chromosomes so that smaller structural changes could be detected. The most important of these early technical developments was the introduction of phytohemagglutinin, which allowed chromosome preparations to be made within 2–3 days from peripheral blood samples [10]. This reagent was originally used to clear red cells from preparations of lymphocytes, but it was found that T lymphocytes underwent transformation and division under its influence. When colchicine was used to accumulate lymphocytes in metaphase during short-term culture, air-dried drop preparations of metaphase

chromosomes could be made far superior to any previous method. The simplicity of the technique, which is still in use almost unchanged, has undoubtedly been responsible for the widespread application of chromosome analysis throughout the world and for the growth of human cytogenetics as a diagnostic procedure in clinical medicine. In the 1960s, individual chromosomes were identified by characteristics such as total length, centromere index (length of short arm divided by total length), the presence of heterochromatic regions, and the pattern of DNA replication as revealed by pulse labeling with tritiated thymidine. These studies revealed considerable normal variation in chromosome size and centromere position, much of which was heritable and of no clinical significance. Only chromosomes 1, 2, 3, 9, and 16 and the Y chromosome could be identified with certainty in any one metaphase by standard techniques. Chromosomal heteromorphisms mainly involved the centromeres of chromosomes 1, 9, 16 (and occasionally chromosomes 3 and 4); the short arms and satellites of chromosomes 13, 14, 15, 21, and 22; and the distal heterochromatic region of the long arm of the Y chromosome.

In 1970, two new techniques were introduced that have had a major impact on modern cytogenetic analysis. The first was the demonstration by Pardue and Gall [11] that isotopically labeled DNA probes could be annealed to complementary DNA sequences in cytologic preparations of chromosomes made by standard techniques, a procedure referred to as in situ hybridization (ISH). Pardue and Gall also noted that when the denatured chromosomes were stained by Giemsa, the paracentric regions were preferentially stained (C-bands; see later). Various modifications of the denaturing and staining process by other workers following Pardue and Gall yielded chromosomes that showed patterns of differential staining along their length, which appeared specific for each chromosome. These experiments were extended and led to the second major development, the establishment of a standard chromosome banding pattern, and this has become the criterion for chromosome identification and classification in many species.

Even before chromosome denaturation was being used to produce various banding patterns, Caspersson and colleagues independently discovered that quinacrine compounds that intercalate in DNA could produce bright fluorescent bands visible along the chromosome using fluorescence microscopy [12]. The quinacrine bands (Q-bands) were at first more reproducible than those produced by denaturing and Giemsa staining but yielded virtually the same banding pattern and were equally useful for chromosome identification.

Cytogenetic analysis has evolved from these beginnings, the main ingredients being cell culture, colchicine treatment to accumulate metaphases, manipulations of the cell cycle to enhance chromosome morphology, hypotonic treatment to separate the chromosomes from one another, fixation in suspension followed by air drying, banding methods for chromosome identification, and ISH for the localization of specific DNA sequences. The growing emphasis on timely management of patients and the detection of chromosome abnormalities beyond the resolution of the light microscopy in recent years has led to the development of targeted molecular cytogenetic techniques freed from the cell culturing and lengthy protocols used for the preparation of high-quality metaphase spreads. These new technological advances allow quantitative evaluation of the chromosomal content and include methods such as quantitative fluorescence ISH (FISH) and polymerase chain reaction (Q-PCR), comparative genomic hybridization (CGH) and array CGH, multiplex amplifiable probe hybridization (MAPH), and multiplex ligation-dependent probe amplification (MLPA) [13,14]. These methods allow higher-resolution chromosome analysis and at the same time are more amenable to automation and high throughput of the samples than are traditional methods.

FISH-based techniques provided a bridge between the chromosomes, as viewed under the microscope and the DNA that composes the chromosomes. FISH vastly improved our ability to diagnose small aberrations and to characterize marker chromosomes not identifiable by banding [15]. FISH was introduced to cytogenetics in the mid 1980s, just as the field of molecular biology was being

revolutionized by advances in DNA analysis, most spectacularly, the PCR. As the human genome project progressed through the 1990s, more and more FISH probes became available, increasing our power to diagnose clinically recognizable syndromes. In general, FISH studies are limited by the need to know which region of the genome should be analyzed (disease-related critical regions, specific chromosomes) and cannot be used to survey the entire genome at high resolution. A partial exception to this was the development of a subtelomere assay, in which the subtelomeric regions of all of the human chromosomes were analyzed sequentially in one assay. The subtelomeric probes were developed in response to the hypothesis that polymorphisms in human telomeric region are more prone to breakage and reunion and could play a role in cryptic translocation events that underlie a significant percent of human pathology [16,17]. As probe sets for each of the human subtelomeric regions became available, they were used in screening cohorts with varying criteria (mental retardation, with and without dysmorphic features, other congenital anomalies, family history of related abnormalities), and varying but significant percentages of patients with subtelomeric imbalances were identified, ranging from 3% to 29% [17–20].

Comparative genomic hybridization (CGH) was the next major advance in genomic analysis, and it provided a tool to determine the amount of DNA in specific genomic regions (thereby diagnosing deletions or duplications) on a molecular level. Initial methods allowed comparison of DNA content from a patient, with a normal control, by fragmenting the two genomes, labeling test subject and control with fluorescent dyes of different wavelengths, and co-hybridizing to chromosomes from a normal individual. The ratio of fluorescence of the two colors is measured along the length of each chromosome, and relative gains or losses were recorded as deviations from the 50:50 mixture anticipated if the test subject is normal [21]. Because this technique uses DNA, there is no need to culture cells, which is an advantage in analyses of tumors or in any case where living tissue is not available. The resolution of CGH done in this fashion, however, was not higher than that of analyses of metaphase

chromosomes when applied to constitutional cytogenetics. Nevertheless, CGH paved the way for array-based CGH, in which DNA from both patient and control is co-hybridized against DNA that has been spotted onto an array [22]. With array CGH, the choice of which clones to place on the array lies in the hands of the investigator and can range from selected clones covering specific regions of the genome to a tiling path of the entire genome [23]. For example, targeted arrays could interrogate known disease regions including subtelomeric regions [24,25]. The array can use large pieces of DNA such as inserts of human DNA into bacterial artificial chromosomes (BACs), smaller DNA fragments (oligonucleotides) or polymorphic regions of the genome such as single nucleotide polymorphisms (SNPs). The use of high-resolution arrays is presenting unique opportunities to explore changes in DNA copy number, in both health and disease. Both array CGH and SNP array analysis have surprisingly identified a significant fraction of the genome that can vary in copy number between apparently healthy individuals [26–29]. The current challenge in the cytogenetics/cytogenomics laboratory is to incorporate these technological advances to identify clinically significant copy number alterations, while avoiding the copy number alterations that are true polymorphisms.

## 5.3. Indications for Cytogenetic Analysis

The history of human cytogenetics during the past 55 years has been punctuated by the introduction of new technology followed by the identification of increasing numbers of aberrations, smaller in extent and often associated with less-striking phenotypic changes. The indications for cytogenetic analysis have thus widened over the years. For example, the occurrence of a monogenic disorder associated with mild mental handicap may indicate the presence of a microdeletion detectable array-based analysis or of DNA disruption at one of the breakage sites of a reciprocal translocation. A list of general indications for cytogenetic analysis includes the following:

1. Confirmation or exclusion of the diagnosis for known chromosomal syndromes
2. Unexplained intellectual disability or developmental delay with or without dysmorphic features
3. Autism spectrum disorders
4. Congenital anomalies
5. Abnormalities of sexual differentiation and development
6. Short stature
7. Infertility/subfertility
8. Recurrent miscarriage or stillbirth
9. Pregnancies shown to be at risk of aneuploidy from the results of maternal serum screening or fetal ultrasound scanning
10. Neoplastic conditions for which the identification of specific chromosomal aberrations may be valuable in diagnosis and management

See chapters on chromosomal syndromes for full descriptions.

## 5.4. Tissue Samples and Cell Culture

Chromosome preparations for cytogenetic analysis are made from dividing cells, either directly from tissue samples (e.g., bone marrow, testis, chorionic villi, neoplastic tissue) or after cell culture (biopsy of skin or almost any other living tissue including amniotic fluid cells). Tissue preserved in a fixative is not suitable for making conventional chromosome preparations. It is imperative to use the correct sterile containers when sending biopsy and other tissues to the cytogenetics laboratory to ensure that the cells remain viable and suitable for cell culture. Paraffin-embedded tumor tissue sections can be "FISHed" for deletions/amplification of tumor suppressor genes and oncogenes to assist in diagnosis, prognosis, and management after therapy.

For array-based techniques, dividing cells are not necessary, and all that is needed is a source of DNA. This can come from peripheral blood or tissue samples, and techniques are improving to obtain the DNA from paraffin-embedded tissue sections.

## 5.5. Chromosome Banding

For identification of normal chromosomes and detection of aberrations, the air-dried chromosome preparations must be stained appropriately. Chromosome banding refers to alternating light and dark regions along the length of a chromosome, produced after staining with a dye. A band is defined as the part of a chromosome that is clearly distinguishable from its adjacent segments by appearing darker or lighter with the use of one or more banding techniques. The fluorescent dye quinacrine was used to produce the first banding patterns as mentioned earlier (Q-banding), and through the years several general banding stains, as well as several techniques for staining specific parts of the genome, have been used. Generalized banding techniques have included Q-banding, reverse banding, and G-banding [12,30,31]. Specific staining protocols include C-banding and staining of the nucleolar organizing regions (NORs) using a silver stain (Ag-NOR). G-banding is the benchmark for the routine analysis of human chromosomes, producing a characteristic light-and-dark banding pattern along the chromosomes (Fig. 5.1). Each chromosome has a unique sequence of bar code–like stripes, allowing identification of individual homologues and the analysis of abnormalities of their structure by disruption of the normal banding pattern. Many methods have been published for the production of G-bands, but most rely on proteolytic digestion of the chromosomes by trypsin followed by staining with either Giemsa or Leishman stain. Little is known of the precise nature of the mechanism of G-banding despite much conjecture. The dark bands contain mainly A-T–rich DNA, and the light bands are G-C rich. Manipulation of the cell cycle to produce prometaphase chromosomes with resolution of >550 G-bands per haploid set provides a mechanism for high-resolution analysis of the structure of the chromosomes.

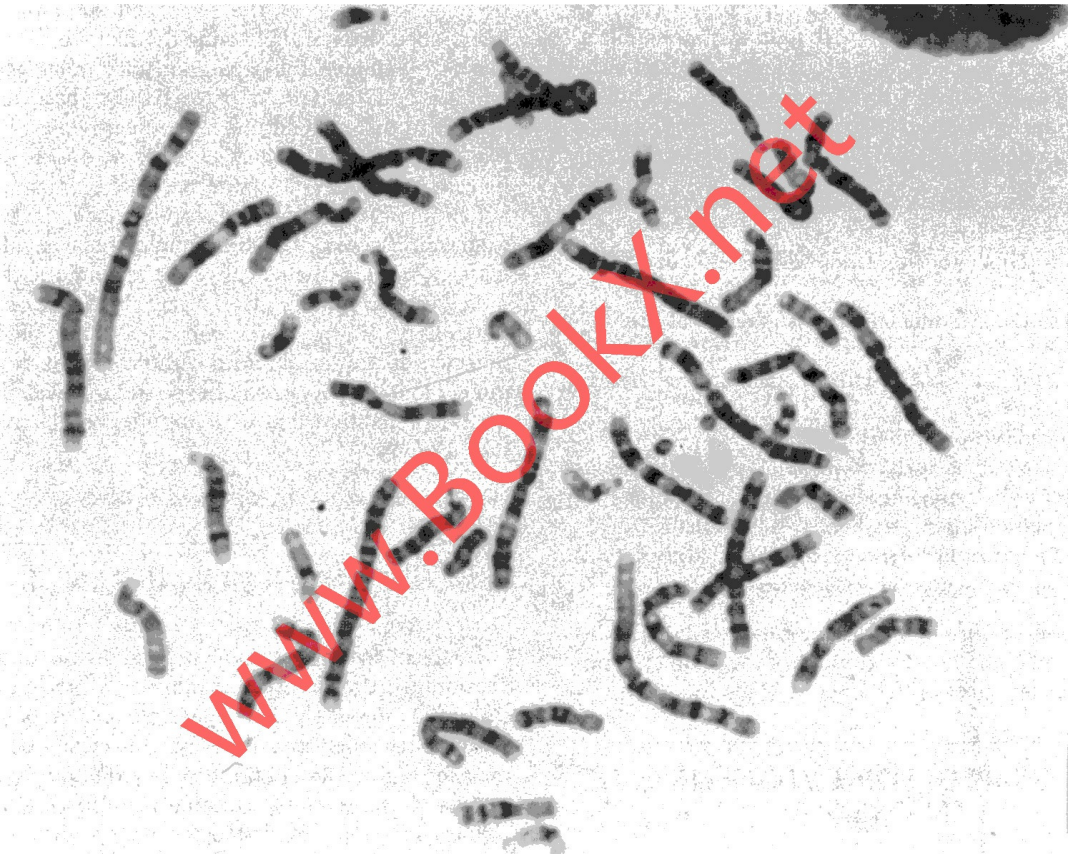

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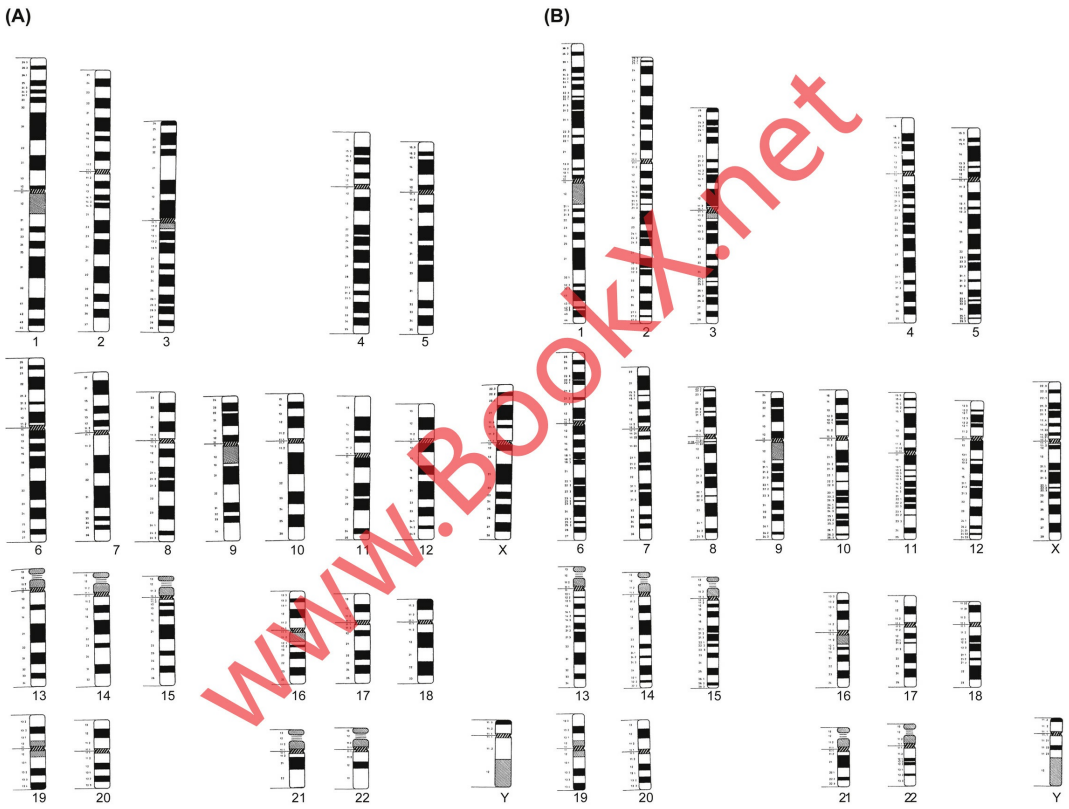
**FIGURE 5.1** G-banded metaphase with three marker chromosomes (whose origin from chromosomes 22, 11, and 14 cannot be identified by this technique).

## 5.6. Normal Human Karyotype

Each species has a chromosome complement, characteristic in number and form. This is known as the karyotype, and the name is also used for a photographic preparation in which the stained chromosomes are arranged approximately in order of decreasing length. Conventional banding allows each normal human chromosome to be given its specific identity number. To make analysis easier, the banding pattern can be diagrammatically represented in the form of an idiogram (Fig. 5.2). About 400 distinct bands per haploid complement usually may be visible in any one metaphase preparation. In prometaphase cells, >550 bands may be resolved because the chromosomes are more extended. The International System for Human Cytogenetic Nomenclature (ISCN) was established in 1978 by a standing

committee to provide a simple shorthand, based on the human banded idiogram, to describe any given chromosome aberration and the karyotype of any individual, and has been updated at regular intervals since that time [32]. In this nomenclature, the number of chromosomes in the karyotype is given first, then the sex chromosome constitution, followed by a shorthand description for any abnormal chromosome. Thus, 46, XY is a normal male karyotype, and 47, XX, +21 is the karyotype of a female with Down syndrome. Parts of chromosomes or sites of chromosome rearrangement are identified by using a simple numbering system specific for each chromosome band or sub-band. In this description, the chromosome identifier is given, followed by the designation for the chromosome arm, p (petit) for the short arm and q for the long arm, and then the number of the chromosome band involved in the rearrangement. The main landmarks of each chromosome are the centromere (cen) and the end of the arm, pter for the short arm and qter for the long arm. The most striking of the bands are the remaining landmarks; these divide the arm into distinct regions. Each region is further subdivided into bands and sub-bands. Thus, band Xp21.2 is to be found in the short arm of the X chromosome in region 2, band 1, and sub-band 2. The shorthand for the exchange of chromosome fragments between 7p21.2 and, for example, 9q34.1 in a female individual would be given as 46, XX, t

(7;9) (p21.2;q34.1), where t = translocation and the semicolon is used to separate the chromosomes and break points. These and other symbols used are listed in Table 5.1. The ISCN provides nomenclature to describe constitutional and acquired chromosome abnormalities and FISH studies (further details of ISH nomenclature are given in the section on FISH). Examples of the use of the shorthand are given in the following section, where the classification of chromosome abnormalities is considered.




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**FIGURE 5.2** Human idiogram based on G-banding pattern and showing the banding nomenclature according to the International System for Human Cytogenetic Nomenclature [32]. (A) Approximately 400 of the main G-bands per haploid complement are shown, as seen in most banded metaphase preparations suitable for cytogenetic analysis. (B) Approximately 550 G-bands per haploid complement are shown, as seen in early metaphase preparations.

## Table 5.1

### International System for Human Cytogenetic Nomenclature (ISCN) Nomenclature: Symbols and Abbreviations Used for Describing Chromosome Aberrations

| Symbol | Definition |
|---|---|
| **p** | Short arm |
| **q** | Long arm |
| **pter** | Terminal of short arm |
| | |

| | |
|---|---|
| **qter** | Terminal of long arm |
| **cen** | Centromere |
| **h** | Heterochromation |
| **add** | Additional material of unknown origin |
| **del** | Deletion |
| **der** | Derivative of a translocation |
| **dic** | Dicentric |
| **dup** | Duplication |
| **fra** | Fragile site |
| **i** | Isochromosome |
| **isodic** | Isodicentric |
| **inv** | Inversion or inverted |
| **r** | Ring chromosome |
| **rec** | Recombinant from inversion or insertion |
| **t** | Translocation |
| **upd** | Uniparental disomy |
| **mat** | Maternal origin |
| **pat** | Paternal origin |
| **mar** | Marker chromosome of unknown origin |
| **dir** | Direct as opposed to inverted |
| **::** | Breakage with reunion |
| **/** | Mosaicism (separates clones) |
| **+/−** | Before a chromosome number, indicates gain or loss of that chromosome |
| **;** | Separates altered chromosomes and break points involved in |

| | |
|---|---|
| | structural rearrangements—indicates from and to |

Chromosome analysis in normal individuals has revealed variation in the karyotype that is without phenotypic effect. It is important to recognize and distinguish this normal variation from the abnormal chromosomal rearrangements that are clinically significant. The most striking of these variations, or heteromorphisms, occur at the centromeric regions of chromosomes 1, 9, and 16, at the short arms of chromosomes 13, 14, 15, 21, and 22, and at the distal end of the long arm of the Y chromosome. All these regions contain varying amounts of highly repetitive DNA (satellite DNA) composed largely of tandemly arranged repeats that are not transcribed. Unequal crossing-over within these repeats during meiosis may account for the variation, but this must occur rarely, because extreme examples of these heteromorphisms are invariably transmitted unchanged in pedigrees. Several euchromatin heteromorphisms have also been described that may present a specific diagnostic concern particularly when detected at prenatal diagnosis or in an individual with an abnormal phenotype. Euchromatic variants at chromosomes 8p23.1, 9p12 and qh, 15q11.2, and 16p11.2 have been characterized on a molecular level and found to comprise region-specific pseudogene cassettes containing processed paralogous sequences frequently dispersed/transposed to or from pericentromeric or protelomeric sites [33,34].

## 5.7. Chromosome Abnormalities

Genetic mutations involve duplication, deletion, or rearrangement of DNA. The extent of the change varies from the gain or loss of a single nucleotide (a point mutation) to the gain or loss of whole chromosomes. When the field of genetic analysis relied on either chromosome analysis or DNA analysis, those changes that were large enough to be detectable under the light microscope were classified as chromosome aberrations, but they differed from molecular mutations involving individual genes only in terms of scale. In general, molecular techniques generally involve analysis of single genes and

focus on mutations that are intragenic, while cytogenetic methods involve larger genomic alterations affecting multiple genes. However, with the introduction of increasingly sensitive techniques into the cytogenetics laboratory, we are able to diagnose single gene deletions, or intragenic deletions or duplications, and the divisions between the cytogenetic and molecular diagnostics are blurring. Historically, it has been possible to identify genomic deletions or duplications that are a minimum of 5–10 Mb with the use of cytogenetic techniques, although there are many cases where even deletions or duplications slightly greater than 10 Mb were missed by analysis of banded chromosomes. Array-based techniques provide resolution that is much finer, and depending on the probe density, some arrays can diagnose single exon changes. Currently, many laboratories use standard cutoffs of 100–200 kb for the diagnosis of deletions and 500 kb to 1 Mb for duplications. There are several chapters in this book that deal with the specifics of chromosome abnormalities, including trisomy and triploidy, structural chromosome abnormalities such as translocations (balanced or unbalanced), Robertsonian translocations, isochromosomes, insertional translocations, deletions, duplications, inversions, marker chromosomes, and ring chromosomes (see chapter on cytogenomic abnormalities). As techniques for cytogenetic analysis have evolved, the types of chromosome abnormalities that are detectable have changed as well. Initially, only larger abnormalities such as gain or loss of an entire chromosome, or large alterations, visible on banding, could be diagnosed.

## 5.8. In Situ Hybridization

The ISH technique is based on the principle that when double-stranded DNA is heated, it denatures into single-stranded DNA. On cooling, the single-stranded DNA reanneals with its complementary

sequence into double-stranded DNA. If an appropriately labeled DNA sequence (probe) is denatured and added to denatured nuclei, or chromosomes during the process of reannealing, some of that labeled DNA will hybridize to its complementary sequence in the chromosomal DNA. Detection of the labeled DNA will identify the site of hybridization and thus the region of chromosomal DNA complementary to the DNA sequence in the labeled probe. Therefore, ISH may be used to map and order genes and other DNA sequences along the length of a chromosome.

In the early days of ISH, DNA probes were labeled with radioisotopes, such as tritium ($^{3}H$) or radioactive iodine ($^{125}I$), and detected with autoradiography using photographic emulsion applied directly to the microscope slide. The scatter of radioactive disintegrations, revealed by silver grains in the developed emulsion, meant that the resolution was poor. The autoradiographs often had to be exposed for several weeks to achieve an adequate signal; therefore, it was not possible to tell whether the probe had hybridized satisfactorily until long after the experiment had been set up. These disadvantages, coupled with the hazards of radioisotopes and the need to count silver grains in a large number of cells to establish significant counts above background levels, led to the search for nonisotopic methods of labeling.

ISH was used first to map the chromosomal location of highly repetitive (satellite) DNA to the centromeric regions of mouse chromosomes and the moderately repetitive ribosomal DNA to the nucleolus organizing regions in the polytene chromosomes of Diptera. Similar studies soon located these repetitive DNAs to the equivalent sites in human chromosomes [35]. However, it was not possible to incorporate sufficient radioactivity in probes derived from single-copy DNA sequences to permit the more extensive application of ISH in gene mapping. This had to await the introduction of recombinant DNA techniques and the development of DNA sequences cloned in plasmids that could be heavily labeled. The early 1980s saw the first chromosomal localizations of single-copy DNA sequences by ISH, and ISH soon became the method of choice for assigning a cloned DNA

sequence to its position in the chromosomal map [36,37]. It was quickly appreciated that ISH also had application in diagnostic cytogenetics, particularly for the detection of structural abnormalities beyond the resolution of conventional banding techniques. The search for nonisotopic alternatives for labeling probes for ISH led first to the introduction of biotin and then other haptens, including acetylaminofluorene and digoxigenin [38,39]. Additional fluors have been introduced into the laboratory, and a variety of methods have been developed to detect these labels in cytologic preparations using fluorescence or enzyme-linked reactions.

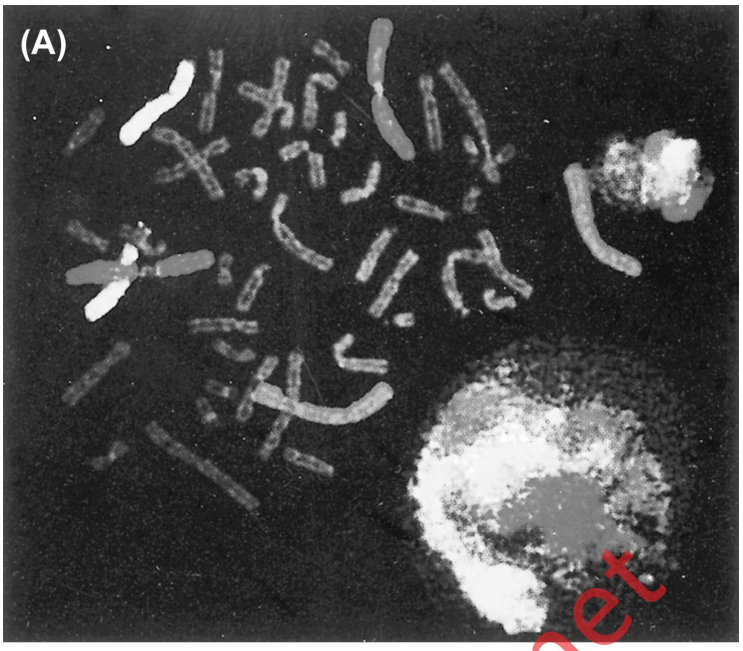
Many types of DNA probes used in FISH applications in diagnostic cytogenetics contain interspersed repetitive elements that will hybridize to complementary sequences throughout the entire genome, leading to a high level of background signals. This background can be suppressed effectively by preannealing unlabeled genomic or Cot-1 (enriched for highly repetitive sequences) DNA to the labeled probe before hybridizing the probe to the target DNA [40]. The unlabeled DNA acts as a competitor and will form duplexes rapidly with the repetitive DNA sequences within the probe. The low-copy DNA remaining in the probe will then be available to hybridize with complementary sequences in the target DNA. With many DNA probes, the use of Cot-1 DNA may be avoided by allowing the probe to preanneal with itself before application to the target.

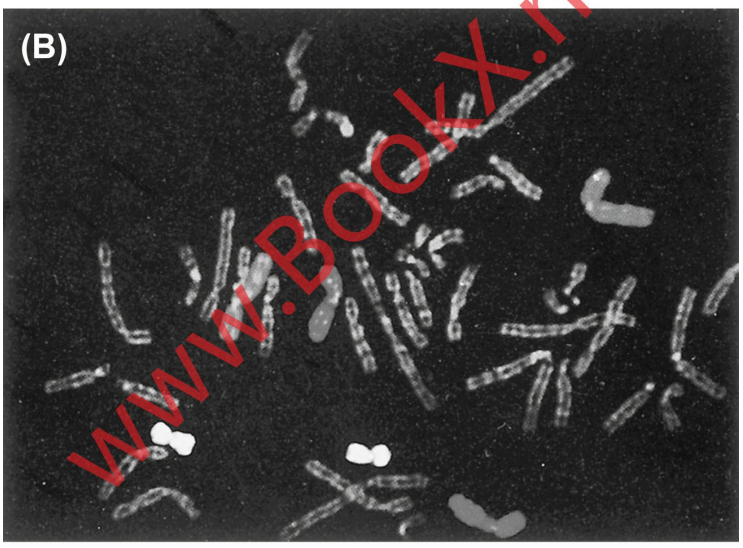
## 5.8.1. DNA Probes and Their Applications

The various classes of DNA probe have wide applications in diagnostic cytogenetics to augment conventional banding analyses of chromosomal rearrangements. When genomic analysis starts with array-based testing, FISH is often needed to obtain positional information, such as the chromosomal location of a duplicated region of the genome.

Collections of chromosome-specific painting probes (Fig. 5.3) derived from each chromosome usually by flow-sorting and less frequently by microdissection, and labeled end-to-end by nick translation or Degenerate oligonucleotide-primed (DOP)-PCR, are

routinely used as a screening tool in the characterization of an abnormal chromosome [41,42]. Centromeric probes on their own and in combination with single-copy DNA sequence probes are widely used in the determination of chromosome copy number in interphase nuclei or as control probes in interphase FISH analysis (Fig. 5.4). The main applications are in rapid aneuploidy tests for prenatal and preimplantation diagnosis, in characterization of marker chromosomes, and in the diagnosis of hematological cancers.



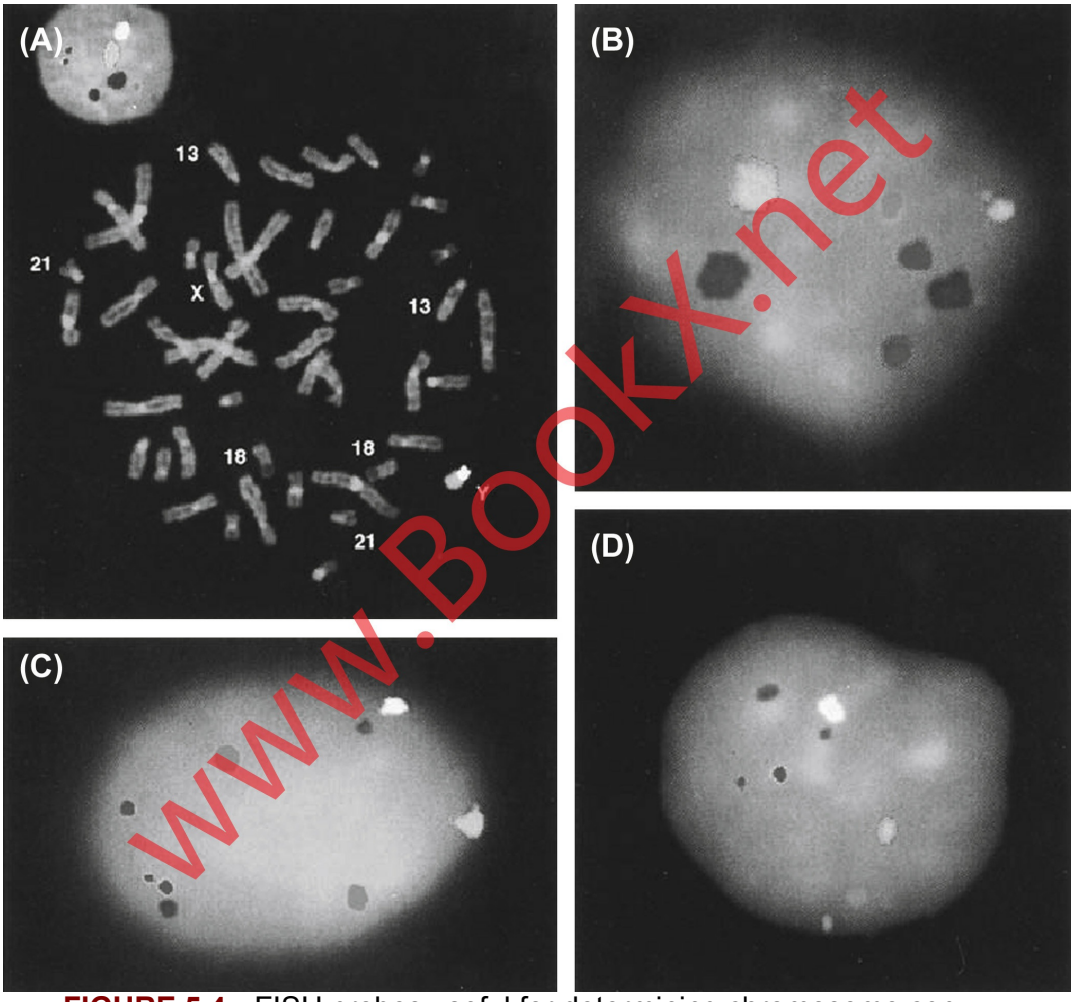







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**FIGURE 5.3** Chromosome painting using chromosome-specific probes (CAMBIO Ltd., Cambridge, England) from flow-sorted chromosomes (DAPI counterstain). (A) Chromosome 1 (red), chromosome 2 (green), and chromosome 6 (yellow). Interphase nucleus reveals chromosome domains within nucleus. (B) Chromosome 7 (green), chromosome 11 (red), and chromosome 20 (yellow). (C) X chromosome (red), Y chromosome (green). Note Y signal (yellow) on XY homologous regions of Xp (tip) and Xq (proximal third) and X signal (yellow) on XY homologous region of Yp (tip).




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**FIGURE 5.4** FISH probes useful for determining chromosome copy number in interphase nuclei. (A), Lymphocyte metaphase showing centromeric probes for X chromosome (lilac), Y chromosome (yellow), and chromosome 18 (blue); a YAC clone marks chromosome 13 (green) and a contig of two overlapping cosmids marks chromosome 21 (red). (B) Uncultured amniotic fluid cell nucleus from a female fetus hybridized with the above probes revealing normal copy number of

each chromosome. (C) As above, from male fetus with trisomy 21 (Down syndrome). (D) As above, from normal male fetus.
From Divane A, Carter NP, Spathas DH, Ferguson-Smith MA. Rapid prenatal diagnosis of aneuploidy from uncultured amniotic fluid cells using five-color fluorescence in situ hybridization. Prenat Diagn 1994;14:1061–69, with permission.

Thanks to the International Human Genome Project, a physical map of the human genome composed of a series of overlapping cloned DNA sequences along each chromosome is available in BAC vectors. These clones act as reference markers along the chromosome and are also suitable for FISH. Many other DNA sequences of interest, including sequences from known genes, have been cloned in yeast artificial chromosome (YAC) and cosmid vectors; both are suitable for FISH. YAC clones can accept inserts of up to 2 million base pairs and have the disadvantages that they may contain sequences from more than one chromosomal region (due to co-ligation) and seem more liable to lose parts of the insert through deletion. Cosmid clones can accept smaller inserts of up to 40 kb and do not have these disadvantages. Both types of clone can be used as markers for gene loci, for the delineation of break points, and for the identification of deletions and duplications associated with chromosomal syndromes. For example, cosmid clones are widely used for the diagnosis of microdeletion syndromes (see Table 5.2 and Fig. 5.5) and in screening for subtelomeric chromosomal imbalances (Fig. 5.6) found in 5%–10% of patients with dysmorphic features and developmental delay [31,34,44,53].

## Table 5.2

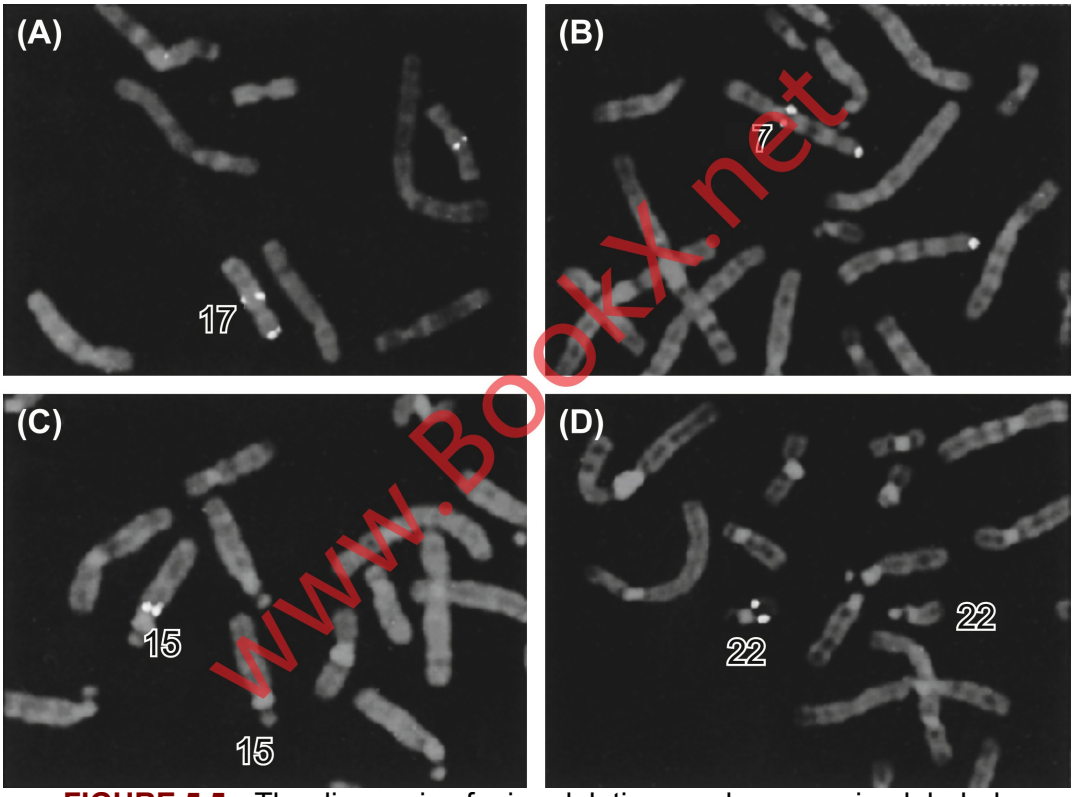
### Recurrent Segmental Deletions/Duplications Associated Syndromes

| Chromosome Band | Syndrome | Del/Dup | Assayed Genes/Loc |
|---|---|---|---|
| **1p36** | Monosomy 1p36 | del | *DVL1* |
| **2q27.3** | Albright hereditary | del | *GPR35* |

| | | | |
|---|---|---|---|
| | osteodystrophy like | | |
| **4p16.3** | Wolf–Hirschhorn syndrome | del | *WHS* |
| **5p15.2** | Cri du chat syndrome | del | *CDC/D5S23/D5S721* |
| **5q35** | Sotos syndrome | del | *NSD1* |
| **7p13** | Greig cephalopolysyndactyly | del | *GLI3* |
| **7q11.23** | Williams syndrome | del | *ELN* |
| **8p23.1** | 8p23.1 deletion | del | *GATA4* |
| **8q24** | Langer–Giedion syndrome | del | *EXT1* |
| **8q24** | Trichorhinophalangeal syndrome | del | *TRPS1* |
| **11p15.5** | Beckwith–Wiedemann syndrome | dup | *IGF2* |
| **11p13** | WAGR syndrome | del | *PAX6/WT1* |
| **11p11.2** | Potocki–Shaffer syndrome 11q deletion syndrome/Jacobsen/Paris-Trousseau | del | *EXT2/ALX4* |
| **11q23.3-q24.1** | syndrome | del | *FLI1* |
| **12q24.1** | Noonan syndrome | del | *PTPN11* |
| **13q14.11** | Retinoblastoma | del | *RB1* |
| **15q12** | Prader–Willi syndrome | del | *SNRPN/D15S10* |
| **15q12** | Angelman syndrome | del | *UBE3A/D15S10* |
| **16p13.3** | $\alpha$-Thalassemia | del | *16ptel* |
| **16p13.3** | Rubinstein–Taybi syndrome | del | *CREBBP* |
| **16p13.3** | Tuberous sclerosis 2 | del | *TSC2* |

| | | | |
|---|---|---|---|
| **16p13.3** | Polycystic kidney disease | del | *PKD1* |
| **17p13.3** | Miller–Dieker syndrome | del | *LIS1* |
| **17p12** | Charcot–Marie–Tooth disease, 1A | dup | *PMP22* |
| **17p12** | Hereditary neuropathy with liability to pressure palsies | del | *PMP22* |
| **17p11.2** | 17p11.2 duplication syndrome | dup | *SMS* |
| **17p11.2** | Smith–Magenis syndrome | del | *SMS* |
| **17q11.2** | Neurofibromatosis 1 | del | *NF1* |
| **20p11.23** | Alagille syndrome | del | *JAG1* |
| **22q11.2** | DiGeorge syndrome/velocardiofacial syndrome | del | *TBX1/TUPLE1/D22S* |
| **22q11.2** | 22q11.2 duplication | dup | *TBX1/TUPLE1/D22S* |
| **22q13.3** | 22q13 deletion syndrome/Phelan–McDermid syndrome | del | *SHAK3/PROSAP2* |
| **Xp22.3** | Steroid sulfatase deficiency | del | *STS* |
| **Xp22.3** | Kallmann syndrome | del | *KAL1* |
| **Xp21.2** | DMD/glycerol kinase deficiency/adrenal hypoplasia congenita | del | *DMD/GK/DAX1* |
| **Yq11.2** | Male infertility | del | **AZFa, AZFb, AZFc** |

*DMD*, Duchenne muscular dystrophy; *WAGR*, Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation.




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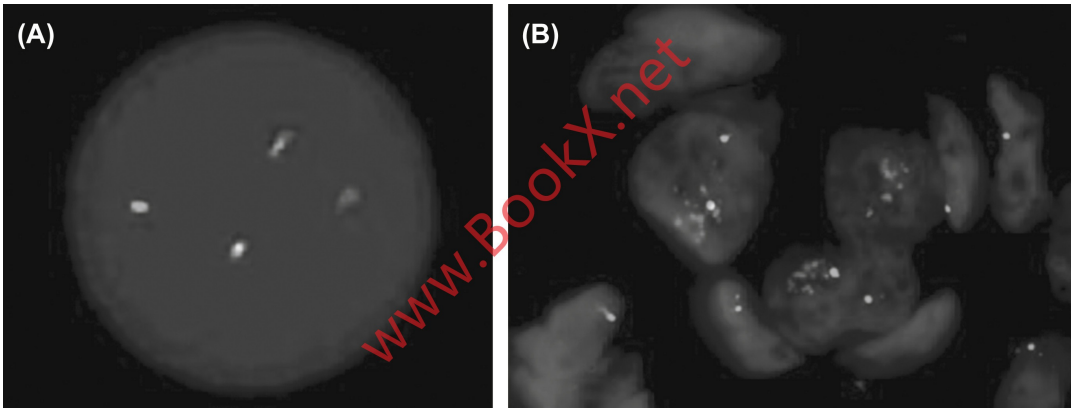
**FIGURE 5.5** The diagnosis of microdeletion syndromes using labeled cosmid clones that map into the region involved in the deletion. (A) Miller-Dieker syndrome. Chromosome 17 identified by cosmid clone in 17q. Note loss of signal at 17q13 in one homologue (right). (B) Williams syndrome. Chromosome 7 identified by cosmid clone at distal end of 7q. Note loss of signal at the elastin locus on 7q11.23 in one homologue (lower). (C) Prader–Willi syndrome. One homologue of chromosome 15 shows loss of signal at 15q12. (D) DiGeorge syndrome. One homologue of chromosome 22 shows loss of signal at 22q11.

Multicolor clone sets for the detection and monitoring of critical genetic aberrations associated with hematopoietic disorders are widely used for the analysis of uncultured bone marrow samples and tumor tissue biopsy specimens. Hybridization targets of these DNA probes have been designed to flank or span common translocation breakpoints on one or both rearranged chromosomes, thus resulting in characteristic signal patterns in normal and abnormal cell populations. The most commonly used probes in the diagnosis and monitoring of hematological malignancies are centromeric probes, dual- and-single fusion probes (Fig. 5.7A), extrasignal probes, and break-apart probes. In solid tumor diagnostic enumeration, probes

complementary to unique sequences of known oncogenes and cancer suppressor genes are being widely used on imprinted fresh tumor samples and paraffin-embedded tissue sections (Fig. 5.7B). Recent developments in automated scanning and image capturing systems together with automated spot counting and image analysis software allow high-throughput, automated interphase FISH analysis of many different sample types as well as simultaneous FISH and conventional immunofluorescence staining.


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**FIGURE 5.6** Human metaphase showing telomeric probes for chromosome 1p (Spectrum Green), 1q (Spectrum Red) and pairing regions of Xp and Yp (Spectrum Orange). X alpha centromeric probe (Spectrum Aqua) identifies the X centromere.



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**FIGURE 5.7** (A) Chronic myeloid leukemia showing detection of the Philadelphia translocation using BCR/ABL dual color dual fusion probe. The ABL gene in red and the BCR region in green map to chromosomes 9 and 22, respectively, and the translocation regions are revealed by the combined signals on each derivative chromosome. (B) Amplification of *HER2 (ERBB2)* oncogene (red) in paraffin-embedded section of breast cancer tissue. Centromeres of chromosome 17 are shown in green.

## 5.8.2. Molecular Substitutes for Multiprobe FISH Tests

Faster, cheaper, and often more precise molecular techniques are adding to an expanding set of tools in the diagnostic cytogenetic laboratories. MAPH is a method that measures copy number (dosage) variation of DNA of almost any size. The method combines hybridization of DNA probes to target genomic DNA immobilized on a solid support. After washing, each specifically bound probe is present in an amount proportional to its target DNA copy number. All probes are then simultaneously amplified using a single primer pair and quantified after electrophoretic separation [43]. MLPA shares the basic principles of primary probe hybridization with subsequent quantitative probe amplification with MAPH. However, instead of a probe hybridization to a filter-bound target DNA, in MLPA two adjacent complementary oligonucleotides hybridize to their target sequence in solution. One of the two oligonucleotides is cloned in an M13-derived vector that contains an inserted stuffer sequence size specific for each probe. In the subsequent ligation step, only the

adjacent oligonucleotides hybridized to their target DNA sequence are ligated. As in MAPH, the ligation-generated template is suitable for PCR amplification using a single primer pair. After the electrophoretic separation that identifies each probe, the PCR products are quantified by comparing the peak areas of the amplification products to a set of amplified controls or to neighboring peaks [44]. Both methods allow reliable screening for DNA copy number changes at ~40 loci simultaneously, with MLPA increasingly used in the diagnostic laboratory to screen for a variety of microdeletion syndrome, subtelomeric deletions/duplications and unbalanced cryptic telomeric translocations, aneuploidies, whole or partial gene duplications, or other deletions [14,45].

## 5.8.3. DNA Fiber-FISH

Because of the condensation of the chromosome fiber at metaphase, the fluorescent signals from two cosmid clones hybridized to the same chromosome can be resolved only if they are >2–3 Mb apart. Because the chromosomes are 10 times more extended in hypotonic preparations of interphase nuclei, the resolution at interphase is much better, and two cosmids >50 kb apart can usually be distinguished from one another [46]. The order of several closely linked cosmids may be determined at interphase, provided they are >50 kb and <1 Mb apart [47]. The latter restriction is due to the tendency of a chromosome to coil back on itself and because the chromosome fiber forms loops radiating in all directions from the central chromosome scaffold. Various techniques have now been developed that release the chromosome fiber from its associated protein within the chromosome scaffold [48,49]. This permits DNA sequences to be hybridized directly onto extended chromosome fibers fixed on a microscope slide and analyzed with the use of FISH (Fig. 5.8). The

resolution of this technique is astonishing; sequences <5 kb apart can be separated readily, and distances down to 1 kb have been claimed [15,50]. The analysis of extended DNA fibers by FISH has application in both the mapping and ordering of contiguous DNA sequences and has been used in the detection of carriers of intragenic gene deletions in X-linked Duchenne muscular dystrophy [51].




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**FIGURE 5.8** Fiber-FISH. Decondensation of chromatin by alkali treatment of fixed nuclei and hybridization of closely linked labeled cosmid clones. Three cosmids from the HLA region labeled with Texas red (lower), FITC (middle), and a 50:50 mixture of FITC and Texas red (upper), respectively. Each cosmid measures 35 kb and there is a 5–10 kb gap between the red and green cosmids.

## 5.8.4. FISH Nomenclature

The Standing Committee for the ISCN has developed nomenclature to describe investigations and abnormalities detected by DNA probes. The observed chromosome banding is described by the standard nomenclature. This is followed by a period (.) and the abbreviation "ish," which precedes the description of the ISH findings. The abnormality identified by ISH is described starting with the abbreviation for the type of abnormality followed, in separate parentheses, by the chromosome(s), break point(s), and loci for which probes were used, designated according to the Genome Data Base (GDB) and ordered from pter to qter. If no GDB locus is available, the name of the probe can be given instead. Capital letters are used for the locus, and + or − is given immediately afterward to indicate whether the locus has been identified as being present or absent, respectively. Thus, ish del (22)(q11.2q11.2)(D22S75–) indicates a deletion in the DiGeorge chromosome region, and ish dup (17)(p11.2p11.2)(CMT1A++) indicates a duplication at the Charcot–Marie–Tooth locus. If no abnormality is detected, the chromosomal location follows ish, and the usual two copies of the probe are confirmed; for example, ish 22q11.2(D22S75x2). The origin of a cryptic balanced translocation identified by forward painting is given as ish t(4;11)(p16.3;p15)(wcp4+;wcp11+), where wcp is whole chromosome paint. Similarly, the origin of an extra marker chromosome revealed by reverse painting is given as 47, XY, +mar.rev ish der (18)t(12;18)(p13.3;p11.2). The ish nomenclature includes symbols appropriate for interphase FISH (nuc ish) and fiber-FISH (fib ish) [32].

## 5.8.5. Comparative Genomic Hybridization

An ingenious FISH technique was developed for the cytogenetic analysis of tumor samples that is capable of identifying DNA amplifications and deletions of ~5 Mb and the 3- to 5-fold

amplification of oncogenes in a single sample of tumor material [21]. The method, CGH, is a development of reverse painting in which test DNA and normal genomic reference DNA are isolated, fragmented, differentially labeled (e.g., in green and red, respectively), mixed, and allowed to compete for hybridization sites in sets of normal high-resolution metaphase spreads. The relative amounts of test and reference DNA that anneal to a particular chromosome region will depend on the number of copies of DNA complementary to that region in the test sample. If the test sample contains relatively more of a particular DNA than the reference sample, this will be revealed by an increased green:red fluorescence ratio in the complementary region; similarly, chromosomal deletion in the test sample will be revealed by a decreased green:red fluorescence ratio (see Fig. 5.9. Significantly, this method uses DNA from the individual to be tested, with no need to culture cells before the analysis. CGH requires digital fluorescence microscopy in which the relative amounts of green and red fluorescence are measured along the length of the chromosome.

## 5.8.6. Array-Based Genomic Analysis

The replacement of metaphase spreads by an array of cloned DNA sequences or PCR products offers much higher resolution and more precise information on the breakpoints of rearranged chromosomes than does conventional metaphase CGH [22,52]. Moreover, each clone and PCR product spotted on an array has known sequence and contains known genes and other expressed sequences, facilitating precise genotype–phenotype correlations and consequently targeted patient management. Initially, chromosomal microarray analysis (CMA) was performed using relatively large insert DNA from BACs, but the technology has evolved with initial use of shorter inserts from cosmids or fosmids to the use of single stranded oligonucleotides, ranging in size from 20 to 70 bp (Fig. 5.9). Currently, the most commonly used platforms use oligonucleotides, SNPs, or a combination of the two [53].

SNP arrays differ from oligonucleotide-based arrays, in that they

capture genotyping information in addition to copy number information. SNP arrays were originally developed for genotyping applications, but it subsequently became clear that they could also reveal genomic copy number information. The genotyping information provided by the SNP array expands the types of abnormalities that can be detected to include detection of regions of homozygosity (which could indicate either uniparental disomy or regions that are identical by descent). This allows the diagnosis of imprinting disorders, which were previously undetectable in the cytogenetics laboratory. In addition, the use of SNP arrays permits diagnosis of low-level mosaicism, chimerism, and incest [54] (Fig. 5.10).

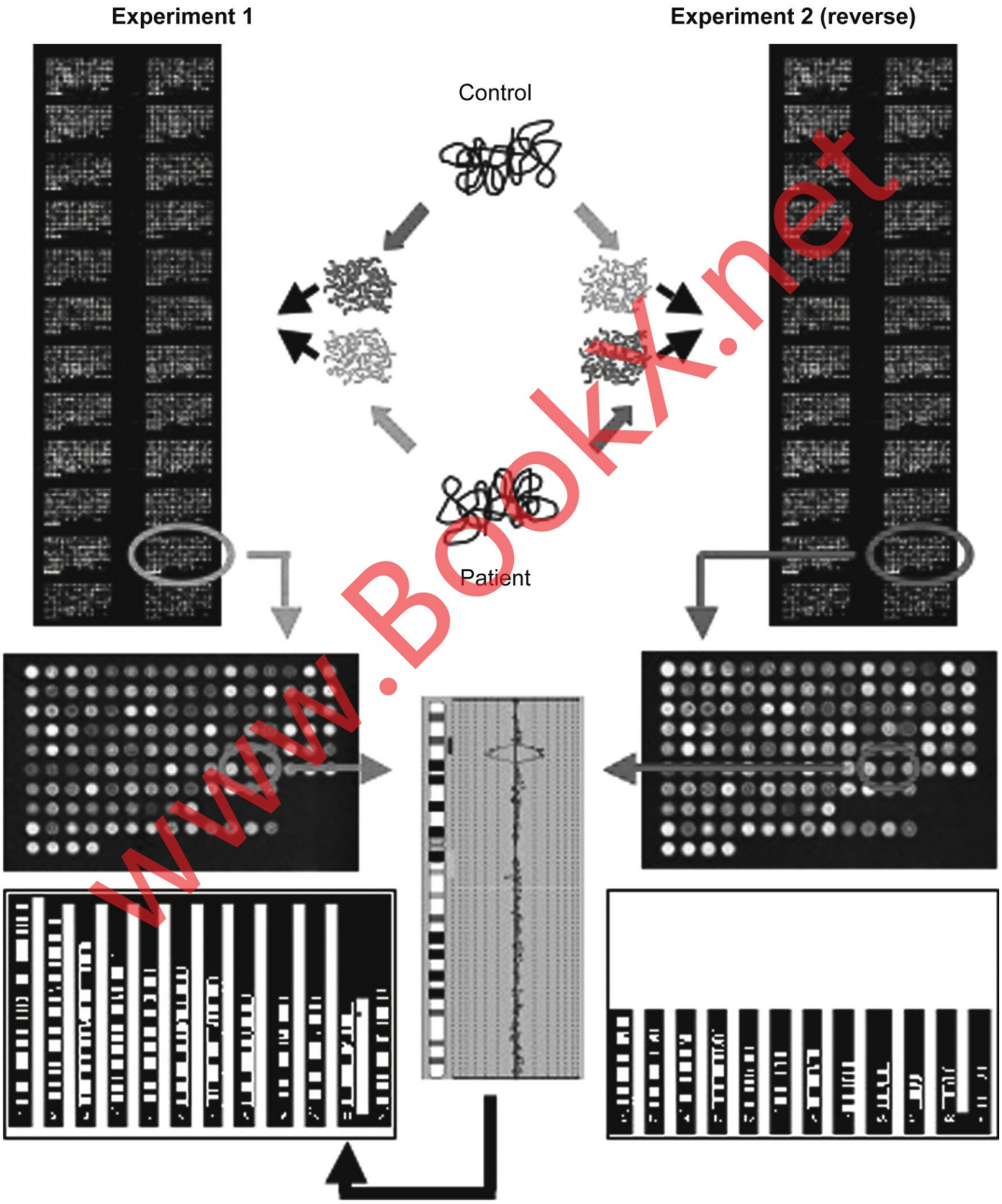
CMA began to be used at clinical laboratories in 2003, and over the next few years it became clear that the technology could identify genomic alterations that were likely to be causative in 5–15% of individuals who had previously had a normal karyotype [55]. This huge success has resulted in almost universal adaptation of CMA, and in 2010 it was recommended that CMA be used as the first tier in clinical diagnostic testing, based on its increased sensitivity [56]. The primary challenge of the clinical utilization of CMA emerged in 2004, with the discovery that deletions and duplications including genes were found in individuals without any known clinical problems (controls). These deletions and duplications have been called copy number variants (CNVs). In fact, the total number of genes in a healthy individual can vary by >100 genes. Therefore, it is crucial to distinguish benign CNVs (deletions or duplications) from those that are pathogenic. Pathogenic variants are those that are not seen in the normal, control population and that are expected to contain gene or DNA sequences that cause disease when deleted or duplicated. Conversely, benign variants are genes that are not dosage sensitive and therefore are found to vary in the normal population. To aid in this distinction, data from a wide variety of studies have been collected and curated in public databases, such as the Database of Genomic Variants (http://projects.tcag.ca/variation/). As our collective experience with CMA has increased, so has our ability to interpret the

data that are generated. But despite the challenges, the success of CMA in the diagnosis of clinically significant genomic alterations has been spectacular. Many new syndromes have been described that are caused by deletions or duplications (e.g., 16p11.2 and 17q21.3 deletions) that were previously undetectable with cytogenetics or FISH (see chapter on common syndromes).

## 5.8.7. ARRAY Nomenclature

The Standing Committee for the ISCN has developed nomenclature to describe investigations and abnormalities detected by chromosomal microarray analysis. A normal female characterized by array testing is indicated as arr(1-22),X) X2, while a male is arr(1-22)x2,(XY)x1, the difference being the relative number and type of sex chromosomes. In the case of deletions or duplications, the aberration can be specified precisely, by presentation of the cytogenetic band location, followed by specification of the genomic nucleotides. For example, an interstitial deletion of the short arm of chromosome 20 might be arr

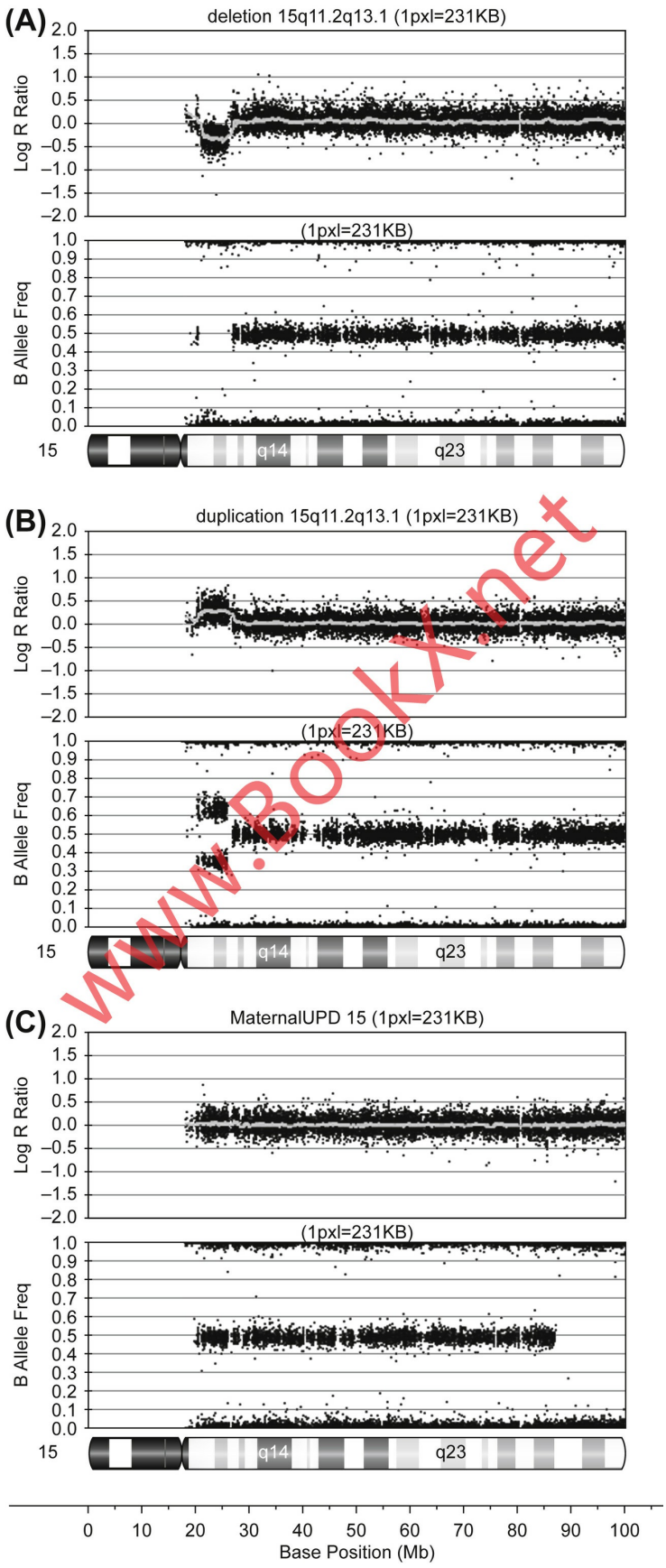
20p12.2(10,454,698-10,818,327)x1. This specifies a 323,630 bp (324 kb) deletion within 20p12.2. It is important to include information on which build of the Human Genome Project is being referenced, as the coordinates change from build to build. This should be included in the body of the report. Note that array studies by themselves do not provide further positional information regarding the imbalance. This deletion could be associated with a translocation, for example, and the only way to determine this would be to carry out accompanying chromosome or FISH analysis. SNP arrays can also identify uniparental disomy; this can be indicated by using the abbreviation "upd," and homozygosity can be indicated by using "hmz." Inclusion of the genomic coordinates for each of these abnormalities allows the laboratory or physician to go to the genomic databases to determine the precise gene content of the deletion or duplication.




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**FIGURE 5.9** Array-CGH analysis in a patient with epilepsy and moderate mental deficiency revealing a cryptic duplication of chromosome 11 short arm. Total labeled genomic DNA from patient is cohybridized with labeled control DNA and unlabeled Cot-1 DNA to an array platform containing approximately 3000 characterized fragments selected from across the human genome. In two experiments using alternative labels for patient and control DNA, the fluorescence ratio is measured for each DNA spot on the array and plotted on chromosome-specific ratio profiles. Significant deviations from the expected 1:1 for a

given DNA fragment indicate a change in copy number for that particular sequence in the patient with respect to the control. True copy number changes are seen as symmetrical deviations from the 1:1 axis in both experiments. The remaining chromosome profiles appear normal with the exception of occasional large-scale copy number polymorphisms.




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**FIGURE 5.10** Genome-wide SNP array results for individuals with three different abnormalities of chromosome 15 (deletion, duplication, and uniparental disomy). Each panel shows the log R ratio (demonstrating SNP signal intensity) and B allele frequency. The B allele frequency indicates genotypes for the biallelic SNPs on the array at each locus, with one allele labeled as A and the other as B. Therefore, BB has a B allele frequency of 1 (2/2 alleles are B); AB has a frequency of 1/2, and AA has a frequency of 0. A chromosome 15 ideogram lies below each patient result. (A) Patient with a deletion of proximal 15q showing the decreased log R ratio and the loss of heterozygosity within the deletion. (B) Patient with a duplication of proximal 15q demonstrating the increased log R ratio and the additional genotypes in the duplication (BBB, ABB, AAB and AAA). (C) P patient with uniparental disomy for chromosome 15, with heterodisomy across much of the long arm and isodisomy towards the telomere, from base

position ~85 Mb (at band 15q26.1) to qter. Note the region of

heterodisomy was confirmed by comparison of the patient with parental genotypes, as it cannot be proved by the SNP array alone, although the region of isodisomy at the telomeric end raises suspicion for UPD of the whole chromosome.

## 5.8.8. Detection of Structural Variation by Genome and Exome Sequencing

Array CGH allowed the identification and diagnosis of CNV, but the resolution of this technique is insufficient to permit precise mapping of the breakpoints of the variants. In addition, array CGH cannot detect copy neutral or balanced rearrangements. To more precisely map CNVs and diagnose balance rearrangements, methods began to be developed to allow the use of next-generation sequencing (NGS) (or massively parallel sequencing) for the detection of CNVs [57]. Another benefit of using NGS for copy number detection is the potential that sequencing will allow elucidation of both single nucleotide variation and CNV in a single assay, leading to decreased costs of genomic testing once sequencing costs are low enough. The use of whole genome sequencing (WGS) has the potential to permit a single test to capture almost all types of genomic variation in a single test, given that the entire genome can be captured in an unbiased

fashion and recent studies have demonstrated an increased diagnostic yield using this technology [58]. Another area that has undergone tremendous innovation is the field of prenatal diagnosis, where diagnostics has moved toward noninvasive testing, based on analysis of fetal cells, with a sweeping shift away from invasive testing. As early as 1997, it was recognized that cell-free DNA from a fetus could be detected in maternal plasma, and during the next 2 decades this finding was refined into a powerful noninvasive test for fetal anomalies, based on screening of maternal serum [59].

The detection of chromosome abnormalities affecting human health continues to evolve, with a continuing shift toward molecular techniques, particularly NGS-based techniques. While the technical changes have been dizzying, the results are profound for our ability to detect chromosome disorders.

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## References

[1] Pinkel D, Gray J.W, Trask B, et al. Cytogenetic analysis by *in situ* hybridization with fluorescently labeled nucleic acid probes. *Cold Spring Harb Symp Quant Biol*. 1986;51(Pt 1):151–157.

[2] Pinkel D, Segraves R, Sudar D, et al. High resolution analysis of DNA copy number variation using

comparative genomic hybridization to microarrays. *Nat Genet*. 1998;20:207–211.
[3] Talkowski M.E, Ernst C, Heilbut A, et al. Next-generation sequencing strategies enable routine detection of balanced chromosome rearrangements for clinical diagnostics and genetic research. *Am J Hum Genet*. 2011;88:469–481.
[4] Koller. The genetical and mechanical properties of sex chromosome III. Man. Proc. Roy. Soc. Edinburgh, B. 57:194
[5] Nowell P.C, Hungerford D.A. Chromosome studies on normal and leukemic human eukocytes. *J Natl Cancer Inst*. 1960;25:85–109.
[6] Tjio J.H, Levan A. The chromosome number of man. *Hereditas*. 1956;42:1–6.
[7] Ford, Hamerton JL. The chromosomes of man. *Nature*. 1956;178 1020-3.
[8] Ford P.A, Jacobs, Lajtha L.G. Human somatic chromosomes. *Nature*. 1958;181 1565-8.
[9] Lejeune J, Gautier M, Turpin R. Les chromosomes somatique des enfants mongoliens. *Comptes Rendus des Academy des Science*. 1959;248:1721–1722 [Paris].
[10] Polani P.E, Hunter W.F, Lennox B. Chromosomal sex in Turner's syndrome with coarctation of the aorta. *Lancet*. 1954;267:120–121.
[11] Jacobs P.A, Strong J.A. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature*. 1959;183(4657):302–303.
[12] Hirschhorn K, Cooper H.L, Firschein I.L. Deletion of short arms of chromosome 4–5 in a child with defects

of midline fusion. *Humangenetik*. 1965;1(5):479–482.
[13] Moorehead P.S, Nowell P.C, Mellman W.J, et al. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res*. 1960;20:613–616.
[14] Pardue M.L, Gall J.G. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proc Natl Acad Scie USA*. 1969;63:378–383. .
[15] Caspersson T, Zech L, Johansson C. Differential banding of alkylating fluorochromes in human chromosomes. *Exp Cell Res*. 1970;60:315–319.
[16] Hollox E.J, Atia T, Cross G, et al. High throughput screening of human subtelomeric DNA for copy number changes using multiplex amplifiable pro hybridisation (MAPH). *J Med Genet*. 2002;39:790–795.
[17] Sellner L.N, Taylor G.R. MLPA and MAPH: new techniques for detection of gene deletions. *Hum Mutat*. 2004;23:413–419.
[18] Van Ommen G.J.B, Breuning M.H, Rapp A.K. FISH in genome research and molecular diagnostics. *Curr Opin Genet Dev*. 1995;5:304–308.
[19] Knight S.J, Flint J. Perfect endings: a review of subtelomeric probes and their use in clinical diagnosis. *J Med Genet*. 2000;37(6):401–409.
[20] Ledbetter D.H. Minireview: cryptic translocations and telomere integrity. *Am J Hum Genet*. 1992;51(3):451–456.
[21] Flint J, Wilkie A.O, Buckle V.J, et al. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat*

*Genet*. 1995;9(2):132–140.
[22] Biesecker L.G. The end of the beginning of chromosome Ends. *Am J Med Genet*. 1992;2002; 107(4):263–266.
[23] De Vries B.B, Winter R, Schinzel A, van Ravenswaaij-Arts C. Telomeres: a diagnosis at the end of the chromosomes. *J Med Genet*. 2003;40(6):385–398.
[24] Kallioniemi A, Kallioniemi O.P, Sudar D, et al. Comparative genomic hybridisation for molecular cytogenetic analysis of solid tumours. *Science*. 1992;258:818–882.
[25] Vissers L.E, de Vries B.B, Osoegawa K, et al. Array-based comparative genomic hybridization for the genome-wide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet*. 2003;73(6):1261–1270.
[26] Snijders A.M, Nowak N, Segraves R, et al. Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet*. 2001;29(3):263–264.
[27] Veltman J.A, Schoenmakers E.F, Eussen B.H, et al. High-throughput analysis of subtelomeric chromosome rearrangements by use of array-based comparative genomic hybridization. *Am J Hum Genet*. 2002;70(5):1269–1276.
[28]
Shaffer L.G, Beaudet A.L, Brothman A.R, Hirsch B, Le al. Microarray analysis for constitutional cytogenetic abnormalities. *Genet Med*. 2007;9:654–662.
[29] Iafrate A.J, Feuk L, Rivera M.N, et al. Detection of large-scale variation in the human genome. *Nat Genet*. 2004;36:949–951.

[30] Redon R, Ishikawa S, Fitch K.R, et al. Global variation in copy number in the human genome. *Nature*. 2006;444(7118):444–454.
[31] Sebat J, Lakshmi B, Malhotra D, et al. Strong association of *de novo* copy number mutations with autism. *Science*. 2007;316(5823):445–449.
[32] Sharp A.J, Hansen S, Selzer R.R, et al. Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. *Nat Genet*. 2006;38(9):1038–1042.
[33] Carter N.P, Ferguson-Smith M.A, Perryman M.T, et al. Reverse chromosome painting: a method for the rapid analysis of aberrant chromosomes in clinical cytogenetics. *J Med Genet*. 1992;29:299–307.
[34] Seabright M. A rapid banding technique for human chromosomes. *Lancet*. 1971;1971(ii):971–972.
[35] ISCN. An international system for human cytogenetics nomenclature, 2016. In: McGowan-Jordan J, Simons A, Schmid M, eds. *Reprint of: cytogenetic and genome research 2016*. vol. 149. Basel: Karger AG; 2016:1–2.
[36] Barber J.C.K, Reed C.J, Dahoun S.P, Joyce C.A. Ampli of a pseudogene cassette underlies euchromatic variation of 16p at the cytogenetic level. *Hum Genet*. 1999;104:211–218.
[37] Hollox E.J, Armour J.A.L, Barber J.C.K. Extensive normal copy variation of a beta-defensin antimicrobial-gene cluster. *Am J Hum Genet*. 2003;73:591–600.
[38] Harper M.E, Saunders G.F. Localization of single

copy DNA sequences of G-Banded human chromosomes by in Situ Hybridization. *Chromosoma*. 1981;83(3):431–439.

[39] Gerhard D.S, Kawasaki E.S, Bancroft F.C, Szabo P. Lo of a unique gene by a direct hybridization *in situ*. *Proc Natl Acad Sci*. 1981;78:3755–3759.

[40] Malcolm S, Barton P, Murphy C, Ferguson-Smith M.A. Chromosomal localization of a single copy gene by *in situ* hybridization—human beta globin genes on the short arm of chromosome 11. *Ann Hum Genet*. 1981;45(Pt 2):135–141.

[41] Landegent J.E, Jasen in de Wal N, Baan R.A, Hoeijmakers J.H, Van der Ploeg M. 2-Acetylaminofluorene-Modified probes for the Indirect Hybridocytochemical detection of specific nucleic acid sequences. *Exp Cell Res*. 1984;153(1):61–72.

[42] Langer P.R, Waldrop A.A, Ward D.C. Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. *Proc Natl Acad Sci USA*. 1981;78(11):6633–6637.

[43] Lichter P, Cremer T, Tang C.J, et al. Rapid detection of human chromosome 21 aberrations by *in situ* Hybridization. *Proc Natl Acad Sci USA*. 1988;85(24):9664–9668.

[44] Meltzer P.S, Guan X.Y, Burgess A, Trent J.M. Rapid generation of region specific probes by chromosome microdissection and their application. *Nat Genet*. 1992;1:24–28.

[45] Telenius H, Pelmear A.H, Tunnacliffe A, et

al. Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer*. 1992;4:257–263. .

[46] Armour J.A, Sismani C, Patsalis P.C, Cross G. Measur of locus copy number by hybridization with amplifiable probes. *Nucleic Acid Res*. 2000;28:605–609.

[47] Schouten P, McElgunn C.J, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acid Res*. 2002;30(12):e57.

[48] Rooms L, Reyniers E, van Luijk R, et al. Subtelomeric deletions detected in patients with idiopathic mental retardation using multiplex ligation-dependent probe amplification. *Hum Mutat*. 2004;23:17–21.

[49] Trask B.J, Massa H, Kenwrick S, Gitschier J. Mapping of human chromosome Xq28 by two-color fluorescence in situ hybridization of DNA sequences to interphase cell nuclei. *Am J Hum Genet*. 1991;48:1–15.

[50] van den Engh G, Sachs R, Trask B.J. Estimating genomic distance from DNA sequence location in cell nuclei by a random walk model. *Science*. 1992;257(5075):1410–1412.

[51] Parra I, Windle B. High resolution visual mapping of stretched DNA by fluorescent hybridization. *Nat Genet*. 1993;1993(5):17–21.

[52] Wiegant J, Kalle W, Mullenders L, et al. High-resolution *in situ* hybridization using DNA halo preparations. *Hum Mol Genet*. 1992;1(8):587–591.

[53] Heiskanen M, Karhu R, Hellsten E, Peltonen L, Kallio resolution mapping using fluorescence in situ hybridization to extended DNA fibers prepared from agarose-embedded cells. *Biotechniques*. 1994;17(5):928–929 932–933.
[54] Florijn R.J, Blonden L.A.J, Vrolijk J, et al. High resolution DNA fibre-FISH genomic DNA mapping and colour bar coding of large genes. *Hum Molec Genet*. 1995;4:831–836.
[55] Solinas-Toldo S, Lampel S, Stilgenbauer S, et al. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromosomes Cancer*. 1997;20(4):399–407.
[56] Schaaf C.P, Wiszniewska J, Beaudet A.L. Copy number and SNP arrays in clinical diagnostics. *Annu Rev Geomics Hum Genet*. 2011;12:25–51.
[57] Conlin L.K, Thiel B.D, Bonnemann C.G, Medne L, Ern of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum Mol Genet*. 2010;19:1263–1275.
[58] Stankiewicz P, Beaudet A.L. Use of array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation. *Curr Opin Genet Dev*. 2007;17(3):182–192.
[59] Miller D.T, Adam M.P, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*. 2010;86(5):749–764.

[60] Korbel J.O, Urban A.E, Affourtit J.P, Godwin B, Grube end mapping reveals extensive structural variation in the human genome. *Science*. 2007;318:420–426.
[61] Stavropoulos D.J, Merico D, Jobling R. Whole-genome sequencing expands diagnostic utility and improves clinical management in paediatric medicine. *NPJ Genom Med*. 2016;1:15012.
[62] Hui L, Bianchi D.W. Noninvasive prenatal DNA testing: the Vanguard of genomic medicine. *Annu Rev Med*. 2017;14(68):459–472.

## Further Reading

Beaudet A.L, Belmont J.W. Array-based DNA diagnostics. Let the Revolutions begin. *Annu Rev Med*. 2008;59:113–129.

Ferguson-Smith M.A. Cytogenetics and the evolution of medical genetics. *Genet Med*. 2008;10:553–559.

Krantz I.D, Spinner N.B. Novel microdeletion syndromes. *Am J Med Genet*. 2007;145C:323–326.

Lupski J.R. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet*. 1998;14:417–422.

Martin C.L, Warburton D. Detection of chromosomal aberrations in clinical practice: from karyotype to genome sequence. *Ann Rev Genom Hum Genet*. 2015;16:309–326.

Pardue M.L, Gall J.G. Chromosomal localization of mouse satellite DNA. *Science*. 1970;168:1356–1358.

Seibl R, Holtke H.J, Ruger R, et al. Non-radioactive labeling and detection of nucleic acids. III. Applications of the digoxigenin system. *Biol Chem Hoppe Seyler*. 1990;371(10):939–951.

Simon-Sanchez J, Scholz S, Fung H.C, Matarin M, Hernandez D, Gibbs J.R, e

al. Genome-wide SNP assay reveals structural genomic variation, extended homozygosity and cell-line induced alterations in normal individuals. *Hum Mol Genet*. 2007;16:1–14.

Speicher M.R, Ballard S.G, Ward D.C. Karyotyping human chromosomes by combinatorial multi-color FISH. *Nat Genet*. 1996;12:368–375.

Trask B.J. Human genetics and disease: human cytogenetics: 46 chromosomes, 46 years and counting. *Nat Rev Genet*. 2010;3:769–778. doi: 10.1038/nrg905.

6

# Diagnostic Molecular Genetics*

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## Abstract

Molecular genetic testing has a unique range of indications, most of which are quite different from the uses of traditional clinical laboratory testing and even molecular biologic testing in other disease classes (e.g., infectious disease, cancer). The technical approaches as well as the psychosocial and ethical implications of molecular genetic tests may vary substantially depending on the reason for testing (e.g., diagnostic, carrier screening, prenatal testing). Just as many of the applications are unique, so, too, the types of patient samples collected for molecular genetic testing may be different from those obtained for other types of clinical laboratory testing. In addition, the choice of technique will depend on the nature of the disease gene being studied (especially its size and mutational heterogeneity), the purpose of the test, and, to some extent, the condition of the specimen. Finally, high-complexity laboratories performing molecular genetic testing need to be aware of the specific regulatory considerations involved.

## Keywords

Clinical genomics; Cystic fibrosis; DNA sequencing; Familial cancers;

Molecular diagnostics; Trinucleotide repeat disorders

Molecular genetic testing has a unique range of indications, most of which are quite different from the uses of traditional clinical laboratory testing and even molecular biologic testing in other disease classes (e.g., infectious disease, cancer). The technical approaches as well as the psychosocial and ethical implications of molecular genetic tests may vary substantially depending on the reason for testing (e.g., diagnostic, carrier screening, prenatal testing). Just as many of the applications are unique, so, too, the types of patient samples collected for molecular genetic testing may be different from those obtained for other types of clinical laboratory testing. In addition, the choice of technique will depend on the nature of the disease gene being studied (especially its size and mutational heterogeneity), the purpose of the test, and, to some extent, the condition of the specimen. Finally, high-complexity laboratories performing molecular genetic testing need to be aware of the specific regulatory considerations involved.

## 6.1. Introduction

There can be no question that we are in the midst of a revolution in clinical medicine, as profound in its own way as the early revelations of human anatomy and physiology and the germ theory of disease. The twenty-first century has already been christened the age of molecular medicine—the molecule in question being, of course, DNA (and other nucleic acids). Molecular biology, which came to dominate basic life sciences research over the second half of the twentieth century, has now become firmly ensconced in clinical medicine as well. The development of robust technologies such as the polymerase chain reaction (PCR) and massively parallel DNA sequencing and the extraordinary productivity of the Human Genome Project in elucidating medically relevant targets for these techniques have firmly established patient DNA as a powerful "analyte" in the clinical laboratory and a valid substrate for therapeutic manipulation in the clinic.

While all areas of medicine have been impacted by these

developments, it is medical genetics, the specialty most directly connected to the human genome, that is the first discipline to be entirely transformed by them. If the promise of gene therapy continues to remain frustratingly elusive through successive editions of this text (though finally within reach thanks to the newer gene-editing techniques), such is not the case for gene-based diagnostics. In no other sector of medical practice does at least the question, if not always the actual execution, of molecular testing arise with virtually every patient seen. Not only is medical genetics by definition concerned with inherited alterations in the patient's genome that are detectable in this way, but much of its attention is also directed at ascertaining recessive carrier states, fetal conditions that will not be expressed until after birth, and predisposition to adult-onset disorders before they are symptomatic; such diagnoses often exhibit no other evidence of their presence than at the DNA level. For these reasons, medical geneticists and genetic counselors, as a group, tend to be significantly more facile and familiar with the applications and interpretation of molecular genetic tests than most other clinicians. Nevertheless, keeping up with a field as rapidly evolving as this one, in which new techniques and disease genes (not to mention their associated ethical and societal dilemmas) are reported every week, can be a challenge for even the most dedicated specialist. These developments have been accelerated exponentially by the introduction of genome-level diagnostic sequencing. That is why close two-way communication between the clinic and the laboratory is so essential, more so in medical genetics practice than in any other.

## 6.2. Indications for Molecular Genetic Testing

Molecular genetics has a unique range of indications, most of which are quite different from the uses of traditional clinical laboratory testing and even molecular biologic testing in other disease classes (e.g., infectious disease, cancer). Most notably, as mentioned earlier, these procedures are often performed on healthy people who have no other signs or symptoms of the disease being tested. Even if they are symptomatic, the assay is not directed at a discrete anatomic site, as

would be the case with a DNA test for an infection or a tumor. Because genetic disorders reflect heritable mutations in the germline, the test in most cases can be performed on any accessible body fluid or tissue. The most common specimen is whole blood, although saliva, buccal swabs, dried blood spots, urine, and other specimens can be used, while amniocytes or chorionic villus samples (CVS) and, most recently, fetal DNA in maternal blood are collected for prenatal diagnosis.

The technical approaches as well as the psychosocial and ethical implications of molecular genetic tests may vary substantially depending on the reason for testing. The major indications are considered individually next.

## 6.2.1. Diagnostic Testing

Most straightforward and least foreign to other areas of laboratory medicine are diagnostic molecular genetic tests performed on symptomatic patients. These are most often performed for diagnostic confirmation of a clinical impression, although they may also assist in differential diagnosis. In the latter regard, however, it should be kept in mind that gene-based tests are specific for the disease gene being tested, and even closely related disorders will not be detected when testing for a single one (e.g., the DNA test for spinocerebellar ataxia (SCA) type 1 will not detect the mutations of SCA type 2). Increasingly, with the advent of high-throughput DNA sequencing, diagnostic genetic tests are not limited to a single gene but expanded to panels of phenotypically related genes or even all the genes in the genome (whole-exome or whole-genome sequencing). These approaches are more open-ended and may produce surprising genetic causation, the discovery of previously unknown gene–disease associations, or unexpected off-target (incidental or secondary) findings. In addition, while diagnostic tests do not typically carry the heavy ethical concerns of predictive tests, the uniqueness of genetic disease comes into play here as well, such that a positive test in the proband places other blood relatives at risk.

## 6.2.2. Newborn Screening

Newborn screening, despite the name, is in a sense actually diagnostic testing performed very early in life, on large populations of infants who are not (yet) symptomatic, although the affected ones may well exhibit at least biochemical signs of the disease, which are the metabolite targets detected by the screening technology. The goal, of course, is to identify affected babies early in life so that treatment (dietary or pharmaceutical) can be initiated before irreversible damage occurs. Unfortunately, most of the classic autosomal recessive disorders screened in this setting, such as phenylketonuria and galactosemia, have so many causative mutations and variants of uncertain significance (VUS) that molecular genetic testing would be less conclusive and far less cost-effective than testing by biochemical or enzymatic means, at least given the current state of DNA sequencing technology. For most such disorders, the molecular tests, if available, are reserved for backup confirmation of indeterminate results, for further genotype–phenotype correlation, or for identification of DNA markers that could be used by the family for prenatal diagnosis in a subsequent pregnancy. However, as DNA-based methods become more efficient with the continued evolution of microarray technologies and massively parallel sequencing, they may eventually become the first-tier approach for certain newborn screens, though we are certainly not at that stage yet.

## 6.2.3. Prenatal Diagnosis

As molecular techniques have become ever more powerful and convenient, the world of prenatal testing has inexorably migrated from the domain of cytogenetics to that of the molecular diagnostic laboratory. We have witnessed this transformation in the diagnosis of single-gene defects for quite some time, because molecular techniques are the only ones with fine enough resolution to discover abnormalities at the nucleotide level. But more recently, molecular approaches have usurped the core targets of cytogenetics, the aneuploidies and large deletions/insertions. For the former, the

introduction of noninvasive prenatal screening (NIPS) to routine obstetric practice has produced a precipitous drop in the volume of prenatal karyotypes (based on amniocentesis or CVS), as patients and practitioners prefer the reduced fetal risk of the peripheral blood approach, its amenability to first-trimester sampling, and its much higher sensitivity and specificity compared with the standard maternal serum biochemical markers. For the latter, chromosomal microarray, having previously replaced karyotype in the postnatal setting as a first-tier test for evaluation of developmental delay, congenital malformations, and autism, is now encroaching on the prenatal setting as well, albeit with some limitations [1]. Moreover, the power of next-generation sequencing (NGS) and its associated bioinformatics is such that we anticipate NIPS will soon be able to target single-gene defects and large rearrangements, potentially replacing amniocentesis for those applications as well [2,3]. Even though some inborn errors of metabolism can be diagnosed by biochemical analysis of amniotic fluid, other disorders, such as muscular dystrophies, do not produce such easily accessible analytes and would require a far more invasive biopsy of the target tissue (e.g., fetal muscle) for examination of the gene product. Molecular genetic techniques in most situations obviate this need, because DNA containing the mutation of interest is readily obtainable from amniocytes or CVS specimens. However, given the time constraints of the prenatal setting, such an approach is usually not attempted unless there is prior knowledge of the identity of the parental mutation(s), at least in the case of disorders with extensive mutational heterogeneity. Needless to say, prenatal molecular genetic testing demands meticulous technique because of the irreversible nature of the intervention provoked by the test results. A key aspect unique to this setting is the detection and avoidance of maternal cell contamination, which is especially crucial in CVS. Laboratory guidelines for addressing this phenomenon using tandem repeat polymorphisms have been published [4].

A more specialized variant of prenatal diagnosis available in certain situations is preimplantation diagnosis, involving single-cell genetic

analysis (using powerful PCR techniques) performed on a blastomere biopsy of embryos produced by in vitro fertilization (IVF). Because of the difficulty and expense of the procedure, it is usually reserved for those couples who would be averse to abortion of an in utero pregnancy or who are already undergoing IVF for other obstetric or infertility indications. Even more so than for traditional prenatal diagnosis, the exact identity of the mutation in the parent (for a dominant disease) or in both parents (for a recessive disease) must be known before embarking on this involved procedure. Still, despite its formidable prerequisites, preimplantation diagnosis has been used many times for pregnancies at risk for cystic fibrosis (CF), thalassemia, and other disorders, including adult-onset diseases like hereditary breast/ovarian cancer [5,6].

Most recently, noninvasive prenatal diagnosis, after several decades of anticipation and disappointment, has finally emerged as a routine service, thanks to the power of NGS. The targeting of cell-free fetal DNA in the maternal blood has proved to be a far more robust and reliable approach than going after circulating fetal cells. By measuring the relative amounts of sequenced fragments (i.e., number of "reads") from the various chromosomes, Down syndrome and other trisomies can be identified, and it is theoretically possible to assess the entire fetal genome in the maternal blood to test for monogenic mutations [7,8]. The uptake among pregnant women and their obstetrical providers has been tremendous, with the result that the volume of prenatal karyotype analysis has dropped precipitously.

## 6.2.4. Carrier Testing and Screening

Carrier screening is another type of clinical laboratory testing unique to the genetics setting. The term is most properly used to denote testing for recessive mutations in the heterozygous state in otherwise healthy individuals. It may be indicated in two quite different situations: one when an individual is at risk because of a positive family history for the disorder in question and the other when the individual is at risk by virtue of belonging to an ethnic group in which the population frequency of one or more mutant alleles is particularly

high. The first application is more accurately termed carrier testing rather than carrier screening, especially when the causative mutation in the family is already known. The second application is true screening, because it is performed on an entire population in the absence of any concerning family history, and the likely mutation in any one individual being tested will not be known (unless the disorder has only a single mutation, as in sickle cell disease).

To be considered a candidate for population carrier screening, a number of criteria must be met by both the disease and the testing technology. The disorder must be frequent enough in the target population to justify the effort, expense, and attendant complexities. The natural history of the disorder must be severe and predictable enough to enable couples to make informed decisions about prenatal diagnosis and pregnancy termination. The available technology must be robust, relatively inexpensive, and capable of detecting a reasonable proportion of the possible disease-causing mutations. The options for intervention in those who test positive must be available, effective, and acceptable to the target population. In addition, the overall screening program must be of sufficient interest and acceptance to the target population to ensure cost-effective uptake. As will be seen, these criteria, or a subset of them, are not met by some of the gene targets that would otherwise appear to be the most obvious. CF carrier screening, although now in widespread practice, is still hampered by mutational complexity and phenotypic variability. Hereditary hemochromatosis, a possible target of adult presymptomatic screening, never gained traction because of the apparently low penetrance of the mutations. Screening for fragile X syndrome is controversial because of questions about prevalence and phenotypic implications of premutation trinucleotide expansions [9,10]. And, again, the ability of NGS to enable analysis of many more recessive genes has resulted in the marketing of greatly expanded carrier screening panels, some targeting as many as 300 diseases, most of which are very rare and therefore poorly understood in their genotype–phenotype correlations and natural history [11].

Because screening tests must be kept inexpensive in order to be

cost-effective, screening strategies for disorders with mutational heterogeneity will often entail complex decisions regarding the number and type of mutations to be included in the test panel. Such debates have been waged for years in the case of CF [12]. A decision must also be made as to whether targeted molecular genetic testing, with its narrow focus on specific mutations, casts a wide enough net within the target population to justify using it in preference to biochemical or enzymatic screening methods, which are often less expensive. In the case of CF, the carriers have no biochemical abnormalities that can be ascertained, so direct mutation testing is the only alternative. In the case of Tay–Sachs disease, even though there are a limited number of hexosaminidase A mutations that account for most carriers in the Ashkenazi Jewish target population, enzymatic testing was long preferred for population screening because of its ease of use, low cost, and ability to capture carriers with uncommon mutations. However, as screening in this ethnic group has expanded to encompass many more additional recessive disorders, it has become easier to perform all of them at once in a multiplexed molecular assay or by NGS [13,14].

### 6.2.5. Presymptomatic/Predisposition Testing

Predictive genetic testing is, in some ways, the most problematic of molecular genetic applications. It is applied primarily to adult- or late childhood-onset autosomal dominant disorders. It is generally not applied as a screening test but rather is reserved for individuals with a strong, usually parental, family history of the disorder, conferring a 50% a priori risk of having inherited the mutant gene. Recently, however, NGS has been invoked as a potentially cost-effective approach to "wellness screening," in which healthy adults might be tested in a nontargeted way to see if they carry deleterious variants in any of the late-onset neoplastic, neurologic, or cardiac disorder genes.

Depending on the penetrance of the mutations being tested, predictive testing is further subdivided into presymptomatic testing and predisposition testing. The former is applied to those disorders with virtually complete penetrance, such as Huntington disease, in

which a positive DNA test result is fully predictive of eventual symptomatology (although not absolutely indicative of severity or age of onset). The latter is applied to diseases with reduced penetrance, such as familial breast/ovarian cancer due to mutations in the *BRCA1* and *BRCA2* genes, in which a positive test result confers increased risk of eventual disease compared with the baseline population risk but does not allow prediction that any particular patient will actually experience the disease. This situation makes genetic counseling and clinical decision-making much more difficult, especially for diseases like familial breast/ovarian cancer, in which risky or irreversible medical or surgical interventions may be initiated based on a positive DNA test result. With both types of predictive testing, there are significant psychosocial risks because otherwise healthy adults are being given potentially devastating news about future disease. There are also theoretical risks of insurance or employment discrimination (supposedly barred by the Genetic Information Nondiscrimination Act in the United States and similar laws in other countries) that beg the question of whether a nucleotide change predictive of future disease in a presently healthy person is to be considered a "preexisting condition." For all these reasons, predictive genetic testing has been the application most scrutinized regarding standards for informed consent, pre- and post-test genetic counseling, and psychosocial supportive services. Because the offspring of patients with autosomal dominant disorders are aware of their risk, many such requests for testing are self-referred, and some of these individuals prefer not to involve their primary care provider or insurance carrier in the testing process. These special situations may place additional demands on genetic counseling services and on the testing laboratory.

## 6.3. Technical Approaches to Molecular Genetic Testing

### 6.3.1. Specimen Collection

Just as many of the applications are unique, so, too, the types of

patient samples collected for molecular genetic testing may be different from those obtained for other types of clinical laboratory testing. Because germline mutations are present in every cell of the body, site-specific biopsy is not required and simple phlebotomy will suffice for most purposes (this is in contrast to the approach for laboratory diagnosis of infectious and neoplastic diseases, where in most cases a specific lesion must be sampled – although this, too, is changing as "liquid biopsy" becomes more routine in cancer diagnosis and monitoring, analogous to noninvasive prenatal testing). Alternatively, the great sensitivity of PCR allows for genetic testing to be performed on minute samples of any readily accessible tissue or body fluid, including saliva and urine. Newborn DNA screening can easily be performed on the same blood spots collected for biochemically based screening. For prenatal diagnosis, either amniocytes or CVS can be used, because both contain the full complement of fetal DNA. In addition, PCR-based single-cell genetic analysis now allows for preimplantation diagnosis, and prenatal targeting of fetal DNA circulating in the maternal blood has become practicable [8], as noted here earlier.

## 6.3.2. Selection of Technique

Once in hand, the specimen can be subjected to any of the molecular genetic techniques in the current armamentarium. The choice of technique will depend on the nature of the disease gene being studied (especially its size and mutational heterogeneity), the purpose of the test, and to some extent the condition of the specimen. Specimens that are extremely small, fixed, or degraded will require some sort of PCR-based analysis, because that technique is the most tolerant of suboptimal samples. Once amplified to an abundant amount, the DNA is then amenable to further analysis by other techniques such as allele-specific oligonucleotide (ASO) hybridization or DNA sequencing. Southern blotting, once the main workhorse in molecular diagnostic laboratories, requires intact DNA of high molecular weight in pristine enough condition to be digested efficiently with restriction endonucleases and to produce reproducible patterns on gel

electrophoresis. It has now largely been replaced by more efficient methods. In either case, the first step is typically the isolation of DNA from the specimen, for which a variety of commonly used methods and commercial reagents are available.

#### 6.3.2.1. Detection of Known Point Mutations

For detection of a known point mutation or microdeletion/insertion, the patient DNA sample can be hybridized with ASO probes that contain either the wild-type or mutant sequence within them. Because hybridization under high stringency can distinguish a single nucleotide change by mismatch of a probe with its target, using these ASO probes in pairs provides an internal control for accurately genotyping both heterozygotes and homozygotes. An alternative strategy capitalizes on the availability of a wide variety of bacterial restriction endonucleases with many different target recognition sequences. With a little investigation (made easy these days through the use of computer DNA sequence analysis programs), it will often be found that the mutation of interest either disrupts a preexisting restriction enzyme cleavage site or creates a new one in the target DNA. In such cases, the mutation can be detected by exposing the patient's PCR-amplified DNA to the specific restriction enzyme and analyzing the cleavage products (sometimes called amplified restriction fragment polymorphisms [AmpFLPs]) by agarose gel electrophoresis. PCR primers can also be designed to hybridize directly at the site of the mutation, in which case a mismatch between a wild-type primer and a mutant target sample will block amplification and produce a blank result. This technique has been called the amplification-refractory mutation detection system (ARMS). While these techniques are relatively easy to perform and not very expensive, they have largely been displaced in recent years by DNA sequencing (Sanger sequencing and later NGS), as its costs and efficiency have improved.

The technical approaches just described are usually developed "in house" by the testing laboratory, often designated in the vernacular of the trade as "home brew" assays or "laboratory-developed tests

(LDTs)." With the increase in test volume in molecular genetics over the past two decades, driven in large part by the advent of population carrier screening for CF and testing for common disease variants such as factor V Leiden, there has been a continuing movement by the laboratories toward more standardized, commercially prepared testing platforms. A few of these have gone through formal US Food and Drug Administration (FDA) review and can then be called "kits." Those that have not are sold as components of an assay and are typically designated "For Investigational Use Only." In either case, these systems have proven robust and reliable and have generally been accepted as a boon to the field. Examples currently available for detection of point mutations include paper strip arrays of ASO hybridization probes, microarrays and multiplex platforms composed of chips or beads or a combination of the two, real-time PCR systems incorporating ASO probes with differential melting curves, oligonucleotide ligation with ASO probes analyzed by capillary electrophoresis, the Invader assay using specific cleavage of ASO hybrids to produce signal amplification by fluorescent resonance energy transfer, and mass spectrometry.

### 6.3.2.2. Detection of Large Deletions

Large deletions, while sometimes detectable by these methods, are usually more easily seen by Southern blot hybridization (signaled by the absence of one or more expected hybridization bands), chromosomal microarray, multiplex ligation-dependent probe amplification (MLPA) [15], or exon array. Alternatively, one can use differential PCR amplification, in which the failure of one or both of a pair of PCR primers to hybridize to the target DNA because of the deletion will result in absence of an expected PCR product. Many primer pairs can be multiplexed to scan for unknown deletions across a gene, as has been done for the diagnosis of Duchenne muscular dystrophy (DMD). The main pitfall of this technique is that there are many other technical reasons for PCR failure (of artifactual origin), and so appropriate internal PCR amplification controls must be included in the assay.

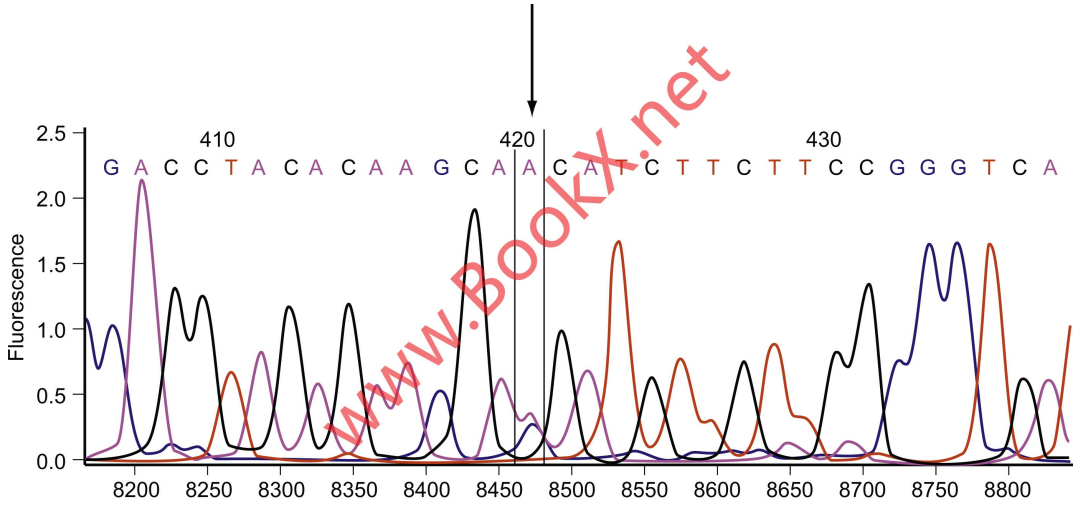
### 6.3.2.3. Detection of Trinucleotide Repeat Expansions

To detect the trinucleotide repeat expansion mutations characteristic of fragile X syndrome, Huntington disease, and other neuromuscular disorders, accurate sizing becomes important. Indeed, this is the most visible area of molecular genetic testing that approaches quantitative assay criteria, as opposed to the more qualitative observational assays for point mutations and deletions. If the expansion size is of moderate length, as in Huntington disease and the SCAs, amplification and sizing of PCR products suffice. This is typically done by capillary electrophoresis or automated DNA analysis instruments. For larger expansions that are difficult or impossible to amplify, such as in fragile X syndrome, sizing by Southern blot analysis or newer long-PCR methods is usually required. The Southern blot method, while not widely used these days, can serve double duty in defining methylation status of the full FMR1 CGG repeat expansion, through the use of methylation-resistant restriction endonucleases; PCR methods using bisulfate treatment can also be applied.

### 6.3.2.4. Detection of Heterogeneous or Unknown Mutations

What about those disorders in which a wide range of point mutations has been reported, often spanning a very large gene? Such disorders exhibiting allelic heterogeneity present special problems to the laboratory in attempting to ensure detection of a majority of the mutations; detection of all potential mutations is virtually impossible, even by complete DNA sequencing of the gene (because mutations may lie in noncoding regions, far upstream enhancers, etc.). Sequencing does cast the widest net and therefore is considered the "gold standard" and is especially indicated in diagnostic or predictive situations in which missing a point mutation could potentially have a lethal outcome, such as predictive testing for *BRCA1/2* mutations associated with familial breast/ovarian cancer. Formerly, it was considered prohibitive for high-throughput screening tests, so most approaches favored a more limited test panel comprising a few of the more commonly recurring mutations in the gene, recognizing at the

outset that some fraction of carriers will go undetected. Now with NGS reducing sequencing costs, it is indeed possible to address the entire coding regions of a great many genes in one cost-effective test; this has formed the basis of larger CFTR screening panels including the very first FDA-approved NGS test [16] and greatly expanded carrier screening panels encompassing hundreds of genes [17]. These days, most laboratories use automated DNA sequencer instruments, usually built on capillary electrophoresis for single-gene Sanger sequencing (Fig. 6.1), and massively parallel platforms for NGS.




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**FIGURE 6.1** Example of automated DNA sequence readout on a capillary electrophoresis instrument, showing a heterozygous G-to-A substitution at position 416 of the connexin-26 (*GJB2*) gene. Any interpretive difficulty in discerning the heterozygous nature of the superimposed peaks can be confirmed by reverse sequencing.

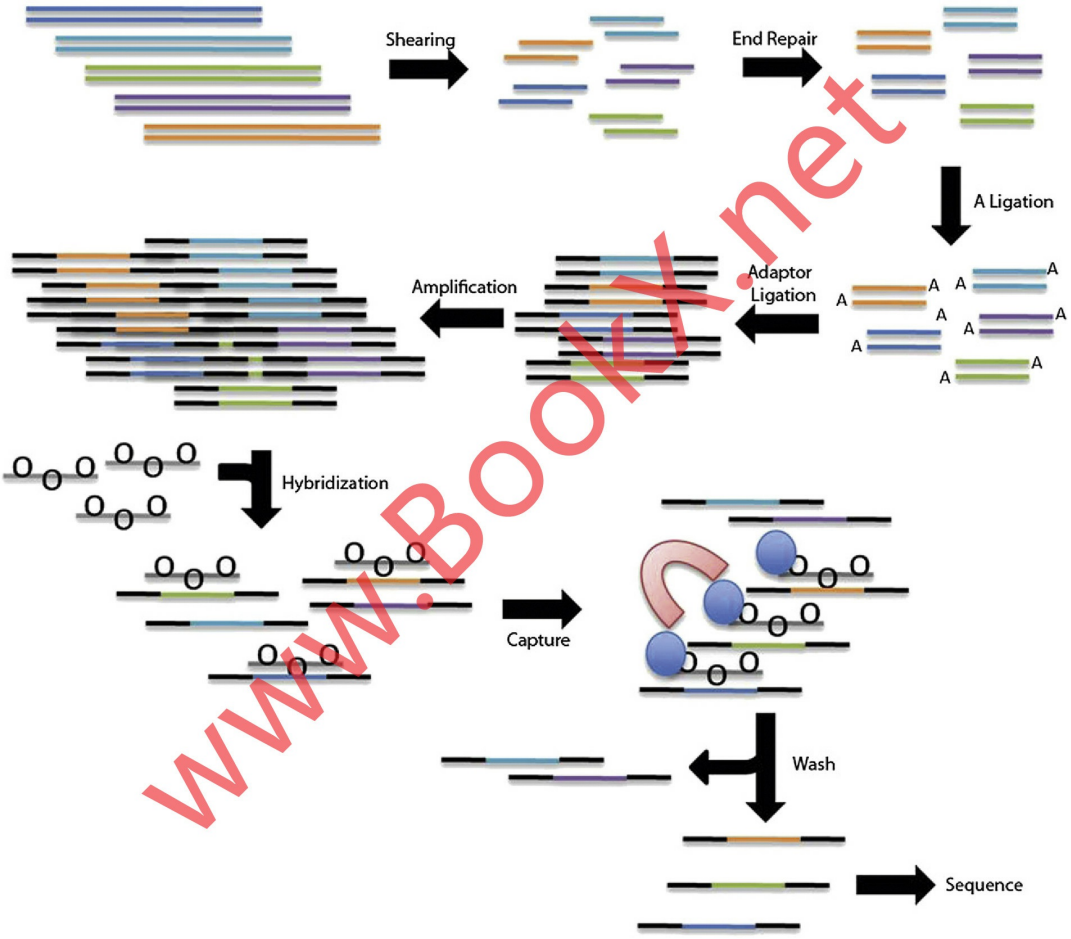
## 6.3.2.5. Detection of Unknown Disease Genes

All the approaches listed earlier are direct mutation detection methods, which can only be used for disorders whose genes have been identified and cloned. Testing for diseases whose genes have not yet been identified formerly required linkage analysis, the use of polymorphic DNA markers to follow segregation of the mutant allele in a family group. This sort of testing requires collection of DNA specimens not only from the proband but also from multiple other

affected and unaffected family members over two or three generations. Also required are polymorphic markers located sufficiently close to (or even inside) the disease gene so that false results due to crossing over are minimized. Although these are now available for most loci in the form of short tandem repeat markers and single-nucleotide variants, linkage analysis is always more cumbersome and involved than direct mutation analysis; it has a higher risk of misleading results and requires family cooperation, which may not always be feasible. Most laboratories were only too happy to leave it behind the instant the identification of the disease gene is reported.

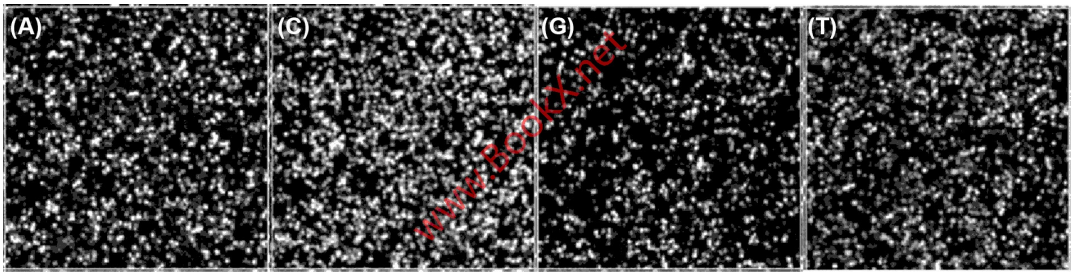
Furthermore, the advent of NGS has rendered linkage analysis even lower on the priority list, because it is now possible to accomplish both gene discovery and mutation identification by whole-exome sequencing of the proband (preferably along with parents, if available). Currently available NGS platforms offer sequencing throughput many orders of magnitude above that which is possible using traditional Sanger (dideoxy chain-termination) sequencing [18]. The technology is based on the ability to perform many parallel DNA synthesis reactions in a manner that allows for the individual products to be analyzed upon each nucleotide addition. This parallelization is achieved through the use of universal primers to DNA fragments that have been derived from the genome by shearing or other methods and immobilized onto a solid support at a dilute concentration (Fig. 6.2). Each DNA molecule is amplified, producing a colony of distinct fragments, and then the colonies are sequenced through the capture of images that reflect the base-by-base addition of nucleotides (Fig. 6.3). Differences in sequencing chemistries of the various platforms result in differences in sequencing capacity, read length, and run time. When sequencing is complete, the resulting sequence reads are aligned against the consensus human genome sequence and all variants are listed, filtered, and annotated. It goes without saying that the computer informatics steps in this form of testing are just as important as the “wet bench” analytical steps. Both the FDA and the College of American Pathologists (CAP) have

recognized this duality by insisting that both arms of the testing be fully validated and reassessed whenever updated [19].




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**FIGURE 6.2** Schematic overview of next-generation sequencing methodology. Adapter ligation to short DNA fragments is followed by amplification, hybridization, and capture of select genomic regions prior to sequencing. The simultaneous capture of specific genomic regions is utilized when intending to test for only a panel of genes (e.g., for all the known genes associated with hypertrophic cardiomyopathy or hereditary hearing loss), as well as for sequencing all of the coding exons in the genome (also known as whole-exome sequencing). Whole-genome sequencing does not require any hybridization and capture steps to be performed.
Photo courtesy of Dr. Hane Lee, UCLA.



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**FIGURE 6.3** Images from one of the commonly used next-generation sequencing platforms (Illumina). With this particular sequencing chemistry, the four nucleotides are each labeled with a different fluorescent dye. After addition of all four nucleotides to the chamber and excitation with a laser, a high-resolution image is captured to identify the newly incorporated nucleotide in a one-base extension step at all of the DNA fragment clusters. The reversible terminators are then removed, and a new cycle of sequencing is initiated.
Photo courtesy of Dr. Hane Lee, UCLA.

## 6.3.2.6. Interpretation of Novel Sequence Variants

Because sequencing will detect any nucleotide change present—not just the previously known mutations in the gene—it becomes important to distinguish benign nucleotide sequence variants (sometimes called polymorphisms) from pathogenic ones. This is not as easy or straightforward as it may seem. While nonsense, frameshift, and splice-site mutations are usually presumed to be pathogenic, especially if they occur prior to the 3′ terminus of the gene, missense variants can bend the rules: even a nucleotide change leading to an apparently conservative or synonymous amino acid substitution may somehow disrupt or inhibit transcription, translation or somatic stability down the line, and a major amino acid substitution may not be deleterious if it lies within a static or nonessential domain of the protein. Furthermore, even if a DNA change is deleterious to the cell, it may be phenotypically neutral if another gene in the genome is programmed to take over the first gene's physiologic function. For these reasons, none of the currently used variant interpretation guidelines, bioinformatics algorithms or functional prediction programs should be taken dogmatically; all are based on assumptions that may not be true in every case. It should also be kept in mind that "VUS" is an invented term reflecting lack of knowledge on our part; it

is not an accurate reflection of biologic behavior. In vivo, the variant is either benign or deleterious, not something in between; although we don't know the answer, nature does.

Given that roughly one in every 500–1000 nucleotides in the genome is polymorphic and that we have only just begun to scratch the surface in our population sequence analysis of most genes, any laboratory performing whole-gene sequencing or whole-exome sequencing is going to have to deal with this conundrum. Both the CAP and the American College of Medical Genetics and Genomics (ACMG) practice guidelines stipulate that it is the laboratory's responsibility to at least attempt an interpretation of the likely clinical significance of any novel variants discovered, rather than simply passing off the burden to the ordering physician. This interpretive discussion, even if admittedly somewhat speculative, must be part of the laboratory test report. Parameters that enter into this analysis include the nature of the amino acid substitution for missense variants (conservative, nonconservative, synonymous), the position of the substitution in the gene and protein product, phenotypic data on the variant if available in the medical literature or mutation databases, degree of evolutionary conservation of the nucleotide and amino acid at that position, presence or absence of the variant in affected and unaffected relatives of the proband and in general "healthy" population databases such as ExAC [20], in vitro functional studies (if available), "tolerance" of the gene to missense and loss-of-function variants in databases, and prediction of the likely impact of the variant on protein function using available programs such as PolyPhen and SIFT [21]—recognizing upfront that none of these criteria or programs is absolutely predictive. These iterative procedures have now been formalized, with relative weights assigned to each piece of evidence, in a detailed guideline on interpretation of sequence variants from a joint working group of the ACMG and Association for Molecular Pathology [22]. However, even when adhering to these guidelines, expert laboratories may reach different conclusions, indicating that there is still some subjectivity in these operations [23].

## 6.4. Molecular Genetic Diagnosis of Some Commonly Tested Diseases

### 6.4.1. Cystic Fibrosis

It seems appropriate to begin the discussion of specific disease examples of molecular genetic testing with CF, because during the 1990s and 2000s it came to be viewed as the prototypic challenge in DNA testing for disorders with high variant heterogeneity. Because it has one of the highest carrier frequencies of any serious autosomal recessive disease, it was considered a high-priority target for population screening ever since the causative gene was identified in 1989 [24,25]. Because CF carriers have no detectable signs or symptoms to distinguish them from other members of the population, such screening could only be accomplished at the DNA level. In addition, DNA testing for CF mutations allows for reliable prenatal diagnosis (provided the identity of both carrier mutations has been established in the parents) and for clinical diagnosis in cases in which the symptomatic manifestations or the sweat chloride levels are atypical or borderline.

However, as is now widely known, the CF gene and in some sense the disease itself have proven resistant to simplistic approaches. While approximately 70% of Caucasian carriers have the common ΔF508 mutation, the other 30% (and an even greater proportion in other racial and ethnic groups) may have any of over 1900 other, much less common, variants. The second most common mutation in non-Jewish Caucasians, G542X, has a frequency of only about 2%, and beyond the first six mutations, the individual carrier frequencies fall below 1% in a general Caucasian/northern European population [26]. Additional problems are raised by the extreme ethnic heterogeneity and admixture of the US target population, the notoriously poor correlation between individual CF mutations and phenotypic expression [27], and evolving ethical concerns surrounding prenatal testing for a disease of considerable clinical variability and potential treatments [28]. As discussed earlier, the most assured way to pick up the greatest number of possible mutations in a large gene like *CFTR*

(which spans 230 kb and 27 exons [29]) is whole-gene DNA sequencing. However, until recently that procedure was considered too expensive for general population screening, and too comprehensive as well, in that missense variants of unknown significance will be found in an appreciable proportion of individuals. For those reasons, screening has been conducted using a more targeted procedure such as allele-specific PCR amplification or microarray hybridization with ASO probes. But which probes to use? Choosing a consensus panel of prevalent CF mutations was not a straightforward task, because all but one are rare, and the prevalence of individual mutations varies greatly according to ethnic group. Over the years, laboratories have used panels of 6, 16, 30, or more than 100 mutations, with remarkable diversity in practice from laboratory to laboratory [30]. After much deliberation, a core, panethnic panel of 25 mutations (along with some associated polymorphisms) was recommended by a subcommittee of the ACMG in order to bring some uniformity to the field and to offer the most efficient strategy for screening in an ethnically diverse population like that of the United States [31]. The criterion used for inclusion in the panel was greater than 0.1% carrier frequency in the affected population as gauged by a large survey of patients with CF [32]; in addition, some mutations especially prevalent in ethnic groups likely to be screened, such as the W1282X mutation in the Ashkenazi Jewish population and the 3120 + 1G → A mutation in African Americans, were included. This core panel produces carrier detectability rates of about 97% in Ashkenazi Jews, 80% in non-Jewish Caucasians, 69% in African Americans, 57% in Hispanic Americans, and an uncertain, although quite low, percentage in Asian Americans.

As in many programs that quickly expanded from a limited pilot cohort to large-scale application, the early years of CF carrier screening revealed a few surprises. One mutation in the core panel, 1078delT, was found to be much rarer in the general population than initially thought and would not have reached the 0.1% threshold for

inclusion. Another one, I148T, was discovered to be a benign variant and an incidental finding in affected patients who invariably had another, more rare, mutation in the same allele. Both of these variants were removed in a revision of the panel announced in 2004 [33]. Conversely, laboratories offering screening with larger panels discovered a number of other mutations with frequencies above the 0.1% threshold, but for reasons of uniformity and parsimony it was decided that these would not be added to the core panel for the time being. Another, perhaps predictable, trend was the tendency among some laboratories, especially on the commercial side, to offer ever larger mutation screening panels, even though the official guidelines discouraged the use of so-called extended panels beyond the core 25 (then 23), and there has been much debate about how much added benefit such panels provide, especially at the cost of including some variants of dubious or poorly characterized clinical significance or extreme rarity [12,34,35].

Laboratories and manufacturers have developed a variety of platforms to encompass whatever mutation panel is chosen, in the form of microarrays, bead arrays, oligonucleotide ligation assays, and single nucleotide variant analysis on automated DNA sequencers. Complete *CFTR* gene sequencing has long been available, although until recently it was primarily used for diagnostic testing [36], where it can help in the differential diagnosis of atypical cases and, in identifying rare parental mutations in an affected child, which can then serve as targets for prenatal diagnosis in a subsequent pregnancy. Now with the advent of NGS, *CFTR* gene sequencing is being applied to carrier screening, either by itself or as part of expanded carrier screening panels. As already mentioned, an FDA-approved NGS platform targets 139 functionally verified [37] pathogenic variants in the gene, while other platforms and services target or visualize many more.

Another lesson from the CF gene story, subsequently revealed in other diseases as well, is the broadening of the clinical phenotype as novel mutations are detected in increasing numbers and varieties of patients, primarily through comprehensive DNA sequencing. It was

already known that some patients with CF may have minimal lung disease [38] or normal sweat chloride levels [39], but it later became apparent that there were individuals with mutations in the *CFTR* gene presenting with phenotypes not at all resembling classic CF. The gene has been implicated in such conditions as pancreatitis [40] and sinusitis [41], but most important has been the association of certain *CFTR* mutation and polymorphism combinations with an isolated congenital malformation, bilateral absence of the vas deferens [42]. Most frequently associated with this condition are the R117H mutation and an intronic polymorphic tandem repeat of either five thymidines in *trans* or seven thymidines in *cis* [43]; five thymidines in *trans* with other mutations can also produce the same malformation [44]. The variable expressivity of the R117H mutation, requiring reflex testing for the poly T tract, has proved to be one of the more problematic aspects of the CF carrier screening program, both in the United States and in Europe [45,46]. However, CF is certainly not unique in this regard, and we are just beginning to appreciate the wide variety of modifier genes and elements affecting the clinical phenotype of supposedly "classic" genetic disorders. That is why the lessons we have learned from the widespread molecular testing in this disease are so relevant to many other applications in diagnostic molecular genetics.

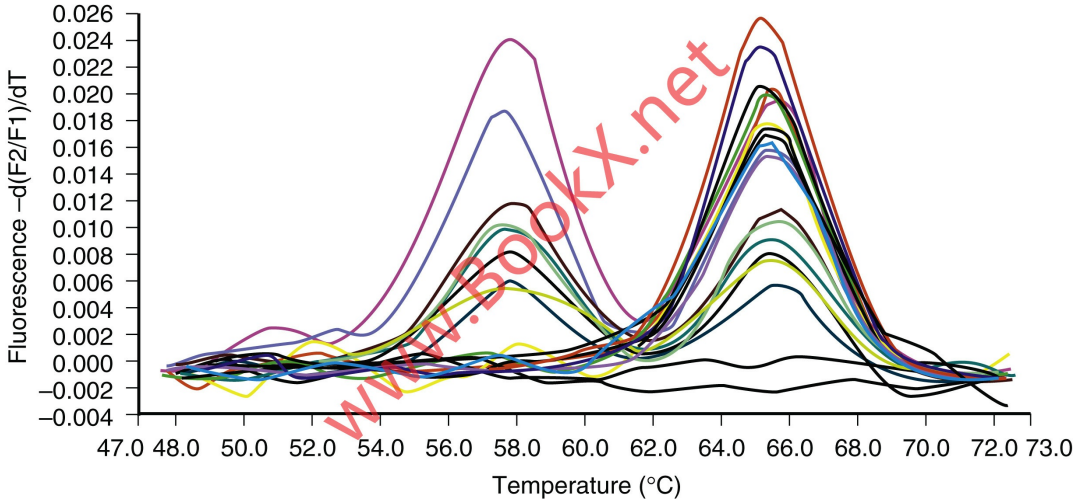
## 6.4.2. Factor V Leiden and the Hereditary Thrombophilias

Similar to the CF carrier state, the Leiden mutation in the clotting factor V gene is a common enough allele (up to 7% in Caucasians [47]) that its DNA assay had been considered for implementation as a screening test, if not in the general population then perhaps at least in those individuals already at risk for thrombosis due to environmental factors (surgery, paralysis, trauma, malignancy, and oral contraceptive use). Indeed, the DNA test itself is very easy, because only a single nucleotide is targeted. There are a variety of automated methods, microarray hybridization, and real-time PCR coupled with

melt-curve analysis (Fig. 6.4) available from commercial vendors [21]. Yet, also analogous to *CFTR*, the biology of this gene in vivo and in large populations is somewhat more complex.

The R506Q substitution renders factor V resistant to cleavage by activated protein C (APC), a key mechanism for keeping the coagulation cascade in check. It is the major (but not the only) cause of thrombophilia due to APC resistance. It is best to think of factor V Leiden as a genotype and APC resistance as the phenotype. As such, the latter can be diagnosed by functional coagulation assays on patient plasma, and some authorities believe that the functional assay is preferable to the DNA test as an initial diagnostic screen because it casts a wider net. On the other hand, technical modifications of the functional assay have made it almost entirely specific for the factor V Leiden etiology [48]. In any event, the DNA test is certainly useful because of its lack of interference by anticoagulant therapy (which so many of the tested patients are already taking) and its ability to reliably distinguish homozygotes from heterozygotes. The latter is important because heterozygotes have a 7- to 10-fold increased risk of venous thrombosis, while homozygotes have about an 80-fold increased risk [49]. While these relative risk estimates may seem high, it is important to remember that idiopathic thrombosis in otherwise healthy adults, especially young adults, is quite rare; so the absolute risk, especially in heterozygotes, is not particularly great. In fact, the lifetime risk of an adverse event in a carrier is much less than the risk of complications from long-term anticoagulant therapy, which is the intervention presumably being considered in those testing positive. A similar risk differential can be stated for female carriers taking oral contraceptives who, as a result of DNA testing, might be obligated to seek other, less effective methods of birth control, leading to the risk of adverse events in unintended pregnancies [50]—although this notion remains somewhat controversial. In view of these low-magnitude relative probabilities, it is understandable that the concept of population screening of asymptomatic individuals for the factor V Leiden mutation has not been endorsed by either of two consensus statements issued by the ACMG [51] and CAP [52]. The test remains

useful, however, in differential diagnosis of otherwise unexplained thromboembolic events in patients under age 50 and in recurrent pregnancy losses. Because its main application is thus diagnosis rather than prediction, factor V Leiden analysis differs from many of the other genetic tests mentioned in this chapter. It is ordered primarily by internists, hematologists, and obstetricians rather than by geneticists and is widely viewed, rightly or wrongly, as a routine blood test rather than a genetic test. The consensus statements recognized this and did not require any pretest informed consent or genetic counseling [51].



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**FIGURE 6.4** Detection of factor V Leiden mutation by real-time PCR and melt curves with allele-specific probes. Each colored curve represents the genotype of a particular patient tested, illustrating a selection of wild-type (right peak), homozygous factor V Leiden (left peak), and heterozygous (both peaks).

Factor V Leiden is but one of a group of disorders classified as hereditary thrombophilias. While some of the others, such as protein S, protein C, and antithrombin III deficiencies, as well as variants in the factor VIII, fibrinogen, thrombomodulin, and platelet glycoprotein genes, are generally thoughtt to be too rare and mutationally heterogeneous to become candidates for widespread testing at the molecular level (especially because they can be identified by less expensive functional coagulation assays) [53], there is one that

approaches factor V Leiden in its indications and ease of testing. The prothrombin 20210A variant (located in the 3′-untranslated region of the gene) leads to increased levels of prothrombin in the circulation and a phenotype similar to that of factor V Leiden [54]. It, too, can be detected by the same automated methods, which can be multiplexed with the factor V Leiden test [55,56]. In addition, because it is also relatively common (1%–2% carrier frequency), it makes some sense to test for it at the same time as factor V Leiden, provided the same indications are present.

In the early days of molecular thrombophilia testing, many laboratories also included in their gene panels the 677C → T variant of the methylenetetrahydrofolate reductase (*MTHFR*) gene, which was thought to be associated with elevated plasma homocysteine levels through inhibition of the folate-mediated remethylation of that compound. This allele is very common, found in 30%–40% of the general population [57]. Another variant, 1298A → C, was also associated with elevated homocysteine levels when in compound heterozygosity with 677C → T. However, the described phenotype was different from that for factor V Leiden and prothrombin 20210A, because elevated homocysteine is also associated with arterial (including coronary) thrombosis, as is well known from patients with classic homocystinuria. However, not everyone with an *MTHFR* variant exhibits elevated homocysteine, and not all cases of elevated homocysteine are caused by *MTHFR* variants. Furthermore, recent studies have called into question the fundamental association of modestly elevated homocysteine levels with thrombotic risk as well as the association with these *MTHFR* variants [57], and such elevation, even if present, is readily lowered by folate ingestion, either as a vitamin supplement or via widespread fortification in foods. For purposes of clinical decision-making, therefore, there does not seem to be a compelling reason to include this test in with the other two, nor even as a freestanding analysis. Indeed, current guidelines explicitly

advise against the use of this test for either thrombophilia screening or diagnosis [58].

### 6.4.3. Hereditary Hemochromatosis

Despite the greater attention paid to CF, hemochromatosis is actually the most common inherited single-gene disorder in people of northwest European ancestry. It is an autosomal recessive disease estimated to affect about 1 in 300 individuals in this population [59,60]. The gene, *HFE*, is located on chromosome 6p, 4.5 megabases telomeric to the *HLA-A* locus, and encodes a major histocompatibility complex class I–like protein that regulates iron absorption from the intestine.

Two missense variants, C282Y and H63D, have been identified within the gene and implicated in iron overload [61]. About 70%–100% of hereditary hemochromatosis cases in various studies are homozygous for the C282Yvariant, which is carried by about 10% of Caucasian individuals [62]. The other variant, H63D, is found in compound heterozygous form with C282Y in 3%–5% of patients. These two mutations are in linkage disequilibrium and have not been found together on the same haplotype. However, considering the high carrier frequency of the H63D mutation in the general population (about 25%), its involvement in the pathogenesis of the disease remains controversial; it might represent a minor mutation of low penetrance or even a benign variant [63]. One large study of more than 3500 affected patients found homozygosity for C282Y in 81%, homozygosity for H63D in about 1%, compound heterozygosity for C282Y and H63D in 6.7%, and combinations with another variant, S65C, in less than 0.5%.

Most laboratories offering *HFE* mutation testing assay for both major variants, while a few also include S65C. Molecular diagnostic testing for these variants can be used to confirm the diagnosis of hereditary hemochromatosis in individuals with symptomatic disease, and potentially to detect those with presymptomatic iron overload in whom future disease manifestations may then be prevented by simple measures (e.g., regular phlebotomy). Because of the high carrier

frequency, easy therapeutic intervention, and irreversibility of organ damage if not diagnosed early, hereditary hemochromatosis has been considered as a target for population screening. It has never been implemented, however, because of the greatly reduced and variable penetrance, even in C282Y homozygotes [64]. Some surveys have suggested that the lifetime penetrance for significant liver disease may be as low as 10% or even 1% [65–68]. It is also not clear whether, analogous to the situation with factor V Leiden, the functional assay (in this case, transferrin saturation and serum ferritin levels) might be the more appropriate initial screening test. In a large survey of 100,000 individuals, assessed for both serum iron measurements and HFE genotype, biochemical differences between C282Y homozygotes and controls were variable, and no consistent deleterious effects of iron overload between the two groups could be demonstrated [68]. It could thus be argued that if widespread population screening is indicated for asymptomatic individuals, testing for transferrin saturation and serum ferritin levels would be a more specific and less expensive strategy than molecular analysis. It should also be noted that there are less common types of hereditary hemochromatosis caused by mutations in other genes, such as hepcidin, hemojuvelin, transferrin receptor-2, and ferroportin-1 [69]. With the availability of NGS, variants in all of the associated genes can be detected readily by gene panel testing or exome sequencing.

## 6.4.4. Hereditary Hemoglobinopathies

Hereditary hemoglobinopathies encompass a vastly heterogeneous group of genetic disorders affecting globin protein structure and/or synthesis. Therefore, while the globin genes are relatively small compared to those associated with other important heritable disorders, the approach to their molecular testing is quite varied.

### 6.4.4.1. Structural Globin Disorders

While the greatest incidence of sickle cell disease occurs in sub-Saharan Africa, the carrier frequency in the African American

population is 10% and presumably a bit higher in the African diaspora around the world, making it potentially a significant target for DNA testing and screening, although socioeconomic factors have impeded such efforts in the past. This is ironic considering that sickle cell disease was the first human genetic disorder for which the molecular basis was described. Later, the β-globin gene was the first human gene to be cloned and its various mutations demonstrated. Several hundred point mutations in the β-globin gene have been identified, with the sickle cell mutation being the most common and most severe. While many of these can be identified by nonmolecular methods as well, it is important to remember that the number of structural variants that can be identified by standard hemoglobin (Hb) electrophoresis represents a minority of the total number in existence, because only approximately one-third of them produce an altered charge in the Hb molecule. In addition, in hyper-unstable Hb syndromes, the protein may be so labile or short-lived that electrophoretic identification is hampered. In these cases, DNA analysis will be preferable. For the same reasons, molecular testing can serve as a useful second-tier backup to newborn sickle cell disease screening by biochemical methods, even using the same blood spots [59].

The original DNA-based method for prenatal diagnosis of sickle cell disease used linkage analysis. An *Hpa*I restriction fragment length polymorphism (RFLP) downstream of the β-globin gene was linked to the sickle cell mutation in 87% of African American HbS genes [70]. Now, of course, direct mutation detection strategies are preferred, with gene sequencing representing the best approach for distinguishing the many other potential β-globin variants, such as HbC, HbE, and others [71].

### 6.4.4.2. Disorders of Globin Synthesis—The Thalassemias

Widespread population-specific screening of carriers for autosomal recessive disorders was initiated first with the thalassemias. Successful thalassemia screening programs have resulted in a marked reduction in the birth rate of affected children in Greece, Cyprus,

continental Italy, Sardinia, Taiwan, China, and Southeast Asia [72]. The thalassemias are a large and heterogeneous group of disorders of imbalanced Hb synthesis with widely varied phenotypic expression. From the laboratory perspective, the most important ones are α- and β-thalassemia (and their respective intermediate subtypes).

#### 6.4.4.2.1. α-Thalassemia

α-Thalassemia is most commonly due to a deletion of one or more of the four α-globin genes ($\alpha_1\alpha_2/\alpha_1\alpha_2$). Deficits relate closely to gene dosage effects, and therefore dosage, and sometimes phase analysis, is an important component of the molecular diagnostic effort. Deletion of one α-globin gene, usually the $\alpha_2$, is most common. These occur predominantly in two types, a 3.7-kb deletion ($-\alpha^{3.7}$) and a 4.2-kb deletion ($-\alpha^{4.2}$). The $-\alpha^{3.7}$ deletion is seen in individuals from Africa, the Mediterranean, the Middle East, and Oceania. The $-\alpha^{4.2}$ deletion is seen in individuals from Southeast Asia and the Pacific Islands [73]. A subset of patients in certain ethnic groups may have point mutations in the α-globin genes.

Because most α-thalassemias are thus due to sizable deletions, Southern blotting of endonuclease-digested genomic DNA was traditionally used for diagnosis, but now more modern techniques such as allele-specific PCR priming and MLPA are usually used [15,74]. The two α-globin genes have divergence in their sequences in the 110-nucleotide-long 3′-untranslated region, allowing the presence of one or the other or both to be distinguished.

#### 6.4.4.2.2. β-Thalassemia

More than 250 different defects in the β-globin gene that result in β-thalassemia have been described, and in contrast to α-thalassemia, most of them are point mutations. Molecular diagnosis of the β-thalassemias is made complicated by this heterogeneity. However, a unique spectrum of mutations is usually prevalent in a given population at risk for β-thalassemia. Fewer than 10 mutations usually encompass the majority of molecular lesions in the target population, making screening and prenatal diagnosis manageable [75]. In fact,

approximately 20 total mutations account for 80% of all the β-thalassemia alleles in the world.

Most β-globin gene defects (about 95%) that result in β-thalassemia syndromes are due to point mutations, along with a few microdeletions (up to 17 bp) and microinsertions. About half these mutations completely inactivate the β-globin gene ($\beta^0$-thalassemia). This portion is caused by loss-of-function mutations. The $\beta^+$-thalassemia mutations mainly affect transcription or messenger RNA (mRNA) processing, usually resulting from mutations located in the promoter region. Large deletions (>24 bp) in the β-globin gene alone are quite rare, with fewer than 20 types seen [76]. The deletion more often involves large segments of the β-globin gene cluster, resulting in $\delta\beta^0$-and $\delta\beta^+$-thalassemias. Depending on the number and variety of mutations/deletions expected, laboratories can choose any of a number of ASO hybridization, sequencing, and/or deletion-detection (e.g., MLPA) strategies.

## 6.4.5. Repeat Expansion Disorders

Trinucleotide repeats are members of the tandem repeat families found throughout the genome. However, unlike the more ubiquitous dinucleotide and tetranucleotide repeat polymorphisms, expansion of these triplet repeats to larger size can have a pathologic effect. Such expansion has been identified as a novel mutation mechanism in at least 15 neuromuscular disorders that result from instability of the expanded repeat once it has reached a certain length. A few additional neurologic disorders are caused by expansion of a repeat of different size, such as tetranucleotide or hexanucleotide. The expansions are unstable mutations that tend to increase in size through successive generations, in contrast to repeat lengths in the normal range that are usually transmitted stably despite their polymorphic nature. The subsequent expansion of the repeat in descending generations does not adhere strictly to the rules of Mendelian inheritance because of the

dynamic nature of the mutation and its variable expressivity. Anticipation is the hallmark of the repeat expansion disorders, where increasing severity and/or earlier onset occur in successive generations and are correlated directly with the length of the repeat. Parent-of-origin effect is also a feature for some of them, with risk of expansion being greater when inherited from either the mother (e.g., myotonic dystrophy) or the father (e.g., Huntington disease). Last, both the sequence and location of the repeat within the gene (e.g., coding region, intron, promoter region) differ among the various disorders [77]. Relatively small repeat lengths can be detected by PCR-based methods, usually followed by accurate sizing by capillary electrophoresis. Very large repeat expansions may require Southern blot or specialized repeat-primed PCR amplification. Note that NGS approaches are of little use for diagnosis of these disorders because of difficulty aligning the short sequence reads across the longer repeat region.

The sequence and expansion ranges of the repeats in the major tandem repeat disorders are summarized in Table 6.1.

## 6.4.5.1. Selected Specific Repeat Expansion Disorders

### 6.4.5.1.1. Spinobulbar muscular atrophy

SBMA, known also as Kennedy disease, is a motor neuron disorder characterized by progressive muscle weakness and atrophy in males. The disease results from an expanded CAG repeat in the androgen receptor gene located at Xq12. The number of CAG repeats ranges from 9 to 33 in normal alleles. Affected males and carrier females have an allele with greater than 38 CAG repeats, readily detectable by PCR.

### 6.4.5.1.2. Huntington disease

The unstable CAG repeat in HD patients lies in exon 1 of the *HTT* gene on chromosome 4p16.3. The CAG repeat length at this locus in the normal population ranges from 10 to 35, whereas in patients with HD, it ranges from 36 to 121, with a reduced penetrance at repeat sizes of 36–39. Strong inverse correlations between repeat length and age of

onset have been observed. Adult-onset patients usually have an expansion in the range of 40–55, whereas juvenile-onset patients have expansions greater than 60 that are often inherited from the fathers in successive generations (due to the increased risk of further expansion with paternal inheritance). Sizing of repeat lengths is readily accomplished by PCR amplification and electrophoresis, best done on a capillary instrument for greatest accuracy (Fig. 6.5).

A problem will arise when only a single PCR product in the normal repeat range is observed. The laboratory is then obligated to determine whether this is due to homozygosity for the normal allele or heterozygosity for a greatly expanded second allele that was missed due to PCR failure. To do this, a second PCR primer set is used that encompasses a neighboring (outside the gene) nonpathologic CCG repeat in addition, to the *HTT* intragenic repeat. Because this second repeat is itself polymorphic, it may allow "splitting" of the homozygous PCR product into two distinguishable secondary products (both in the normal range). However, in a minority of cases, the second repeat will be homozygous as well, in which case reflex testing by Southern blot or triplet-primed PCR to detect a potential greatly expanded repeat is required [78]. In actual practice, if the individual being tested is an adult with no sign of juvenile onset of symptoms, the chance of carrying an expanded allele so large that it would cause PCR failure is negligible. Needless to say, accurate sizing of the repeat number in this disease is extremely important, not only because of the emotionally charged nature of the test but also because the difference in size between affected, intermediate, or normal alleles is as little as three nucleotides.

## Table 6.1

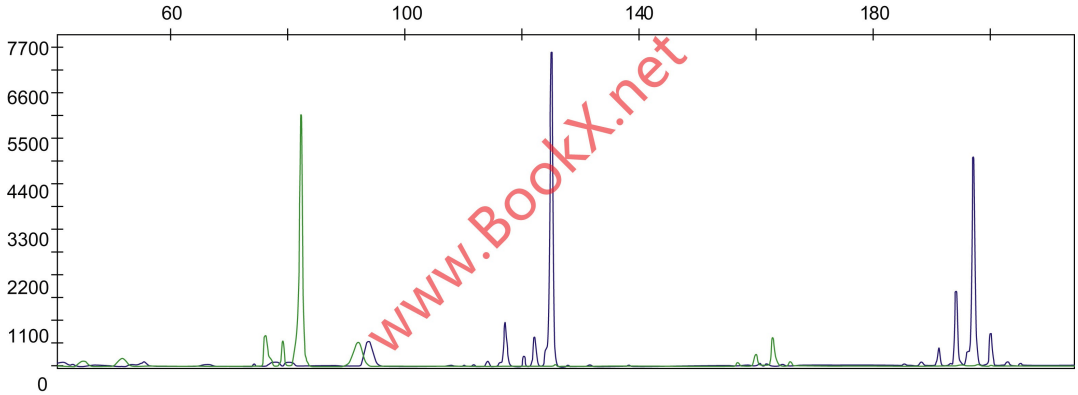
### Mutation Ranges for Selected Tandem Repeat Disorders

| Disease (Gene) | Repeat Unit | Normal Range | Premutation or Partial Penetrance Range[a] | Affected Range |
|---|---|---|---|---|
| **ALS/FTD** | GGGGCC | <23 | – | 100–1000s |

| | | | | |
|---|---|---|---|---|
| **(*C9orf72*)** | | | | |
| **DM1 (*DMPK*)** | CTG | 5–37 | 38–49 | 50–3000+ |
| **DM2 (*ZNF9*)** | CCTG | <26 | – | >75 |
| **DRPLA (*ATN1*)** | CAG | 6–35 | – | 49–88 |
| **Fragile X (*FMR1*)** | CGG | 5–54 | 55–200 or 230 | 200 or 230–1000+ |
| **Friedreich ataxia (*FXN*)** | GAA | 6–33 | – | 67–1700 |
| **HD (*HTT*)** | CAG | 10–35 | 36–39 | 40–121 |
| **SBMA (*AR*)** | CAG | 9–33 | – | 38–62 |
| **SCA1 (*ATXN1*)** | CAG | 6–3 | – | 39–81 |
| **SCA2 (*ATXN2*)** | CAG | 14–31 | 32–35 | 36–64 |
| **SCA3 (*ATXN3*)** | CAG | 12–44 | – | 52–87 |
| **SCA6 (*CACNA1A*)** | CAG | 4–17 | – | 21–33 |
| **SCA7 (*ATXN7*)** | CAG | 10–19 | 28–35 | 37–200+ |
| **SCA8 (*ATXN8, ATXN8OS*)** | CTG<br>CAG | 15–50<br><50 | –<br>– | 71–1300<br>>80 |
| **SCA10 (*ATXN10*)** | ATTCT | 10–32 | – | >800 |
| **SCA12 (*PPP2R2B*)** | CAG | 8–23 | – | 51–78 |
| **SCA17 (*TBP*)** | CAG or CAA | 25–44 | – | 47–63 |

[a] Absence of a listing in this column does not necessarily mean that the disorder has no premutation range, only that it has not yet been documented or well defined.

Adapted from Wallace SE, Bird TD. Molecular genetic testing for hereditary ataxia: what every neurologist should know. Neurol Clin Pract 2018;8:27–32; Fogel BL, Geschwind DH. Clinical neurogenetics. In: Daroff R, Jankovic J, Mazziotta J, Pomeroy S, editors. Neurology in clinical practice. 7th ed. Philadelphia (PA): Elsevier; 2015. pp. 648–75.



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**FIGURE 6.5** Positive test result for HD trinucleotide repeat expansion. Fluorescently labeled primers (green) are used to amplify the HD CAG repeat region by PCR, and the fragments are separated and sized using capillary electrophoresis. One normal allele (<27 repeats) and one full mutation allele (>40 repeats) are evident on the above example. A second set of fluorescently labeled primers that encompass a neighboring polymorphic short-tandem repeat (blue) are run simultaneously in order to confirm the successful amplification of both alleles in cases when only a single peak is seen with the original set of green primers.

HD was the first genetic disorder to be a target of presymptomatic molecular testing. Because of the virtually 100% penetrance of the full CAG expansion and the lack of any preventive therapy (though there are some ongoing clinical trials), a positive result in this test represents particularly devastating news, carrying risks of depression, suicide, and discrimination [79]. For these reasons, standard practice for predictive testing emphasizes pre- and post-test psychosocial support and genetic counseling, and many centers keep the results strictly confidential and not accessible through the medical record.

#### 6.4.5.1.3. Dentatorubral-pallidoluysian atrophy

DRPLA results in both ataxia and choreoathetosis, along with myoclonus, epilepsy, and dementia, so it is sometimes considered in the differential diagnosis of HD. The gene encodes a cytoplasmic protein with a polyglutamine tract in the middle. The number of CAG repeats in unaffected individuals ranges from 6 to 35, whereas in patients with DRPLA it ranges from 49 to 88. Molecular sizing is done as for HD.

#### 6.4.5.1.4. Spinocerebellar ataxias

The SCAs are a group of genetically diverse neurologic conditions that share progressive deterioration in balance and coordination due to degeneration of the cerebellum and its afferent and efferent pathways. Linkage studies identified multiple subtypes, eight of which have thus far been found to have unstable CAG (or CTG) expansions (types 1, 2, 3, 6, 7, 8, 12, and 17). The relatively small size of the SCA trinucleotide expansions (Table 6.1) allows for PCR-based testing as for HD. An additional subtype, SCA10, is due to an intronic pentanucleotide repeat (ATTCT) with pathologic expansions above 800 repeats, requiring repeat-primed PCR or Southern blot.

#### 6.4.5.1.5. Fragile X syndrome

Fragile X syndrome is caused by a large expansion of CGG repeats in the 5′-untranslated region of the fragile X mental retardation (*FMR1*) gene. The larger expansions are associated with increased methylation of both the repeat and the adjacent CpG island of the promoter region, leading to transcriptional silencing of the gene. The expansion and the subsequent methylation account for the presence of a fragile site (FRAXA) at chromosome Xq27.3 that results from a failure of normal chromatin condensation during mitosis.

Alleles of the CGG repeat can be classified as normal, premutation, or full mutation based on the number of the repeats. In the normal population, this repeat is highly polymorphic, ranging from 5 to 54 repeats, while in affected individuals the expansions range from between 200 and 230 to more than 1000 repeats and are referred to as full mutations. The full mutation alleles are usually methylated and

fail to express the *FMR1* mRNA and protein. Southern blot sizing of the *FMR1* expansion also includes use of a methylation-sensitive restriction enzyme to determine methylation status (Fig. 6.6(a)), which is especially important because some males with full but unmethylated expansion and no clinical symptoms have been reported [80]. In addition, use of the methylation-sensitive enzyme allows separation of the two *FMR1* alleles in females, because the one located on the inactive X chromosome will be resistant to digestion because of intrinsic methylation due to the lyonization process. This approach helps to avoid overlooking a very large expansion in a female when only a single normal band is observed on the first-pass Southern blot (using a methylation-insensitive enzyme) or PCR. On the other hand, PCR with capillary electrophoresis is preferred for accurate sizing of premutations in carrier females, because the risk of expansion in offspring increases incrementally with increasing premutation lengths [81]. Difficulty in amplifying the GC-rich premutation alleles of fragile X patients can be overcome by the addition of dimethylsulfoxide, 7-deaza-dGTP, and betaine to the PCR, and more recent innovations in primer design now allow for amplification and even methylation analysis of full mutations [82,83] (Fig. 6.6(b)).

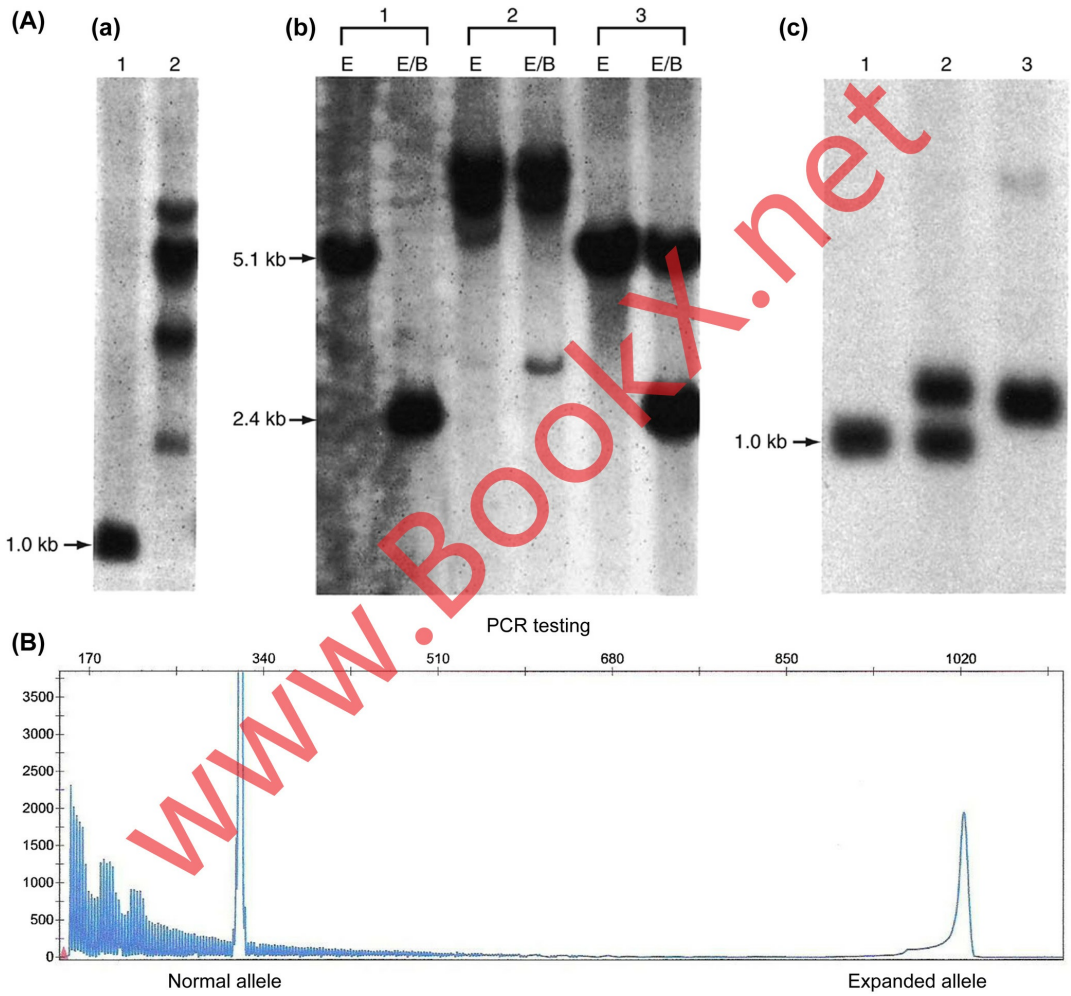



**FIGURE 6.6** (A) Southern blot analysis of the *FMR1* gene of fragile X syndrome. (a) *Pst*I digestion and probing of genomic DNA: 1, normal control showing unexpanded allelic band at 1.0 kbp and 2, patient with full CGG expansion mutation showing a spread of high-molecular-weight bands. (b) Southern blot methylation analysis by *Eco*RI and *Eco*RI/*Bss*HII double digestion (*Bss*HII is a methylation-sensitive restriction enzyme): (1) normal male showing single unexpanded, unmethylated allele; (2) affected male showing a single expanded and almost completely methylated allele; and (3) normal female showing two unexpanded alleles, one of which is methylated due to X-inactivation. (c) *Pst*I digestion and probing of genomic DNA: (1) normal male; (2) female with one normal allele and one premutation allele; and (3) premutation male. (B) Amplicon profile of female DNA containing an FMR1 CGG expansion into the full-mutation range, using triplet-primed PCR.

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Large expansions display mitotic instability, resulting in a mixture

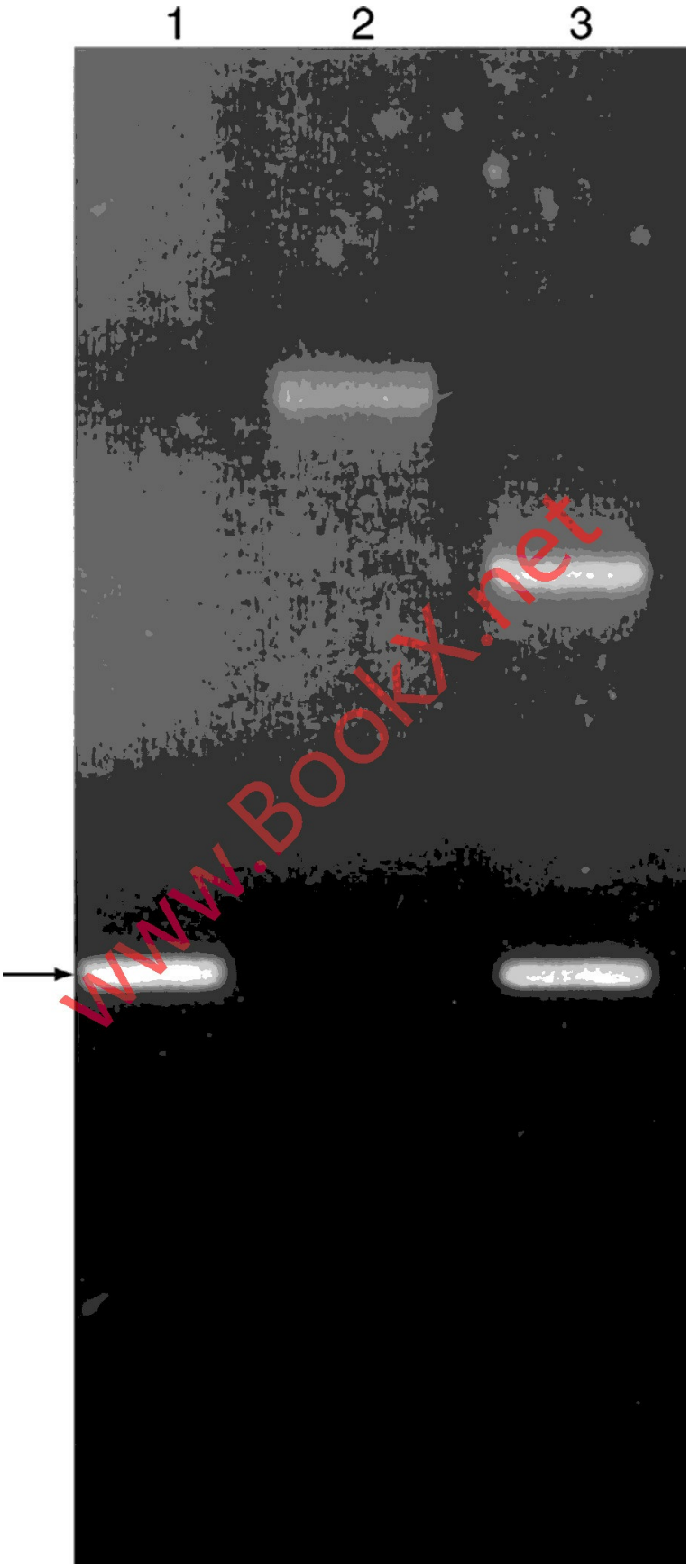
of allele sizes within a single individual. Premutation alleles ranging from about 55 to 200 are not typically associated with methylation, but they display instability in subsequent generations. These alleles may change in size when transmitted from either sex, invariably remaining within the premutation size range in male transmission but expanding to full mutation size in female transmission. (Alleles in the so-called "gray zone" of 45–54 repeats have a slight chance of expanding incrementally into the premutation range but not into the full mutation range.) The presence and location of AGG triplets interspersed within the CGG repeats in the *FMR1* gene also play an important role in the stability of the repeat. An uninterrupted pure CGG repeat of greater than about 33–39 triplets appears to increase the instability of maternal alleles [84,85]. While it was long assumed that premutation carriers have a normal phenotype, we now know that they can cause late-onset effects, including premature ovarian failure in women and a middle-aged tremor-ataxia-dementia syndrome in men [86]. Needless to say, the awareness of these phenomena now raises ethical concerns in the reporting of premutation results in fetuses and children, particularly if discovered as a consequence of population screening [9].

Identification of some families that were cytogenetically positive for the fragile site on chromosome Xq27-28 but negative for *FMR1* expansion led to the discovery of two more fragile sites, FRAXE and FRAXF, both located distal to FRAXA [87]. Both involve GCC repeat expansion, with FRAXE exhibiting mild mental retardation in the absence of the characteristic physical stigmata of FRAXA, and FRAXF showing no apparent clinical phenotype. In practice, these two genes are not often investigated.

#### 6.4.5.1.6. Myotonic dystrophy

Myotonic dystrophy (DM) is the most common inherited muscle disorder in adults, with effects in many other organ systems as well. The genetic basis for the major type, DM1, is an expanded CTG repeat in the 3′-untranslated region of a dystrophica myotonica protein kinase gene (*DMPK*). The size of the expanded repeat and the severity

of the disease tend to increase in successive generations. The normal repeat size is 5–37 copies, while expansions range from 50 to more than 3000 in affected individuals. CTG repeat lengths in the range of 38–49 are considered "premutations." In the range of 50–150 repeats, patients develop mild DM with cataract, mild myotonia, or diabetes mellitus, and they may have fully active lives and a normal or minimally shortened life span. In the range of 100–1500 repeats, patients usually develop classic DM with muscle weakness and wasting, myotonia, cataracts, and often cardiac conduction abnormalities, with the age of onset typically in the 20s and 30s. Most infants with congenital DM have more than 1000 CTG repeats, with mental retardation (in 50%–60% of cases) and early death. Infants with congenital DM nearly always inherit the expanded *DMPK* allele from the mother, illustrating a parent-of-origin effect on the anticipation phenomenon. Because of the large size of some DM expansions, "long PCR" or Southern blotting may be required for diagnosis. DM2 is caused by expansion of an intronic tetranucleotide repeat (CCTG) in the zinc finger protein 9 (*ZNF9*) gene.




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**FIGURE 6.7** PCR analysis of Friedreich ataxia GAA trinucleotide repeat expansion. *Arrow* indicates the size of PCR products generated from normal alleles. The samples shown are (1) normal; (2) homozygous expanded; and (3) heterozygous.

#### 6.4.5.1.7. Friedreich ataxia

Friedreich ataxia, the only autosomal recessive condition among the major tandem repeat disorders, is caused in 97% of patients by the expansion of a GAA repeat in the first intron of the frataxin (*FXN*) gene. The level of frataxin protein expression is decreased in affected individuals with expanded repeats, presumably by an inhibitory effect of the intronic repeat on transcription [88]. The larger the repeat, the greater is the effect on mRNA and protein levels. Normal individuals have 6–33 uninterrupted GAA repeats, and affected patients have 67–1700 repeats. Sizing can be accomplished by PCR even for the larger expanded repeats, because the repeat sequence is not GC-rich (in contrast to fragile X) (Fig. 6.7). Compound heterozygotes have been identified in a small percentage of cases with a triplet expansion in one allele and either a nonsense mutation, missense point mutation, or initiation codon mutation in the other allele. This makes the results reporting rather complicated, because the methods used for repeat sizing will not pick up point mutations, and referral to a specialized laboratory offering full gene sequencing may be required.

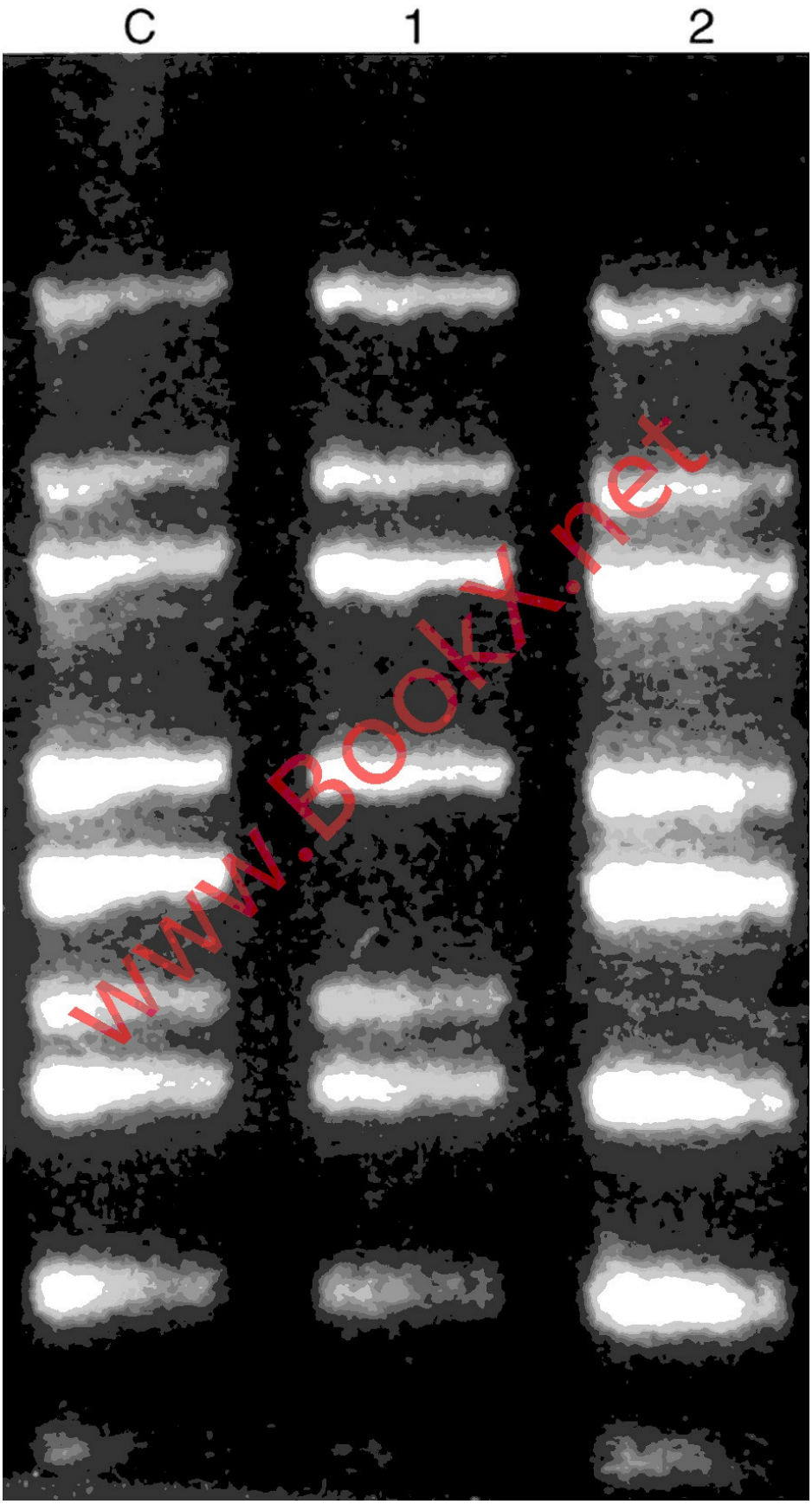
#### 6.4.5.1.8. Amyotrophic lateral sclerosis/frontotemporal dementia

Perhaps the most important of the nontriplet repeat disorders is dominantly inherited ALS and/or FTD caused by expansion of a hexanucleotide repeat (GGGGCC) in the first intron of a poorly understood gene teasingly called *chromosome 9 open reading frame-72* (*C9orf72*). The normal repeat length in healthy individuals is 2–22 repeats, while affected patients show repeat lengths in the hundreds or thousands [89].

### 6.4.5.2. Duchenne and Becker Type Muscular Dystrophies

Duchenne (DMD) and Becker (BMD) muscular dystrophies are allelic forms of an X-linked neuromuscular disorder that affects about 1 in 3500 live male births. Both are caused by mutations arising in the gene encoding dystrophin, a cytoskeletal protein that underlies the plasma membrane of normal skeletal muscle. The dystrophin gene is located on the short arm of the X chromosome (Xp21) and is one of the largest genes ever characterized, spanning 2.4 Mbp and encoding 79 exons [90]. The gene has an unusually high rate of intragenic recombination of about 10%–12% in normal pedigrees [91]. Recombination events appear to occur mostly in two hotspots located between exons 1–8 and exons 44–51 [92]. The majority of the mutations are intragenic deletions (~65%) or duplications (5%) [93,94]. The remaining one-third of cases are due to point mutations such as microdeletions, insertions, and substitutions. According to the frameshift hypothesis proposed by Monaco et al. [95], a patient most likely develops a DMD phenotype if the mutation disrupts the reading frame, whereas a BMD phenotype results if the reading frame is maintained. This would explain the apparent paradox of even fairly large BMD deletions producing a mild phenotype. At the protein level (e.g., on Western blot), DMD is seen to be caused by the absence of detectable dystrophin, whereas muscle from BMD patients shows a protein of reduced size at less than 40% of normal levels. These observations have inspired targeted therapeutic strategies for DMD based on gene editing and exon skipping [96].

The current strategy for DNA testing in this disorder was developed to attempt to circumvent problems arising from the unusual features of the gene, such as its very large size and the great variety of mutations. The approach will vary somewhat depending on the intent and indications for testing, as described in the following paragraphs.



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**FIGURE 6.8** Multiplex PCR for detection of dystrophin deletions in Duchenne/Becker type muscular dystrophy, by the method of Beggs et al. [55]. The samples shown are C, normal control showing PCR products corresponding to (from top to bottom of electrophoretic gel) promoter and exons 3, 43, 50, 13, 6, 47, 60, and 52; 1, patient positive for a deletion involving exon 13; and 2, patient positive for a deletion involving exon 6.

#### 6.4.5.2.1. Detection of large deletions in patients and carriers

The original method for detecting large deletions was the Southern blot. This approach used full-length or partial complementary DNA probes to detect deletions by the absence of hybridization or appearance of novel hybridizing bands (junction fragments) [97]. It was a laborious method, requiring several weeks to complete if multiple probes needed to be tested sequentially. It has because been supplanted by a PCR method that uses two sets of multiplex primers, each covering deletion hotspots in various exons of the dystrophin gene [55,98]. Deleted exons fail to be amplified and are recognized as absent bands on the electrophoretic gel of the PCR products (Fig. 6.8). Because the PCR method screens many regions of the gene simultaneously, it is dramatically faster than the Southern blot approach, with results possible in 1 day. In turn, this approach can be confirmed or supplanted by targeted MLPA [99], which has the additional advantage of reliably identifying deletions in carrier females.

#### 6.4.5.2.2. Detection of microlesions in patients and carriers

One-third of mutations in the dystrophin gene are base substitutions or microdeletions/insertions. Because of the large size of the gene, in years past a number of indirect methods had to be employed to screen the gene for these microlesions, including heteroduplex analysis, chemical mismatch cleavage, protein truncation assay, or linkage analysis [100,101]. More recently, the advent of DNA NGS has rendered the huge dystrophin gene amenable to complete sequencing and direct detection of point mutations [102]. Once the mutation is

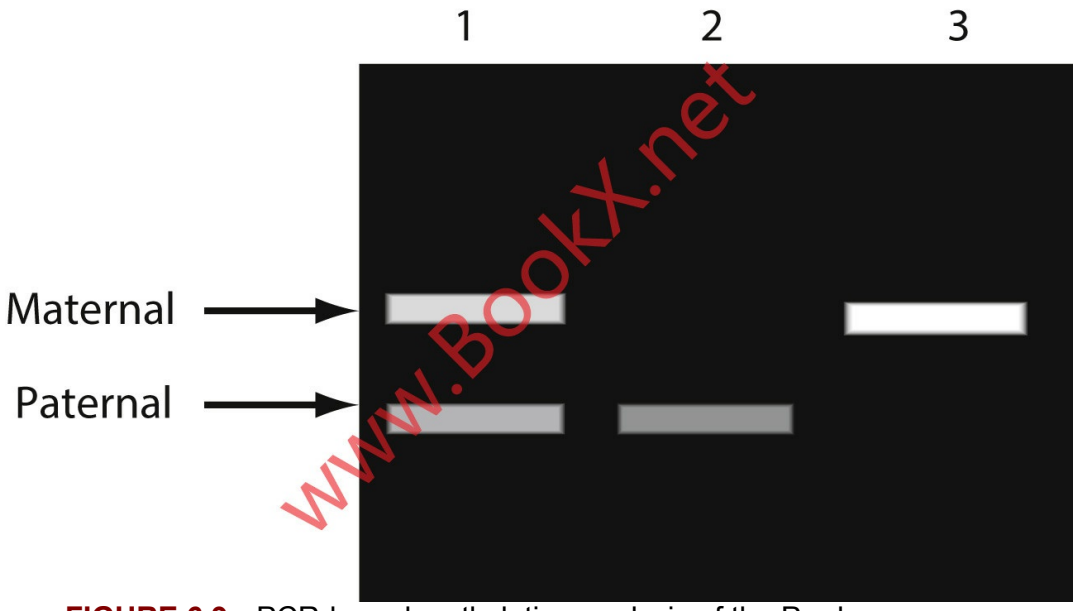
found in a proband, reliable carrier detection is possible by sequencing only the region containing that particular mutation using the Sanger technique.

### 6.4.6. Prader–Willi and Angelman Syndromes

Despite their disparate clinical phenotypes and completely independent genes, these two disorders are forever linked in cytogenetics and molecular genetics laboratories because of their coincident deletion sites on chromosome 15q11–q13 and mirror-image imprinting mechanisms. About 70% of cases of both disorders are due to deletions at this locus, some of which are too small to be visible by chromosome banding, although they may be detected by fluorescence in situ hybridization. Most of the remainder are due to uniparental disomy (UPD) in Prader–Willi syndrome (PWS) and point mutations in Angelman syndrome (AS). About 10% of AS cases are due to an imprinting defect, while a smaller proportion (3%–5%) are due to UPD. The small fraction of PWS cases not due to deletion or UPD apparently involve an imprinting defect. Owing to the imprinting phenomenon active at this locus, the AS gene (*UBE3A*) is expressed only on the maternally inherited chromosome 15, while the PWS gene (which is still not identified) is expressed only on the paternally inherited chromosome. Thus, loss of maternal *UBE3A* gene expression by either deletion or UPD for the paternal allele will produce AS, while loss of the paternal PWS gene by either deletion or UPD for the maternal allele will produce AS. As mentioned, loss of *UBE3A* function through point mutation is also seen in AS, but whether or not it occurs in PWS will not be known until the gene is identified. Either disorder can also be produced by aberrant parental gene expression due to a primary imprinting defect [103].

Molecular genetic testing for these disorders is aimed at tracking the maternal and paternal alleles and determining whether one or the other is either missing (deletion) or duplicated (UPD); obviously, the UPD mechanism results in both duplication of one allele and loss of the other. The two parental alleles can be distinguished from one another because of the differential methylation that occurs at this

locus, using methylation-sensitive PCR or real-time PCR [104]. The PCR technique capitalizes on the property that exposure of DNA to sodium bisulfite converts cytosine to uracil except when it is methylated. Primer or restriction endonuclease digestion can be designed to distinguish this difference at a site within the critical locus, such as the *SNRPN* gene. A missing maternal allele is diagnostic of AS, while a missing paternal allele indicates PWS (Fig. 6.9). The real-time PCR technique relies on differential melting curves of methylated versus nonmethylated CpG sequences. None of these approaches can distinguish between the deletion and UPD mechanisms, although in actual practice the difference is not critical because both have very low recurrence risks. Documentation of UPD requires microsatellite analysis of the chromosome 15 region in the patient and parents, demonstrating homozygosity for the polymorphic alleles, or observation of long stretches of homozygosity on chromosomal microarray analysis [105]. PWS or AS caused by imprinting defects (presumably involving aberrant methylation) will also not be distinguished by these methods (although the tests will still be positive), which is unfortunate because those cases do have a much higher recurrence risk [106]. This mechanism is usually established by exclusion of the other molecular defects and is otherwise difficult to prove. However, a microdeletion of the putative imprinting center can be demonstrated in some cases [107].




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**FIGURE 6.9** PCR-based methylation analysis of the Prader–Willi/Angelman region on chromosome 15, using the sodium bisulfite method. The upper band corresponds to the maternal allele and the lower band to the paternal allele. The samples shown are (1) normal; (2) AS; and (3) Prader–Willi syndrome.

## 6.4.7. Familial Cancer Syndromes

The emergence of genetic testing and counseling for hereditary cancer syndromes represented something of a paradigm shift for the specialty of medical genetics. Among other features, it led the way toward the widespread offering of adult genetic services and molecular tests for determining cancer risks in the context of extreme mutational heterogeneity and variable penetrance. These factors, along with the perception of risks of insurance discrimination and adverse psychosocial impact, have led to practice guidelines mandating pre- and post-test genetic counseling. Indeed, there are documented cases of serious misinformation ensuing when these procedures are not followed [108].

There are at least 35 strongly heritable syndromes in which various cancers are a primary manifestation [109]. This section considers only those autosomal dominant forms for which DNA testing has become prevalent. All of them have in common a pathogenetic mechanism

fitting the classic tumor suppressor gene model.

#### 6.4.7.1. Familial Breast/Ovarian Cancer

While it was the retinoblastoma gene many years before in which Knudson first developed the "two-hit" model of dominantly inherited cancer, it was the discovery of the *BRCA1* and *BRCA2* genes that really put familial cancer on the map for the medical genetics community and led to only the second routinely offered presymptomatic genetic test after HD. In fact, the two tests are often discussed as instructive analogies for one another, yet they could hardly be more different. The triplet repeat expansion mutation in the HD gene shows virtually 100% penetrance, giving the DNA test an extraordinarily high predictive value, while *BRCA1/2* mutations may only be 50%–85% penetrant for breast cancer and 25%–45% penetrant for ovarian cancer in various families; these penetrance ranges are a continually source of debate and revision [110]. HD has no prevention or treatment (yet), while breast cancer (and to a lesser extent ovarian cancer) can be screened for early detection and prevented in some cases by prophylactic surgery and/or medication. Breast cancer is one of the most common serious diseases in medicine, affecting roughly 1 in 9 women, although it is important to keep in mind that the *BRCA1/2* genes account for only 10% of this incidence at best. Finally, and most importantly, from the perspective of this chapter, HD shows only a single species of easily detectable mutation (the CAG triplet repeat expansion), while *BRCA1/2* mutations and variants are extremely heterogeneous and still not yet completely characterized.

Because of its difficulty and expense, the potential risk of stigmatization or other adverse psychosocial reactions, and the not uncommon finding of nucleotide changes of unknown significance (VUS) within the gene imparting even more uncertainty to the patient than she had before the test, *BRCA* testing should never be undertaken lightly. Initially there was a general consensus that testing not be encouraged unless the a priori risk of the proband testing positive exceeds 10% (although this strict numerical cutoff has since been relaxed somewhat [111]), and there are several formulas for

making this estimation based on personal and family history, ethnicity, and other factors [112,113]. Of course, testing of minors should not be considered outside of exceptional circumstances, because there is a risk of misunderstanding and stigmatization for a condition that has no symptomatic onset or clinical intervention until adulthood.

The *BRCA1/2* genes are very large, and thousands of pathogenic and benign variants have been reported, spread diffusely across the length of both genes. In this sense they are similar, from a molecular testing point of view, to *CFTR*. However, while the clinical impact of missing a carrier of a rare *CFTR* mutation in a population screening program does not justify the cost of sequencing the gene, the adverse consequences of missing a *BRCA* mutation in a presymptomatic test are great enough that full sequencing of both genes is justified despite the cost (assuming there is a strong enough personal or family history to consider testing in the first place). Patients testing negative must be cautioned that even complete sequencing could potentially overlook a mutation in an unusual location (outside the coding region) and that it is possible the cancer in their family was due to a non-*BRCA* etiology. One class of mutations known to be missed by DNA sequencing consists of large deletions. Because it is now recognized that these constitute a significant minority of *BRCA* mutations, screening for exon dosage by MLPA or exon array has become an important component of the testing process [114].

There are two major exceptions to the need for complete gene sequencing in *BRCA* testing. One is the situation in which the mutation in the family is already known from prior testing of an index case (something that should always be sought if the affected relative is available). The other is in the Ashkenazi Jewish population, in which three recurring mutations account for the vast majority (at least 90%) of *BRCA* carriers: 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2* [115]. Individuals in this ethnic group can be offered a limited screening panel (Fig. 6.10) at much reduced cost, although if they test negative in the absence of a known mutation in the index case, the question of pursuing more comprehensive testing of lower yield will

arise.

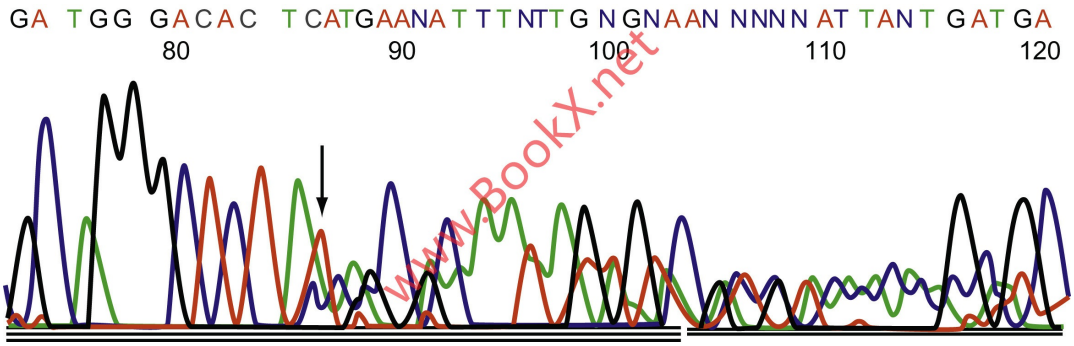



**FIGURE 6.10** Targeted detection of the Ashkenazi Jewish *BRCA1* founder mutation 185delAG by automated sequencing analysis. Because this is a heterozygous two-nucleotide deletion, it results in a heterozygous frameshift on the sequencing readout, causing mismatched nucleotides and overlapping peaks following the mutation site (*arrow*).

While clinical follow-up is continuing, existing outcomes data indicate dramatic, although not absolute, reduction in breast and ovarian cancer incidence in women testing positive for *BRCA* mutations presymptomatically, who then opted for prophylactic surgery and/or medical risk reduction (e.g., tamoxifen). Importantly, in premenopausal women, oophorectomy reduces not only the incidence of ovarian cancer but that of breast cancer as well [116,117].

While *BRCA1* and *BRCA2* are the most commonly involved genes in hereditary breast/ovarian cancer, and the ones with the highest penetrance, there are a number of others that should be considered, such as *BARD1, CHEK2, PALB2, MUTYH*, etc. Now with the availability of NGS, it has become more practicable to include those additional genes in a sequencing panel, with the result that ordering *BRCA* gene sequencing in isolation (in the absence of a known mutation in the family) is becoming less common [118].

### 6.4.7.2. Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome)

Colon cancer, like breast cancer, is a common disease, and similarly,

about 5%–10% of cases have a strong familial component. The dominant form of adult-onset colon cancer, as distinguished from familial adenomatous polyposis, is hereditary nonpolyposis colon cancer (HNPCC, also known as Lynch syndrome), characterized by predisposition to early-onset (average age 44 years) tumors mostly proximal to the splenic flexure. The syndrome is also associated with other adenocarcinomas of the endometrium, ovary, small bowel, stomach, pancreas, and other organs. Ascertainment of Lynch families based on history is done using the Amsterdam criteria or their subsequent modifications [119,120], again recognizing, just as in hereditary breast/ovarian cancer, that overly strict adherence to such guidelines results in falsely excluding some affected families [121].

At least five genes are known to be responsible for HNPCC: *MSH2*, *MLH1*, *PMS1*, *PMS2*, and *MSH6*; the first two account for about 70% of the cases [122]. The protein products of these genes are involved in DNA mismatch repair, producing the so-called replication error or mutator phenotype (also known as mismatch repair deficiency) in tumor cells. The latter can be assayed in the molecular diagnostic laboratory by observing novel, non-germline peaks in capillary electrophoretic analysis of short tandem repeat loci, a phenomenon called microsatellite instability (MSI). Indeed, this is an easier test than searching for the many possible mutations in the five causative genes, although it is not entirely sensitive or specific for familial colon cancer (about 15% of sporadic colon cancers also exhibit the phenomenon). However, it may be helpful in estimating whether a colon tumor is due to HNPCC when the family history is incomplete or ambiguous [123]. The presence of MSI also has some prognostic and management value, as these tumors tend to be more responsive to various chemotherapeutic agents [124,125]. Direct mutation testing of the five dominant genes by the same approaches used for the *BRCA* genes is available, and certainly made easier by the use of NGS. It can be offered to patients and relatives meeting the family history criteria and to those whose tumors demonstrate MSI. As for *BRCA* testing, the possibility of large deletions invisible to standard sequencing, and

missense variants of uncertain clinical significance, must be considered. In index cases from whom tumor tissue is available, analysis of the mismatch repair gene products by immunohistochemistry may offer a less expensive initial screen to allow selection of the particular impaired mismatch repair gene for sequencing. Also as for familial breast cancer, NGS has encouraged testing with expanded gene panels beyond the initial five [126].

### 6.4.7.3. Familial Adenomatous Polyposis

This autosomal dominant syndrome is characterized by early onset (most often in childhood) of very large numbers of colonic polyps with high malignant potential, along with benign and malignant lesions in a number of other tissues. It is due to mutations in *APC*, which are quite numerous and heterogeneous, although the majority are of the nonsense or frameshift class. Individuals with the "attenuated" form of FAP (<100 polyps) tend to have mutations near the 5′ or 3′ ends of *APC* [127]. This is one of the few predictive genetic tests acceptable for performance in children, because of the early onset of polyps and the onerous clinical surveillance procedures (which can be dispensed with in those children who test negative). Because of the possible overlap with the attenuated form of FAP, the *APC* gene is often included in expanded gene panels applied to Lynch syndrome [126].

### 6.4.7.4. Multiple Endocrine Neoplasia

Multiple endocrine neoplasia (MEN) type 1 is characterized predominantly by neoplasms in the parathyroid, pancreas, adrenals, and pituitary. MEN type 2 features medullary thyroid cancer, pheochromocytoma, and hyperparathyroidism. A subtype, MEN2B, is further characterized by marfanoid habitus, ganglioneuromas, and mucosal neuromas.

The gene for MEN2, the *RET* proto-oncogene, was discovered first, and DNA testing has proved quite valuable for at-risk family members. Indeed, the *RET* gene seems to be everything the *BRCA* genes are not: it contains a limited number of mutations localized to a

few exons, and the penetrance is virtually 100%. In fact, most of the mutations in MEN2A are found in exons 10 and 11, with a single cystine codon (number 634) most commonly affected with every possible missense substitution of its three nucleotides. Less common mutations have also been found in exons 13 and 14 [128]. Furthermore, almost all cases of MEN2B, which have a unique presentation, show a single missense mutation at codon 918 in exon 16. Thus, molecular genetic testing for MEN2 is very straightforward, typically involving limited sequencing of the mutable exons to identify almost all carriers, though NGS has made it just as easy to examine the entire coding sequence of the gene (albeit with the revelation of more VUSs. Because the penetrance is so high and the extremely aggressive medullary thyroid cancers can occur in childhood, *RET* gene analysis is another predictive genetic test indicated for use in presymptomatic children.

The gene for MEN1 (designated simply *MEN1*) was identified later and has been less subject to testing because of the large number of mutations (>400) described. Most of them are of the nonsense, frameshift, or deletion variety [129] and are detected by sequencing.

We now know of a number of other genes associated with heritable endocrine neoplasia syndromes, including MEN4 and familial pheochromocytoma [130]; these, too, are readily encompassed by gene panel testing or whole-exome sequencing.

### 6.4.7.5. Other Dominantly Inherited Cancer Syndromes

Molecular tests are available for the less prevalent familial cancers, either alone or as part of larger familial cancer gene panels [131]. Just a few of the more prominent examples will be mentioned here.

*Li–Fraumeni syndrome*, characterized by a wide variety of tumors in affected families (breast, brain, sarcoma, leukemia, lung, pancreas, etc.), is caused by germline mutations in the *p53* (also designated *TP53*) tumor suppressor gene. The mutations are diverse and must be distinguished from the somatic *p53* mutations that are found in many nongenetic tumors [132].

*Familial melanoma* accounts for about 5%–10% of all melanomas and

may be associated with the dysplastic nevus syndrome. Sequencing of the implicated *CDK4* and *CDKN2A* (also designated *p16*) genes is available, though the yield is not particularly high even in observably family cases [133].

*Von Hippel–Lindau syndrome* exhibits a variety of benign and malignant tumors, most prominently renal clear cell carcinoma. The causative *VHL* gene contains only three exons and so can be sequenced readily, but about a fourth of the mutations are large deletions that must be detected by another method such as Southern blot or MLPA [134].

*Retinoblastoma*, the cancer that set the stage for our understanding of tumor suppressor gene action, remains one of the more difficult to test for, owing to the large size of the *RB1* gene, the complexity of the mutations, and the rarity of the disorder. However, NGS-based approaches are now available in a few laboratories [135].

## 6.5. Mitochondrial DNA Disorders

Last, we must not forget about "the other human genome," the one that exists outside the nucleus, in those energy-generating organelles called mitochondria. The mitochondrial genome is subject to pathogenic mutations just as is the nuclear genome and thus is fair game for molecular diagnostic attention. In fact, the mutation or replication error rate of mitochondrial DNA is substantially higher than that of nuclear DNA, making the mitochondrial genome highly polymorphic between individuals and heteroplasmic within different cells and tissues of the same individual. This latter property in particular makes DNA-based diagnosis of mitochondrial disorders tricky and subtle. Because pathogenic variants are often heteroplasmic, mitochondrial DNA diagnostic techniques must be of sufficient sensitivity to detect alterations in a portion, or even a minority, of cells, yet of sufficient specificity and clinical predictive value to distinguish them from benign polymorphisms. DNA NGS, because of its ability to sample even minor subpopulations in parallel with the majority, without being diluted out, is particularly amenable to this application, and the serendipitous observation that mtDNA is

sequenced in whole-exome NGS despite not intentionally being captured has added to its appeal, especially because the majority of "mitochondrial" disorders are actually caused by mutations in nuclear genes [136,137].

The 16.5-kbp mitochondrial genome carries genes for 13 polypeptides (components of the respiratory chain), 22 transfer RNAs, and two ribosomal RNAs, and mutations may be found in any one of them [138]. Because a number of these mutations are recurrent in particular disorders, one approach is to test first for some of these as a panel, such as the G11778A mutation, which is found in 50%–70% of patients with Leber's hereditary optic atrophy, or the A3243G mutation, found in a large proportion of patients with myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). Laboratories may offer one or more core mutation panels to be employed as a first-tier test depending on the clinical presentation of the patient, to be followed by another panel or a wider sequencing method if the first test is negative [139,140]. The protocol requires close communication with the referring physician in order to decide which panel to use and just how far to pursue a mitochondrial etiology that may in fact never be found. In embarking on such testing, the phenomenon of heteroplasmy must always be at the top of one's mind, even from the point of specimen collection: mitochondrial DNA testing may require sampling of the affected tissue, rather than simple phlebotomy, in order to increase the chances of finding the causative mutation.

## 6.6. Other Targets of Molecular Genetic Screening

As of 2018, the Genetic Testing Registry, the current online directory of molecular genetic testing laboratories (https://www.ncbi.nlm.nih.gov/gtr/), lists more than 10,000 different disorders and more than 15,000 genes for which DNA-based tests are available. Many of these disorders are quite rare, and testing for some may be offered by only a single laboratory. At the other end of the

spectrum are diseases resembling CF in that they have high carrier frequencies but for one reason or another they have not yet been taken up as targets for large-scale screening. One such example is *spinal muscular atrophy*, with a carrier frequency of about 1 in 50 for mutation in *SMN1*. Given the severity of the disorder and a mutation carrier frequency that approaches that of CF, one might think it an obvious target for population screening. But molecular testing, especially for carriers, is quite challenging because of the presence of a closely homologous adjacent gene, *SMN2*, which can be present in multiple copies and can have a mitigating effect on the phenotype [141]. Despite a recommendation from ACMG that population carrier screening be implemented [142], the uptake has been slow, at least outside of larger expanded carrier screening panels (see later). Another example is *congenital hearing loss*, which is now a target of universal phenotypic screening of newborns in the United States and some other countries. Because the audiologic screening techniques used are not uniformly reliable for the desired early diagnosis and intervention, ancillary molecular genetic testing has been proposed [143]. The most obvious target for such screening is *GJB2*, encoding the cochlear hair cell protein connexin-26; mutations in this gene are responsible for about half the cases of nonsyndromic autosomal recessive hearing loss. The mutation carrier frequency in the general population is about 3%, and there are predominant European Caucasian, Ashkenazi Jewish, and Asian mutant alleles. Molecular screening can be done using allele-specific techniques for these three high-frequency mutations or by complete gene sequencing for the more than 100 other variants, because the entire gene is only two exons. Some laboratories also test for a deletion in the neighboring *GJB6* (connexin-30) gene, which can interact with mutations in *GJB2* to cause hearing loss even in the double heterozygote form. However, there are at least 100 other genes associated with hereditary hearing loss, and to develop a test encompassing them all requires comprehensive NGS of the entire gene panel [144,145].

Because of the presence of a founder mutation, 167delT, in *GJB2* carried in as many as 10% of individuals of Ashkenazi Jewish descent,

connexin mutation testing is sometimes added to the *Ashkenazi Jewish carrier screening panels* that have long been offered to that ethnic community in a variety of forms. These panels have traditionally contained anywhere from 3 to 15 diseases, the most common ones being CF (carrier frequency in this population, 1/29), Tay–Sachs disease (1/27), Gaucher disease (1/15), Canavan disease (1/36), and familial dysautonomia (1/30) [146]. As in *GJB2*, the genes for these disorders feature between two and five predominant Ashkenazi mutations each, making screening technically easy and producing an aggregate yield of at least one positive carrier per six individuals tested [147]. As in so many other applications, NGS has made it technically easy to expand this panel, along with other ethnicity-based carrier screening panels, to a much larger number of disorders [148]. Eventually, however, the carrier frequencies of the added disorders become so low that they blend into the background frequencies in other ethnic groups and the larger population. Also, as noted earlier, with the sequencing of full genes, as opposed to targeted mutation detection, and the inclusion of genes and disorders that are not well characterized, the incidence of VUS results inevitably increases – results, it should be kept in mind, that are overlaid upon the anxiety, time-pressure and irreversible decision-making of a pregnancy [11]. Their appropriate use is still being discussed and addressed by the relevant professional organizations [149]. Should molecular techniques be applied to the single largest segment of genetic screening, namely newborn screening? Traditionally the assays used for newborn screening have been biochemical in nature. However, DNA-based tests have served as second-tier or follow-up tests for infants showing positive primary screens for CF or sickle cell disease in some states, and increasingly for some of the metabolic disorders in the expanded newborn screening panel [150]. Nevertheless, any stand-alone molecular newborn screening test would have to meet the criteria of well-defined predictive value, a manageable number of predominant and well-characterized mutations, availability of a relatively inexpensive test methodology to pick up a significant proportion of them, and effective clinical interventions for those

testing positive. Theoretically, applying molecular techniques to newborn screening could deliver some advantages, such as better yield for conditions known to be subject to false-negatives by tandem mass spectrometry (e.g., VLCAD) and simultaneous prediction of therapeutics and prognosis (e.g., the BH4-responsive form of PKU). But on the negative side, particularly if sequencing of full genes is applied, would be the high number of VUSs that would need to be managed, poor genotype–phenotype correlations for many of the disorders, even among members of the same family, and potential false-negatives caused by missing large deletions, insertions, and rearrangements. At the end of the day, the big advantage of biochemically based newborn screening is that it detects a phenotype – and it is the phenotype that needs early treatment, not the genotype.

## 6.7. Pharmacogenetic Testing

Another application of molecular genetic testing that could potentially involve large segments of the population is pharmacogenetic screening. At its broadest definition, the goal of pharmacogenetic testing is to identify sequence variants throughout the genome that determine response and reaction to drugs. Presumably by their effects on drug metabolism and excretion, these genetic variants will predict which patients are likely to respond or not respond to varying doses of the drug or to manifest adverse reactions to standard doses. Characterization of these genotypes prior to drug administration could hopefully enable individualized therapeutic approaches to maximize early efficacy at the lowest possible dosage while avoiding side effects and toxicity. Existing pharmacogenetic tests target either germline variants, delineating heritable differences in drug response, or acquired, somatic mutations in tumors to predict response to chemotherapy; only the former application falls within the domain of medical genetics and is considered here.

The largest single class of enzymes involved in drug metabolism is the cytochrome P450 family of microsomal enzymes. SNPs in various members of this family, such as CYP2D6, CYP2C9, and CYP2C19, have been associated with impaired metabolism and hence toxicity of

many important drugs, including codeine, haloperidol, propranolol, omeprazole, diazepam, amitriptyline, tamoxifen, clopidogrel, and warfarin [151]. Testing for these variants is now possible on a variety of multiplex platforms, some of which are FDA approved. However, uptake by treating physicians has been quite low, owing in part to unfamiliarity but, more importantly, to the low predictive value of many of these tests [152].

Other potential pharmacogenetic targets are more narrowly drug specific. For example, the mitochondrial DNA mutation A1555G is known to confer increased sensitivity to the ototoxic side effects of aminoglycoside antibiotics [153]. While not in routine practice because of other considerations surrounding the emergent use of these agents in the critical care setting, one might imagine pretreatment mitochondrial DNA testing for this variant if a rapid assay and alternative therapeutic strategies were available. A similar case could be made for preoperative testing of patients for mutations in the ryanodine receptor gene, *RYR1*, as a screen for potential risk of malignant hyperthermia in response to common surgical anesthetics. The practicality of this approach is limited by the genetic heterogeneity of the disorder and evolution of anesthesiology practice beyond the former gas anesthetics like halothane. Still, a panel of 15 mutations has been recommended by a European consortium for testing at-risk family members [154], and pathogenic *RYR1* variants are included in the "recommended-to-report" list of incidental findings in genomic sequencing compiled by the ACMG [155].

The conceptualization of pharmacogenetic testing may even be expanded beyond traditional drug response to encompass other forms of therapy, such as radiotherapy. Heterozygote carriers of mutations in the *ATM* and related genes have increased sensitivity to therapeutic radiation, mirroring the more extreme radiosensitivity that is a hallmark of the affected patients with ataxia telangiectasia. With a carrier frequency of at least 1% in the general population, and the possibility that these carriers also have increased susceptibility to cancer, many of these individuals may find themselves facing radiotherapy sometime in their lives. A pretreatment screen for *ATM*

and related mutations could theoretically identify those whose radiation dosage should be decreased in order to avoid severe tissue damage.

## 6.8. Quality Assurance, Reimbursement, and Regulatory Issues

All clinical laboratories in the United States must adhere to the CLIA guidelines as a minimal level of quality assurance (and similar structures are in place in Canada, Europe, and other regions). Because all molecular genetic tests are considered "high complexity," the regulations for that category must be followed. A problematic loophole has existed from the fact that molecular genetic tests had not been listed specifically in the existing CLIA regulations when written. For this reason, and because the CLIA regulations are seen as minimal standards, professional organizations have developed more detailed and rigorous practice guidelines. The most important are those of the ACMG and CAP, along with more general policy statements by the Association for Molecular Pathology (AMP). The CAP Molecular Pathology Checklist is particularly crucial because it is used for inspection and accreditation of those laboratories that choose to enroll with this agency, which has been granted "deemed" status under CLIA. This Checklist and the ACMG Standards and Guidelines for Clinical Genetics Laboratories are continually updated to reflect changes in techniques and maturation of the discipline, most recently with the incorporation of NGS procedures and informatics [19,156], and the most recent versions are available on the respective organizations' websites (see next section). They contain many guidelines unique to molecular genetic testing, addressing issues such as PCR contamination, communication with genetic counselors, informed consent, interpretation of sequence variants, laboratory personnel qualifications, technical procedures, appropriateness of test requests, results reporting and so on. For the ultra-rare or "orphan" diseases, lacking the test demand to be of interest to mainstream clinical molecular genetics laboratories, research laboratories often

fulfill a vital service in making this testing available to families at risk. At the same time, it must be recognized that they are operating outside the legally accepted domain of clinical laboratory testing (at least in the United States), because most of them do not have certification or licensure under the Clinical Laboratory Improvement Amendments (CLIA) guidelines or by other regulatory bodies. Given that most research laboratories would not find it practical or desirable to pursue such certification, while the established clinical laboratories are not interested in assuming the burden of such low-volume and esoteric tests, the best solution seems to lie in the establishment of a network of orphan testing dedicated to covering the aggregate of these rare disorders [157].

*Quality assurance* (QA) encompasses all aspects of testing, from requisitions to specimen receipt through performance, interpretation, and reporting. In addition, recent initiatives by government and professional organizations have focused increasing attention on the need to include preanalytic and postanalytic factors within overall QA for genetic tests, especially those of a predictive nature. Tests must be validated both analytically and clinically before introduction, although that is often easier said than done, particularly for predisposition tests that may take decades to establish clinical predictive value. On the other hand, documentation of analytic validity has been assisted greatly by a continuing effort in partnership with the Centers for Disease Control and Prevention (CDC), AMP, and the Coriell Cell Repositories to develop, characterize, and make generally available genomic DNA reference materials representing the most important alleles for genetic diseases and pharmacogenetic testing [158,159].

Another important component of QA is *proficiency testing*. This involves the regular shipment to laboratories of specimen unknowns that are analyzed, reported, and graded. CLIA mandates that laboratories participate in proficiency testing programs for each analyte they test, and if an organized formal program is not available, they must revert to other means such as informal sample exchanges with another laboratory or blinded retesting of previous samples. A

successful and ever-expanding national program exists, codeveloped by CAP and ACMG and evolving in scope to address newer technologies like NGS [160]. The performance of all participating laboratories is aggregated and provides a useful snapshot of the state of the art at any given time [161].

*Reimbursement* for molecular genetic testing has been a particularly frustrating issue for the laboratories, especially in the United States. These tests tend to be relatively expensive and labor-intensive to perform and are often considered esoteric or experimental by third-party health insurers. Furthermore, because they are frequently performed on healthy individuals (e.g., carrier screening for recessive mutations or presymptomatic testing for dominant disorders), the usual diagnostic/symptomatic indications and billing (CPT) codes for clinical laboratory testing are often absent. The acceptance and updating of essential procedural billing codes that more realistically represented the range and workload effort of modern molecular techniques was a step in the right direction, beginning with the addition of codes for such techniques as DNA sequencing, multiplex PCR, and DNA hybridization some years ago [162]. However, method-specific codes have a lack of transparency, in that they do not reflect the testing indication of patient diagnosis, and they are often bundled or "stacked" to reflect the ingredients of multiplex assays. More recently more of the coding was transitioned to disease-specific codes [163], as had always been the practice in molecular microbiology. Last, for many years there was great concern that the whole field might be priced out of existence if mandatory royalties for use of *patented genes* in clinical laboratory tests inordinately added to the cost. Many of the genes discussed in this chapter had been patented, either by the discoverer or a sublicensee, and various levels of exclusivity or sublicensing royalties were enforced. A number of lawsuits on this matter have been brought in U.S. and European courts, perhaps the most publicized one contesting the constitutionality of the patents on the *BRCA1* and *BRCA2* genes held by Myriad Genetics, Inc [164]. In a groundbreaking unanimous decision, the U.S. Supreme Court ruled in 2013 that genes and gene

sequences are products of nature and therefore not patentable under the conditions put forth in Article 1 of the U.S. Constitution [165]. The effect, particularly in the United States, was an immediate increase in offerors of the previously restricted tests, and a dramatic decrease in prices. Given the trend in molecular genetic testing toward massively parallel sequencing of thousands of genes, many of which had formerly been "owned" by patent-holders, this decision came none too soon.

## 6.9. Internet Resources for Molecular Genetic Testing

With clinical genomic testing producing an overwhelming information load, with new gene-disease associations appearing almost every week in the literature, print sources in this field can never be up to date, and even if they were, it is difficult and time-consuming to extract the information needed, especially to answer an urgent clinical question or to evaluate the clinical significance of a novel sequence variant in real time. Fortunately, the genetics revolution has occurred at the same time as the informatics revolution, so computer technology can be used for rapid searching of relevant information that is constantly updated. The Internet offers many sites with useful medical genetics content; listed here are some of the key resources available to assist those ordering or performing modern molecular genetic and genomic tests.

### 6.9.1. Online Mendelian Inheritance in Man (OMIM)

(https://www.omim.org/)—Most readers of this text will already be familiar with OMIM, the most comprehensive and up-to-date catalog of human genetic disorders available, currently boasting over 24,000 entries. Each disease entry includes, in addition, to phenotypic features, historical review of the condition, symptomatology, and information on the mapping and structure of the involved gene

(where known) and its variants.

## 6.9.2. Genetic Testing Registry

(https://www.ncbi.nlm.nih.gov/gtr/)—GTR is now the primary listing of genetic testing laboratories, having superseded the well-used GeneTests database. It is searchable by disease (>10,000 currently listed, as noted earlier), tested genes (>15,000), or testing laboratories. It is extremely useful for those facing a patient in need of an "orphan" genetic test for a rare disease, when one does not know where in the country (or the world) it might be offered. It is also an excellent referral source for more routine molecular genetic tests. Contact information for each laboratory is provided in order to address indications and requirements for testing prior to sending a specimen.

## 6.9.3. GeneReviews

(https://www.ncbi.nlm.nih.gov/books/NBK1116/)—GeneReviews is the go-to site for quick answers and summaries pertaining to all of the major disorders subject to molecular genetic testing, and many others as well (including chromosomal deletion and duplication syndromes). There are currently more than 700 entries, each featuring the most pertinent and up-to-date information on clinical manifestations, the molecular pathology of the gene, the meaning and limitations of existing DNA test strategies, clinical management, and genetic counseling issues.

## 6.9.4. GeneCards

(http://www.genecards.org/)—GeneCards is structured like an encyclopedia, with entries on human genes, their protein products, variants and mutations, and their associated genetic disorders. It is one of the best-organized "meta-sites" that combines data from a wide range of sources. It contains entries on over 21,000 protein-coding genes and more thaan 100,000 noncoding elements (microRNAs, etc.).

## 6.9.5. Human Gene Mutation Database

(http://www.hgmd.cf.ac.uk/ac/index.php)—Providing even finer detail, HGMD is a comprehensive, continually updated listing of molecular alterations with phenotypic associations, currently cataloging over 157,000 reported mutations in about 6500 human genes. This database is one of the first sources routinely checked against novel variants discovered in the course of clinical exome sequencing. Keep in mind that the power of the evidence for determination of the pathogenicity of variants will not be the same across all genes and variants, and is dependent on the evidence provided by the submitting laboratories.

## 6.9.6. ClinVar

(https://www.ncbi.nlm.nih.gov/clinvar/)—ClinVar is another database, publicly accessible, that catalogues human genetic variants along with phenotypic associations. As in HGMD, there will be varying weights of evidence and some discrepancies among multiple submitters of the same variant. The database boasts over 600,000 submissions covering over 27,000 genes.

## 6.9.7. College of American Pathologists

(http://www.cap.org/web/home)—The CAP's home website contains the most up-to-date version of the Molecular Pathology Checklist for laboratory inspection, along with many other products and services relevant to QA, proficiency testing, lab accreditation, and education. Some content is open only to members.

## 6.9.8. American College of Medical Genetics and Genomics

(https://www.acmg.net/)—The ACMG home website features its own updated "Standards and Guidelines" document and many policy statements related to molecular genetic testing, along with educational

resources in various media. These cover both general testing issues and guidelines specific for individual diseases and include detailed descriptions of the available methodologies, appropriate use of the test, results interpretation, risk calculations, and reporting protocols.

### 6.9.9. Association for Molecular Pathology

(http://www.amp.org/)—The only organization devoted exclusively to molecular diagnostics, it is a source of information on technical and regulatory issues in the field. It also features its own laboratory test directory, focusing on non-genetic molecular tests not covered by GeneTests.

### 6.9.10. ExAC and gnomAD

These are two searchable databases containing sequence data on large cohorts of reportedly healthy individuals. ExAC (http://exac.broadinstitute.org/) contains exome data on over 60,000 individuals compiled by the Exome Aggregation Consortium, while gnomAD (http://gnomad.broadinstitute.org/) contains over 120,000 exomes and over 15,000 whole genomes. Both are tremendously helpful in eliminating VUSs from clinical consideration on the basis of being too common in the general population (though one should still use caution when dealing with diseases of later onset or reduced penetrance).

## 6.10. Societal Impact of the New Genetic Technology

The dawn of the twenty-first century has been called the beginning of the age of molecular medicine, and now with the widespread clinical application of NGS and chromosomal microarrays, we have entered the age of genomic medicine. Surely there can be no doubt that virtually every patient undergoing diagnosis or treatment in the coming decades will be involved in some sort of molecular genetic

procedure. Even if it is on the treatment end (gene replacement therapy or gene editing), it will have been preceded by a molecular diagnostic procedure, because no patient can become a candidate for gene therapy until the precise molecular defect is known. Also certain is the fact that the diagnostic methods outlined in this chapter will become increasingly automated. As DNA microarrays and other multiplex platforms become available as completely closed systems, free of complex hands-on manipulations and expert troubleshooting, they could just as easily move into the automated clinical chemistry laboratory as stay in the molecular genetics laboratory. Such a development would not necessarily serve the patients well, however, for even as the technical aspects of DNA tests become easier, their interpretation becomes more complex. Professional organizations have emphasized repeatedly that preanalytic and postanalytic aspects of genetic tests are just as important as the technical procedures themselves. Even if the technical portion is highly accurate and robust, much harm can ensue if the test was ordered inappropriately or the results information conveyed improperly. The emergence of numerous start-up bioinformatics companies offering clinical interpretation of exome or genome data if you send them the raw sequence is also cause for some concern. The authors' own experience with clinical exome sequencing over a span of many years has convinced us of the essential need for in-house clinical/molecular expertise in deciding which variants to report to the ordering physician; computer algorithms alone cannot do this job optimally.

With PCR, microarray, massively parallel sequencing, and other powerful technologies allowing ever more comprehensive genetic analyses on ever smaller and less invasive specimens, many complex issues surrounding genetic privacy and informed consent have arisen. Both governmental and professional organizations are attempting to address these concerns through safeguards in the testing process as stipulated in professional practice guidelines and legislation. Especially thorny have been the concerns surrounding predictive genetic testing in presently healthy individuals, because a positive test result carries risks of stigmatization, discrimination, anxiety, and

depression. For these reasons, most professionals believe that specific informed consent and strict confidentiality are prudent for predictive testing. The goals of privacy and confidentiality become even more challenging with genomic sequencing tests, because the data produced are essentially unique (and potentially identifiable) to the individual being tested. Furthermore, to assure appropriate ordering and results counseling for complex predictive tests, a case can be made that the laboratory should accept such orders primarily from genetics professionals or others with specific expertise in the field.

In the United States, more so than in other Western countries, the specter of insurance discrimination, which has been documented in limited numbers of patients [166,167], is often raised, impelling some patients to pay out of pocket for expensive tests so that their insurance carrier will not be aware of the result or even the fact that the patient was being tested. Yet, this raises other ethical issues such as inequitable access to testing, because only the economically well-off can afford to pay the cost themselves. Sometimes, however, these concerns become so blown out of proportion as to appear slightly irrational. Despite the hypothetical risk, most medical geneticists and genetic counselors would have a difficult time recalling even a single case in their experience of such blatant discrimination as loss of health insurance because of a genetic test result. Furthermore, we (and our patients) must always keep in mind the motivation for the test in the first place. For example, a woman may wish to pay for a predictive *BRCA* test out of pocket to keep the results secret from the insurance carrier, but if she tests positive and then desires a prophylactic mastectomy or oophorectomy, is she intending to pay for the costs of surgery out of pocket also? Probably not, and yet she cannot expect the insurance company to pay unless it is aware of the indication for surgery, which is the positive test result itself. Such patients can be somewhat reassured by the passage of the Genetic Information Nondiscrimination Act by the U.S. Congress in 2008, although the actual extent of protection from this legislation remains to be tested in actual cases.

These concerns arise not only with targeted predictive testing for

risk alleles like *BRCA*, but now also in the context of "off-target" or incidental findings revealed unintentionally in the course of whole-exome or genome- sequencing. There has been passionate debate about how such findings should be handled, ever because the beginning of clinical genomic sequencing. On one side are those who feel, as health care providers, that we have a "duty to warn" the patient of any significant revealed risk, whether or not it was deliberately searched for. Analogies are made to unexpected findings on physical examination or "incidentalomas" observed on routine chest radiographs. On the opposing side are those who worry about burdening patients with information that is not relevant to their immediate concerns, potentially exposing them to stigmatization or discrimination, and creating lifetime anxiety and potentially risky or irreversible interventions over a risk that may not even be that high (due to low or unknown penetrance). The ACMG position has generally fallen more in the first camp, albeit with some nuance. An updated list of disorders considered potentially lethal, relatively common and of high penetrance has been propounded as reportable in most cases, even if the patient is a child and the risk allele discovered is for an adult-onset disease [155,168]. Needless to say, the recommendation generated much discussion and controversy [169]. In the end, it is up to the individual laboratory to decide how to deal with incidental findings, which ones to report, and whether to offer patients and parents an "opt out" from receiving them.

## 6.11. Future Directions

All the ethical/societal dilemmas discussed in the preceding section remain moving targets because of the continuing march ahead of technology. The advent of NGS, placing the entire genome or exome of a patient within reach for less than the price of sequencing of two or three individual genes, probably represents the greatest sea-change in molecular diagnostics since the innovation of PCR in the mid-1980s. Will comprehensive sequencing soon supplant all other targeted gene tests and screens? Will a drop of blood from every newborn be used for whole-genome sequencing in addition, to or instead of the

traditional biochemical screens for metabolic disorders? Or, taking it one step further, will pan-genomic screening be done in utero or noninvasively using free fetal DNA circulating in the maternal blood (beyond its current use for detecting aneuploidies) [7,8,170]? Could the availability of these less physically invasive methods of prenatal diagnosis, as well as less emotionally invasive methods such as preimplantation genetic diagnosis, lead to a more cavalier attitude toward the use of molecular genetic technology to select for traits not directly related to disease?

Aside from the obvious ethical concerns in such scenarios, we should always remain aware of the limitations of molecular genetic testing, no matter how sophisticated the technology. Single genes and mutations rarely act entirely alone, and complex disorders clearly involve hundreds or perhaps thousands of genes all interacting at the DNA, RNA, and protein levels and with the outside environment. Thus, no DNA test, no matter how comprehensive, can ever tell the whole story. As we move on to the "postgenomic" or "proteomics" era, we will need to begin to think of genetic disease pathogenesis on a more "three-dimensional" level, taking into account the many interacting gene products in the nucleus and cytoplasm, as opposed to merely the "one-dimensional" string of nucleotides on a stretch of DNA. All groups experienced with whole-exome sequencing can produce many cases testing negative that nevertheless clearly appear to be genetic or syndromic. We usually ascribe the "false negative" results to our inability to query the "dark matter" of the genome: introns, vast intergenic regions, various forms of noncoding RNA, and epigenetic marks. Already this frustration is the impetus for triaging such cases to whole-genome sequencing, RNA sequencing, promoter methylation studies, and so on. Without question, the next edition of this text will contain far more accrued experience with those approaches. But as we await such advances, we can remain confident that the molecular genetic tests of today, and those added to the menu with each passing month, already offer a tremendous and irreplaceable service to many patients and their families, providing information and choices for their own lives and those of their children

that had never been available before, in any form, in the entire history of medicine.

## References


[1] Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. *Fertil Steril*. 2018;109:201–212.

[2] Liu L, Li K, Fu X, Chung C, Zhang K. A forward look at noninvasive prenatal testing. *Trends Mol Med*. 2016;22:958–968.

[3] Gerson K.D, O'Brien B.M. Cell-free DNA: screening for single-gene disorders and determination of fetal Rhesus D genotype. *Obstet Gynecol Clin N Am*. 2018;45:27–39.

[4] Nagan N, Faulkner N.E, Curtis C, Schrijver I. Laborat guidelines for detection, interpretation, and reporting of maternal cell contamination in prenatal analyses a report of the association for molecular pathology. *J Mol Diagn*. 2011;13:7–11.

[5] Simpson J.L. Preimplantation genetic diagnosis at 20 years. *Prenat Diagn*. 2010;30:682–695.

[6] Derks-Smeets I.A, de Die-Smulders C.E, Mackens S, van Golde R, Paulussen A.D, Dreesen J, Tournaye H, Verc Heijnen V.C, Meijer-Hoogeveen M, De Greve J, Geraedts J, De Rycke M, Bonduelle M, Verpoest W.M. Hereditary breast and ovarian cancer and reproduction: an observational study on the suitability of preimplantation genetic diagnosis for both

asymptomatic carriers and breast cancer survivors. *Breast Cancer Res Treat*. 2014;145:673–861.

[7] Lo Y.M, Chan K.C, Sun H, Chen E.Z, Jiang P, Lun F.M al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. *Sci Transl Med*. 2010;2:61ra91.

[8] Palomaki G.E, Kloza E.M, Lambert-Messerlian G.M, Haddow J.E, Neveux L.M, Ehrich M, den Boom D, Bombard A.T, Deciu C, Grody W.W, et al. DNA sequencing of maternal plasma to detect down syndrome: an international clinical validation study. *Genet Med*. 2011;13:913–920.

[9] Grody W.W. Expanded Carrier screening and the law of unintended consequences: from cystic fibrosis to fragile X. *Genet Med*. 2011;13:996–997.

[10] Hantash F.M, Goos D.M, Crossley B, Anderson B, Zha premutation Carrier frequency in patients undergoing routine population-based Carrier screening: insights into the prevalence of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, and fragile X-associated primary ovarian insufficiency in the United States. *Genet Med*. 2011;13:39–45.

[11] Grody W.W. Where to draw the boundaries for prenatal Carrier screening. *J Am Med Assoc*. 2016;316:717–719.

[12] Grody W.W, Cutting G.R, Watson M.S. The cystic fibrosis mutation “arms race”: when less is more. *Genet Med*. 2007;9:739–744.

[13]
Shi L, Webb B.D, Birch A.H, Elkhoury L, McCarthy J, population screening in the Ashkenazi Jewish population for recurrent disease-causing variants. *Clin Genet*. 2017;91:599–604.
[14] Arjunan A, Litwack K, Collins N, Charrow J. Carrier screening in the era of expanding genetic technology. *Genet Med*. 2016;18:1214–1217. .
[15]
Cao M, Liu Z, Jia X, Tang N, Cai R, Wang L, Chen C, X of $\alpha$-globin gene deletions using denaturing high-performance liquid chromatography and multiplex ligation-dependent probe amplification. *J Clin Lab Anal*. 2011;25:426–431.
[16]
Grosu D.S, Hague L, Chelliserry M, Kruglyak K.M, Le investigational studies for validation of a next-generation sequencing in vitro diagnostic device for cystic fibrosis testing. *Expert Rev Mol Diagn*. 2014;14:605–622.
[17] Gregg A.R. Expanded Carrier screening. *Obstet Gynecol Clin N Am*. 2018;45:103–112.
[18] ten Bosch J.R, Grody W.W. Keeping up with the next generation: massively parallel sequencing in clinical diagnostics. *J Mol Diagn*. 2008;10:484–492.
[19]
Aziz N, Zhao Q, Bry L, Driscoll D.K, Funke B, Gibson of American Pathologists' laboratory standards for next-generation sequencing clinical tests. *Arch Pathol Lab Med*. 2015;139:481–493.
[20]

Lek M, Karczewski K.J, Minikel E.V, Samocha K.E, Ba
Luria A.H, Ware J.S, Hill A.J, Cummings B.B, Tukiain
Hoffman E, Berghout J, Cooper D.N, Deflaux N, DePr
Rubio V, Rose S.A, Ruderfer D.M, Shakir K, Stenson F
Luna M.T, Weisburd B, Won H.H, Yu D, Altshuler D.
Aggregation Consortium. Analysis of protein-coding
genetic variation in 60,706
humans. *Nature*. 2016;536:285–291.

[21] Louis M, Dekairelle A.A.F, Gala J.L. Rapid and
combined genotying of factor V, prothrombin and
methylenetetrahydrofolate reductase single
nucleotide polymorphisms using minor groove
binding DNA oligonucleotides (MGB probes) and
real-time polymerase chain reaction. *Clin Chem Lab
Med*. 2004;42:1364–1369.

[22] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-
Foster J, Grody W.W, Hegde M, Lyon E, Spector E, Vo
laboratory quality assurance committee. Standards
and guidelines for the interpretation of sequence
variants: a joint consensus recommendation of the
American College of medical genetics and genomics
and the association for molecular pathology. *Genet
Med*. 2015;17:405–424.

[23]
Amendola L.M, Jarvik G.P, Leo M.C, McLaughlin H.M
of ACMG-AMP variant-interpretation guidelines
among nine laboratories in the clinical sequencing
exploratory research consortium. *Am J Hum
Genet*. 2016;98:1067–1076.

[24] Kerem B.-S, Rommens J.M, Buchanan J.A, et
al. Identification of the cystic fibrosis gene: genetic

analysis. *Science*. 1989;245:1073–1080.
[25] Riordan J.R, Rommens J.M, Kerem B.-S, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science*. 1989;245:1066–1073.
[26] Cystic Fibrosis Genetic Analysis Consortium. Population variations of common cystic fibrosis mutations. *Hum Mutat*. 1994;4:167–177.
[27] Cystic Fibrosis Genotype–Phenotype Consortium. Correlation between genotype and phenotype in patients with cystic fibrosis. *N Engl J Med*. 1993;329:1308–1313.
[28] Massie J, Castellani C, Grody W.W. Carrier screening for cystic fibrosis in the new era of medications that restore CFTR function. *Lancet*. 2014;383:923–925.
[29] Zielenski J, Rozmahel R, Bozon D, et al. Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics*. 1991;10:214–228.
[30] Grody W.W, Desnick R.J, Carpenter N.J, Noll W.W. D of cystic fibrosis mutation screening practices. *Am J Hum Genet*. 1998;62:1252–1254.
[31] Grody W.W, Cutting G, Klinger K, et al. Laboratory standards and guidelines for population-based cystic fibrosis Carrier screening. *Genet Med*. 2001;3:149–154.
[32] *Cystic fibrosis Foundation cystic fibrosis Foundation patient Registry Annual data report*. Bethesda: Cystic Fibrosis Foundation; 1998.
[33] Watson M.S, Cutting G.R, Desnick R.J, et al. Cystic fibrosis couple Carrier screening: 2004 revision of the

American College of medical genetics mutation panel. *Genet Med*. 2004;6:387–391. .
[34] Strom C.M, Crossley B, Buller-Buerkle A, Jarvis M, Quan F, Peng M, Muralidharan K fibrosis testing 8 Years on: lessons learned from Carrier screening and sequencing analysis. *Genet Med*. 2011;13:166–172.
[35] Strom C.M, Redman J.B, Peng M. The dangers of including nonclassical cystic fibrosis variants in population-based screening panels: p.L997F, further genotype/phenotype correlation data. *Genet Med*. 2011;13:1042–1044.
[36] Strom C.M, Huang D, Chen C, et al. Extensive sequencing of the cystic fibrosis regulator gene: assay validation and unexpected benefits of developing a comprehensive test. *Genet Med*. 2003;5:9–14.
[37] Sosnay P.R, Siklosi K.R, Van Goor F, Kaniecki K, Yu H, Sharma N, Ramalho A.S, A the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet*. 2013;45:1160–1167.
[38] Cutting G.R, Kasch L.M, Rosenstein B.J, et al. Two patients with cystic fibrosis, nonsense mutations in each cystic fibrosis gene, and mild pulmonary disease. *N Engl J Med*. 1990;323:1685–1689.
[39] Highsmith W.E, Burch L.H, Zhou Z, et al. A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. *N Engl J Med*. 1994;331:974–980.
[40] Cohn J.A, Bornstein J.D, Jowell P.S. Cystic fibrosis mutations and genetic predisposition to idiopathic

chronic pancreatitis. *Med Clin N Am*. 2000;84:621–631.
[41]
Coltrera M.D, Mathison S.M, Goodpaster T.A, Gown A
expression of the cystic fibrosis transmembrane regulator in chronic sinusitis in cystic fibrosis and non-cystic fibrosis patients. *Ann Otol Rhinol Laryngol*. 1999;108:576–581.
[42] Anguiano A, Oates R.D, Amos J.A, et al. Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. *J Am Med Assoc*. 1992;267:1794–1797.
[43] Kiesewetter S, Macek M.J, Davis C, et al. A mutation in CFTR produces different phenotypes depending on chromosomal background. *Nat Genet*. 1993;5:274–277.
[44] Groman J.D, Hefferon T.W, Casals T, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmemberane conductance regulator gene is pathogenic or benign. *Am J Hum Genet*. 2004;74:176–179.
[45] Watson M.S, Desnick R.J, Grody W.W, et al. Cystic fibrosis Carrier screening: issues in implementation. *Genet Med*. 2002;4:407–409.
[46]
Castellani C, Macek Jr. M, Cassiman J.J, Duff A, Massi
Kate L.P, Barton D, Cutting G, Dallapiccola B, Dequek
for cystic fibrosis carrier screening: a European consensus document. *J Cyst Fibros*. 2010;9:165–178.
[47]
Ridker P.M, Miletich J.P, Hennekens C.H, Buring J.E.
distribution of factor V Leiden in 4047 men and

women: implications for venous thromboembolism screening. *J Am Med Assoc*. 1997;227:1305–1307.
[48] Sun X, Evatt B, Griffin J.H. Blood coagulation factor va abnormality associated with resistance to activated protein C in venous thrombophilia. *Blood*. 1994;83:3120–3125.
[49] Ridker P.M, Hennekens C.H, Lindpaintner X, et al. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med*. 1995;332:912–917.
[50] Kupferminc M.J, Eldor A, Steinman N, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med*. 1999;340:9–13.
[51] Grody W.W, Griffin J.H, Taylor A.K, et al. American College of medical genetics consensus statement on factor V Leiden mutation testing. *Genet Med*. 2001;3:139–148.
[52] Press R.D, Bauer K.A, Kujovich J.L, Heit J.A. Clinical utility of factor V Leiden (R506Q) testing for the diagnosis and management of thromboembolic disorders. *Arch Pathol Lab Med*. 2002;126:1304–1318.
[53] Kottke-Marchant K. Genetic polymorphisms associated with venous and arterial thrombosis. *Arch Pathol Lab Med*. 2002;126:295–304.
[54] Poort S.R, Rosendaal F.R, Reitsma P.H, Bertina R.M. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase

in venous thrombosis. *Blood*. 1996;88:3698–3703.
[55] Beggs A.H, Koenig M, Boyce F.M, et al. Detection of 98% of DMD/BMD gene deletions by polymerase chain reaction. *Hum Genet*. 1990;86:45–48.
[56] Baris I, Koksal V, Etlik O. Multiplex PCR-RFLP assay for detection of factor V Leiden and prothrombin G20210A. *Genet Test*. 2004;8:381–383.
[57] Moll S, Varga E.A. Homocysteine and MTHFR mutations. *Circulation*. 2015;132:e6–9.
[58] Hickey S.E, Curry C.J, Toriello H.V. ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing. *Genet Med*. 2013;15:153–156.
[59] McCabe L.L, McCabe E.R.B. Genetic screening: carriers and affected individuals. *Annu Rev Genomics Hum Genet*. 2004;5:57–69. .
[60] Merryweather-
Clarke A.T, Pointon J.J, Shearman J.D, Robson K.J. Glo prevalence of putative haemochromatosis mutations. *J Med Genet*. 1997;34:275–278.
[61] Feder J.N, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*. 1996;13:399–408.
[62] Press R.D. Hereditary hemochromatosis: impact of molecular and iron-based testing on diagnosis, treatment, and prevention of a common chronic disease. *Arch Pathol Lab Med*. 1999;123:1053–1059.
[63] Risch N. Haemochromatosis, HFE and genetic complexity. *Nat Genet*. 1997;17:375–376.
[64] Alexander J, Kowdley K.V. HFE-associated hereditary hemochromatosis. *Genet Med*. 2009;11:307–

313.
[65] Grosse S.D, Gurrin L.C, Bertalli N.A, Allen K.J. Clinica penetrance in hereditary hemochromatosis: estimates of the cumulative incidence of severe liver disease among HFE C282Y homozygotes. *Genet Med*. 2018;20 383-9.
[66] Beutler E, Felitti V.J, Koziol J.A, et al. Penetrance of 845G-A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet*. 2002;359:211–218.
[67] Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. *N Engl J Med*. 2004;350:2383–2397.
[68] McLaren G.D, McLaren C.E, Adams P.C, Barton J.C, F al. Hemochromatosis and iron overload screen (HEIRS) study research Investigators clinical manifestations of hemochromatosis in HFE C282Y homozygotes identified by screening. *Can J Gastroenterol*. 2008;22:923–930.
[69] Gerhard G.S, Paynton B.V, DiStefano J.K. Identificatio of genes for hereditary hemochromatosis. *Methods Mol Biol*. 2018;1706:353–365.
[70] Kan Y.W, Dozy A.M. Antenatal diagnosis of sickle-cell anaemia by DNA analysis of amniotic-fluid cells. *Lancet*. 1978;2:910–912.
[71] Hassan S.M, Vossen R.H, Chessa R, den Dunnen J.T, Bakker E, Giordano P.C, Harteveld C.L. M diagnostics of the HBB gene in an Omani cohort using bench-top DNA Ion Torrent PGM

technology. *Blood Cells Mol Dis*. 2014;53:133–137.
[72] Alter B.P. Antenatal diagnosis of thalassemia: a review. *Ann NY Acad Sci*. 1985;445:393–407.
[73] Flint J, Hardin R.M, Boyce A.L, et al. The population genetics of the haemoglobinopathies. *Baillieres Clin Haematol*. 1998;11:1–51.
[74] Sabath D.E. Molecular diagnosis of thalassemias and hemoglobinopathies: an ACLPS critical review. *Am J Clin Pathol*. 2017;148:6–15.
[75] Naja R.P, Kaspar H, Shbaklott X, et al. Accurate and rapid prenatal diagnosis of the most frequent East mediterranean beta-thalassemia mutations. *Am J Hematol*. 2004;75:220–224.
[76] Cao A, Galanello R. Beta-thalassemia. *Genet Med*. 2010;12:61–76.
[77] Wallace S.E, Bird T.D. Molecular genetic testing for hereditary ataxia: what every neurologist should know. *Neurol Clin Pract*. 2018;8:27–32.
[78] Zhao M, Lee C.G, Law H.Y, Chong S.S. Enhanced detection and sizing of the HTT CAG repeat expansion in Huntington disease using an improved triplet-primed PCR assay. *Neurodegener Dis*. 2016;16:348–531.
[79] Almqvist E.W, Bloch M, Brinkman R, et al. A worldwide assessment of the frequency of suicide, suicide attempts, or psychiatric hospitalization after predictive testing for Huntington disease. *Am J Hum Genet*. 1999;64:1293–1304.
[80] Smeets H.J, Smits A.P, Verheij C.E, et al. Normal phenotype in two brothers with a full FMR1 mutation. *Hum Mol Genet*. 1995;4:2103–2108.

[81] Nolin S.L, Brown W.T, Glicksman A, et al. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. *Am J Hum Genet*. 2003;72:454–464.
[82] Chen L, Hadd A.G, Sah S, Houghton J.F, Filipovic-Sadic S, Zhang W, Hagerman P.J, Tassone F, Latham G resolution methylation polymerase chain reaction for fragile X analysis: evidence for novel FMR1 methylation patterns undetected in Southern blot analyses. *Genet Med*. 2011;13:528–538.
[83] Lyon E, Laver T, Yu P, Jama M, Young K, Zoccoli M, M simple, high-throughput assay for Fragile X expanded alleles using triple repeat primed PCR and capillary electrophoresis. *J Mol Diagn*. 2010;12:505–511.
[84] Chen L, Hadd A, Sah S, Filipovic-Sadic S, Krosting J, Sekinger E, Pan R, Hagerman P.J, S al. An information-rich CGG repeat primed PCR that detects the full range of fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diagn*. 2010;12:589–600.
[85] Eichler E.E, Richards S, Gibbs R.A, Nelson D.L. Fine structure of the human FMR1 gene. *Hum Mol Genet*. 1994;3:684–685.
[86] Jacquemont S, Hagerman R, Leehey M.A, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation Carrier population. *J Am Med Assoc*. 2004;291:460–469.
[87] Nelson D.L. The fragile X syndromes. *Semin Cell Biol*. 1995;6:5–11. .

[88] Sakamoto N, Chastain P.D, Parniewski P, et al. Sticky DNA: self-association properties of long GAA.TTC repeats in R.R.Y triplex structures from Friedreich's ataxia. *Mol Cell*. 1999;3:465–475.

[89] Fenoglio C, Scarpini E, Serpente M, Galimberti D. Rol of genetics and epigenetics in the pathogenesis of Alzheimer's disease and frontotemporal dementia. *J Alzheimers Dis*. 2018;62:913–932.

[90] Roberts R.G, Coffey A.J, Bobrow M, Bentley D.R. Exor structure of the human dystrophin gene. *Genomics*. 1993;16:536–538.

[91] Abbs S, Roberts R.G, Mathew C.G, et al. Accurate assessment of intragenic recombination frequency within the Duchenne muscular dystrophy gene. *Genomics*. 1990;7:602–606.

[92] Oudet C, Hanauer A, Clemens P, et al. Two hot spots of recombination in the DMD gene correlate with the deletion prone regions. *Hum Mol Genet*. 1992;1:599–603.

[93] Forrest S.M, Cross G.S, Flint T, et al. Further studies of gene deletions that cause Duchenne and Becker muscular dystrophies. *Genomics*. 1988;2:109–114.

[94] Hu X.Y, Burghes A.H, Ray P.N, et al. Partial gene duplication in Duchenne and Becker muscular dystrophies. *J Med Genet*. 1988;25:369–376.

[95] Monaco A.P, Bertelson C.J, Liechti-Gallati S, et al. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. *Genomics*. 1988;2:90–95.

[96] Lim K.R, Maruyama R, Yokota T. Eteplirsen in the treatment of Duchenne muscular dystrophy. *Drug Des Dev Ther*. 2017;11:533–545.
[97] Darras B.T, Francke U. Normal human genomic restriction-fragment patterns and polymorphisms revealed by hybridization with the entire dystrophin cDNA. *Am J Hum Genet*. 1988;43:612–619.
[98] Chamberlain J.S, Gibbs R.A, Ranier J.E, et al. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acids Res*. 1988;16:11141–11156.
[99] Itto A.B, Hamzi K, Bellayou H, Itri M, Slassi I, Nadifi S of molecular diagnosis of Duchenne muscular dystrophy. *J Mol Neurosci*. 2013;50:314–316.
[100] Mendell J.R, Buzin C.H, Feng J, et al. Diagnosis of Duchenne dystrophy by enhanced detection of small mutations. *Neurology*. 2001;57:645–650.
[101] Prior T.W, Bartolo C, Pearl D.K, et al. Spectrum of small mutations in the dystrophin coding region. *Am J Hum Genet*. 1995;57:22–23.
[102] Lim B.C, Lee S, Shin J.Y, Kim J.I, Hwang H, Kim K.J, H diagnosis of Duchenne and Becker muscular dystrophy using next-generation sequencing technology: comprehensive mutational search in a single platform. *J Med Genet*. 2011;48:731–736.
[103] Hartin S.N, Hossain W.A, Weisensel N, Butler M.G. T siblings with Prader-Willi syndrome caused by imprinting center microdeletions and review. *Am J*

*Med Genet A*. 2018;176:886–895.
[104] Kosaki K, McGinniss M.J, Veraksa A.N, et al. Prader–willi and Angelman syndromes: diagnosis with a bisulfite-treated methylation-specific PCR method. *Am J Med Genet*. 1997;73:308–313.
[105] Smith A, Hung D. The dilemma of diagnostic testing for Prader-Willi syndrome. *Transl Pediatr*. 2017;6:46–56.
[106] Burger J, Buiting K, Dittrich B, et al. Different mechanisms and recurrence risks of imprinting defects in Angelman syndrome. *Am J Hum Genet*. 1997;61:88–93.
[107] Raca G, Buiting K, Das S. Deletion analysis of the imprinting center region in patients with Angelman syndrome and Prader–Willi syndrome by real-time quantitative PCR. *Genet Test*. 2004;8:387–394.
[108] Giardello F.M, Brensinger J.D, Persen G.M, et al. The use and interpretation of commercial APC gene testing for familial adenomatous polyposis. *N Engl J Med*. 1997;336:823–827.
[109] Lindor N.M, Greene M.H. For the Mayo familial cancer program the concise handbook of family cancer syndromes. *J Natl Cancer Inst*. 1998;90:1039–1071.
[110]
Metcalfe K.A, Lubinski J, Gronwald J, Huzarski T, Mc Hereditary Breast Cancer Clinical Study Group. The risk of breast cancer in BRCA1 and BRCA2 mutation carriers without a first-degree relative with breast cancer. *Clin Genet*. 2018;93 1063-8.
[111] American Society of Clinical Oncology. American

society of clinical oncology policy statement update: genetic testing for cancer susceptibility. *J Clin Oncol*. 2003;21:2397–2406.

[112] Euhus D. Risk modeling in breast cancer. *Breast J*. 2004;10(Suppl. 1):S10–S12.

[113] Panchal S.M, Ennis M, Canon S, Bordeleau L.J. Selecti a BRCA risk assessment model for use in a familial cancer clinic. *BMC Med Genet*. 2008;9:116.

[114] Bunyan D.J, Eccles D.M, Sillibourne J, et al. Dosage analysis of cancer predisposition genes by multiplex ligation-dependent probe amplification. *Br J Cancer*. 2004;91:1155–1159.

[115] Struewing J.P, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med*. 1997;336:1401–1408.

[116] Olopade O.I, Artioli G. Efficacy of risk-reducing Salpingo-oophorectomy in women with BRCA-1 and BRCA-2 mutations. *Breast J*. 2004;10(Suppl. 1):S5–S9. .

[117] Cao A, Huang L, Shao Z. The preventive intervention of hereditary breast cancer. *Adv Exp Med Biol*. 2017;1026:41–57.

[118] O'Leary E, Iacoboni D, Holle J, Michalski S.T, Esplin E gene panel use for women with breast cancer: identification and intervention beyond breast cancer risk. *Ann Surg Oncol*. 2017;24:3060–3066.

[119] Lipton L.R, Johnson V, Cummings C, Fisher S, Risby I Sadat A.T, Cranston T, Izatt L, Sasieni P, Hodgson S.V

al. Refining the Amsterdam criteria and Bethesda guidelines: testing algorithms for the prediction of mismatch repair mutation status in the familial cancer clinic. *J Clin Oncol*. 2004;22:4934–4943.

[120] Umar A, Boland C.R, Terdiman J.P, Syngal S, de la Chapelle A, Ruschoff J, Fishel R, Lindor N.M, Burgart al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*. 2004;96:261–268.

[121] Reyes C.M, Allen B.A, Terdiman J.P, Wilson L.S. Com of selection strategies for genetic testing of patients with hereditary nonpolyposis colorectal carcinoma: effectiveness and cost-effectiveness. *Cancer*. 2002;95:1848–1856.

[122] Lynch H.T, Lynch J.F, Lynch P.M, Attard T. Hereditar colorectal cancer syndromes: molecular genetics, genetic counseling, diagnosis and management. *Fam Cancer*. 2008;7:27–39.

[123] Lamberti C, Kruse R, Ruelfs C, et al. Microsatellite instability—a useful diagnostic tool to select patients at high risk for hereditary nonpolyposis colorectal cancer: a study in different groups of patients with colorectal cancer. *Gut*. 1999;44:839–843.

[124] Gryfe R, Kim H, Hsieh E.T.K, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med*. 2000;342:69–77.

[125]

Sepulveda A.R, Hamilton S.R, Allegra C.J, Grody W, C
Vokoun A.M, Funkhouser W.K, Kopetz S.E, Lieu C, L
Smolkin R, Ventura C.B, Nowak J.A. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American society for clinical pathology, College of American Pathologists, association for molecular pathology, and American society of clinical oncology. *J Mol Diagn*. 2017;19:187–225.

[126] Lorans M, Dow E, Macrae F.A, Winship I.M, Buchana on hereditary colorectal cancer: improving the clinical utility of multigene panel testing. *Clin Colorectal Cancer*. 2018;17:e293–e305.

[127] Nieuwenhuis M.H, Vasen H.F. Correlations between mutation site in APC and phenotype of familial adenomatous polyposis (FAP): a review of the literature. *Crit Rev Oncol Hematol*. 2007;61:153–161.

[128] Decker R.A, Peacock M.L. Update on the profile of multiple endocrine neoplasia type 2a Ret mutations: practical issues and implications for genetic testing. *Cancer*. 1997;80:557–568.

[129] Klein R.D, Salih S, Bessoni J, Bale A.E. Clinical testing for multiple endocrine neoplasia type 1 in a DNA diagnostic laboratory. *Genet Med*. 2005;7:131–138.

[130] Guerin C, Romanet P, Taieb D, Brue T, Lacroix A, Seb beyond the thyroid: advances in the understanding of pheochromocytoma and hyperparathyroidism phenotypes in MEN2 and of non-MEN2 familial

forms. *Endocr Relat Cancer*. 2018;25:T15–T28.
[131] Stanislaw C, Xue Y, Wilcox W.R. Genetic evaluation and testing for hereditary forms of cancer in the era of next-generation sequencing. *Cancer Biol Med*. 2016;13:55–67.
[132] Gonzalez K, Noltner K, Buzin C, Gu D, Wen-Fong C, Nguyen V, Han J, Lowstuter K, Longmate J, S al. Beyond Li–Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol*. 2009;27:1250–1256.
[133] Hawkes J.E, Truong A, Meyer L.J. Genetic predisposition to melanoma. *Semin Oncol*. 2016;43:591–597.
[134] Nordstrom-O'Brien M, van der Luijt R.B, van Rooijen E, van den Ouweland A.M, Majoor-Krakauer D.F, Lolkema M.P, van Brussel A, Voest E.E, Giles R.H. Genetic Analysis of von Hippel–Lindau Disease. *Hum Mutat*. 2010;31:521–537.
[135] Li W.L, Buckley J, Sanchez-Lara P.A, Maglinte D.T, Viduetsky L, Tatarinova T.V, Generation S. Sequencing method to detect RB1 mutations Improves care for retinoblastoma patients and their families. *J Mol Diagn*. 2016;18:480–493.
[136] Graham B.H. Diagnostic challenges of mitochondrial disorders: complexities of two genomes. *Methods Mol Biol*. 2012;837:35–46.
[137] Wong L.J. Next generation molecular diagnosis of mitochondrial disorders. *Mitochondrion*. 2013;13:379–387.
[138] Servidei S. Mitochondrial encephalomyopaties: gene

mutation. *Neuromuscul Disord*. 2002;12:524–529.
[139] Simon D.K, Johns D.R. Mitochondrial disorders: clinical and genetic features. *Annu Rev Med*. 1999;50:111–127.
[140] Tang S, Halberg M.C, Floyd K.C, Wang J. Analysis of common mitochondrial DNA mutations by allele-specific oligonucleotide and Southern blot hybridization. *Methods Mol Biol*. 2012;837:259–279.
[141] Ojino S, Wilson R.B. Spinal muscular atrophy: molecular genetics and diagnostics. *Expert Rev Mol Diagn*. 2004;4:15–29. .
[142] Prior T.W, Professional Practice and Guidelines Committee. Carrier screening for spinal muscular atrophy. *Genet Med*. 2008;10:840–842.
[143] Schimmenti L.A, Warman B, Schleiss M.R, Daly K.A, I of newborn screening bloodspot-based genetic testing as second tier screen for bedside newborn hearing screening. *Genet Med*. 2011;13:1006–1010.
[144] Kothiyal P, Cox S, Ebert J, Husami A, Kenna M.A, Gre throughput detection of mutations responsible for childhood hearing loss using resequencing microarrays. *BMC Biotechnol*. 2010;10:10.
[145] Shearer A.E, DeLuca A.P, Hildebrand M.S, Taylor K.F genetic testing for hereditary hearing loss using massively parallel sequencing. *Proc Natl Acad Sci USA*. 2010;107:21104–21109.
[146] Grody W.W. Molecular genetic risk screening. *Annu Rev Med*. 2003;54:473–490.

[147] Eng C.M, Schechter C, Robinowitz J, et al. Prenatal genetic Carrier testing using triple disease screening. *J Am Med Assoc*. 1997;278:1268–1272.
[148] King J.R, Klugman S. Ethnicity-based Carrier screening. *Obstet Gynecol Clin N Am*. 2018;45:83–101.
[149] Lazarin G.A, Goldberg J.D. Current controversies in traditional and expanded carrier screening. *Curr Opin Obstet Gynecol*. 2016;28:136–141.
[150] Mak C.M, Lee H.C, Chan A.Y, Lam C.W. Inborn errors of metabolism and expanded newborn screening: review and update. *Crit Rev Clin Lab Sci*. 2013;50:142–162.
[151] Sweeney B.P. Watson and Crick 50 Years on: from double helix to pharmacogenetics. *Anaesthesia*. 2004;59:150–165.
[152] Rosove M.H, Grody W.W. Should we Be applying warfarin pharmacogenetics to clinical practice? No, not now. *Ann Intern Med*. 2009;151:270–273.
[153] Fischel-Ghodsian N, Prezant R, Chaotraw W.E, et al. Mitochondrial gene mutation is a significant predisposing factor in aminoglycoside ototoxicity. *Am J Otolaryngol*. 1997;18:173–178.
[154] Urwyler A, Deufel T, McCarthy T, West S. Guidelines for the molecular genetic detection of malignant hyperthermia susceptibility. *Br J Anaesth*. 2001;86:283–287.
[155] Green R.C, Berg J.S, Grody W.W, Kalia S.S, Korf B.R, M College of Medical Genetics and Genomics. ACMG recommendations for reporting of incidental findings

in clinical exome and genome sequencing. *Genet Med*. 2013;15:565–574.

[156] Rehm H.L, Bale S.J, Bayrak-Toydemir P, Berg J.S, Brown K.K, Deignan J.L, Friez M group of the American College of medical genetics and genomics laboratory quality assurance Commitee. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*. 2013;15:733–747.

[157] Das S, Bale S.J, Ledbetter D.H. Molecular genetic testing for ultra rare diseases: models for translation from the research laboratory to the CLIA-Certified diagnostic laboratory. *Genet Med*. 2008;10:332–336.

[158] Pratt V.M, Everts R.E, Aggarwal P, Beyer B.N, Broeck Baak R, Hujsak P, Kornreich R, Liao J, Lorier R, Scott S of 137 genomic DNA reference materials for 28 pharmacogenetic genes: a GeT-RM Collaborative Project. *J Mol Diagn*. 2016;18:109–123.

[159] Kalman L.V, Tarleton J.C, Percy A.K, Aradhya S, Bale Toydemir P, Bridges C, Buller-Burckle A.M, Das S, Iyer R.K, Vo T.D, Zvereff V.V, To of a genomic DNA reference material panel for Rett syndrome (MECP2-related disorders) genetic testing. *J Mol Diagn*. 2014;16:273–379.

[160] Hegde M, Santani A, Mao R, Ferreira-Gonzalez A, Weck K.E, Voelkerding K.V. Developme and validation of clinical whole-exome and whole-genome sequencing for detection of germline variants in inherited disease. *Arch Pathol Lab*

*Med*. 2017;141:798–805.
[161] Palomaki G.E, Richards C.S. Assessing the analytic validity of molecular testing for Huntington disease using data from an external proficiency testing survey. *Genet Med*. 2012;14:69–75.
[162] Grody W.W, Watson M.S. Those elusive molecular diagnostics CPT codes. *Diagn Mol Pathol*. 1997;6:131–133.
[163] Klein R.D. Reimbursement in molecular pathology: bringing genomic medicine to patients. *Clin Chem*. 2015;61:136–138.
[164] Matloff E.T, Brierley K.L. The double-helix derailed: the story of the BRCA patent. *Lancet*. 2010;376:314–315.
[165] Binnie I, Park-Thompson V. Baseball bats and chocolate chip cookies: the judicial treatment of DNA in the Myriad genetics litigation. *Cold Spring Harb Perspect Med*. 2015;5:1–11.
[166] Billings P.R, Kohn M.A, de Cuervas M, et al. Discrimination as a consequence of genetic testing. *Am J Hum Genet*. 1992;50:476–482.
[167] Billings P.R. Genetic nondiscrimination. *Nat Genet*. 2005;37:559–560. .
[168] Kalia S.S, Adelman K, Bale S.J, Chung W.K, Eng C, Ev for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2017;19:249–255.
[169]

Mackley M.P, Fletcher B, Parker M, Watkins H, Ormo views on secondary findings in whole-genome and whole-exome sequencing: a systematic review of quantitative and qualitative studies. *Genet Med*. 2017;19:283–293.

[170] Larrabee P.B, Johnson K.L, Lai C, et al. Global gene expression analysis of the living human fetus using cell-free messenger RNA in amniotic fluid. *J Am Med Assoc*. 2005;293:836–842.

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7

# Therapies for Lysosomal Storage Diseases

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## Abstract

The various approaches to the treatment of Lysosomal Storage Diseases (LSDs) are reviewed. These include bone marrow transplantation (BMT), hematopoietic stem cell transplantation (HSCT), enzyme replacement therapy (ERT), substrate reduction therapy (SRT), pharmacologic chaperone therapy (PCT), and gene therapy including genome-editing. For each of those therapeutic approaches, the methodology as well as the relative safe and effectiveness in various LSDs are reviewed. With respect to gene therapy and various genome-editing techniques, the methodology and current experimental approaches are presented.

## Keywords

Bone marrow therapy (BMT); Enzyme replacement therapy (ERT); Gene therapy; Genome editing; Hematopoietic stem cell transplantation (HSCT); Lysosomal storage diseases (LSDs); Pharmacologic chaperone therapy (PCT); Substrate reduction therapy

(SRT)

## 7.1. Introduction

In previous editions of this text, we reviewed the history of enzyme replacement therapy (ERT) and bone marrow transplantation (BMT), including the early animal model and human studies that identified the therapeutic successes and limitations of ERT and BMT, as well as the potential of gene therapy [1–7]. Here, we provide an update of the various approaches used to treat these diseases, which include ERT, BMT, substrate reduction therapy (SRT), and pharmacologic chaperone therapy (PCT), all of which have been approved for specific lysosomal storage diseases (LSDs) by the United States Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA). In addition, we briefly highlight emerging approaches, including recent gene therapy clinical trials and the application of gene editing technology.

## 7.2. Enzyme Replacement Therapy

Over 50 years ago, Nobel Laureate Christian de Duve first suggested that lysosomal diseases could be treated by replacing the defective enzyme with its normal counterpart [8]. Subsequent experiments demonstrated that when the appropriate normal enzyme was added to the media of enzyme-deficient cultured fibroblasts from individuals with various lysosomal storage diseases, the exogenous enzyme gained access to and degraded the substrates accumulated in the lysosomes [e.g., 9,10]. Of note, only 1%–5% of normal intracellular enzyme activity was required to correct the metabolic defects in the enzyme-deficient cells [11]. The subsequent discovery that lysosomal enzyme glycoproteins are targeted to the lysosome by the mannose 6-phosphate residues on their oligosaccharide chains provided further rationale for the treatment of lysosomal disorders by ERT [12,13]. The pathway mediated by the mannose 6-phosphate receptors is responsible for the intracellular delivery of newly synthesized

lysosomal enzymes to the lysosomes, as well as for the delivery of exogenously supplied normal lysosomal enzymes to the lysosomes via the mannose 6-phosphate receptors on the plasma membranes of most cell types [12,13].

These studies indicated the feasibility of ERT and, in particular, that exogenous lysosomal enzymes could gain access to intracellular lysosomal sites and that low levels of enzyme activity could metabolize the accumulated substrate(s) in cellular lysosomes. Thus, these findings provided the rationale for the early clinical studies of ERT in lysosomal storage diseases.

Many investigators were skeptical about the prospects for enzyme therapy. It was not until the early 1990s that ERT became a reality with the demonstration of its safety and effectiveness in patients with Type 1 Gaucher disease [14,15]. Roscoe Brady's group at the NIH reported that weekly or biweekly intravenous infusions of large doses (2–3 mg/kg) of the mannose-terminated enzyme markedly reduced the hepatosplenomegaly, improved the hematologic values, and led to substantial improvements in bone density and other clinical manifestations in these patients [14,15]. Their demonstration that biweekly ERT was safe and well tolerated, and that the enzyme could reverse years of substrate accumulation in these patients, provided the first clinical "proof of principle" for ERT in lysosomal disorders without primary neurologic involvement.

Since a viable murine or naturally occurring animal model for Gaucher disease did not exist, investigators experimented with the dose and dose schedule to determine the minimum effective dose of the enzyme to decrease the cost of therapy. Eventually, it was appreciated that clinical response was dose-dependent and that the maintenance dose was not significantly different from the dose initially used to reverse years of substrate accumulation. During the past 25 years, worldwide experience with ERT in thousands of patients with Type 1 Gaucher disease has clearly documented its safety and effectiveness (for reviews, see Grabowski and associates

[16] and Weinreb and colleagues [17]).

Early lessons were learned from treating Gaucher disease by ERT. Notably, most investigators initially thought that the reversal of lysosomal storage by ERT was unexpected, as most presumed that the storage was irreversible, and that, at best, stabilization of the disease process would be obtainable. This proved not to be the case, as the accumulated substrates were metabolized and the organomegaly in several disorders markedly decreased. The lessons learned included the following: (1) enzyme delivery was receptor-mediated and dose-dependent, (2) substrate clearance was dose-dependent, (3) decreased dose or cessation of ERT resulted in rapid substrate reaccumulation, (4) the earlier that ERT was initiated, the more effective it was, even preventive, since irreversible damage (e.g., bone disease, fibrosis, etc.) could not be altered by ERT (Table 7.1), and (5) the neurologic manifestations of Types 2 and 3 Gaucher disease were not altered by the intravenous administration of the normal enzyme, even at high doses, as the enzyme did not cross the blood–brain barrier (Table 7.1).

Over the past 10 years, alternate sources of recombinant β-glucocerebrosidase have been approved for use in treating Type 1 Gaucher patients. These recombinant enzymes are made in human fibrosarcoma cells through gene activation technology (velaglucerase alfa,VPRIV, Shire Biotechnology company) [18] and transgenic carrot root cells (taliglucerase alfa, Eleyso, Protalix Biotherapeutics) [19,20]. Although these enzyme versions contain minor differences in amino acid sequences, comparison of the forms made in Chinese hamster ovary cells and human fibrosarcoma cells showed no major differences with respect to their pharmacokinetics and efficacy in preclinical and clinical studies [19–22]. Longer-term experience has shown these alternative forms of recombinant β-glucocerebrosidase to be safe and effective.

## Table 7.1

**ERT Lessons Learned From Gaucher Disease**

| **Proof of Concept: ERT Works** |
| --- |
| Safe and generally well tolerated |
| Minimal immunologic complications (cross-reactive immunologic material positive ($CRIM^+$) patients) |
| Dose-dependent enzyme delivery |
| Mannose receptor-mediated enzyme uptake |
| Inability to cross blood–brain barrier |
| Dose-dependent substrate clearance |
| Unexpected reversal of lysosomal storage in organs |
| Rapid reaccumulation off therapy: lifetime therapy |
| Need to treat early to prevent irreversible disease |

The success of ERT in Type 1 Gaucher disease stimulated investigators to develop and evaluate enzyme replacement for other lysosomal diseases. These efforts were facilitated by the cloning of the complementary DNAs (cDNAs) and genes encoding the normal human lysosomal enzymes that are deficient in various disorders, the development of eukaryotic expression systems to produce large quantities of recombinant enzymes [i.e., 23], and the use of gene-targeting techniques to generate "knockout" murine models for preclinical studies [e.g., 24]. Importantly, it was discovered that the overexpression of a lysosomal cDNA in CHO cells resulted in the delivery of its glycosylated enzyme to the lysosomes, and, more importantly, that the majority of the recombinant human enzyme was selectively secreted into the culture media [23], thereby facilitating large-scale production of the critical highly glycosylated human enzyme. Of note, the secreted enzymes retained their terminal mannose 6-phosphate and sialic acid residues, whereas the enzyme's oligosaccharide chains were trimmed by glycosidases in the lysosome. Other expression systems using transgenic animals and plant systems have been employed to produce therapeutic enzymes [19,20,25]. These advances abrogated two of the early recognized major obstacles to

ERT: the lack of sufficient amounts of human enzyme and the need for animal models for preclinical studies.

### 7.2.1. Factors Affecting Selection of Candidate Lysosomal Disorders for ERT

Several factors influence the selection of lysosomal disorders for ERT. These include the target site(s) of pathology, the poor likelihood of reversing certain manifestations (e.g., skeletal, connective tissue, and neural pathologies), and the presence or absence of residual enzymatic activity. For example, in Type 1 Gaucher disease, the major pathologic cell type is the easily targeted macrophage-monocyte system, i.e., liver, spleen, bone marrow. However, treatment must begin early to prevent the progressive bone disease secondary to marrow involvement, as severe bone complications appear to be irreversible. In addition, ERT did not reverse the severe neurologic manifestations in patients with Types 2 and 3 Gaucher disease. These results in the neuropathic subtypes of Gaucher disease further supported the initial findings that infused enzymes could not cross the blood–brain barrier [26] and that CNS manifestations were not treatable by ERT. In Fabry disease, the major sites of pathology are the vascular endothelium, cardiomyocytes, and renal podocytes, all which can be accessed by exogenous enzyme, whereas the dramatic bone and joint abnormalities in the mucopolysaccharidoses are due to substrate accumulation in connective tissue matrix and cells (e.g., chondrocytes), which take up little, if any, exogenous enzyme. Thus, the effectiveness of ERT in lysosomal disorders depends on the delivery of sufficient amounts of the infused enzyme to the target sites of pathology and the reversibility of certain clinical manifestations.

Animal model studies have also revealed organ-specific variations in response to ERT, which are primarily due to the biodistribution of the infused enzyme. For example, in various mouse models of the lysosomal storage diseases, the tissue distribution of intravenously infused enzyme and the amount and duration of substrate clearance (i.e., pharmacodynamics) from target sites of pathology were clearly

dose-dependent [e.g., 24]. Thus, for each disease, the infused enzymes must be administered in high-enough doses to be delivered to specific and unique cell types.

In addition, therapeutic responsiveness is related to disease subtype and disease-specific mutations. For most lysosomal disorders, patients who inherit mutations that render the gene product absent or nonfunctional manifest the severest form of the disease, often causing a more rapid onset, more severe manifestations, and/or a neurodegenerative course. Alternatively, mutations that alter the protein's stability and/or kinetics, but retain some residual enzyme function, have a less severe phenotype. Thus, certain disease subtypes may be more amenable to ERT (or SRT or PCT; see later). For example, the MPS IH subtype (Hurler disease) results in early onset of disease manifestations, including severe neurologic involvement, whereas Types IH/S and IS (Hurler–Scheie and Scheie subtypes, respectively) are characterized by later onset and less-severe disease manifestations, including the absence of mental retardation in the Scheie subtype.

The presence or absence of residual enzymatic activity, even if less than 1% of normal, primarily determines the immunologic response to ERT. In Type 1 Gaucher disease, all patients have residual β-glucocerebrosidase activity, and experience with thousands of Type 1 patients has documented that less than 15% raise nonneutralizing immunoglobulin G (IgG) antibodies against the recombinant enzyme [16,27]. These antibodies have no effect on efficacy and rarely cause infusion-associated reactions [28]. In contrast, about 30%–50% of patients with Type 1 classic Fabry disease, who have no or very little residual $\alpha$-galactosidase A ($\alpha$-Gal A) activity, develop non-neutralizing IgG antibodies after four to six infusions [29,30]. Up to half of these patients may experience infusion-associated reactions, including chills, rigors, and/or fevers, which do not affect efficacy and can be managed conservatively by premedication with nonsedating antihistamines and antipyretics and by slowing the infusion rate, since these reactions are directly related to the rate of the infusion and protein load. Importantly, patients who seroconvert decrease their antibody titers with time and may eventually become tolerant. Of

note, even in patients with no residual enzyme, the intravenous infusion of the recombinant normal enzyme has been well tolerated and has not precluded treatment. The only exception is patients with infantile Pompe disease who have certain mutations develop significant neutralizing antibodies that markedly decrease the effectiveness of ERT [31,32].

Clinical trials of ERT using recombinant human enzymes have led to FDA and EMA approvals of this treatment for patients with Fabry disease, MPS I, and MPS II, MPS IVA, MPS VI, Pompe disease, lysosomal acid lipase deficiency, and most recently MPS VII and ceroid lipofuscinosis Type 2 (CLN2) (Table 7.2). In addition, clinical trials are currently underway in Neimann-Pick disease (NPD) Type B, mannosidosis, metachromatic leukodystrophy, and galactosialidosis, among others.

## Table 7.2

### FDA and EMEA Approval of ERT for Lysosomal Storage Diseases

| Disease | Company | Status in 2018 |
|---|---|---|
| **Gaucher** | Genzyme<br>Shire<br>Protalix/Pfzier | Approved 1991 (USA)[a]<br>Approved 2010 (EU[b]& US)<br>Approved 2012 (US) |
| **Fabry** | Genzyme &<br>Shire<br>Genzyme | Approved 2001 (EU)<br>Approved 2003 (US) |
| **MPS I** | BioMarin/Genzyme | Approved 2003 (EU & US) |
| **MPS VI** | BioMarin | Approved 2005 (US & EU) |
| **Pompe** | Genzyme | Approved 2006 (US & EU) |
| **MPS II** | Shire | Approved 2006 (US & EU) |
| **MPS II** | Shire | Approved 2006 (US & EU) |
| **MPS IVA** | BioMarin | Approved 2014 (US & EU) |

| | | |
|---|---|---|
| **LAL-Deficiency** | Synageva/Alexion | Approved 2015 (US & EU) |
| **CLN2** | BioMarin | Approved 2017 (USA) |
| **MPS VII** | Ultragenyx | Approved 2017 (USA) |

[a] Approval by United States Food and Drug Administration (FDA).

[b] Approval by European Union (EU), European Agency for Evaluation of Medical Products (EMEA).

More recently, efforts have focused on the treatment of the neurologic symptoms of the LSDs by direct intrathecal or intraventricular delivery of the therapeutic enzyme in MPS I and III B, metachromatic leukodystrophy, Neimann-Pick C, and CLN2. In neuronal CLN2, a rare lysosomal disorder that causes progressive dementia in children, recombinant peptidase I (cerliponase alfa)was administered intraventricularly every 2 weeks. The primary endpoint, the median 2-point decline in the motor-language score, was not reached in treated patients, and was 345 days for historical controls. The mean (±SD, standard deviation) unadjusted rate of decline on the motor-language score per 48-week period was 0.27 ± 0.35 in the treated patients and 2.12 ± 0.98 in the 42 historical controls ($P < .001$) [33]. Intraventricular ERT for CLN2 was the first FDA-approved brain-targeted ERT [34]. Preclinical studies in animal analogs are underway for several other neurodegenerative disorders.

## Table 7.3

### Principles for Effective Treatment for Lysosomal Storage Diseases

| |
|---|
| Enzyme delivery is receptor mediated and dependent on the number of |

| |
|---|
| receptors on plasma membrane |
| Mannose 6-phosphate receptor for most lysosomal enzymes |
| Mannose receptor for β-glucocerebrosidase uptake (Gaucher disease) |
| Enzyme: available mannose 6-phosphate residues |
| Avoid galactose residues for asialoglycoprotein receptor |
| Inability to cross the blood–brain barrier |
| Enzyme delivery is dose dependent |
| Substrate clearance is dose dependent |
| Adequate dose required for delivery to critical organs |
| Off ERT, rapid substrate reaccumulation, and clinical exacerbation |
| Immune reactions depend on cross-reactive immunologic material (CRIM) status; infusion-associated reactions are manageable |
| Treat early to prevent irreversible damage; even in advanceddisease, treat to slow/prevent progression |

## 7.2.2. ERT: Principles for Effective Treatment

As noted earlier, the clinical trials and subsequent clinical experience in lysosomal disorders have demonstrated or indicated the therapeutic effectiveness of ERT. From these studies, certain general principles for effective treatment by ERT have become evident (Table 7.3): (1) Intravenously administered recombinant lysosomal enzymes are cleared from the circulation primarily by the pathway mediated by the mannose 6-phosphate (mannose in Gaucher disease) receptor for cellular uptake and lysosomal delivery. Thus, the administered enzymes must have their full complement of mannose 6-phosphate residues and be fully silylated for maximal lysosomal delivery to organs other than the liver, where the hepatocytes will rapidly take up asialo- and galactose-terminated glycoproteins via the asialofetuin receptor. (2) Dose is important. The higher the dose, the greater is the

biodistribution, especially to cell types or sites that have limited uptake, such as the heart and kidney. Substrate clearance is dose-dependent. (3) There is rapid reaccumulation of substrate if ERT is interrupted or stopped. This was surprising since ERT cleared decades of substrate accumulation, but in the absence of continued treatment, the substrate rapidly reaccumulates. This may be related in part to cell turnover or the proclivity of enlarged "hungry" lysosomes. (4) Infusion-associated reactions occur, presumably when patients develop IgG antibodies against the infused recombinant enzyme, but these reactions are transient, can be managed conservatively, and are more frequent in patients with little or no residual enzyme activity. With time, patients may reduce their antibody titers and become tolerized to these enzymes.

Until gene therapy, or another therapy, proves effective in these diseases, ERT provides a safe and effective means to reverse years of substrate accumulation and to control further deposition. A clear need for each of these diseases will be the identification and monitoring of biomarkers that will assess disease progression or improvement, and permit therapeutic monitoring, such as the use of plasma chitotriosidase activity in Type 1 Gaucher disease [e.g., 35] or plasma lyso-globotriosylsphingosine levels in Fabry disease [36,37]. Such biomarkers may determine if the initial dose of the recombinant enzyme used to debulk years of substrate accumulation can be adjusted to a maintenance dose in individual patients.

## 7.3. Bone Marrow Transplantation

The first case of a lysosomal disease patient being treated by BMT was by Hobbs and colleagues in 1981 in a child with severe MPS IH (Hurler disease) [38]. These investigators reported positive biochemical (substrate reduction) and clinical effects (reduced hepatosplenomegaly, corneal clouding), leading to the evaluation of this therapeutic approach in numerous MPS I and other lysosomal disease patients over the next three decades. The rationale underlying BMT for the lysosomal storage diseases is that the myeloid stem cells in healthy donor bone marrow would be able to engraft and

repopulate various tissues following transplantation, providing a source of normally functioning enzymes for therapy. This concept was further supported by the pioneering work of Neufeld and colleagues showing that many lysosomal enzymes, including the MPS I enzyme, alpha-L-iduronidase, were released from cells at low levels and could "cross-correct" neighboring cells [39,40]. Thus, the successfully engrafted bone marrow-derived cells also could serve as a source of normal enzymes for other established cell types in the patient's tissues, especially that glial cells from the bone marrow could gain access to the brain [41].

To date, many hundreds of lysosomal disease patients have undergone BMT. More recently, the transplants have been performed using purified populations of stem cells from the bone marrow or peripheral blood, rather than the entire marrow cell populations, hence leading to the terminology hematopoietic stem cell transplantation (HSCT). Unfortunately, due to the variable protocols and the lack of natural history data for many lysosomal diseases, interpretation of the results has been difficult to evaluate. Many factors influence the successful engraftment of the stem cells, including the histocompatibility of the donor cells, the preconditioning regime of the host, the type of stem cell population used, etc. These variables are often different in the reported cases, or in some cases are not reported at all. In addition, the clinical outcome is highly dependent on the age at which the transplant is performed and the clinical status of the patient, which in many studies has not been controlled. The lack of adequate natural history data for many lysosomal disorders has added to this uncertainty.

MPS I remains the most extensively studied and best example of HSCT among the lysosomal disorders. Based on experience from hundreds of patients, The European Bone Marrow Transplantation Group recommends HSCT as a first-line treatment for patients affected with MPS I diagnosed before the age of 2.5 years with clinically presumed Hurler Syndrome (MPS IH) [42]. Under such conditions, HSCT has been shown to be clinically effective, increasing

life expectancy and improving various clinical outcomes. However, residual disease burden is present in the transplanted patients, including in the skeleton and heart, and the CNS remains partially refractory to correction posttransplant. Therefore, in many European centers, ERT is used as a bridging therapy to help stabilize and prepare patients for transplant, and increase its safety and overall outcome [43].

The results in other lysosomal disorders have been less clear than MPS 1H, in part due to the same lack of controlled studies and outcome evaluation. In addition, although most lysosomal enzymes have the ability to "cross-correct," the extent to which they are able to do so is highly variable, which directly impacts the clinical outcome of the transplant and which diseases are likely candidates for this approach. As with MPS I, age at which the transplant is performed and the clinical status of the patient also are key factors, as well as the preconditioning of the recipient and preparation of the donor cells used for transplant. Although the safety of the procedure has been greatly improved overall, in a small number of cases morbidity and/or mortality are still limiting factors, and secondary transplants may be required. Other than MPS IH, lysosomal diseases in which HSCT is considered potentially therapeutic and for which supportive clinical data exists include alpha-mannosidosis[44], MPS II (Hunter disease) [45], MPS VI (Maroteaux-Lamy disease) [46], Farber disease (acid ceramidase deficiency) [47], and several others.

## 7.4. Substrate Reduction Therapy

The fact that ERT does not correct all the manifestations of the lysosomal diseases or does not effectively address the neuropathic manifestations has prompted investigations into the use of small molecule drugs that are orally administered [e.g., 48]. These alternate therapies could be deployed either as stand-alone or adjuvant therapies to ERT. Two small molecule approaches that involve oral administration are SRT and PCT (see Section 7.5). SRT is designed to reduce the rate of biosynthesis of the toxic accumulating substrate.

The aim of SRT is to reduce the amount of noncatabolized

substrates in the lysosomes by inhibiting the synthesis of their precursors [49–52]. For the glycolipidoses, the concept seeks to inhibit the enzyme glucosylceramide synthase that catalyzes the first step in the synthesis of glycosphingolipids [49–51]. Since its inception, significant progress has been made in identifying small molecule inhibitors of this enzyme with the appropriate safety and efficacy profiles for use in patients with Gaucher disease as well as other glycosphingolipidoses. The potential to deploy small molecule drugs offers the convenience of oral delivery and the potential to have a pharmacodynamic response that is different and potentially complementary to ERT.

As SRT seeks to restore the balance between the rate of biosynthesis and catabolism of the substrates, this therapy is ideally suited for disease mutations that retain some residual enzymatic activity. Subjects with Type 1 Gaucher disease as well as others with lysosomal diseases, which have residual enzymatic activity and a less severe disease course typically, exhibit this feature. In patients with Type 1 Gaucher disease, these small molecule inhibitors of glucosylceramide synthase directly reduce the synthesis of glucosylceramide, thereby allowing the residual activity of the mutant β-glucocerebrosidase to hydrolyze the accumulated, but reduced amount of the glycolipid substrate. In these patients, SRT can be deployed as a monotherapy [e.g., 53]. In subjects who harbor mutations that extinguish the relevant enzymatic activity, SRT could be considered in the context of an adjunctive therapy to ERT [e.g., 54–56].

Two chemical classes of inhibitors of glucosylceramide synthase have been approved to treat Type 1 Gaucher disease. Miglustat (Zavesca, Actelion Pharmaceuticals) is based on the imino sugar, *N*-butyldeoxynojirimycin (NB-DNJ) and Eliglustat, (Cerdelga, Sanofi Genzyme) on D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (eliglustat, Genzyme Corporation) [51,57].

Early indications that miglustat could effect substrate reduction came from cell culture studies in the presence of conduritol-β-epoxide, an agent that inhibits glucocerebrosidase activity and induces the accumulation of glucosylceramide. Treatment of these cells with

miglustat abrogated lysosomal storage of the substrate [50]. Subsequent preclinical studies in mouse models of lysosomal diseases confirmed the use of these inhibitors in treating a variety of glycosphingolipidoses, including those with CNS involvement [52,58,59]. As miglustat reportedly can traverse the blood–brain barrier into the CNS, it has also been used to treat neuropathic Type 3 Gaucher disease and NPD type C (see later).

Characterization of the inhibitory activity of eliglustat on glycosphingolipid synthesis in vitro showed that it was more potent and selective than miglustat[51]. However, as it is a substrate for the P-glycoprotein multidrug transporter, it does not accumulate in brain tissue and therefore is unlikely to be useful for treating neuropathic lysosomal disorders. Testing of eliglustat in a mouse model of Type 1 Gaucher disease showed that it reduced the number of Gaucher cells in the liver and the accumulation of glucocerebroside in the liver, spleen, and lung. Moreover, Gaucher mice treated sequentially by ERT (Cerezyme) and eliglustat exhibited significantly lower accumulation of glucocerebroside than animals treated with either agent alone [55]. Testing of eliglustat either as a monotherapy or combination therapy with ERT (Fabrazyme) in Fabry mice showed similar benefits [54]. These data supported the notion that SRT could function as an adjunctive therapy to ERT for these glycosphingolipidoses. In subjects whose disease has been stabilized by ERT, SRT could potentially be used as a convenient maintenance therapy. In subjects naïve to treatment, ERT followed by SRT could potentially accelerate clearance of the accumulated substrates.

### 7.4.1. SRT for Type 1 Gaucher Disease

Several clinical trials of miglustat have been carried out in Type 1 Gaucher disease [56,60,61]. Significant improvements were noted, including a decrease in liver and spleen volume, clinically significant increases in hemoglobin and platelet counts, and a progressive decline in the biomarker chitotriosidase. The most frequent adverse event was diarrhea due to inhibition of intestinal disaccharidases, which occurred in 79% of the patients. Tremor was relatively common (25%

of patients), but this tended to resolve spontaneously or became attenuated with lower doses. Based on the safety and efficacy profile of miglustat, the drug was approved by the EMA in November 2002 and the FDA in July 2003 for the treatment of adult Type 1 Gaucher patients with mild or moderate disease who are unwilling or unable to receive or to continue ERT.

Based on preclinical studies, eliglustat was shown to have a higher specificity for glucosylceramide synthase than miglustat, and it did not inhibit the gut glycosidases. This oral inhibitor has undergone a series of clinical trials in Type 1 Gaucher patients [62–64].

Significant decreases in spleen and liver volumes, increased hemoglobin concentrations and platelet counts, and improved bone mineral density were demonstrated [63,64]. Patients were administered 50 or 100 mg of eliglustat twice daily based on plasma drug concentrations, the latter dose to ensure that CYP2D6 rapid metabolizers attained concentrations of approximately 10 ng/mL. The magnitude of the hematologic and visceral responses was within the ranges observed with ERT over the same period. However, the increase in lumbar spine bone mineral density was more rapid than with ERT, suggesting that cortical bone might have greater access to eliglustat, presumably because of its smaller size. Plasma glucocerebroside and $GM_3$ ganglioside levels were normalized, and chitotriosidase, CCL18, angiotensin-converting enzyme, and tartrate-resistant acid phosphatase levels were decreased by 35%–50% relative to baseline. Treatment was generally well tolerated. Unlike miglustat, gastrointestinal events were low with eliglustat, which is consistent with its lack of inhibition of intestinal disaccharidases, and there was no treatment-related tremor.

Three Phase 3 randomized controlled clinical trials of eliglustat further documented its safety and effectiveness [65–67]. These trials included (1) naïve patients who had not been treated with ERT in the previous 9 months, (2) as a maintenance therapy in Type 1 Gaucher

patients previously stabilized with ERT for at least 3 years, and (3) a third trial that compared the effects of once daily versus twice daily dosing of the drug [65–67]. These clinical trials demonstrated that orally administered eliglustat was generally well tolerated and therapeutically effective.

### 7.4.2. SRT for Other Lysosomal Storage Disorders

As glucosylceramide synthase catalyzes the first committed step in the synthesis of glycosphingolipids, inhibitors of this enzyme may also be considered for treating other glycosphingolipidoses, such as Fabry disease. Preclinical studies in murine models of Fabry disease showed that a dose-dependent reduction in its major accumulated substrate, globotriaosylceramide, could be attained in several visceral organs using an eliglustat-related drug[54,68]. Moreover, when used in combination with ERT, the effects were shown to be additive. An early clinical trial using miglustat (100 mg daily or twice daily) in Fabry disease was stopped as a result of untoward neurologic toxicity. Of the 16 males who received the drug, 13 developed tremor and 2 complained of paresthesias [69].

As noted before, miglustat is able to traverse the blood–brain barrier into the CNS and has also been used for treating lysosomal storage diseases with neurologic involvement. While initial clinical testing in neuropathic Type 3 Gaucher disease patients suggested a positive effect of miglustat, a randomized controlled trial in 30 Type 3 Gaucher patients receiving ERT showed no effect on neurologic manifestations [70]. Measurements of the aberrant saccadic eye movement that is characteristic of this group of patients as well as neurologic and neuropsychologic evaluations were not significantly different between the drug-treated and untreated groups. It has been suggested that this failure may be due to the limited potency of miglustat or due to the irreversible nature of the neurologic disease in these subjects.

Moreover, despite encouraging preclinical data obtained with miglustat in animal models of $GM_2$ gangliosidosis [71], an open-label study in five patients with juvenile $GM_2$ gangliosidosis showed that the treatment did not alter the progression of neurologic disease over a 24-month period [72]. However, there was evidence of reduction of intracytoplasmatic inclusions in peripheral blood white cells. In a separate study of miglustat in two patients with the infantile form of Tay–Sachs disease, there was failure to arrest neurologic deterioration over 12 months [73].

### 7.4.3. SRT With Miglustat

SRT with miglustat is reportedly modestly effective in patients with NPD type C [74,75]. The disorder is characterized by neuronal accumulation of complex gangliosides and neutral glycosphingolipids resulting from a defect in cholesterol and ganglioside trafficking. Treatment of murine and feline models of the disease with miglustat reduced ganglioside accumulation and neuropathology, delayed neuronal dysfunction, and increased their lifespan [76]. A randomized controlled clinical trial conducted in 29 patients who were 12 years or older showed an improvement in the horizontal saccadic eye movement velocity in patients who received 200 mg miglustat three times daily when compared to those receiving standard care [77]. Improvements in swallowing capacity, stable auditory acuity, and a slower deterioration in an ambulatory index were also noted at 12 months, which were sustained in the majority of patients when analyzed after 24 months [74]. Two additional clinical studies in NPD type C patients also showed a stabilization of the neurologic disease, although the effects were more modest among younger patients who were at a more advanced stage of disease [75,78]. Based

on these findings, miglustat was approved by the EMA for treating NPD type C in January 2009 and by the US FDA on May 24, 2010.

#### 7.4.3.1. SRT With Cyclodextrins

Another approach to SRT for NPC disease, although not by inhibiting an enzyme in the biosynthesis of the accumulated substrate, is the use of cyclodextrins (CDs) to bind to and reduce the intracellular cholesterol accumulation [79,80]. This disease is an autosomal recessive lysosomal disorder due to mutations in the genes encoding two lipid transport proteins, NPC 1 and NPC 2. They work cooperatively to traffic intracellular lipids, and the loss-of-function of either leads to cholesterol accumulation. CDs are cyclic oligosaccharides that contain six, seven, or eight glucose units ($\alpha$-CDs, β-CDs, and ϒ-CDs, respectively). These compounds are often used to solubilize and/or stabilize various therapeutic drugs and other more complex compounds, and they serve as very effective drug delivery agents. In fact, they were used to deliver CD drugs to potentially treat

NPC 1 cells and mice and also were used as the sham control. Unexpectedly, the control was most effective in reducing the cholesterol accumulation, which lead to their further evaluation as potent compounds for the treatment of NPC in their own right. The molecular mechanism of CD-mediated cholesterol extraction has been investigated [81]. According to Lopez and colleagues, the molecular mechanism of β-CDs extraction from a cholesterol monolayer involves several sequential steps: (1) formation of CD dimers, (2) binding of CDs at the membrane interface, (3) absorption of cholesterol into the CD, (4) tilting of the CD, and (5) desorption of the CD/cholesterol complex from the interface.

Studies in NPC 1 cells [82,83] and mice [e.g., 84] proved the effectiveness of various CDs. However, even at high doses, the CDs do not readily cross the blood–brain barrier [85]. In NPC 1 cats, the intraperitoneal or subcutaneous administration of CDs had therapeutic effects on visceral cholesterol accumulation, but also

caused deafness [85,86]. These findings led to Phase 1/2a and 2b/3 clinical trials of an intrathecally administered mixture of hydroxypropyl-β-cyclodextrins (HPβCD) [87]. Also a Phase1 clinical study using high-dose intravenous administration of a different HPβCD is underway [85]. Recent results of NPC 1 patients treated by intrathecal administration of an HPβCD for 2.5–3 years indicated stable or improving neurologic assessments [88]. These studies are ongoing at this writing and will provide information on the safety, efficacy, dose, and route of administration for future trials of this substrate chelating and depleting technique to treat this progressive neurologic and fatal disease.

## 7.4.4. Conclusions

Clearly, SRT has emerged as a competitive alternate therapeutic to ERT. The convenience that this oral therapy offers coupled with the positive clinical studies indicate that SRT represents an advancement in the standard of care for Type 1 Gaucher patients [65–67] and possibly other lysosomal storage disorders. The promise of this technology lies in the development of molecular entities that are able to traverse the blood–brain barrier in quantities that are therapeutic for subjects with neuropathic lysosomal diseases. This will require an investment either in medicinal chemistry to improve the characteristics of the existing entities or in additional high-throughput screens for new entities with the appropriate drug profile. Now that the concept has been validated, it is also imperative that efforts are expanded to identify drugs for SRT for lysosomal disorders that extend beyond the glycosphingolipidoses.

Although SRT is an attractive strategy, long-term outcomes must be assessed to determine its ultimate safety, as certain compounds can cross the blood–brain barrier and possibly can alter the homeostasis of the more complex glycolipids, gangliosides, and related lipids in the brain.

## 7.5. Pharmacologic Chaperone Therapy

"Pharmacologic chaperones" are specific, preferably low molecular weight, ligands that bind to and rescue misfolded and/or mistargeted proteins and augment the cell's molecular chaperones to enhance correct folding and organelle targeting of the misfolded mutant conformers. By stabilizing the corrected conformation of the mutant protein, they prevent its degradation. These chaperones may also function by stabilizing a specific conformation of the misfolded protein, reducing aggregation, and/or preventing nonproductive interactions with other resident proteins (for reviews on protein folding and pharmacologic chaperones, see [89–93]).

As proposed for enzymes by Fan and coworkers [94,95], pharmacologic chaperones can diffuse into the cell and bind site-specifically to folding intermediates of a mutant enzyme protein, thereby stabilizing the intermediate that is rate limiting for the folding and/or trafficking of the mutant enzyme and rescuing a portion of it from degradation. Competitive reversible enzyme inhibitors can be effective pharmacologic chaperones because of their high affinity to the catalytic domain. Such inhibitors serve as a folding template for those mutant proteins with fragile conformational structures during the protein folding process and induce proper folding. In this way, the pharmacologic chaperone prevents excessive degradation of the misfolded mutant enzyme, which may retain full or partial activity if it can be properly folded, processed, and transported to its normal site of action.

For lysosomal storage diseases, pharmacologic chaperones (active-site-directed reversible enzyme inhibitors) would rescue the misfolded/mistrafficked enzyme that would otherwise be transported by the endoplasmic reticulum–associated protein degradation (ERAD) machinery to the proteasome for degradation. If even a small percentage of the mutant misfolded/mistrafficked enzyme is rescued, it can be transported to the Golgi for oligosaccharide processing and then delivered to the lysosomes, where the chaperone is displaced by the high concentration of the accumulated substrate(s) (Fig. 7.1). It is anticipated that even a small increment in enzymatic activity can

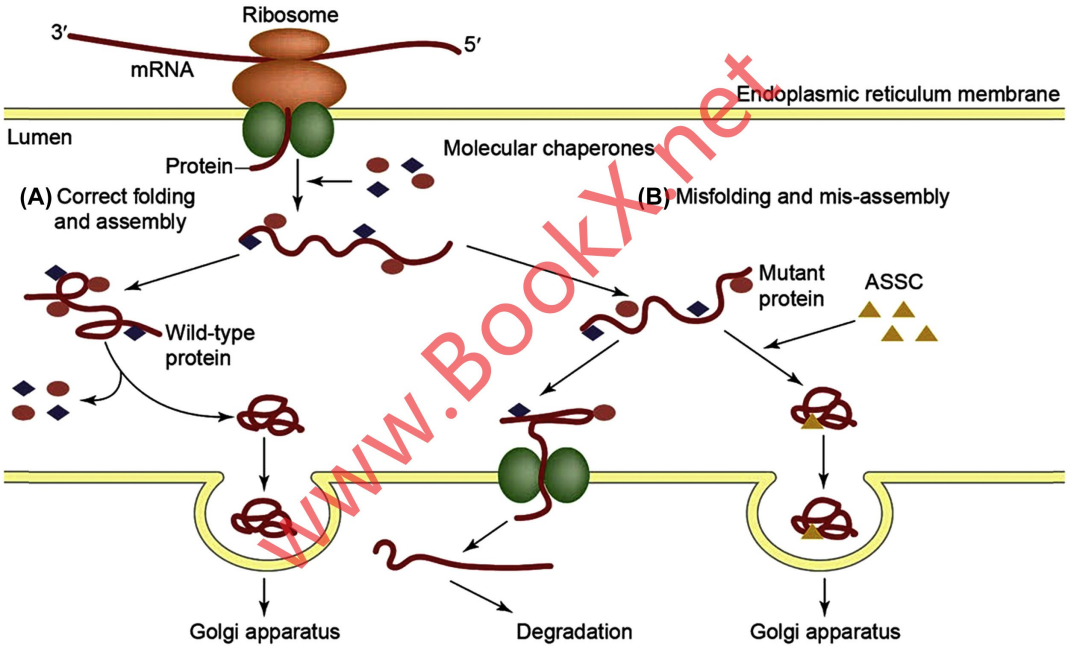
markedly attenuate a severe phenotype, as illustrated by the fact that the less-severe Type 1 subtype of Gaucher disease and other lysosomal disorders are due to the presence of low levels of mutant enzyme that have very low levels of residual activity (even <1% of wildtype). Thus, this therapeutic strategy offers the potential to prevent or effectively reverse the disease manifestations in lysosomal diseases resulting from mutant enzyme misfolding and/or mistrafficking.

The first studies of PCT for lysosomal diseases, carried out in Fabry disease (deficient $\alpha$-Gal A activity), demonstrated that mutant $\alpha$-Gal A enzyme forms, encoded by various missense mutations, could be effectively rescued by various $\alpha$-Gal A inhibitors, including 1-deoxygalactonojirimycin HCl (DGJ, also AT1001, now known as migalastat, Galafold, Amicus Therapeutics) [94,95]. Subsequently, specific pharmacologic chaperones have been identified for Gaucher, Tay–Sachs, and Sandhoff diseases and $GM_1$ gangliosidosis [96–100]. In a murine model of $GM_1$ gangliosidosis, studies have shown that a pharmacologic chaperone can cross the blood–brain barrier and decrease the neural lysosomal substrate accumulation [99].

## 7.5.1. Lysosomal Enzyme Biosynthesis and Degradation

Lysosomal proteins are synthesized in the cytoplasm and then secreted into the lumen of the endoplasmic reticulum (ER) in a largely unfolded state. In general, protein folding is governed by the principle of self-assembly. Newly synthesized polypeptides and glycopolypeptides fold into their native (active) conformation based on their amino acid sequences in a thermodynamic manner with help from the resident molecular chaperones [(e.g., binding protein (BiP), calnexin, heat shock proteins, etc.)]. To monitor the in vivo folding process, the ER has evolved a "quality-control" mechanism, termed ERAD, which uses molecular chaperones to bind and rescue unstable misfolded conformers to facilitate their proper folding and assembly and to prevent the aggregation of nonnative forms through binding

and release cycles [13,92,101]. This machinery ensures that only properly folded and assembled proteins are transported to the Golgi complex for further maturation. Improperly folded proteins are retained in the ER by molecular chaperones and then transported to the cytosol for ubiquitination and degradation within the cytosolic proteasomes [102]. In this way, misfolded or unstable (normal or mutant) proteins are eliminated from the cell [102–106]. However, even after interaction with molecular chaperones, it is estimated that up to 30% of certain normal proteins do not achieve their functional state, misfold and/or aggregate, and are rapidly degraded within minutes of their synthesis by the cell's quality-control machinery [107].




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**FIGURE 7.1** (A) Schematic of the processing, correct folding, and assembly of newly synthesized lysosomal enzymes. The newly synthesized enzyme is translocated into the endoplasmic reticulum (ER), where molecular chaperones facilitate its proper folding and subunit assembly. The molecular chaperones then dissociate from the folded assembled enzyme, which moves to the Golgi apparatus and then to lysosomes, where the enzyme is stable and active in the acidic environment of these organelles. (B) Misfolding and misassembly of mutant lysosomal enzymes. The mutant proteins are retained in the ER and are eventually degraded by the ERAD machinery. Pharmacologic chaperones (*yellow triangles*) bind to the active site of the enzyme,

promote folding, and stabilize the mutant enzyme for proper subunit assembly. This promotes transport of the mutant enzymes to the Golgi apparatus and the lysosomes, thereby increasing the concentration of the mutant enzyme and its residual enzyme activity.
From Fan JQ. A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. Trends Pharmacol Sci 2003;24:355–60.

## 7.5.2. Mutations Causing Lysosomal Diseases

Most lysosomal disorders result from a variety of mutations in their respective disease gene that renders the encoded enzyme or protein nonfunctional. Nonsense and frameshift mutations encode truncated and markedly altered polypeptide sequences that cannot function and are rapidly transported, presumably by the ERAD machinery, to the cytosol for proteasome degradation. Splicing defects result either in no enzyme protein or in an insufficient amount of normal enzyme, which results in substrate accumulation. Certain missense mutations and some small in-frame deletions that do not (or minimally) impair the functional domains of the mutant protein (i.e., active site, receptor binding site, etc.), but cause polypeptide misfolding, aggregation, instability, and/or altered trafficking to the lysosome are candidates for pharmacologic chaperones. Presumably, mutations that cause misfolding slow the normal folding process, thereby resulting in higher concentrations of "folding intermediates," which can self-aggregate [92,108]. Such mutant lysosomal proteins are retained in the ER, where they become associated with molecular chaperones (e.g., calnexin, BiP, etc.) that attempt to restore the native conformation or, having failed to form a functional state, undergo rapid degradation by the quality control system.

In many lysosomal disorders, certain missense mutations produce mutant enzymes that retain a small amount of residual enzymatic activity (even <1%). These missense mutations are associated with the less-severe disease phenotypes. The presence of residual activity presumably results from the small amount of the mutant glycopeptide that was properly folded, assembled, posttranslationally modified,

and trafficked to the lysosome. Such mutations are excellent candidates for PCT. Indeed, even mutations that totally misfold are potentially rescuable by pharmacologic chaperones.

## 7.5.3. Criteria for Rescuable Mutations by PCT

Ulloa-Aguirre and associates [92] have proposed general guidelines to identify rescuable and nonrescuable missense mutations: (1) The missense mutation should not alter critical residues essential for ligand binding or substrate/cofactor binding in the case of enzymes that are, predictably, nonfunctional. (2) Loss or gain of a cysteine residue may potentially disrupt required sulfuryl bridges or form inappropriate bridges that may be so significantly disruptive to the protein's structure that rescue cannot occur. (3) Loss or gain of a proline residue may limit or even impede pharmacologic rescue, since this amino acid typically causes forced turns in the protein sequence that may dramatically alter the structure, rendering it nonrescuable. In some proteins, an abrupt turn is likely a requisite for correct structure and cannot be corrected by pharmacologic chaperones. (4) Substitutions that impede or promote hydrogen bond formation may reduce the ability of the pharmacologic chaperone to rescue because of the inability of the mutant protein to establish correct interactions between its different domains. (5) Substitutions by larger amino acid residues (valine, tryptophan, threonine, and cysteine) may or may not be destabilizing, depending on their position, whereas replacements with smaller residues (glycine or alanine) may allow for more steric freedom and may be accommodated in the folding process, and, therefore, are potentially strong candidates for pharmacologic chaperone rescue. (6) If the enzyme or protein has been crystallized, molecular modeling studies can be used to predict the effect of the amino acid substitution on the conformation of the mutant enzyme and the impact on its active site.

## 7.5.4. Criteria for Selection of Pharmacologic Chaperones

For lysosomal enzymes, pharmacologic chaperones must bind reversibly, so that, when the rescued protein arrives in the lysosomes, the chaperone bound to the enzyme will be displaced by the high concentration and greater affinity of the already accumulated natural substrate(s). In contrast to ERT with recombinant lysosomal enzymes, the small hydrophobic molecules given orally may cross the blood–brain barrier, diffuse through connective tissue matrices, and reach target sites of pathology that infused lysosomal enzymes cannot, or only can when administered at very high doses. For neurodegenerative disorders, the compound must be able to reach sufficient concentration in the brain. Thus, PCT is particularly attractive for the treatment of neurodegenerative lysosomal diseases. Finally, clinical studies must demonstrate long-term safety, biochemical effectiveness (increased enzymatic activity and/or substrate clearance), and clinical benefit without adverse reactions.

Implicit in the selection of a pharmacologic chaperone for the treatment of patients with a lysosomal or other disease is the demonstration that the compound is nontoxic, is readily eliminated in the urine so it or its metabolites do not accumulate, and optimally rescues a variety of disease-causing mutations. In addition, molecules that could function as pharmacologic chaperones should be cell permeable and have the ability to reach the ER and remain undegraded/unmetabolized long enough to stabilize the target mutant protein and transport it to the lysosome.

## 7.5.5. Experimental Studies of PCT in Lysosomal Disorders

Most lysosomal storage diseases have less-severe subtypes, resulting from mutations encoding altered enzymes with a small amount of residual activity. Such mutant enzymes are targets for PCT, as noted earlier. To identify potential pharmacologic chaperones for lysosomal disorders, investigators have evaluated known or chemically modified substrate inhibitors and analogs or have performed high-throughput screens of chemical libraries [e.g., 109]. These compounds can be

evaluated in tissue culture systems or transgenic mice to determine their in vitro and in vivo ability to rescue certain mutant enzymes (see examples later). PCT may be particularly useful in disorders resulting from common missense mutations that are rescuable, such as β-glucocerebrosidase c.1226 A > G (p.N370S) in Type 1 Gaucher disease [98,110], β-hexosaminidase α-chain p.G269S in late-onset Tay–Sachs disease [100], and α-Gal A p.N215S in Fabry disease. However, the use of a specific enzyme inhibitor that binds to the active site has the potential to rescue a variety of missense mutations whose mutant proteins retain residual activity, even less than 1% of the wildtype.

#### 7.5.5.1. PCT for Fabry Disease

This X-linked disorder, due to deficient α-Gal A activity, has two major subtypes: the severe childhood-onset "classical phenotype" and the later-onset phenotypes [111,112]. To date, over 925 α-Gal A mutations have been published, including missense, nonsense, splicing, and frameshift mutations due to small and large deletions and insertions (Human Gene Mutation Database) [113]. In males affected with the Type 1 classic phenotype, all types of mutations have been reported. However, most patients with the Type 2 later-onset phenotype had missense mutations that encoded mutant enzymes with low levels of residual α-Gal A activity [114]. Expression studies have been used to characterize the missense mutations and to predict or assess their residual activities [114,115].

Fan and colleagues evaluated a series of α-Gal A substrate analogs and identified the imino sugar migalastat, a compound that bound the α-Gal A active site most effectively, increased the residual activities of various missense mutations [94,95,116,117]. These investigators generated transgenic mice carrying the rescuable c.902 G > A (p.R301Q) mutation and demonstrated that the oral administration of migalastat caused a dose-dependent increase in α-Gal A activity in the tissues of the mice [118]. No toxic effect was observed in transgenic

mice treated with migalastat for 140 days [119]. Independent studies in p.R301Q transgenic mouse fibroblasts demonstrated that migalastat bound to the rescuable p.R301Q mutant enzyme from the molecular chaperone BiP in the ER at pH 7.4 [117]. The rescued mutant enzyme was then trafficked via the pathway mediated by mannose 6-phosphate receptors to the lysosome, where it released the pharmacologic chaperone, bound to the accumulated glycosphingolipid substrate, and cleared it [120]. More recently, structural analyses demonstrated that migalastat binds tightly to $\alpha$-Gal A residue p.D170 in the ER, and then in the acidic lysosome the binding becomes weaker, so the enzyme can interact with the accumulated substrate [121]. Thus, these "in vitro" studies indicated that migalastat stabilized the mutant $\alpha$-Gal A glycoprotein, so more of the enzyme was transported to the lysosome where it functioned.

#### 7.5.5.1.1. Clinical trials of PCT for Fabry disease

Based on the preclinical studies in Fabry cultured cells [117], and in transgenic p.R301Q mice [118], efforts were directed to determine the safety and effectiveness of PCT with migalastat for Fabry disease. Under the sponsorship of Amicus Therapeutics, Inc., a Phase 1 safety study conducted in normal individuals indicated the safety of the pharmacologic chaperone migalastat in humans and also demonstrated that it increased the $\alpha$-Gal A activity of many missense mutations in a dose-responsive manner [119,120]. Phase 2 clinical testing of migalastat in 26 Fabry patients showed measurable increases in enzyme activity in the leukocytes of 24 patients with various missense mutations [122]. In subjects with increased enzyme activity, a reduction in substrate load was noted in kidney biopsies. Another Phase 2 trial evaluated urinary substrate, globotriaosylceramide (Gb3) and renal capillary inclusion levels in Fabry heterozygotes [123]. A double blind, randomized placebo-controlled Phase 3 trial is ongoing to evaluate the efficacy, safety, and pharmacodynamics of migalastat in affected males and female heterozygotes. These trials [124,125] indicated the safety and

effectiveness of migalastat in patients with amenable mutations. Based on these findings,migalastat was approved by the EMA for the amenable mutations in July 2016 [126]. Subsequently, migalastat was shown to reduce podocyte inclusions [127] and improve diarrhea [128] in amenable patients.

## 7.5.6. PCT for Other Lysosomal Diseases

As noted before, efforts have been directed to develop PCT for other lysosomal disorders, including Gaucher disease (defective β-glucocerebrosidase), $G_{M1}$ gangliosidosis (defective β-galactosidase), Tay–Sachs and Sanhoff diseases (defective β-hexosaminidase A or B, respectively), and Pompe disease (defective *α*-glucosidase). Among these disorders, the only experimental PCT to enter clinical trials was the imino sugar chaperone isofagamine for Gaucher disease. The prevalence of the Type 1 subtype, and particularly the frequency of the p.N370S mutation, makes Type 1 Gaucher disease an excellent candidate for PCT. Moreover, if a pharmacologic chaperone could be identified to safely and effectively rescue the c.1448 T > C (p.L444P) mutant enzyme, and if this chaperone could cross the blood–brain barrier, PCT with such a chaperone might stabilize or reverse the neurologic manifestations in Type 3 Gaucher disease patients, in whom the p.L444P mutation is relatively frequent.

Based on the encouraging preclinical data with isofagamine[129–131], an active site inhibitor of β-glucocerebrosidase, Amicus Therapeutics initiated a Phase 1 clinical trial for Type 1 Gaucher disease in 2006. Administration of isofagamine (Plicera) to 54 healthy volunteers was well tolerated and a dose-dependent increase in β-glucocerebrosidase activities was observed in peripheral leukocytes. However, an uncontrolled Phase 2 study in 19 treatment-naïve adult Gaucher type I disease patients failed to demonstrate clinically meaningful improvements in key measures of the disease. Two dosing regimens were studied over a 6-month period. All patients enrolled experienced an increase in the level of β-

glucocerebrosidase activity in their leukocytes, whereas clinical improvements were noted in just one of the 18 patients who completed the study. In 2009, Amicus Therapeutics announced that it did not plan to advance Plicera into Phase 3 development.

### 7.5.7. Cotherapy: PCT and ERT

Another use of chaperones is cotherapy with ERT. In patients who have nonamenable mutations, the appropriate treatment is to replace the missing enzyme with the recombinant enzyme. In such patients, cotherapy with the chaperone can potentially enhance the effectiveness of ERT. Coadministration of migalastat and recombinant $\alpha$-Gal A in Fabry fibroblasts resulted in greater intracellular enzyme activity and lower substrate levels than the recombinant enzyme alone, indicating that the chaperone stabilized the recombinant enzyme for enhanced cell uptake [132]. When migalastat was coadministered with recombinant $\alpha$-Gal A to Fabry mice, the enzyme activities in the organs were markedly increased, and the accumulated substrate was reduced more than administration of the enzyme alone [133,134]. A Phase 2a clinical trial of cotherapy in male Fabry patients demonstrated increased activities in the plasma, skin, and blood mononuclear cells compared to ERT alone [135]. Thus, cotherapy with migalastat could be used to enhance the effectiveness of ERT in nonamenable patients as well as in patients with amenable mutations. In the future, cotherapy with a chaperone may be used in other lysosomal diseases in which ERT or SRT have proven effective.

## 7.6. Emerging Therapies: Gene Therapy and Genome Editing

The past 35 years have seen remarkable advances in the treatment of lysosomal diseases including the development, evaluation, and approval of BMT, ERT, SRT, and PCT. These treatments have led to greater awareness of these disorders and significant clinical benefits

for patients. ERT is available for 10 disorders (Gaucher, Fabry, and Pompe diseases; MPS I, II, IVA, VI, and VII; lysosomal acid lipase deficiency; and most recently CLN2), and various treatments are under development for other lysosomal disorders. Experimental and clinical studies with other approaches, including gene and stem cell therapies, are underway. However, despite these remarkable advances, significant hurdles still remain for the treatment of lysosomal diseases with neurodegenerative manifestations. As noted earlier in the text, ERT for CLN2 was recently approved, and efforts have been undertaken to use hematopoietic stem cells as carriers of viral vectors that cross the blood–brain barrier and express the needed recombinant enzyme in neural tissues [e.g., 136, 137].

Recent advances in gene therapy using viral vectors and gene editing techniques have led to the future possibility of "cures" for lysosomal diseases [138]. There are several gene therapy strategies underway: (1) use of HSCT to deliver viral vectors expressing normal enzymes to the viscera and specially to the brain, (2) direct administration of viral vectors, typically adeno-associated viral (AAV) vectors with tissue-targeted serotypes, to deliver high-expressing cDNAs encoding the normal enzyme or protein to disease-specific sites of pathology, including the brain, and (3) use of genome editing as a means to either correct a mutant gene in the patients' hematopoietic stem cells or induced pluripotent stem cells, or to deliver the normal cDNA to an organ such as the liver where it would be therapeutically "edited," for example in intron 1 of the albumin gene, so it would produce and secrete gram quantities of the normal enzyme into the circulation for mannose-6-phosphate tissue uptake into the lysosomal apparatus for substrate clearance. These experimental approaches are under development and preclinical evaluation in animal models and in clinical trials.

## 7.6.1. HSCT for ExVivo Gene Therapy

Despite some of the aforementioned uncertainties (see Section 7.1), the rationale behind HSCT for the treatment of certain lysosomal disorders is strong and has provided a basis for developing "ex vivo"

gene therapy approaches. In the simplest such approach, HSCs are isolated from the patients themselves and transduced with a viral vector to express normal or even higher than normal levels of enzyme, followed by an "autologous" transplantation. There are several advantages of this approach compared to HSCT itself, including the use of autologous cells, which allows for the use of less invasive preconditioning regimes and more successful engraftment, and the fact that the vectors often result in higher than normal levels of expression, releasing more enzyme for cross-correction. Currently the most commonly used vectors for this approach are based on the lentivirus, and there are several clinical trials underway, including Fabry disease [139] and metachromatic leukodystrophy [140]. Whether high-level enzyme expression and engraftment can be sustained with this approach for the life of the patient remains a question, but primate studies that, in some cases, have been carried out for several years, support this potential.

## 7.6.2. In Vivo Gene Therapy

An emerging technology platform for treating lysosomal diseases is gene therapy. This approach seeks to genetically modify a subset of the patient's cells in situ to produce and secrete the normal enzyme into the systemic circulation to correct the enzyme defect. As the enzyme is expressed continuously, the higher bioavailability might facilitate improved efficacy, particularly for those organs that are not well served by periodic infusions of the exogenous normal enzyme. This modification can be realized using a number of gene transfer systems of viral and nonviral origin. The most advanced of these delivery systems is that based on AAV vectors. Over the past

20 years, there have been many gene therapy experiments in animal models of lysosomal diseases [e.g., 136, 141–143], but little translation to human trials due to issues of sufficient delivery of the vectors to sites of pathology and adequate expression of active enzyme. Recently, however, gene therapy to certain easily delivered sites, such as direct injection in the eye, has proven effective [e.g., 138, 144, 145].

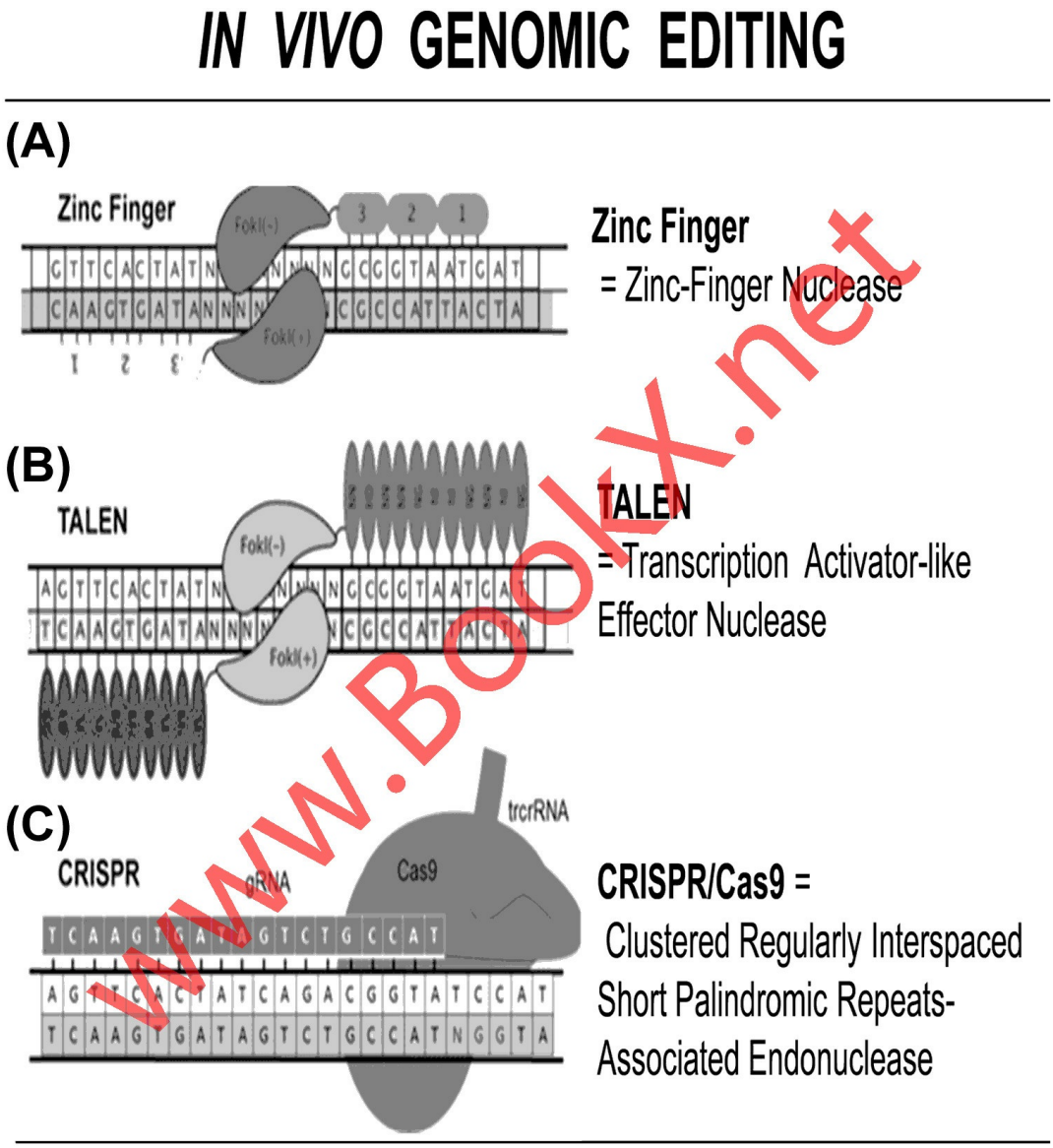
Various companies have initiated gene therapy trials in lysosomal disorders, but to date, no trials are in Phase 3, and there are no approved gene therapies for any lysosomal disease.

## 7.6.3. Gene Editing and Genomic Therapy

Genome editing is a molecular engineering technique in which the genome sequences are directly changed by adding, replacing, or removing DNA bases using nucleases or "molecular scissors." These nucleases create site-specific double-stranded breaks at desired locations in the genome, which can be repaired to result in the targeted changes. For genome editing to be specific, the nuclease must be directed to the desired location where a DNA break is to be introduced. To do so, various types of nucleases have been developed to specifically cut a DNA sequence. All nucleases consist of two components: (1) the nuclease itself that is responsible for DNA cleavage and (2) a secondary component that is responsible for recognizing a specific DNA sequence, DNA protein binding elements, or a complementary RNA; for reviews see [144,145]. There are three major nuclease techniques (Fig. 7.2):

(1) Zinc finger nucleases (ZFNs): ZFNs consist of a nuclease component (FOK1) linked to a DNA-binding component derived from an array of zinc finger proteins. Each finger protein can bind three nucleotides, so combinations of zinc fingers linked together can be designed to recognize specific genomic sequences [e.g., 146]. (2) Transcription activator-like effector nucleases (TALENs): TALENs are very similar to ZFNs in that they also have a (FOK1) nuclease domain linked to a DNA recognition domain. In TALENs, the DNA recognition domain is a series of amino acid repeats. Each repeat corresponds to a single nucleotide base, and TALENs can be designed to have different combinations of repeats to recognize specific genomic sequences [e.g., 147]. (3) Clustered regularly interspaced short palindromic repeats-CRISPR associated endonuclease system (CRISPR/Cas9): CRISPR is a naturally occurring bacterial defense system, which was developed to manipulate mammalian cellular DNA in 2012. The major advantages and disadvantages of CRISPR

include the following: (1) the advantages are it is relatively easy to perform, is a two-component system, efficiently edits different human cells, and can multiplex gene editing targets in a single application; (2) and the disadvantages are that there are a relatively few protospacer adjacent motif sequences, off-targeting issues [148], and antibodies to Cas9. The CRISPR/Cas9 system employs a nuclease called Cas9 to introduce a DNA double-strand break. Unlike ZFNs or TALENs, this approach does not use a protein-based DNA recognition domain. To guide the Cas9 nuclease to a specific DNA binding site, an RNA sequence is designed to precisely bind to a complimentary DNA sequence, allowing for the Cas9 nuclease to make a cut [e.g., 149]. However, recent reports indicated that this technique can cause large deletions and rearrangements near the target site [148].




http://www.addgene.org/CRISPR/guide/

**FIGURE 7.2** The three major genome-editing techniques. For a more detailed explanation, see www.addgene.org/CRISPR/guide.

Genomic editing with the CRISPR/Cas9 system was successfully used to correct a disease in a murine model of tyrosinemia Type 1 [150]. Subsequently, site-specific endonucleases, such as ZFNs and the CRISPR/Cas9 system, in combination with delivery vectors engineered to target disease tissues, have enabled correction of mutations in disease models of hemophilia, hereditary tyrosinemia

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type 1, orthenine transcarbamylase deficiency, and lysosomal storage disorders [151].

A novel approach has been to use gene editing to insert the normal lysosomal enzyme-encoding cDNA in intron 1 of the murine or human albumin gene. Albumin is only produced in the liver ~100 g/week in a 70 kg person, so it is safe to co-opt a small percentage (1%–2%) of the hepatic albumin alleles to produce and secrete a therapeutic lysosomal enzyme into the circulation for mannose-6-phosphate-mediated uptake into the endosomal/lysosomal apparatus to clear the accumulated lysosomal substrate. This approach can also be used to replace circulating proteins such as coagulation factors for hemophilia, etc. [152].

This approach has recently been used in knockout mice with MPS II and Fabry disease [e.g., 153]. The zinc fingers and enzyme cDNAs were delivered to the hepatocytes using AAV2/8 vectors. In each of these murine models, there was high expression of the relevant enzyme in the circulation (~200–250 times normal) and clearance of the accumulated substrate in the organs of the treated mice. Currently, the first clinical trial of this gene editing approach has been undertaken in adult patients with MPS II.

In summary, the future for the treatment of lysosomal diseases appears bright. There will be continued applications of HSCT, ERT, SRT, and PCT strategies to additional lysosomal diseases. Gene therapy and gene editing of pluripotent stem cells from the patient, to delivery of genes to the albumin or similar high expression gene for secretion into the circulation, will be undertaken in the future to provide a continuous production, secretion, and delivery of the enzyme or therapeutic protein to sites of pathology. It is even likely that mutation-specific gene therapies will be developed for diseases with common mutations like Type 1 Gaucher disease. A huge advantage of gene therapy approaches is the likely possibility that they will be one-time, life-long treatments. Clearly, our therapeutic armamentarium for lysosomal and other diseases is undergoing another therapeutic revolution, which will likely lead to safe,

convenient, and more effective, life-long treatments.

## References


[1] Desnick R.J, Thorpe S.R, Fiddler M.B. Toward enzyme therapy for lysosomal storage diseases. *Physiol Rev*. 1976;56:57–99.

[2] Desnick R.J, Bernlohr R, Krivit W. Enzyme therapy in genetic diseases. Preface. *Birth Defects Original Article Series*. 1973;9 [viii-x.].

[3] Desnick R.J. *Enzyme therapy in genetic diseases*. 1980 New York.

[4] Desnick R.J, Schuchman E.H. Enzyme replacement and enhancement therapies: lessons from lysosomal disorders. *Nat Rev Genet*. 2002;3:954–966.

[5] Desnick R.J. Enzyme replacement and enhancement therapies for lysosomal diseases. *J Inherit Metab Dis*. 2004;27:385–410.

[6] Ellinwood N.M, Vite C.H, Haskins M.E. Gene therapy for lysosomal storage diseases: the lessons and promise of animal models. *J Gene Med*. 2004;6:481–506.

[7] Eto Y, Shen J.S, Meng X.L, Ohashi T. Treatment of lysosomal storage disorders: cell therapy and gene therapy. *J Inherit Metab Dis*. 2004;27:411–415.

[8] De duve C. From cytases to lysosomes. *Fed Proc*. 1964;23:1045–1049.

[9] Cantz M, Kresse H. Sandhoff disease: defective glycosaminoglycan catabolism in cultured fibroblasts and its correction by beta-N-acetylhexosaminidase. *Eur J Biochem*. 1974;47:581–590.

[10] Porter M.T, Fluharty A.L, Kihara H. Correction of abnormal cerebroside sulfate metabolism in cultured metachromatic leukodystrophy fibroblasts. *Science*. 1971;172:1263–1265.

[11] O'Brien J.S, Miller A.L, Loverde A.W, Veath M.L. San disease type B: enzyme replacement and metabolic correction in cultured fibroblasts. *Science*. 1973;181:753–755.

[12] Gary-Bobo M, Nirde P, Jeanjean A, Morere A, Garcia M. Ma 6-phosphate receptor targeting and its applications in human diseases. *Curr Med Chem*. 2007;14:2945–2953.

[13] Braulke T, Bonifacino J.S. Sorting of lysosomal proteins. *Biochim Biophys Acta*. 2009;1793:605–614.

[14] Barton N.W, Brady R.O, Dambrosia J.M, Di Bisceglie A.M, Doppelt S.H, Hill S.C, Mankin H.J, Mu al. Replacement therapy for inherited enzyme deficiency–macrophage-targeted glucocerebrosidase for Gaucher's disease. *N Engl J Med*. 1991;324:1464–1470.

[15] Brady R.O, Barton N.W. Enzyme replacement therapy for type I Gaucher disease. In: Desnick R.J, ed. *Treatment of genetic diseases*. New York: McGraw-Hill; 1991:153.

[16] Grabowski G.A, Leslie N, Wenstrup R. Enzyme therapy for Gaucher disease: the first 5 years. *Blood Rev*. 1998;12:115–133.

[17] Weinreb N.J, Charrow J, Andersson H.C, Kaplan P, K of enzyme replacement therapy in 1028 patients with

type 1 Gaucher disease after 2 to 5 years of treatment: a report from the Gaucher Registry. *Am J Med*. 2002;113:112–119.

[18] Brumshtein B, Salinas P, Peterson B, Chan V, Silman I of gene-activated human acid-beta-glucosidase: crystal structure, glycan composition, and internalization into macrophages. *Glycobiology*. 2010;20:24–32.

[19] Aviezer D, Brill-Almon E, Shaaltiel Y, Hashmueli S, Bartfeld D, Mizrac plant-derived recombinant human glucocerebrosidase enzyme–a preclinical and phase I investigation. *PLoS One*. 2009;4:e4792.

[20] Grabowski G.A, Golembo M, Shaaltiel Y. Taligluceras alfa: an enzyme replacement therapy using plant cell expression technology. *Mol Genet Metab*. 2014;112:1–8.

[21] Xu Y.H, Sun Y, Barnes S, Grabowski G.A. Comparativ therapeutic effects of velaglucerase alfa and imiglucerase in a Gaucher disease mouse model. *PLoS One*. 2010;5:e10750.

[22] Zimran A, Altarescu G, Philips M, Attias D, Jmoudiak 1/2 and extension study of velaglucerase alfa replacement therapy in adults with type 1 Gaucher disease: 48-month experience. *Blood*. 2010;115:4651–4656.

[23]

Ioannou Y.A, Bishop D.F, Desnick R.J. Overexpressior of human alpha-galactosidase A results in its intracellular aggregation, crystallization in lysosomes, and selective secretion. *J Cell Biol*. 1992;119:1137–1150.

[24] Ioannou Y.A, Zeidner K.M, Gordon R.E, Desnick R.J. I disease: preclinical studies demonstrate the effectiveness of alpha-galactosidase A replacement in enzyme-deficient mice. *Am J Hum Genet*. 2001;68:14–25.

[25] Van den Hout H, Reuser A.J, Vulto A.G, Loonen M.C, Cromme Dijkhuis A, Van der Ploeg A.T. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet*. 2000;356:397–398.

[26] Johnson W.G, Desnick R.J, Long D.M, Sharp H.L, Kriv injection of purified hexosaminidase A into a patient with Tay-Sachs disease. *Birth Defects Orig Artic Ser*. 1973;9:120–124.

[27] Richards S.M, Olson T.A, McPherson J.M. Antibody response in patients with Gaucher disease after repeated infusion with macrophage-targeted glucocerebrosidase. *Blood*. 1993;82:1402–1409. .

[28] Rosenberg M, Kingma W, Fitzpatrick M.A, Richards S of alglucerase enzyme therapy for Gaucher patients: induction of humoral tolerance in seroconverted patients after repeat administration. *Blood*. 1999;93:2081–2088.

[29] Eng C.M, Guffon N, Wilcox W.R, Germain D.P, Lee P, International Collaborative Fabry Disease Study. Safety and efficacy of recombinant human alpha-galactosidase A replacement therapy in Fabry's disease. *N Engl J Med*. 2001;345:9–16.

[30] Schiffmann R, Kopp J.B, Austin 3rd. H.A, Sabnis S, M replacement therapy in Fabry disease: a randomized controlled trial. *J Am Med Assoc*. 2001;285:2743–2749.

[31] Kishnani P.S, Goldenberg P.C, DeArmey S.L, Heller J, reactive immunologic material status affects treatment outcomes in Pompe disease infants. *Mol Genet Metab*. 2010;99:26–33.

[32] Banugaria S.G, Prater S.N, Ng Y.K, Kobori J.A, Finkel impact of antibodies on clinical outcomes in diseases treated with therapeutic protein: lessons learned from infantile Pompe disease. *Genet Med*. 2011;13:729–736.

[33] Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, Dyke J.P, Cahan H, Slaso of intraventricular cerliponase alfa for CLN2 disease. *N Engl J Med*. 2018;378:1898–1907.

[34] Markham A. Cerliponasealfa: first global approval. *Drugs*. 2017;77:1247–1249.

[35] Hollak C.E, van Weely S, van Oers M.H, Aerts J.M. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. *J Clin Invest*. 1994;93:1288–1292.

[36] Aerts J.M, Groener J.E, Kuiper S, Donker-Koopman W.E, Strijland A, Ottenhoff R, van Roomen C, Mirzaian M, Wijburg F.A, Linthorst G.E, V Brinkman J, Somerharju P, Boot R.G, Hollak C.E, Brad globotriaosylsphingosine is a hallmark of Fabry disease. *Proc Natl Acad Sci U S A*. 2008;105:2812–2817.

[37] Nowak A, Mechtler T, Kasper D.C, Desnick R.J. Corre of Lyso-Gb3 levels in dried blood spots and sera from patients with classic and Later-Onset Fabry disease. *Mol Genet Metab*. 2017;121:320–324.

[38] Hobbs J.R, Hugh-Jones K, Barrett A.J, Byrom N, Chambers D, Henry K, of clinical features of Hurler's disease and biochemical improvement after treatment by bone-marrow transplantation. *Lancet*. 1981;2:709–712.

[39] Fratantoni J.C, Hall C.W, Neufeld E.F. Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. *Science*. 1968;162:570–572.

[40] Neufeld E.F, Cantz M.J. Corrective factors for inborn errors of mucopolysaccharide metabolism. *Ann N Y Acad Sci*. 1971;179:580–587.

[41] Krivit W, Sung J.H, Shapiro E.G, Lockman L.A. Micro the effector cell for reconstitution of the central nervous system following bone marrow transplantation for lysosomal and peroxisomal storage diseases. *Cell Transplant*. 1995;4:385–392.

[42] de Ru M.H, Boelens J.J, Das A.M, Jones S.A, van der Lee J.H, Mahlaoui N, Mengel E, Offringa M, O'Meara replacement therapy and/or hematopoietic stem cell

transplantation at diagnosis in patients with mucopolysaccharidosis type I: results of a European consensus procedure. *Orphanet J Rare Dis*. 2011;6:55.

[43] Eisengart J.B, Rudser K.D, Xue Y, Orchard P, Miller W der Ploeg A, Mercer J, Jones S, Mengel K.E, Gokce S, Guff Souza C.F.M, Shapiro E.G, Whitley C.B. Long-term outcomes of systemic therapies for Hurler syndrome: an international multicenter comparison. *Genet Med*. 2018.

[44] Ceccarini M.R, Codini M, Conte C, Patria F, Cataldi S, mannosidosis: therapeutic strategies. *Int J Mol Sci*. 2018;19.

[45] Selvanathan A, Ellaway C, Wilson C, Owens P, Shaw of early hematopoietic stem cell transplantation in preventing neurocognitive decline in mucopolysaccharidosis type II: acase series. *JIMD Rep*. 2018.

[46] Turbeville S, Nicely H, Rizzo J.D, Pedersen T.L, Orcha Seraihy A. Clinical outcomes following hematopoietic stem cell transplantation for the treatment of mucopolysaccharidosis VI. *Mol Genet Metab*. 2011;102:111–115.

[47] Ehlert K, Levade T, Di Rocco M, Lanino E, Albert M.H, Fuhrer M, Jarisch A, hematopoietic cell transplantation in Farber disease. *J Inherit Metab Dis*. 2018.

[48] Cox T, Lachmann R, Hollak C, Aerts J, van Weely S, Hrebicek M, Platt F, Butters T, Dwek R, Moy oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet*. 2000;355:1481–1485. .

[49] Platt F.M, Jeyakumar M. Substrate reduction therapy. *Acta Paediatr*. 2008;97:88–93.

[50] Platt F.M, Neises G.R, Dwek R.A, Butters T.D. N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. *J Biol Chem*. 1994;269:8362–8365.

[51] Abe A, Radin N.S, Shayman J.A, Wotring L.L, Zipkin and stereochemical studies of potent inhibitors of glucosylceramide synthase and tumor cell growth. *J Lipid Res*. 1995;36:611–621.

[52] Platt F.M, Neises G.R, Reinkensmeier G, Townsend M of lysosomal storage in Tay-Sachs mice treated with N-butyldeoxynojirimycin. *Science*. 1997;276:428–431.

[53] Pastores G.M, Barnett N.L. Substrate reduction therapy: miglustat as a remedy for symptomatic patients with Gaucher disease type 1. *Expert Opin Investig Drugs*. 2003;12:273–281.

[54] Marshall J, Ashe K.M, Bangari D, McEachern K, Chua reduction augments the efficacy of enzyme therapy in a mouse model of Fabry disease. *PLoS One*. 2010;5:e15033.

[55] Marshall J, McEachern K.A, Chuang W.L, Hutto E, Sie

management of lysosomal glucosylceramide levels in a mouse model of type 1 Gaucher disease using enzyme and substrate reduction therapy. *J Inherit Metab Dis*. 2010;33:281–289.

[56] Elstein D, Dweck A, Attias D, Hadas-Halpern I, Zevin S, Altarescu G, Aerts J.F, van Weely S, Zimran A. Oral maintenance clinical trial with miglustat for type I Gaucher disease: switch from or combination with intravenous enzyme replacement. *Blood*. 2007;110:2296–2301.

[57] McEachern K.A, Fung J, Komarnitsky S, Siegel C.S, Cl specific and potent inhibitor of glucosylceramide synthase for substrate inhibition therapy of Gaucher disease. *Mol Genet Metab*. 2007;91:259–267.

[58] Jeyakumar M, Butters T.D, Cortina-Borja M, Hunnam V, Proia R.L, Perry V.H, Dwek R.A, symptom onset and increased life expectancy in Sandhoff disease mice treated with N-butyldeoxynojirimycin. *Proc Natl Acad Sci U S A*. 1999;96:6388–6393.

[59] Heare T, Alp N.J, Priestman D.A, Kulkarni A.B, Qasba endothelial dysfunction in the aorta of a mouse model of Fabry disease; partial prevention by N-butyldeoxynojirimycin treatment. *J Inherit Metab Dis*. 2007;30:79–87.

[60] Elstein D, Hollak C, Aerts J.M, van Weely S, Maas M, Cox T.M, Lachmann R.H, Hrebicek therapeutic effects of oral miglustat (Zavesca, N-butyldeoxynojirimycin, OGT 918) in type I Gaucher

disease. *J Inherit Metab Dis*. 2004;27:757–766.
[61] Pastores G.M, Barnett N.L, Kolodny E.H. An open-label, noncomparative study of miglustat in type I Gaucher disease: efficacy and tolerability over 24 months of treatment. *Clin Therapeut*. 2005;27:1215–1227.
[62] Peterschmitt M.J, Burke A, Blankstein L, Smith S.E, Pu tolerability, and pharmacokinetics of eliglustat tartrate (Genz-112638) after single doses, multiple doses, and food in healthy volunteers. *J Clin Pharmacol*. 2011;51:695–705.
[63] Lukina E, Watman N, Arreguin E.A, Banikazemi M, D phase 2 study of eliglustat tartrate (Genz-112638), an oral substrate reduction therapy for Gaucher disease type 1. *Blood*. 2010;116:893–899.
[64] Lukina E, Watman N, Arreguin E.A, Dragosky M, Ias in hematological, visceral, and skeletal manifestations of Gaucher disease type 1 with oral eliglustat tartrate (Genz-112638) treatment: 2-year results of a phase 2 study. *Blood*. 2010;116:4095–4098.
[65] Mistry P.K, Lukina E, Ben Turkia H, Amato D, Baris H, Dasouki M, Ghosn M, M of oral eliglustat on splenomegaly in patients with Gaucher disease type 1: the ENGAGE randomized clinical trial. *J Am Med Assoc*. 2015;313:695–706.
[66] Cox T.M, Drelichman G, Cravo R, Balwani M, Burrow compared with imiglucerase in patients with

Gaucher's disease type 1 stabilised on enzyme replacement therapy: a phase 3, randomised, open-label, non-inferiority trial. *Lancet*. 2015;385:2355–2362.

[67] Mistry P.K, Lukina E, Ben Turkia H, Shankar S.P, Baris H, Ghosn M, Mehta A, P after 18 months of eliglustat therapy in treatment-naive adults with Gaucher disease type 1: the phase 3 ENGAGE trial. *Am J Hematol*. 2017;92:1170–1176. .

[68] Abe A, Gregory S, Lee L, Killen P.D, Brady R.O, Kulka of globotriaosylceramide in Fabry disease mice by substrate deprivation. *J Clin Invest*. 2000;105:1563–1571.

[69] Cox T.M, Platt F.M, Aerts J. *Medicinal use of iminosugars. Iminosugars: from synthesis to therapeutic application*. West Sussex: John Wiley & Sons Ltd; 2007:295–326.

[70] Schiffmann R, Fitzgibbon E.J, Harris C, DeVile C, Dav Schaik I.N, Benko W, Timmons M, Ries M, Vellodi A. controlled trial of miglustat in Gaucher's disease type 3. *Ann Neurol*. 2008;64:514–522.

[71] Elliot-Smith E, Speak A.O, Lloyd-Evans E, Smith D.A, van der Spoel A.C, Jeyakumar M, Butters T.D, Dwek R.A, d'A effects of substrate reduction therapy in a mouse model of GM1 gangliosidosis. *Mol Genet Metab*. 2008;94:204–211.

[72] Maegawa G.H, Banwell B.L, Blaser S, Sorge G, Toplak reduction therapy in juvenile GM2

gangliosidosis. *Mol Genet Metab*. 2009;98:215–224.
[73] Bembi B, Marchetti F, Guerci V.I, Ciana G, Addobbati Guillen L, Butters T, Pittis M.G. Substrate reduction therapy in the infantile form of Tay-Sachs disease. *Neurology*. 2006;66:278–280.
[74] Wraith J.E, Vecchio D, Jacklin E, Abel L, Chadha-Boreham H, Luzy C, Giorgino R, Patterson M.C. Migl in adult and juvenile patients with Niemann-Pick Disease type C: long-term data from a clinical trial. *Mol Genet Metab*. 2010;99:351–357.
[75] Patterson M.C, Vecchio D, Jacklin E, Abel L, Chadha-Boreham H, Luzy C, Giorgino R, Wraith J.E. Long-term miglustat therapy in children with Niemann-Pick Disease type C. *J Child Neurol*. 2010;25:300–305.
[76] Zervas M, Somers K.L, Thrall M.A, Walkley S.U. Criti role for glycosphingolipids in Niemann-Pick Disease type C. *Curr Biol*. 2001;11:1283–1287.
[77] Patterson M.C, Vecchio D, Prady H, Abel L, Wraith J.l for treatment of Niemann-Pick C disease: a randomised controlled study. *Lancet Neurol*. 2007;6:765–772.
[78] Pineda M, Perez-Poyato M.S, O'Callaghan M, Vilaseca M.A, Pocovi M, la Barca O.B, Silva M.T, Macias-Vidal J, Coll M.J. Clinical experience with miglustat therapy in pediatric patients with Niemann-Pick Disease type C: a case series. *Mol Genet Metab*. 2010;99:358–366.

[79] Patterson M.C, Vanier M.T, Suzuki K, Morris J.A, Car Mackie J.E, Pentchev P.G. Niemann-Pick Disease type C: alipid trafficking disorder. In: Beaudet A.L, Vogelstein B, Kinzler K.W, *online metabolic and molecular bases of inherited disease*. New York, NY: The McGraw-Hill Companies, Inc.; 2014.

[80] Burlina A. Niemann-Pick Disease type C: introduction and main clinical features. *J Neurol*. 2014;261(Suppl 2):S525–S527.

[81] Lopez C.A, de Vries A.H, Marrink S.J. Molecular mechanism of cyclodextrin mediated cholesterol extraction. *PLoS Comput Biol*. 2011;7:e1002020.

[82] Szente L, Singhal A, Domokos A, Song B. Cyclodextri assessing the impact of cavity size, occupancy, and substitutions on cytotoxicity and cholesterol homeostasis. *Molecules*. 2018;23.

[83] Li R, Hao J, Fujiwara H, Xu M, Yang S, Dai S, Long Y, characterization of methyl-beta-cyclodextrin for pharmacological activity to reduce lysosomal cholesterol accumulation in Niemann-Pick Disease type C1 cells. *Assay Drug Dev Technol*. 2017;15:154–166.

[84] Tamura A, Yui N. Polyrotaxane-based systemic delivery of beta-cyclodextrins for potentiating therapeutic efficacy in a mouse model of Niemann-Pick type C disease. *J Control Release*. 2018;269:148–158.

[85] Calias P. 2-Hydroxypropyl-beta-cyclodextrins and the blood-brain barrier: considerations for Niemann-Pick Disease type C1. *Curr Pharm Des*. 2017;23:6231–6238.

[86] Crumling M.A, King K.A, Duncan R.K. Cyclodextrins and iatrogenic hearing loss: new drugs with significant risk. *Front Cell Neurosci*. 2017;11:355.

[87] Yergey A.L, Blank P.S, Cologna S.M, Backlund P.S, Pc of hydroxypropyl-beta-cyclodextrins used in the treatment of Niemann-Pick Disease type C1. *PLoS One*. 2017;12:e0175478.

[88] Berry-Kravis E, Chin J, Hoffmann A, Winston A, Stoner R, L term treatment of Niemann-Pick type C1 disease with intrathecal 2-hydroxypropyl-beta-cyclodextrin. *Pediatr Neurol*. 2018;80:24–34.

[89] Perlmutter D.H. Chemical chaperones: a pharmacological strategy for disorders of protein folding and trafficking. *Pediatr Res*. 2002;52:832–836.

[90] Hampton R.Y. ER-associated degradation in protein quality control and cellular regulation. *Curr Opin Cell Biol*. 2002;14:476–482.

[91] Cohen F.E, Kelly J.W. Therapeutic approaches to protein-misfolding diseases. *Nature*. 2003;426:905. .

[92] Ulloa-Aguirre A, Janovick J.A, Brothers S.P, Conn P.M. Pha rescue of conformationally-defective proteins: implications for the treatment of human disease. *Traffic*. 2004;5:821–837.

[93] Meusser B, Hirsch C, Jarosch E, Sommer T. ERAD: the long road to destruction. *Nat Cell Biol*. 2005;7:766–772.
[94] Fan J.Q, Ishii S, Asano N, Suzuki Y. Accelerated transport and maturation of lysosomal alpha-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. *Nat Med*. 1999;5:112–115.
[95] Fan J.Q. A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. *Trends Pharmacol Sci*. 2003;24:355–360.
[96] Alfonso P, Pampin S, Estrada J, Rodriguez-Rey J.C, Giraldo P, Sancho J, Pocovi M. Miglustat (NB-DNJ) works as a chaperone for mutated acid beta-glucosidase in cells transfected with several Gaucher disease mutations. *Blood Cells Mol Dis*. 2005;35:268–276.
[97] Lin H, Sugimoto Y, Ohsaki Y, Ninomiya H, Oka A, Ta octyl-beta-valienamine up-regulates activity of F213I mutant beta-glucosidase in cultured cells: a potential chemical chaperone therapy for Gaucher disease. *Biochim Biophys Acta*. 2004;1689:219–228.
[98] Sawkar A.R, Cheng W.C, Beutler E, Wong C.H, Balch chaperones increase the cellular activity of N370S beta -glucosidase: a therapeutic strategy for Gaucher disease. *Proc Natl Acad Sci U S A*. 2002;99:15428–15433.
[99] Matsuda J, Suzuki O, Oshima A, Yamamoto Y, Noguc chaperone therapy for brain pathology in G(M1)-

gangliosidosis. *Proc Natl Acad Sci U S A*. 2003;100:15912–15917.

[100] Tropak M.B, Reid S.P, Guiral M, Withers S.G, Mahura enhancement of beta-hexosaminidase activity in fibroblasts from adult Tay-Sachs and Sandhoff patients. *J Biol Chem*. 2004;279:13478–13487.

[101] Hartl F.U, Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science*. 2002;295:1852–1858.

[102] Ellgaard L, Molinari M, Helenius A. Setting the standards: quality control in the secretory pathway. *Science*. 1999;286:1882–1888.

[103] Fewell S.W, Travers K.J, Weissman J.S, Brodsky J.L. Tl action of molecular chaperones in the early secretory pathway. *Annu Rev Genet*. 2001;35:149–191.

[104] Oda Y, Hosokawa N, Wada I, Nagata K. EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. *Science*. 2003;299:1394–1397.

[105] Princiotta M.F, Finzi D, Qian S.B, Gibbs J, Schuchman protein synthesis, degradation, and endogenous antigen processing. *Immunity*. 2003;18:343–354.

[106] Schubert U, Anton L.C, Gibbs J, Norbury C.C, Yewdel degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature*. 2000;404:770–774.

[107] Yewdell J.W. Not such a dismal science: the economics of protein synthesis, folding, degradation and antigen processing. *Trends Cell Biol*. 2001;11:294–

297.
[108] Horwich A. Protein aggregation in disease: a role for folding intermediates forming specific multimeric interactions. *J Clin Invest*. 2002;110:1221–1232.
[109] Pedemonte N, Lukacs G.L, Du K, Caci E, Zegarra-Moran O, Galietta L.J, Verkman A.S. Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. *J Clin Invest*. 2005;115:2564–2571.
[110] Sawkar A.R, Adamski-Werner S.L, Cheng W.C, Wong C.H, Beutler E, Zimm disease-associated glucocerebrosidases show mutation-dependent chemical chaperoning profiles. *Chem Biol*. 2005;12:1235–1244.
[111] von Scheidt W, Eng C.M, Fitzmaurice T.F, Erdmann E, Hu atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med*. 1991;324:395–399.
[112] Arends M, Wanner C, Hughes D, Mehta A, Oder D, W of classical and nonclassical Fabry disease: amulticenter study. *J Am Soc Nephrol*. 2017;28:1631–1641.
[113] Stenson P.D, Mort M, Ball E.V, Evans K, Hayden M, H human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet*. 2017;136:665–677.

[114] Wu X, Katz E, Della
Valle M.C, Mascioli K, Flanagan J.J, Castelli J.P, Schiff
pharmacogenetic approach to identify mutant forms of alpha-galactosidase A that respond to a pharmacological chaperone for Fabry disease. *Hum Mutat*. 2011;32:965–977.
[115] Benjamin E.R, Della
Valle M.C, Wu X, Katz E, Pruthi F, Bond S, Bronfin B, validation of pharmacogenetics for the identification of Fabry patients to be treated with migalastat. *Genet Med*. 2017;19:430–438. .
[116]
Asano N, Ishii S, Kizu H, Ikeda K, Yasuda K, Kato A, inhibition and intracellular enhancement of lysosomal alpha-galactosidase A activity in Fabry lymphoblasts by 1-deoxygalactonojirimycin and its derivatives. *Eur J Biochem*. 2000;267:4179–4186.
[117]
Yam G.H, Bosshard N, Zuber C, Steinmann B, Roth J. chaperone corrects lysosomal storage in Fabry disease caused by trafficking-incompetent variants. *Am J Physiol Cell Physiol*. 2006;290:C1076–C1082.
[118] Young-
Gqamana B, Brignol N, Chang H.H, Khanna R, Soska HCl reduces globotriaosylsphingosine (lyso-Gb3) in Fabry transgenic mice and in the plasma of Fabry patients. *PLoS One*. 2013;8:e57631.
[119]
Johnson F.K, Mudd Jr. P.N, Bragat A, Adera M, Boude and safety of migalastat HCl and effects on

agalsidase activity in healthy volunteers. *Clin Pharmacol Drug Dev*. 2013;2:120–132.

[120] Johnson F.K, Mudd Jr. P.N, DiMino T, Vosk J, Sitaram open-label study to determine the pharmacokinetics and safety of migalastat HCl in subjects with impaired renal function and healthy subjects with normal renal function. *Clin Pharmacol Drug Dev*. 2015;4:256–261.

[121] Guce A.I, Clark N.E, Rogich J.J, Garman S.C. The molecular basis of pharmacological chaperoning in human alpha-galactosidase. *Chem Biol*. 2011;18:1521–1526.

[122] Germain D.P, Giugliani R, Hughes D.A, Mehta A, Nic and pharmacodynamic effects of a pharmacological chaperone on alpha-galactosidase A activity and globotriaosylceramide clearance in Fabry disease: report from two phase 2 clinical studies. *Orphanet J Rare Dis*. 2012;7:91.

[123] Giugliani R, Waldek S, Germain D.P, Nicholls K, Bich Phase 2 study of migalastat hydrochloride in females with Fabry disease: selection of population, safety and pharmacodynamic effects. *Mol Genet Metab*. 2013;109:86–92.

[124] Germain D.P, Hughes D.A, Nicholls K, Bichet D.G, Gi Rasmussen U, Nedd K, Sharaf El
Din U, Lourenco C.M, Banikazemi M, Charrow J, Das Alpan O, Longo N, Scott C.R, Torra R, Tuffaha A, Jova

of Fabry's disease with the pharmacologic chaperone migalastat. *N Engl J Med*. 2016;375:545–555.

[125] Hughes D.A, Nicholls K, Shankar S.P, Sunder-Plassmann G, Koeller D, Nedd K, Vockley G, Hamaza Alpan O, Hachulla E, Jovanovic A, Lourenco C.M, Na Rasmussen U. Oral pharmacological chaperone migalastat compared with enzyme replacement therapy in Fabry disease: 18-month results from the randomised phase III ATTRACT study. *J Med Genet*. 2017;54:288–296.

[126] Markham A. Migalastat: first global approval. *Drugs*. 2016;76:1147–1152.

[127] Mauer M, Sokolovskiy A, Barth J.A, Castelli J.P, Willia of podocyte globotriaosylceramide content in adult male patients with Fabry disease with amenable GLA mutations following 6 months of migalastat treatment. *J Med Genet*. 2017;54:781–786.

[128] Schiffmann R, Bichet D.G, Jovanovic A, Hughes D.A, Rasmussen U, Shankar S.P, Barisoni L, Colvin R.B, Jer improves diarrhea in patients with Fabry disease: clinical-biomarker correlations from the phase 3 FACETS trial. *Orphanet J Rare Dis*. 2018;13:68.

[129] Steet R, Chung S, Lee W.S, Pine C.W, Do H, Kornfeld action of the iminosugarisofagomine, a pharmacological chaperone for mutant forms of acid-beta-glucosidase. *Biochem Pharmacol*. 2007;73:1376–1383.

[130]

Sun Y, Ran H, Liou B, Quinn B, Zamzow M, Zhang W in vivo effects in a neuronopathic Gaucher disease mouse. *PLoS One*. 2011;6:e19037.

[131]
Sun Y, Liou B, Xu Y.H, Quinn B, Zhang W, Hamler R, and in vivo effects of isofagomine on acid beta-glucosidase variants and substrate levels in Gaucher disease. *J Biol Chem*. 2012;287:4275–4287.

[132]
Porto C, Pisani A, Rosa M, Acampora E, Avolio V, Tu Marca G, Andria G, Parenti G. Synergy between the pharmacological chaperone 1-deoxygalactonojirimycin and the human recombinant alpha-galactosidase A in cultured fibroblasts from patients with Fabry disease. *J Inherit Metab Dis*. 2012;35:513–520. .

[133]
Benjamin E.R, Khanna R, Schilling A, Flanagan J.J, Pel administration with the pharmacological chaperone AT1001 increases recombinant human alpha-galactosidase A tissue uptake and improves substrate reduction in Fabry mice. *Mol Ther*. 2012;20:717–726.

[134]
Xu S, Lun Y, Brignol N, Hamler R, Schilling A, Frasce of a novel human alpha-galactosidase awith the pharmacological chaperone AT1001 leads to improved substrate reduction in Fabry mice. *Mol Ther*. 2015;23:1169–1181.

[135] Warnock D.G, Bichet D.G, Holida M, Goker-Alpan O, Nicholls K, Thomas M, Eyskens F, Shankar S migalastat HCl leads to greater systemic exposure

and tissue levels of active alpha-galactosidase a in Fabry patients when co-administered with infused agalsidase. *PLoS One*. 2015;10:e0134341.

[136] Hughes M.P, Smith D.A, Morris L, Fletcher C, Colaco intracerebroventricular gene therapy improves lifespan, locomotor function and pathology in a mouse model of Niemann-Pick type C1 disease. *Hum Mol Genet*. 2018.

[137] Markmann S, J J.C.-R, Rosenberg J.B, De B.P, Kaminsky S.M, Crystal R.G, of the Niemann-Pick type C2 disease phenotype by intracisternal administration of an AAVrh.10 vector expressing Npc2. *Exp Neurol*. 2018;306:22–33.

[138] Dunbar C.E, High K.A, Joung J.K, Kohn D.B, Ozawa K therapy comes of age. *Science*. 2018;359.

[139] Huang J, Khan A, Au B.C, Barber D.L, Lopez-Vasquez L, Prokopishyn N.L, Boutin M, Rothe M, Rip Blais C, Foley R, Medin J.A. Lentivector iterations and pre-clinical scale-up/toxicity testing: targeting mobilized CD34(+) cells for correction of Fabry disease. *Mol Ther Methods Clin Dev*. 2017;5:241–258.

[140] Sessa M, Lorioli L, Fumagalli F, Acquati S, Redaelli D, Carro U, Sora M.G, Martino S, Quattrini A, Montini E, Serio C, Ciceri F, Roncarolo M.G, Aiuti A, Naldini L, F haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. *Lancet*. 2016;388:476–487.

[141] Penati R, Fumagalli F, Calbi V, Bernardo M.E, Aiuti A therapy for lysosomal storage disorders: recent advances for metachromatic leukodystrophy and mucopolysaccaridosis I. *J Inherit Metab Dis*. 2017;40:543–554.

[142] Hinderer C, Katz N, Louboutin J.P, Bell P, Yu H, Naya of an adeno-associated virus vector into cerebrospinal fluid attenuates central nervous system disease in mucopolysaccharidosis type II mice. *Hum Gene Ther*. 2016;27:906–915.

[143] Belur L.R, Temme A, Podetz-Pedersen K.M, Riedl M, Vulchanova L, Robinson N, H adeno-associated virus mediated gene delivery and expression of human iduronidase in the central nervous system: a noninvasive and effective approach for prevention of neurologic disease in mucopolysaccharidosis type I. *Hum Gene Ther*. 2017;28:576–587.

[144] Song M, Ramakrishna S. Genome editing in stem cells for disease therapeutics. *Mol Biotechnol*. 2018;60:329–338.

[145] Maeder M.L, Gersbach C.A. Genome-editing technologies for gene and cell therapy. *Mol Ther*. 2016;24:430–446.

[146] Porteus M.H, Carroll D. Gene targeting using zinc finger nucleases. *Nat Biotechnol*. 2005;23:967–973.

[147] Joung J.K, Sander J.D. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol*. 2013;14:49–55.

[148] Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nat Biotechnol*. 2018.

[149] Wang H, La Russa M, Qi L.S. CRISPR/Cas9 in genome editing and beyond. *Annu Rev Biochem*. 2016;85:227–264.

[150] Yin H, Xue W, Chen S, Bogorad R.L, Benedetti E, Gro editing with Cas9 in adult mice corrects a disease mutation and phenotype. *Nat Biotechnol*. 2014;32:551–553.

[151] Schneller J.L, Lee C.M, Bao G, Venditti C.P. Genome editing for inborn errors of metabolism: advancing towards the clinic. *BMC Med*. 2017;15:43. .

[152] Sharma R, Anguela X.M, Doyon Y, Wechsler T, DeKel genome editing of the albumin locus as a platform for protein replacement therapy. *Blood*. 2015;126:1777–1784.

[153] Laoharawee K, DeKelver R.C, Podetz-Pedersen K.M, Rohde M, Sproul S, Nguyen H.O, Ngu Martin S.J, Ou L, Tom S, Radeke R, Meyer K.E, Holme dependent prevention of metabolic and neurologic disease in murine MPS II by ZFN-mediated in vivo genome editing. *Mol Ther*. 2018;26:1127–1136.

**Relevant Websites**

http://www.fda.gov/ohrms/dockets/ac/cder03.html#endocrinologicmet

http://www.amicustherapeutics.com.

http://www.clinicaltrials.gov/ct/show/nct00214500?order=1.

8

# Transplantation Genetics

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## Abstract

In 1912 George Schöne concluded his studies defining the "laws of transplantation" as by staing that (1) autografts generally succeed, (2) allografts usually fail, and (3) xenografts invariably fail. The locus responsible for transplantation outcome became known as the major histocompatibility complex, and its products in humans are known as the human leukocyte antigens (HLAs). These molecules can be considered the molecular embodiment of Schöne's laws of transplantation. "Tissue typing" refers to the genotypic and phenotypic determination of the human leukocyte antigen determinants of both the potential donor(s) and the recipient, because the matching of HLA alleles between the two of them is essential for the successful outcome of many tissue transplantation interventions.

## Keywords

Allotransplantation; Donor/Recipient matching; Donor-specific antibody; Graft-versus-host-diseaseHuman Leukocyte Antigens; Laws of transplantation; Major Histocompatibility Complex; United Network for Organ Sharing

## 8.1. The Major Histocompatibility Complex

The major histocompatibility complex (MHC) in humans encompasses the genes encoding for the molecules so defined historically as human leukocyte antigens (HLAs) since originally they were recognized as the targets (i.e., antigens) of alloreacting antibodies. HLA molecules are grouped into two classes based on their structure, function, and cellular expression [1,2] (Fig. 8.1). Class I (HLA-A, -B, and -C molecules) are characterized by a heavy (43 kDa) $\alpha$-chain with three domains ($\alpha$1, $\alpha$2, and $\alpha$3) encoded by a gene located on chromosome 6 that dimerizes with the nonpolymorphic $\beta_2$-microglobulin, a short (12 kDa) protein that provides the fourth domain necessary to stabilize the HLA molecule even if it is encoded by a gene present on chromosome 15 [3]. Class I molecules are coexpressed on nearly all nucleated cells and platelets and are anchored on them via the transmembrane portion and the short cytoplasmic tail of the $\alpha$-chain only. Class II molecules (HLA-DR, -DQ, and -DP) consist of $\alpha$- (34 kDa) and $\beta$- (29 kDa) chains, each with two domains both encoded in humans on chromosome 6. Class II molecules are anchored on the cell surface by both chains, each of which can communicate across the membrane with cytoplasmic structures. These molecules are expressed primarily on antigen presenting cells (APCs), such as B lymphocytes and dendritic cells, or activated T cells and endothelial cells.

The two outermost domains of each molecule ($\alpha_1$ and $\alpha_2$ in class I; $\alpha_1$ and $\beta_1$ in class II) fold together to form the cleft in which antigenic peptides can find appropriate lodging (Fig. 8.2). The antigen-combining site (also known as the Bjorkman "groove," from the scientist who interpreted the HLA molecule structure via crystallography [4]) is composed of a $\beta$-pleated sheet with eight antiparallel strands forming the floor of the groove and sides assuming the shape of $\alpha$-helices. The amino acid residues forming this

cleft are very polymorphic and are particularly immunologically relevant in the $\alpha$-helical regions. As the protein folds into the tertiary structure, some of the hypervariable regions assume a location pointing into the cleft in an optimal position for interaction with the processed antigen. Other hypervariable segments are instead located on the external part of the molecule and constitute the determinants potentially involved with T-cell receptor (TCR) recognition. These same hypervariable regions form the molecular basis of alloreactivity in tissue transplantation (i.e., variable donor amino acids can be recognized as "foreign," that is, different, by the recipient who immunologically reacts against them). These antigenically relevant differences constitute the human leukocyte antigens originally recognized by Dausset and Payne (see "Historical *Iter* towards Histocompatibility Definition").

The HLA class I and class II molecules constitute the most highly polymorphic genetic system in humans. A stable, inherited polymorphism gives rise to alternative forms of the protein, the *alleles*. Nearly all the HLA molecules have various alleles, with *HLA-B* being the most polymorphic, having on average 86 single nucleotide polymorphisms (SNPs)/kb [5] and more than 1800 reported alleles, with the numbers consistently rising [6]. The molecular basis for the HLA polymorphism resides in nucleotide sequence differences present in the coding regions of the *HLA* genes. The polymorphism is clustered into three or four discrete hypervariable regions, which become more evident when the amino acid sequences of these alleles are aligned. These nucleic acid sequence differences define the antigenic *HLA* alleles, which occur in all ethnic groups and generally vary in frequency from group to group [7,8].

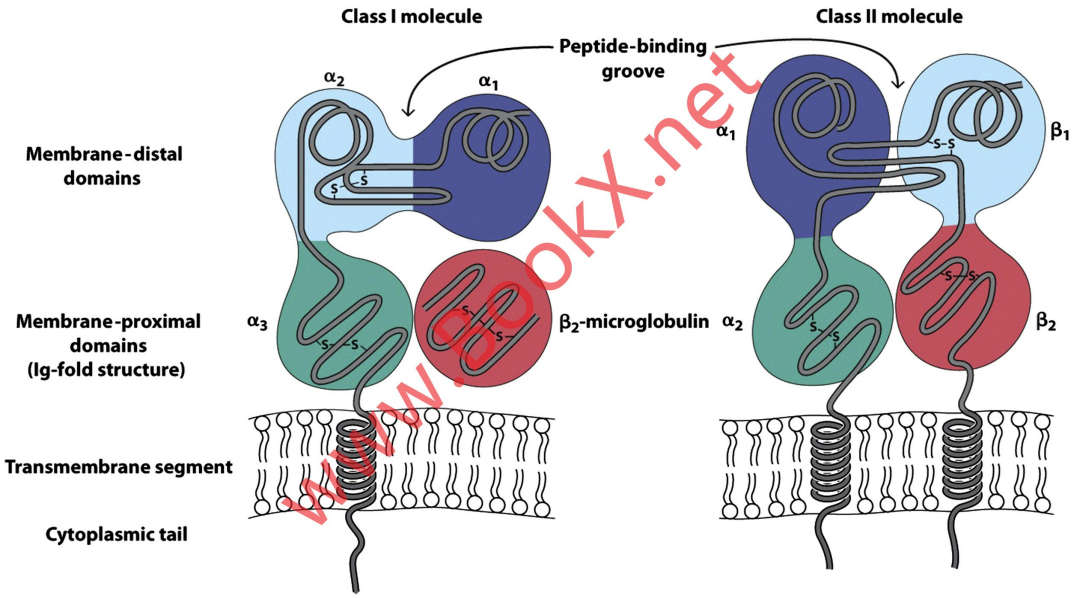
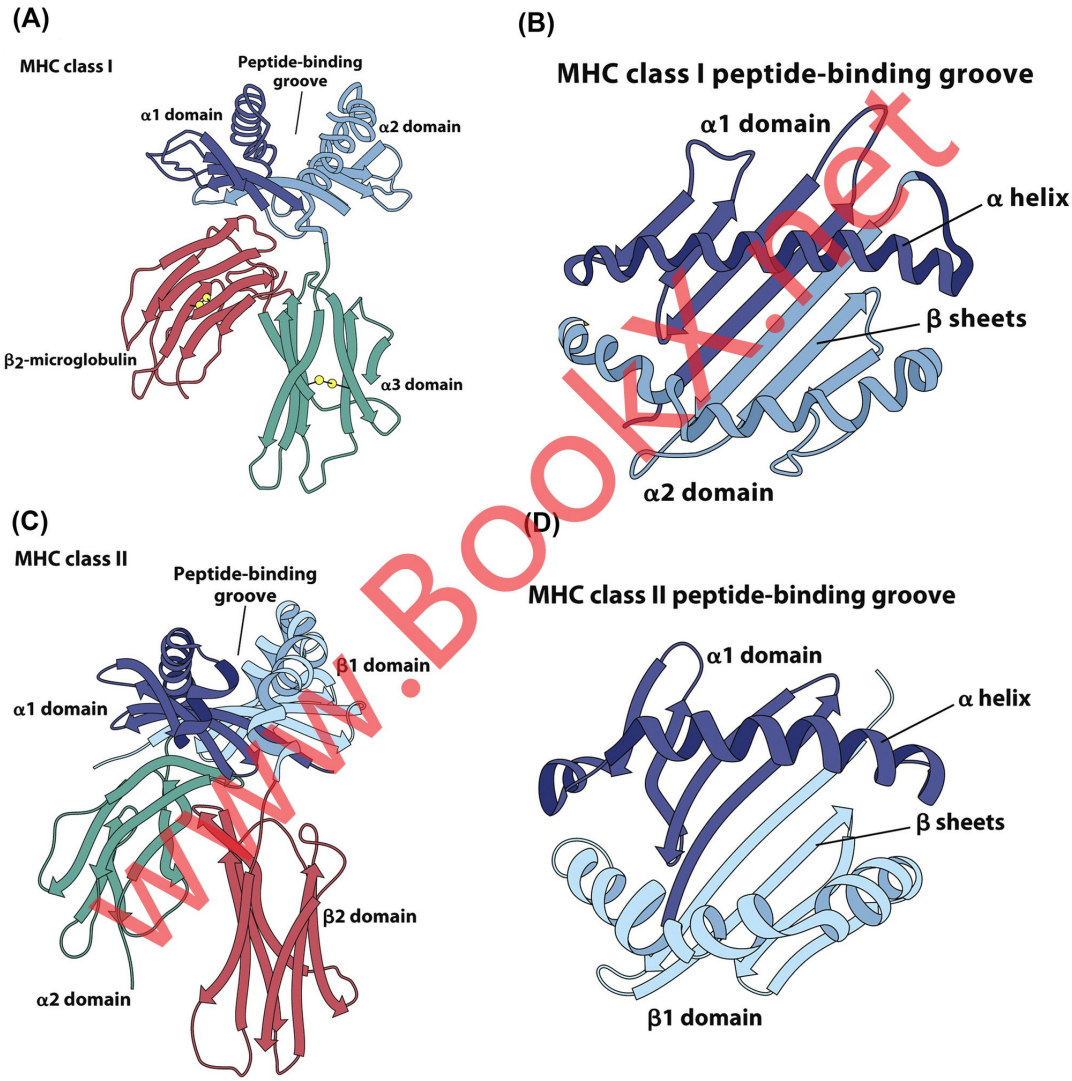



**FIGURE 8.1** Schematic diagrams of class I and class II MHC molecules showing the external domains, transmembrane segments, and cytoplasmic tails. The peptide-binding groove is formed by the membrane-distal domains in both class I and class II molecules (blue). The membrane-proximal domains (green and red) possess the basic immunoglobulin-fold structure; thus, both class I and class II MHC molecules are classified as members of the immunoglobulin superfamily.
From: The major histocompatibility complex and antigen presentation, Chapter 8. In: Owen, Punt, Stranford, editors. Kuby immunology. 7th ed. New York: W.F. Freeman and Company; 2013. pp. 261–96.

As previously stated, the genes encoding HLA class I and class II molecules are located in a greater than 4000-kilobase (kb)-long DNA segment present on the short arm of chromosome 6 [5,9,10]. This DNA segment can be subdivided into three major regions that encode: class I molecules (2000 kb), class III molecules (1000 kb), and class II molecules (1000 kb). The organization of the genes included in the HLA complex is shown in Fig. 8.3. The class I and class II regions are physically separated on the chromosome by genes encoding MHC class III molecules. Class III genes encode components of the complement cascade, *C2*, *Bf*, *C4A*, *C4B*, the functional steroid hydroxylase gene *CYP21*, a CYP21 pseudogene, and the genes for tumor necrosis factors (*TNF*α and *TNF*β) that are not relevant in the

context of histocompatibility. The physical location, the *locus*, of each HLA gene determines its order on the chromosome, while the nomenclature instead recapitulates the chronologic characterization of the gene products at the various loci.

Each individual has one maternal and one paternal chromosome 6 and consequently one maternal and one paternal allele at any *HLA* locus. If the maternal allele and the paternal allele differ, as they often do, the person is said to be *heterozygous* at that *HLA* locus. If the inherited maternal and paternal alleles happen to be the same, the person is *homozygous* at that locus. Homozygosity is rarely found and is frequently generated by descent in couples in which the two partners are somewhat related. As the maternal and paternal alleles are expressed codominantly, each cell of an individual will express two sets of each HLA molecule (e.g., two HLA-A molecules, two HLA-B molecules, etc.). The combined expression of both sets of molecules constitutes the HLA *phenotype* of the cells of an individual. The *genotype* of an individual is instead constituted by two (i.e., one paternal and one maternal) sets of arrays of closely linked genes, many with multiple alleles, normally inherited as a unit.




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**FIGURE 8.2** Representations of the three-dimensional structure of the external domains of human MHC class I and class II molecules based on X-ray crystallographic analysis. (A and C) Side views of class I and II, respectively, in which the β-strands are depicted as thick arrows and the α-helices as spiral ribbons. Disulfide bonds are shown as two interconnected spheres. The α1 and α2 domains of class I and the α1 and β1 domains of class II interact to form the peptide-binding groove. Note the immunoglobulin-fold structure of each membrane proximal domain, including the $\beta_2$-microglobulin molecule. (B and D) The α1 (dark blue) and α2 domains (light blue) of class I and the β1 (dark blue) and α1 (light blue) domains of class II as viewed from the top, showing the peptide-binding grooves, consisting of a base of antiparallel β-strands and sides of α-helices for each molecule. This groove can accommodate peptides of 8–10 residues for class I and 13–18 residues for class II.

From: The major histocompatibility complex and antigen presentation, Chapter 8. In: Owen, Punt, Stranford, editors. Kuby immunology. 7th

In 1967, Ceppellini coined the term *haplotype* (*haplous* in Greek for "single") to define each genetic unit inherited from one or the other parent [11]. As originally defined, an HLA haplotype includes the class I, III, and II genes present on one chromosome 6 of an individual. The *HLA* genotype of an individual is composed of one paternal and one maternal HLA haplotype. Each HLA haplotype is transmitted from the parent to the child in mendelian fashion so that each child shares one haplotype with each parent (Fig. 8.4). Within a family, one can find siblings that share two haplotypes (i.e., they are HLA identical), one haplotype (i.e., they are HLA haploidentical), or no haplotypes (i.e., they are HLA different) with the probability of 25%, 50%, and 25%, respectively. Haplotype recombination may occur during parental gametogenesis by crossing over during meiosis. However, due to the relative proximity of the HLA genes, recombination events between loci within a region, or even between regions, though possible, are quite rare. In addition, the selective pressure seems to maintain the genes of each established HLA haplotype together, that is, in *linkage disequilibrium* (LD) [12,13]. With LD, we define the nonrandom association of the alleles at loci physically present in one haplotype. For example, among Europeans, the most frequent HLA-A and HLA-B haplotype, if we disregard all other loci in the complex, encodes HLA-A*101; HLA-B*801 molecules. This combination occurs in about 9.5% of the population. However, the allele frequencies are 0.172 and 0.125, respectively. If the population were in Hardy–Weinberg equilibrium, that is, noninfluenced by external forces and then in a situation occurring when the genes considered are independently segregating through successive generations, the HLA-A1; B8 haplotype frequency would be $0.172 \times 0.125 = 0.022$, or about one-fourth the observed frequency. In its oldest and simplest measure, LD can be determined by calculating the difference between the observed haplotype frequency and the expected frequency. At equilibrium, $D = 0$.

Whether D is significantly different from 0 is assessed using Fisher's exact test. If it is significant, then that LD is said to exist. As a logical consequence, an HLA haplotype composed in part of the paternal and in part by the maternal genes (i.e., a recombinant haplotype) is only rarely observed.

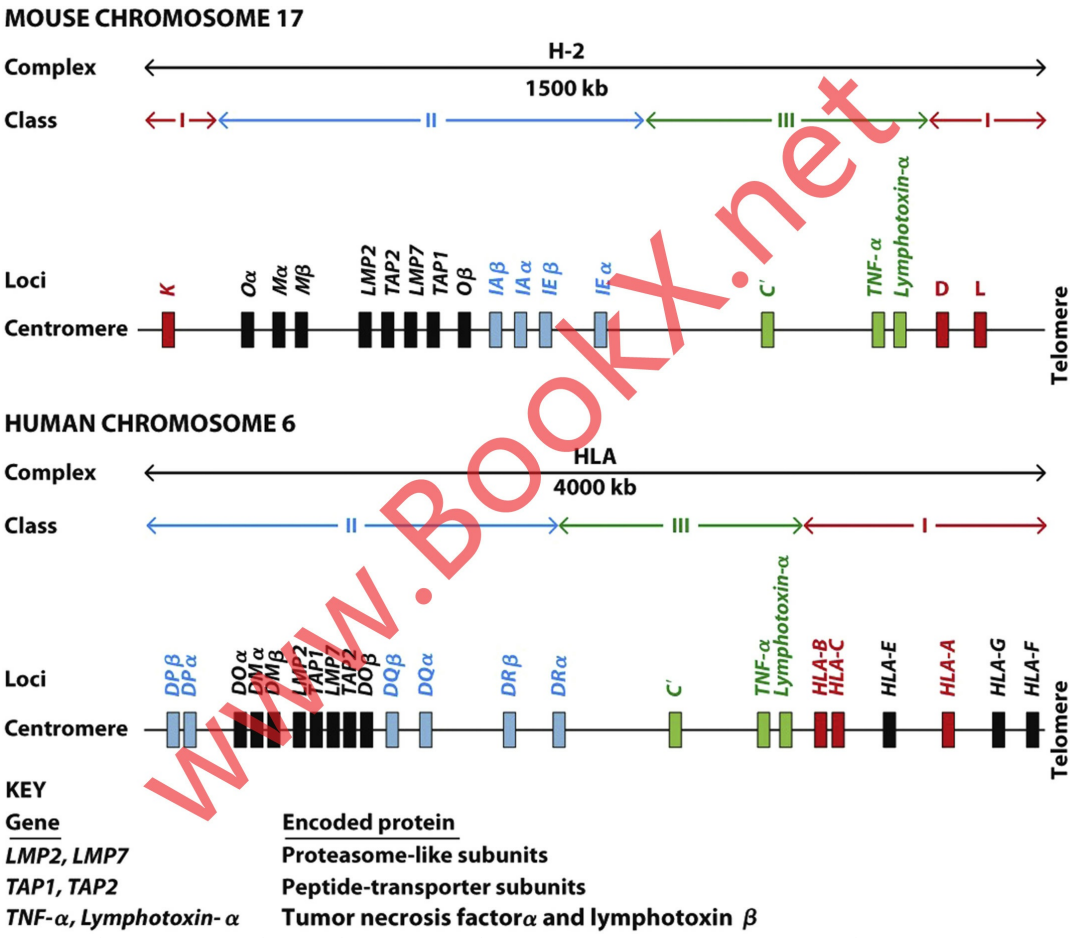
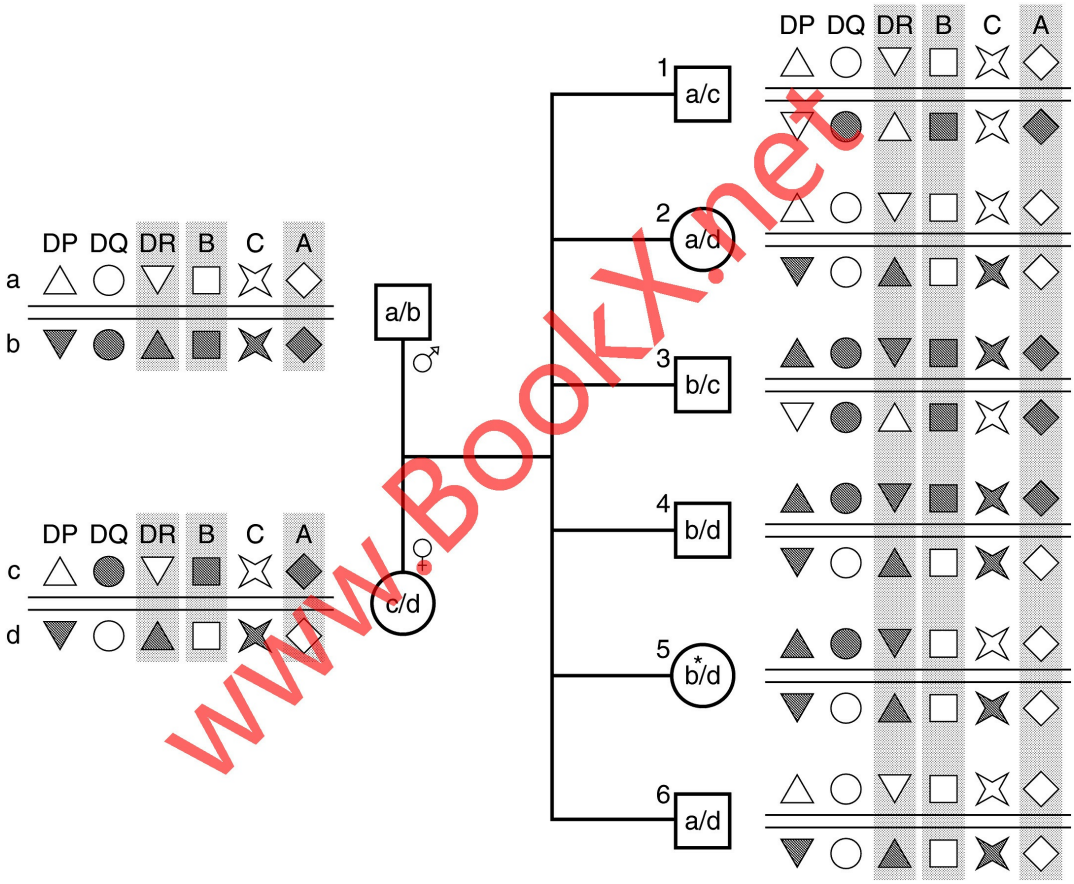



**FIGURE 8.3** Simplified map of the mouse and human MHC loci. The MHC class I genes are colored red, MHC class II genes are colored blue, and genes in MHC III are colored green. Classical class I genes are labeled in red, classical class II genes are labeled in blue, and the nonclassical MHC genes are labeled in black. The concept of classical and nonclassical does not apply to class III. Only some of the nonclassical class I genes are shown; the functions of only some of their proteins are known.

From: The major histocompatibility complex and antigen presentation, Chapter 8. In: Owen, Punt, Stranford, editors. Kuby immunology. 7th ed. New York: W.F. Freeman and Company; 2013. pp. 261–96.

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**FIGURE 8.4** With HLA typing we determine the HLA phenotype of an individual. The determination of the HLA phenotypes on at least the two parents and a child or on three HLA-different members of the same family permits segregation analysis, useful to determine the four haplotypes (normally called *a*, *b*, *c*, and *d*) present in the family, or the genotype of each individual. Consequently, the definition of individuals as heterozygous or homozygous at certain loci (e.g., sibling 1 is homozygous at *-DP, -DR*, and *-C*, both alleles are white; but heterozygous at *-DQ, -B*, and *-A* loci, one white and one black allele), together with the recognition of individuals who share one haplotype only (e.g., siblings 1 and 2 share the "*a*" haplotype and are HLA haploidentical) or two haplotypes (e.g., siblings 2 and 6 share the "*a*" and the "*d*" haplotypes and are HLA identical), or none (e.g., siblings 2 and 3 and are HLA different) becomes possible, as well. BMT always offers the best chance of success if it is performed between HLA-identical siblings. "Six antigen matching" refers to the situation in which two individuals share both alleles at the loci *-A, -B*, and *-DR* (gray background) and to the unique situation in which national sharing of kidneys is considered mandatory. In this simplistic example, father and mother are phenotypically the same because they are sharing the alleles at the *-A, -B*, and *-DR* loci and constitute a six-antigen matching pair, although they are not HLA "identical," like siblings 2 and 6, because their haplotypes are actually different. Although it is

considered a very rare event, it is possible to find individuals, represented here by sibling 5, in which a crossing over between class I and class II gene regions, involving the paternal and maternal haplotypes of the father, cause an *a-b* recombination flagged here with an asterisk: *b*∗.
From Rosner G, Martell J, Trucco M, Histocompatibility. In: Lister J, Ball ED, editors. Hematopoietic stem cell therapy. Philadelphia: Churchill Livingstone; 2000. pp 233–51.

By convention, the paternal haplotypes are designated as *a* and *b* and the maternal haplotypes as *c* and *d*. Children in a family are designated as genotypes *ac*, *bc*, *ad*, or *bd*. If *a* = HLA-A1; B8; C7; DR3; DQ2; DP4 while *b* = A2; B7; C7; DR2; DQ6; DP1, and *c* = A3; B65; C3; DR7; DQ2; DP2, or *d* = A11; B35; C4; DR1; DQ1; DP4, the phenotype of a child *b*/*d* will be A2,11; B7,35; C4,7; DR1,2; DQ1,6; DP1,4, and the recombinant haplotype resulting from, for example, a crossing over that took place somewhere in between class I and class II regions of the paternal *a* and *b* haplotypes, will be *b*∗ A1; B8; C7; DR2; DQ6; DP1 as described in Fig. 8.4 in simplified terms. Family studies are therefore recommended, both to confirm the alleles present in each of the patient's haplotypes and to rule out the possibility of recombination. Only a family study and the analysis of allele segregation in it can positively elucidate both haplotypes of an individual.

The class I genes present at the *A*, *B*, and *C* loci encode molecules that can efficiently present "endogenous" antigenic peptides to cells matured in the thymic environment ("T" cells) promoting positive and negative selection. The class I region also contains nonfunctional genes (i.e., pseudogenes HLA-H, -J, -K, and -L) and genes that are potentially functional, but whose gene products are not yet fully elucidated like HLA-E and -F. HLA-G, a protein preferentially expressed at the surface of cytotrophoblasts at the maternal-fetal interface of the placenta, has been shown by Pazmany and colleagues [14] to sufficiently protect otherwise susceptible target cells from lysis promoted by activated natural killer (NK) cells.

The class II are heterodimers, that is molecules consisting of two MHC-encoded polypeptide chains: the $\alpha$ (encoded by "A" genes), noncovalently bound to the $\beta$ (encoded by "B" genes). For -DQ molecules, only the -DQA1 and -DQB1 gene products are expressed as functional -DQ $\alpha/\beta$ heterodimers. The *-DQA2* and *-DQB2* genes are pseudogenes. Likewise, only the *-DPA1* and *-DPB1* genes encode the chains of functional -DP molecules. In contrast, more than one type of -DR molecule can be encoded on the same chromosome, and all are coexpressed at the surface of the same cell. Different *-DRB* genes are present in different number and in different combinations on chromosome 6. Each type of -DR$\beta$–chain pairs with the single nonpolymorphic $\alpha$-chain encoded by the *-DRA1* gene. In addition to the ones at the *-DR*, *-DQ*, and *-DP* loci, the class II region includes genes encoding proteins involved in antigen processing and in peptide transport from the cytoplasm to the cell surface [15]. Besides the large multifunctional proteases and transporter associated with antigen processing–encoding genes, others (i.e., the $\alpha$-chain encoding genes *-DMA* and *-DOA*, or the $\beta$-chain encoding genes *-DOB* and *-DMB*) are present in this region. These nonclassic class II loci likely function by regulating peptide loading onto HLA-DR, -DQ, and -DP molecules [16,17].

Class II molecules normally present to naïve T cells "foreign" antigens processed through their "exogenous" pathway. Although with different efficiency, macrophages, monocytes, dendritic cells, and mature B cells are all able to phagocytose foreign molecules, thus capturing "non-self" proteins invading our body and enzymatically cleaving them into smaller segments called peptides. This function is called "processing" of the antigenic protein. The peptides generated as the result of antigen processing are then protected from further cleavage and brought to the cell surface by class II HLA molecules able to accommodate the peptides in their antigen-combining site [18]. The peptide complexed with the so-called binding groove is then exposed at the cell surface (i.e., "presented") to a T lymphocyte that recognizes and engages it by its TCR molecule. The APC thus activates T lymphocytes, causing them to divide and secrete specific

factors. The signal from the engaged TCR to the inside of the cell is transmitted by a chain of molecules, the CD3 complex, consisting of a series of dimers such as the CD3 δε, γε, and ζζ chains, that ultimately activate the enzymes necessary to stimulate cell division, cytokine receptor upregulation, and cytokine production [19]. Cytokines are secreted cellular hormones that can inhibit or stimulate other cells to become activated.

Before the physiologic functions of the HLA were defined, these molecules were discovered as the agents limiting the possibility of transplanting cells, tissues, or entire organs from one individual to another one (i.e., allotransplantation). The search for histo- (*histos* in Greek for "tissue") compatibility became the original goal of these studies.

## 8.2. Historical *Iter* Toward Histocompatibility Definition

### 8.2.1. HLA Antigens Can Be Recognized by Antisera

The idea of replacing a diseased or damaged part of the body of an individual, the "recipient," with the same part provided by another healthy individual, the "donor," has always been present in the human mind. Greek mythology is full of examples of *chimeras* (i.e., imaginary animals composed of parts coming from individuals of different species). If, on the one hand, chimeras can represent an artistic version of a successful xenotransplantation intervention (i.e., a transplant between individuals of different species), on the other hand, picturesque examples of dreamed allotransplantation (i.e., a transplant between two genetically different individuals of the same species) are also available [20].


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**FIGURE 8.5** The healing of Justinian by Saint Cosmas and Saint Damian as portrayed by Fra Angelico in his painting conserved in the Museo di San Marco, Florence, Italy.

The most elegant representation of this mental concept is perhaps the famous altarpiece in the San Marco Church in Florence, Italy (Fig. 8.5). Fra Angelico (1387–1455), the painter, here represented the miracle performed by Saints Cosmas and Damian in Constantinople as narrated in a third-century legend. According to the legend, these twin brothers, one a physician and the other a surgeon, replaced the cancerous leg of a white church sacristan with a healthy limb from a recently deceased Moor. The story is told that the sacristan walked around for the rest of his life with one white leg and one black leg [20–22].

There is, however, evidence that transplantation surgery was successfully performed more than 2000 years ago by ancient Hindu *vaidya* [23]. The reported clinical interventions that involved the reconstruction of the nose using pedicle flap grafts from the patient's own forehead were similar in approach to the one described by the

historian Calinzio, who, in 1503, wrote in praise of the surgeon Branca who reconstructed the nose of a gentleman using skin removed from his slave [24]. Calinzio added that: "for a time the new nose functioned well," then "suddenly grew cold," "turned blue," and "eventually fell off." Because the slave died on the same day, the slave's death was blamed for the loss of the engrafted nose. Today we know that the difference between these successful and unsuccessful transplants is that the first was an autologous transplant (i.e., a transplant using different tissues coming from the same individual) and the other an allotransplant. Whereas Branca's patient actually suffered a typical graft rejection, the physician Gaspare Tagliacozzi was instead successful when, 100 years later (in 1597), he tried to reproduce the *vaidya*'s results by performing similar reconstructive rhinoplasty using skin flaps collected from the patient's arm (i.e., another example of autotransplantation) [20,22].

We had to wait until the beginning of the twentieth century to find evidence of a fresh new look to the transplantation problem. In 1912 George Schöne clearly summarized conclusions he gathered from different transplant attempts mostly performed in animals. The "laws of transplantation" were so defined: (1) autografts generally succeed, (2) allografts usually fail, and (3) xenografts invariably fail. "Blood relationship" was proposed as the biological barrier that separates "donor" from "recipient": The closer the relationship, the more likely it is that the graft will be accepted [20,22].

Twenty-five years later Peter Gorer, an expert on tumor transplantation in mice, defined the scientific basis of the postulated Schöne blood relationship [25]. Gorer was interested in explaining why tumors that could often be transplanted from one mouse to another of the same strain failed to grow when transplanted into recipient mice of another strain. To determine what was different between the accepting versus the rejecting strains, Gorer performed tumor transplants in $F_1$, $F_2$, and backcross progeny of the three inbred strains of mice. The permissive trait was attributed to two independent genetic loci. On the basis of his knowledge of the blood

groups, Gorer also attempted to distinguish the tumor-susceptible from the tumor-resistant animals on the basis of mouse erythrocyte agglutination driven by human serum. The successful experiments also proved that, like for the red cell ABO system, agglutination behaved as a simple mendelian dominant trait. Furthermore, rabbit anti-mouse erythrocyte sera were sufficient to define three mouse red blood cell antigens. The detection in the serum of the mice of anti–type II antigen antibodies coincided with rejection propensity [26]. It was on this basis that Gorer joined forces with George Snell at the Jackson Laboratory in Bar Harbor, Maine, where they expanded the studies to Snell's "congenic" inbred mouse strains [27]. Congenic are genetically identical strains except at a single chromosomal locus. Using congenic strains of mice, Gorer and Snell were able to determine that engrafted tumors were most likely able to "take" when the recipient mouse was of the same strain as the donor [28]. More importantly, they also realized that survival of donor tissue was not limited to tumors but applied to any graft including skin. Also, donor-specific antibodies first noted by Gorer in the graft-rejecting recipient were induced not only by tumor grafting but also by any other tissue graft or blood transfusion [21]. By appropriate genetic crosses and backcrosses among their congenic inbred strains, Snell and Gorer identified a number of genetically independent loci controlling these immune responses, which they designated H for histocompatibility, followed by an Arabic numeral in the order of discovery. The locus designated *H-2* appeared to be more immunogenic than the others and corresponded to Gorer's original type II antigen. This locus became known with time as the murine MHC. The MHC can be considered the molecular embodiment of Schöne's "blood relationship" [20].

Sir Peter Medawar tried to apply these concepts to help soldiers burned during World War II with skin grafts [29,30]. He also came to the conclusion that, while autografts succeed, allografts actually fail after an initial period of survival. A second allograft from the same donor did not experience the latent period of the first; rather, it rejected in an accelerated fashion. The survival of a second graft from

a different donor was instead similar to that of the original graft, rejecting only after a latent period. Because the accelerated rejection could also be induced by injection into the recipient of donor leukocytes prior to engraftment, he hypothesized that "destruction of the foreign epidermis was brought about by a mechanism of active immunization." Specific antigens analogous to those of the ABO blood groups were supposed to be shared by skin and leukocytes and were responsible for the rejection response. Medawar's conclusions constituted the theoretical basis to postulate that immunosuppression might overcome the laws of transplantation.

In 1954, the first successful kidney transplant was performed between monozygotic twins for which immunosuppression was not necessary [31]. In 1959, the same team used an efficient immunosuppressive regimen to perform the first kidney graft between unrelated individuals [32]. The graft survived for 20 years.

The observations that antibodies against transplantation antigens could be generated as the result of an immune response against transfused blood components suggested the possibility of identifying human histocompatibility antigens through serologic methods. In 1958, Jean Dausset produced the first human histocompatibility antiserum through deliberate immunization of one blood donor with leukocytes from another [33]. This antiserum agglutinated the white blood cells of about 60% of his French donor panel. Concurrently, Payne and Rolfs showed that the sera from multiparous women often contained leukoagglutinins [34]. This important finding promised readily available sources of reagent antisera with which to characterize HLAs.

## 8.2.2. Serologically Characterized *HLA* Alleles and Loci

Using a battery of selected antisera from multiparous women, it became possible to perform serologic HLA typing of patients with use of the microlymphocytotoxicity assay [35]. This technique uses as

target cells a suspension of pure and viable lymphocytes that have been separated from whole peripheral blood by using density gradient separation. The lymphocytes are incubated with the panel of alloreactive antisera and a source of complement (C′), usually rabbit serum. The anti-HLA antibodies present in the various sera are quite specific for the individual structural determinants that characterize the polymorphism of the HLA antigens. When a specific antigen–antibody complex is formed, C′ is fixed and activated, resulting in cell lysis. Following the addition of a vital dye, the percentage of dead versus live cells can be visualized with phase contrast microscopy. A positive reaction is scored when at least half of the cells are killed. The HLA type is assigned by interpreting the patterns of reactivity of dozens of sera whose specificities are known [36,37]. This is the basis of serologic typing.

Originally, it was the responsibility of the individual tissue typing laboratory to procure, test, and maintain quality control of the antisera used in testing. The pioneering HLA investigators soon realized, however, the need for standardizing their own reagents. This was achieved by organizing international workshops that have been held every 3–4 years since 1963. These international workshops, originally aimed at sharing and consequently standardizing alloantisera, target cells or cell lines, and technical procedures, soon became the forum for recognition of new loci, the definition of new alleles, the adoption of the standard methods, and the acceptance and then the use of a common nomenclature. Serologically, two different loci were first defined during the Third International Histocompatibility Workshop held in Torino, Italy, in 1967 and called *HLA-A* and *HLA-B*, respectively [36,37]. Eventually a third locus was also recognized, the *HLA-C*.

Today, sera can be obtained locally or through national or international serum exchange programs. Commercial trays with well-characterized sera are most widely used. All accredited histocompatibility laboratories are required to adhere to rigorous standards regarding reagents, protocols, and quality assurance set

forth by the American Society for Histocompatibility and Immunogenetics. In addition, most laboratories participate in several proficiency testing programs administered by such agencies as the College of American Pathologists.

In 1977, the development of mouse anti-human monoclonal antibodies was seen as the best alternative source to increase specificity and have an unlimited supply of standardized reagents [38]. Their use, however, is not yet widespread due to the limited number of "private" specificities recognized by the mouse, which reacts preferentially against "public" specificities, that are different between mice and humans. Trays containing only monoclonal antibodies that allow a relatively informative typing are commercially available.

Despite its limitations, the microlymphocytotoxicity test remained for years the most popular test for clinical use to provide a moderate level of typing resolution in one working day. A drastic improvement in the analysis of percentages of reactive antibodies present in the recipient serum versus the cells of the donor improved the results of transplant (kidney, in particular) interventions, eliminating the negative conditions frequently found associated with fulminant or hyperacute rejections.

## 8.2.3. Cellularly Characterized *HLA* Alleles and Loci

Even with all the refinement and standardization promoted through the years, many problems still confounded the results obtainable by serologic methods. Serology is encumbered by the requirement for relatively pure populations of viable lymphocytes used as targets. This has the greatest impact on molecules expressed most abundantly on B lymphocytes and less significantly on activated T cells and macrophages, which, all together, comprise at best 20% of the peripheral blood lymphocyte population.

Once it was clear that serologically so defined "compatible" individuals could still reject allotransplants, a cellular-based approach

was proposed to solve this still present impediment to allotransplantation. The first cellular approach successfully used for recognition of additional (i.e., not *HLA-A* nor *-B* or *-C*) alleles was the so-called mixed lymphocyte culture. To perform this test, lymphocytes from two individuals are coincubated for several days in tissue culture. If the two individuals are disparate for something other than serologically defined alleles, both cell types undergo lymphoblastogenesis, synthesize DNA, and proliferate, the so-called mixed lymphocyte reaction (MLR). In the presence of $^{3}$H-thymidine, the degree of newly synthesized DNA can be estimated. Cells that are HLA-identical are nonreactive in mixed lymphocyte culture, and the level of $^{3}$H-thymidine incorporation is similar to that of the negative controls. Higher than controls $^{3}$H-thymidine incorporation was considered instead evidence of additional antigenic differences. In 1969, in order to separately monitor the response of the recipient (host-versus-graft) and the donor (graft-versus-host), Fritz Bach proposed to perform two separate unidirectional MLRs [39]. The cells chosen as "stimulators" are irradiated to inhibit DNA synthesis and $^{3}$H-thymidine incorporation. The nonirradiated "responders" are instead still able to incorporate the thymidine. Every MLR typing test is set up as a complicated matrix of many one-way reactions. In general, three unrelated individuals are used as positive controls of the maximum proliferative ability of the responder cells. Every cell should be tested as both stimulator and responder. In addition, autologous controls (i.e., each individual's cells incubated with its own irradiated cells) are used to normalize the response of each cell type to its stimulators. The results are expressed as a relative response or as a stimulation index. Ideally, when two individuals are identical, the proliferative responses are less than 20% of the positive controls [40]. The MLR test was used most widely in bone marrow transplantation (BMT) and was developed primarily to help select the most compatible (i.e., least stimulatory) donor when several compatible family members or matched unrelated donors were available [39].

The MLR was used to recognize non–serologically defined alleles in

the Sixth International Workshop held in Arhus, Denmark, in 1975, in which the stimulators were cell lines obtained from Epstein–Barr virus–transformed lymphocytes of individuals recognized with serologic typing to be chromosome-6 homozygous by descent. Cellular specificities recognized by MLR testing were originally designated as *HLA-Dw* ("D" because these specificities were considered to be encoded by a different locus than A, B, and C; "w" because they were still to be officially recognized in the context of an international workshop). HLA-Dw antigens were in reality immunogenic combinations recognized as such by alloreactive T cells. However, by absorbing the antisera with platelets to remove anti–class I antibodies and using purified B cells as cytotoxicity test targets, the *HLA-Dw* determinants were also recognized by certain antisera. They were then defined as "D-related" serologic specificities and eventually "*-DR*" alleles. The MLR test proved extremely useful for research purposes and allowed the characterization of additional loci encoding molecules preferentially expressed on B lymphocytes different than *DR*: *DQ* and *DP*. However, the MLR test proved too complex and the results too difficult to standardize to be included in routine typing procedures. The MLR lacks sensitivity and specificity, particularly when used for patients with hematologic disorders. Spontaneous proliferation of malignant blast cells in culture and/or poor HLA antigen expression can contribute to false-positives or false-negatives, respectively. The MLR was technically cumbersome and as many as 40% of the tests were uninterpretable. Furthermore, it did not have good predictive value for either rejection or graft-versus-host disease (GVHD). Therefore, despite being the only functional test for assessing nonserologic histocompatibility, the National Marrow Donor Program (NMDP) eventually no longer required it before performing BMT.

A functional and geographical distinction between *HLA-A, -B,* and *-C* and *HLA-DR, –DQ,* and *-DP* loci was then formally characterized in what were defined as class I and class II genes/molecules.

## 8.2.4. Molecularly Characterized *HLA* Alleles

## and Loci

The practical limitations of both serologic and cellular assays, as well as their inability to resolve clinically relevant subtypes, prompted the rapid development of molecular biologic techniques for HLA typing. Molecular techniques can precisely define differences missed by serologic typing and/or those suggested by an incompatible MLR.

In the 1980s, molecular biology technologies, such as gene cloning and sequencing, became simple to perform. This facilitated the isolation and characterization of the HLA genes at different loci from hundreds of individuals. By comparing sequence variations from person to person, the nature and locations of the polymorphism of the HLA complex were revealed.

The first attempts to perform molecular HLA typing were based on restriction fragment length polymorphism (RFLP) analysis [41]. RFLP testing uses a specific DNA probe to recognize the presence of a certain *HLA* gene on a set of DNA fragments. These fragments are generated by digestion of genomic DNA with commercially prepared bacterial restriction endonucleases (i.e., restriction enzymes) that recognize short, specific nucleotide sequences. The resulting array of restriction fragments are separated on agarose gels by electrophoresis, transferred by capillary action to a nitrocellulose or nylon membrane (Southern blotting), and sequentially hybridized with radiolabeled DNA probes specific for each *HLA* locus. After repetitive washings, the radioactive probes specifically hybridized to their DNA fragment are revealed by exposing the membranes to X-ray film. The positive fragments differ in size and number from an individual to another, thus creating a polymorphism useful for typing. Although by RFLP it was possible to generate results more precisely and completely than serologic typing [42,43], in some laboratories, the use of radioactivity was problematic in that high-quality Southern blots could not be achieved. Also, the turnaround time for even a small number of tests was very long, thus precluding the use of these DNA tests in a clinical setting.

The Tenth International Histocompatibility Workshop was organized with the specific aim of transferring RFLP technology to

laboratories specialized in HLA serology. All participant laboratories received a common set of homozygous typing cells and a panel of DNA probes. Although the proceeds of this workshop, published in 1987, were useful in establishing standards for RFLP analysis of *DR* and *DQ* loci, and the collective data obtained revealed strong associations between certain -*DR* and -*DQ* alleles and the RFLPs [44], the results were not definitive, largely due to strong homology and cross-reactivity between different alleles and even different loci. These obvious limiting aspects did not preclude, however, their application to fulfill specific aims, like the characterization of *HLA-DQ* alleles that, for example, confer susceptibility to type 1 diabetes [45], or the comparison between serologic and molecular typing in promoting kidney graft survival [46,47]. As other techniques that directly analyze HLA polymorphism were being developed, RFLP analysis was, however, quickly deemed unsuitable for general clinical use.

The introduction of polymerase chain reaction (PCR) [48] during the late 1980s revolutionized HLA molecular typing. PCR is a powerful and versatile technique for generating millions of copies of specific genomic DNA fragments from minute amounts of starting material once the nucleotide sequence of a certain gene is known. Amplification is accomplished automatically in a DNA thermal cycler by incubating nanogram quantities of DNA, isolated from any possible source in solution with buffer, free deoxynucleotide triphosphates (dNTPs), specific oligonucleotide primers, and thermostable DNA polymerase [49]. The most common analyses were performed by dot blotting a few microliters of the amplified DNA onto replicate nylon membranes. Each membrane is then hybridized with a different sequence-specific oligonucleotide probe (SSOP) labeled with a reporter moiety. After washing away the unbound excess, the retention of specific probe can be visualized by a variety of methods, the most common of which is chemiluminescence. This approach allowed typing of hundreds of samples with a relatively small number of probes to determine, for example, that variants of sequences involving codon 57 of the second exon of the *HLA-DQB1* gene were reliable markers for insulin-dependent diabetes mellitus

susceptibility [50,51].

As the extremely large sequence diversity of the class II genes' second exon was revealed, the possibility of using this technique for complete HLA class II typing seemed difficult, yet feasible. This daunting task was promoted and organized by the Eleventh International Histocompatibility Workshop, which provided standardized conditions and a battery of probes to each of its participant laboratories [52].

The advantages of this technique, often referred to as "oligotyping," are many. The requirement for viable, purified lymphocytes expressing HLA antigens is eliminated. Reagents can be standardized and synthesized in unlimited quantities. Functionally relevant subtypes and polymorphisms as small as one nucleotide substitution can be defined precisely, as stringent conditions for hybridization can be perfected for each probe. However, for example, to provide a high-resolution *-DRB* and *-DQB1* typing alone, up to seven separate PCR amplifications were needed. A complete set of results, no matter how large or small the set, required 4–5 days to be finalized. Therefore, this technique was not well suited for routine clinical applications in which a relatively small number of samples should be tested as quickly as possible.

A different format referred to as the "reverse dot blot" has proved advantageous in reducing the amount of technical manipulation and in improving turnaround time [53]. Reverse dot blots feature a premade membrane to which the entire battery of probes has been fixed. The DNA to be tested incorporates the reporter moiety during amplification and is then hybridized against the probes present on the membrane. Advantages included labeling one or few DNA samples per donor instead of many probes, performing an entire test with only one membrane, and obtaining results in 1 working day.

The canonical size of specific oligonucleotides normally used as probes was about 20 nucleotides, a size that has been determined as best for discriminating even 1-bp change. For the reverse dot blot, the direct attachment of probes this size was inefficient and involved

formations of chemical bonds to portions of the probe, resulting in reduced hybridization capability. These difficulties have been solved by enzymatically adding a large number of thymidine nucleotides to the end of the probe [poly(dT) tailing] [53]. The increased length facilitated binding efficiency and favored formation of chemical bonds in the poly(dT) tails, thus leaving the sequence-specific parts of the probe available for hybridization. This approach was then used in some commercial kits for HLA typing.

An alternative method [54] used a different approach to bind nucleotide probes to the membrane. Long polymers, usually greater than 25 repeats of the specific nucleotide sequence, are synthesized and bound to the membrane with high efficiency [55]. Although chemical bonds between the membrane and the DNA will prevent the hybridization to some sequences, the result is that many more specific sequences remained available for hybridization. Another advantage of this method was that both complementary strands of probe sequence were bound to the membrane (i.e., the probes are double-stranded). This allowed both strands of amplified DNA to hybridize to the probe, further increasing the sensitivity of this assay. Finally, a large quantity of polymers of the same size could be generated from those cloned into plasmid vectors. This level of reproducibility was not obtainable with poly(dT) tailing and would permit standardization of this technique for intralaboratory use. Other "reverse" techniques involved fixing probes to 96-well plastic trays with either colorimetric or fluorescent detection [56,57]. Some of these techniques are, however, no longer in use.

Another technique based on the use of sequence-specific primer (SSP) pairs and PCR has been proved more suitable for clinical use. SSP consists of a battery of primer pairs, each capable of only amplifying a specific gene segment. The numerous PCR products obtained are then loaded onto an agarose gel. After electrophoresis and staining, the presence of the specifically amplified DNA segments can be visualized on a UV lightbox so to allow correct allele definition. The presence of determined band combinations allowed the attribution of each allele. SSP offers the possibility of obtaining

moderate-to high-resolution HLA typing in a few hours. First, techniques for *-DRB* molecular typing using 32 sequence-specific pairs of primers [58] and *-DQB1* typing using 14 primer pairs [59] were published. Subsequently, several commercial companies offered SSP testing kits for all the *HLA* alleles.

SSP methodology was also applied to class I typing but proved to be a much more formidable task due to the high degree of widespread polymorphism compared with the class II genes and the complications presented by the presence of pseudogenes. One method for SSP typing [60] used nine coding strand and seven noncoding strand primers in various combinations to generate *HLA-A*–specific amplification products. The specificity of PCR priming was increased by taking advantage of the amplification refractory mutation system (ARMS), in which nonspecific reactions are inhibited by introducing a nucleotide mismatch near the 3′ end of the primer [61]. Fifteen *HLA-A* group specificities can be successfully characterized using this method, which appears to be comparable to routine serology with respect to identification and resolution of *HLA-A* specificities. Subtypes of each group can be more specifically resolved using additional primer pairs [62]. Low-resolution typing of the *HLA-B* alleles was described [63] using a similar approach. Twenty-four PCRs using 34 primers were sufficient to determine the majority of, but not all, serologically defined specificities at the *HLA-B* locus. ARMS-PCR has also been used to detect alleles at the *HLA-C* locus [64].

The SSP approach is especially amenable for use in clinical laboratories that do not have a great deal of molecular biology experience, as the equipment and special techniques required are minimal. Because reliable and easily interpretable results can be generated in less than 4 h from DNA extraction to allele identification, SSP typing is an especially attractive alternative to serology for clinical laboratories. However, for laboratories performing large-scale molecular typing, the SSP method may not be amenable.

Substantial progress has been made in converting the methods for

molecular HLA typing to microchip technology [65]. What is generally known as SSOP typing was used, for example, to test *HLA-B* alleles as a model system. An oligonucleotide array has been used to identify alleles occurring within a set of 100 samples obtained from unrelated individuals [66]. Probes were optimized that represented all known polymorphisms encoded in HLA-B exons 2 and 3. Hybridization of PCR products allowed unambiguous detection of complex heterozygous SNP combinations, including some (e.g., *HLA-B*1502+*2702* and *HLA-B*1513+*2708*) in which combinations of nearby SNPs cause ambiguous genotyping results when analyzed by conventional sequencing-based methods [67,68]. More recently, the International MHC and Autoimmunity Genetics Network (IMAGEN) has exploited parallel genotyping of 1472 SNPs using microarrays to determine complete HLA class I and class II genotype from more than 10,000 subjects [69]. The accuracy of the method for determining two- and four-digit resolution at *HLA* class I and II loci has been estimated to be as great as 95% [68]. Among its advantages, microarray technology can potentially accommodate thousands of perfectly distributed DNA spots on microarray platforms where hybridization can take place, saving reagent quantities, avoiding time-consuming manipulations, and providing automatically read results. Moreover, oligonucleotide array-based strategies can provide high-throughput genotyping by virtue of parallel analysis of multiple genetic regions [67,69]. A limitation of the microarray-based method, however, is the need for sophisticated, robust data handling protocols that are likely out of reach of the typical clinical typing laboratory. Thus, current use of this approach is limited to genome sequencing centers and a handful of specialized laboratories with interest in the HLA region.

### 8.2.5. Microsphere-Based Technology for *HLA* Typing

In contrast to the microarray approach, microsphere technology has been widely adopted by the *HLA* typing community. The method exploits the use of fluorescently color-coded beads developed in order

to advance DNA hybridization-based typing methods [70]. The major components of the system are benchtop flow cytometer, two-color microspheres, and biotinylated PCR amplified product for probing specific oligonucleotides attached to individually color-coded beads. Flow cytometry analyzes individual microspheres by size and fluorescence, distinguishing three fluorescent colors (i.e., green at 530 nm, orange at 585 nm, and red at wavelengths above 650 nm). The ratio of two of the fluorescent signals are used for microsphere classification so that different two-color coded beads, prelabeled with probe DNAs, can be identified along with a third fluorescent signal to be used for quantification of hybridization signal. In this methodology, first applied toward HLA for genotyping of *HLA-DQA1* alleles but later applied to class I as well as *HLA-DRB1, -DQB1*, and *-DPB1* alleles, targeted DNA is PCR amplified using group-specific SSP biotinylated primers [70–72]. The biotin-labeled PCR product allows detection of hybridization between probe and amplified sequence by using *R*-phycoerythrin (PE)-conjugated streptavidin. Flow cytometry analysis enables quantification of the fluorescent signals on each microsphere. Assignment of HLA genotype is based on the hybridization reaction pattern compared with the pattern associated with published *HLA* gene sequences. In this manner it is conceptually similar to traditional hybridization-based HLA typing approaches. At present, several hundred individually color-coded microspheres each containing a different allelic oligonucleotide probe can be analyzed in a single mixture of oligonucleotide-coupled microspheres. The HLA loci are amplified from genomic DNA using SSP mixes that are optimized for PCR amplification of particular HLA alleles and have been used with dried blood spots as well as DNA isolated from blood draws and buccal swabs.

## 8.2.6. DNA Sequencing Technology for *HLA* Typing

Advances to sequence-based typing methods have been applied to

*HLA* genotyping. Pyrosequence-based typing (PSBT) has been developed to allow accurate sequencing of short stretches of DNA, initially for the analysis of expressed sequence tags [73,74], and has been applied to *HLA* genotyping [75–80]. Sequencing of lengths between 50 and 150 nucleotides have been reported during analysis of *HLA* genotypes of loci *HLA-DQB1* and *HLA-DR*. The method is suited for use during SNP analysis of HLA due to its ability to accurately resolve heterozygous nucleotides by enabling out-of-phase sequencing [79,81]. Pyrosequencing is performed by addition of dNTPs individually, in a predefined dispensation order, so that the nascent nucleotide chain is extended one nucleotide residue per dispensation event. Detection of nucleotide sequence is performed by way of a chain of enzymatic reactions involving the activities of DNA polymerase, apyrase, ATP sulfurase, as well as luciferase, respectively allowing for the incorporation of complementary nucleotide, degradation of unused dNTP, generation of luciferase-substrate from pyrophosphate and adenosine 5′-phosphosulfate, and emission of light from the ATP-driven conversion of luciferin to oxyluciferin [73,82]. Incorporation of a particular nucleotide is displayed graphically in the form of a chart recording of nucleotide dispensation event versus the intensity of emitted light. This cascade of enzyme reactions is quantitative, in that increased light intensity is produced on incorporation of multiple nucleotides [83] (Fig. 8.6).

PSBT provides distinct advantages for genetic typing in that samples can be assayed in 96-well trays making it compatible with common laboratory automation instruments, thus increasing the rate of sample analysis, and the sensitivity of the technology requires little PCR-amplified material for each sequencing reaction. PSBT strategies can be designed using a minimal number of nucleotide dispensation events so that an entire 96-well tray can be assayed at a rate of roughly

1 min per base and approximately 20 min for a 20-nucleotide sequence, sufficient for analysis of most *HLA* alleles when primers are placed adjacent to polymorphic regions. Moreover, out-of-phase pyrosequencing of alleles [79,81] allow unequivocal genotyping of

allelic combinations that are ambiguous by conventional sequence-based typing techniques as well as those spaced too far apart to allow resolution by hybridization approaches. This results from the pyrosequencing system accepting nucleotides individually at each dispensation event, which directs the phase of primer extension of individual alleles. Out-of-phase pyrosequencing enables the detection of sequencing signal from each haplotype individually. This in turn enhances the ability to distinguish between genotypes for allelic combinations involving polymorphisms associated with crossing-over events [77–79]. These sequences can be determined with greater accuracy because they can be sampled at multiple independent dispensation events tailored to provide allele discrimination from each haplotype.

### 8.2.7. Next-Generation DNA Sequencing for *HLA* Typing

In the past few years, pyrosequencing technology has advanced substantially allowing it to be used as an important method within the stable of next-generation sequencing technologies [84]. Initially the method was reengineered by the 454 Life Sciences in ways that enable efficient preparation of thousands of individually prepared clones for analysis within a single sequencing run, scaling of the reaction to picoliter volumes, and increasing read lengths to as great as 400 nucleotides. As the length of the polymorphic exons of HLA class I (exons 2 and 3) and class II loci (exon 2) are between 264 bp for HLA-DPB1 exon 2 and 276 bp for exon 3 of *HLA-A*, *-B*, and *-C*, the reengineered pyrosequencing method was capable of sequence based typing of *HLA* alleles. In fact, a number of research laboratories reported achieving high resolution typing of HLA class I and class II loci using genomic DNA isolated from human cell lines as well as blood samples [85–87] and from cDNA created from isolations of mRNA [88]. The main focus of research was then concerned with preparing high quality template from DNA obtained using typical patient samples, including venous blood draws, dried blood spots,

and buccal swabs, with the latter two examples being especially challenging to prepare due to the small amounts of DNA that are typically obtained.

Along with translating the methodology from the research-based setting to the clinical *HLA* typing laboratory, there was great need to develop computational methods for analyzing the data. For example, using only half of the 454 sequencer's capacity, Galan and colleagues reported obtaining complete *HLA-DRB* exon 2 sequencing from 1407 samples [89]. This resulted from analysis of greater than 220,000 sequence-reads and required substantial computational support in order to organize the data by subject, align the sequences, and identify the alleles involved. It seemed likely that some of the alignment and assembly methods developed for the Human Genome Project [90] along with advanced statistical methodology for sorting data obtained from heterozygous subjects would be required in order to fully achieve the goal of using next-generation sequencing as a paradigm for routine *HLA* typing [91,92]. Given the proven ability of the methodology to generate high resolution *HLA* typing from thousands of samples in a single sequencing run and at relatively low cost, these final hurdles were eventually overcome.

At the beginning it was published the availability of software for genotyping of *HLA* alleles using data generated by the 454 next-generation sequencer. CONEXIO ATF was used in a study conducted by Holcomb et al. [92], where the same set of genomic DNA samples were analyzed by eight different laboratory sites, and the results compared well, showing the consistency of called genotypes. Although the methodology underling the CONEXIO was not fully explained, it seems likely that the main step was the identification of the most abundant sequences generated, followed by the assignment of the *HLA* genotype.

Another *HLA* typing algorithm was subsequently developed by Elrich et al. [91] and integrated the Genome Analysis Toolkit (GATK), a data processing tool for next-generation sequencing data [93]. Given samples from heterozygous subjects, this methodology focused on computing the posterior probability for all possible pairs of four-digit

*HLA* alleles and assigned as the genotype the most likely pair. In particular the posterior probability was computed as the product of the genotype, the phase, and the frequency probability. The genotype probability was defined as the product of the probabilities of observing a base at a particular position given the data, as determined by the GATK. The phase probability was computed as the product of the binomial probabilities that the phase orientation for each allele pair was consistent with the sequence data at a pair of adjacent polymorphic sites. Finally the frequency probability is the expected allele frequency in the population of each pair, where the allele frequency was obtained from the Allele Frequency Net Database [94].

Genotype A:
B*4418 5′ – G**GAC**AGAGCCTACCTGGAGGGCCTGTGCGTGGAGT**C**GCTCCG – 3′
B*780202 5′ – G**CTG**AGAGCCTACCTGGAGGGCCTGTGCGTGGAGT**G**GCTCCG – 3′

Genotype B:
B*5002 5′ – G**CTG**AGAGCCTACCTGGAGGGCCTGTGCGTGGAGT**C**GCTCCG – 3′
B*5108 5′ – G**GAC**AGAGCCTACCTGGAGGGCCTGTGCGTGGAGT**G**GCTCCG – 3′

Ambiguous conventional sequence based typing pattern:
Genotype A is equivalent to Genotype B
5′ – G (**C/G**) (**A/T**) (**C/G**) AGAGCCTACCTGGAGGGCCTGTGCGTGGAGT (**C/G**) GCTCCG – 3′

Unequivocal out-of-phase pyrosequence based typing pattern:
Genotype A is not equivalent to Genotype B
Out-of-phase pyrosequence
Genotype A:
B*4418 GG–ACAGA–GCCTA– – –CCTGGA– – –GGGCCTG–TG– –C– –GTGG–AGT– –CG– – – – – –**C–T–CC**– – – –G
B*780202 G–C– – – – –TG– – –AGAGCCT– –ACCTGG– – – – –A–GGGCCTGTG–C–GT**GG**– – –AGT**GGC–T–CC**– – – –G

Genotype B:
B*5002 G–C– – – – –TG– – –AGAGCCT– –ACCTGG– – – – –A–GGGCCTGTG–C–GT**GG**– – –AGT– –**C**– –**GC–T–CC**G
B*5108 GG–ACAGA–GCCTA– – –CCTGGA– – –GGGCCTG–TG– –C– –GTGG–AGT**GG**C–**T**– – – – –**CC–GC**– –**A**– –G

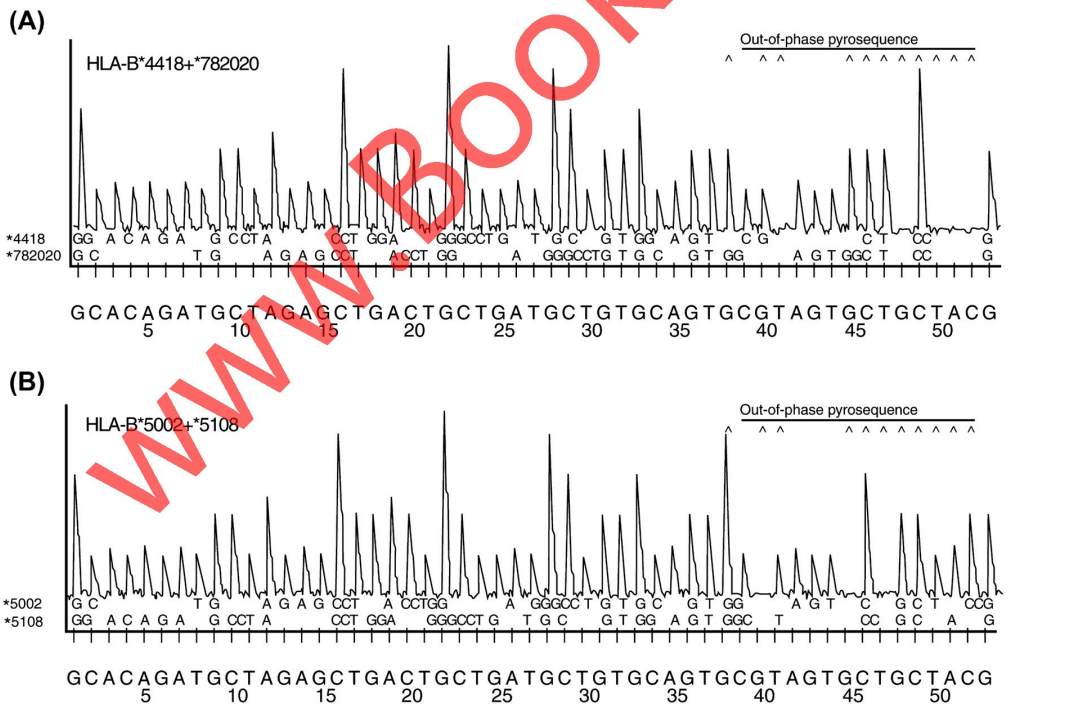



**FIGURE 8.6** An example of ambiguous *HLA-B* allelic pairs resolved by pyrosequence based typing. (A) *HLA-B* genotypes *HLA-B∗4418+∗780202* and *HLA-B∗5002+∗5108* share modular polymorphisms that confound genotyping by conventional sequence-based typing. Partial sequences from exon 3 of these alleles are shown along with the polymorphism, indicated by an asterisk above the sequence. While sequence-based typing provides a sum of the nucleotide signals from these allelic pairs, out-of-phase pyrosequence-based typing provides unique signals for each genotype, indicated by the symbol ^. (B) Simulation of the data expected from pyrosequencing of these genotypes. The allelic sequences are indicated in the chart recording.
Modified from Rinquist S, Alexander AM, Styche et al. HLA class II-DRB high resolution genotyping by pyrosequencing: comparison of

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group specific PCR and pyrosquencing primers. Hum Immunol 2004;65:163-84.

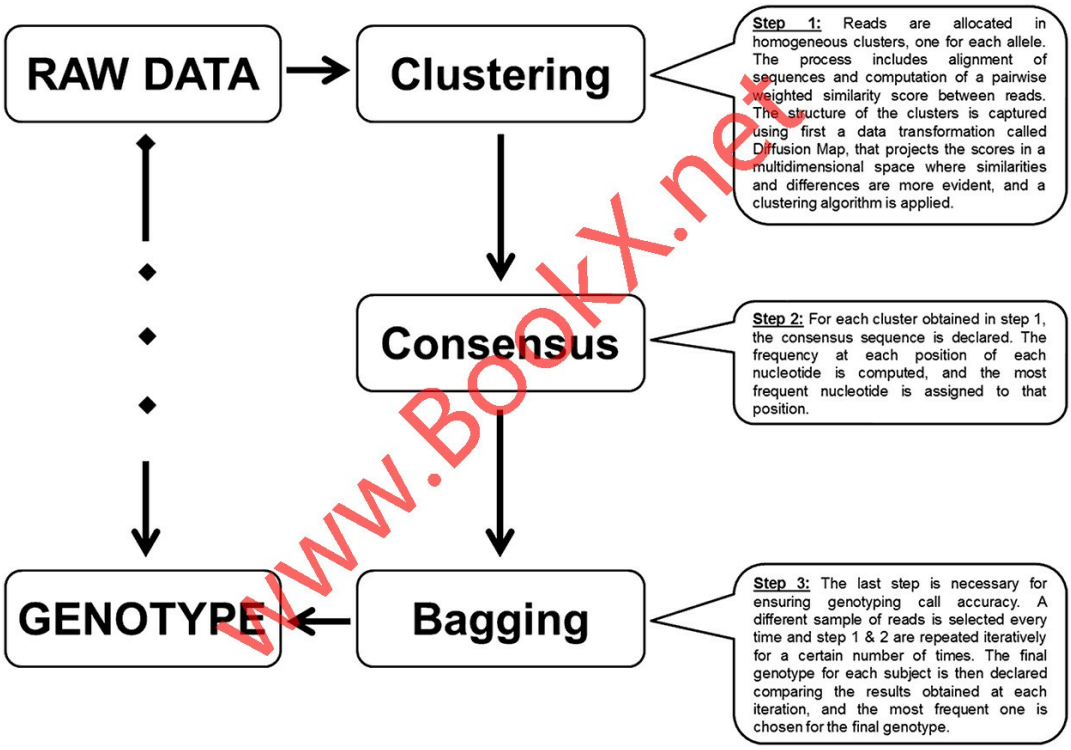



**FIGURE 8.7** Clustering and alignment of polymorphic sequences (CAPSeq) is a software application designed to genotype MHC alleles. Using next-generation data as input, the application enables clustering of similar sequences (i.e., those reflecting different alleles present in a heterozygous individual) and generation of a consensus sequence for each cluster. The consensus sequences are then compare with a list of known HLA allele sequences in order to identify the best match. The process is repeated in an iterative fashion using statistical method "bagging" in order to increase the accuracy of the genotype call.

We developed an additional method, called CAPSeq (clustering and alignment of polymorphic sequences). It makes use of sophisticated statistical analysis for calling each genotype with a precision of six digits [95–97]. The genotyping process can be divided in three main steps (Fig. 8.7). The first step consists of identifying the clusters of reads generated by the different alleles. To do this, a pairwise weighted similarity score is computed between aligned reads belonging to the same subject and exon. At this step the information

about the error-prone regions are incorporated into the overall sequence quality score, giving lower weight to the mismatches belonging to these regions. Next, diffusion mapping [98] is used to generate a data transformation that can simplify cluster structures and protect from the presence of outliers (e.g., very different sequences originating from pseudogenes). Diffusion maps are used to measure the "connectivity" of a dataset. The data are projected into diffusion space in which the Euclidean distance between two points is small if the points are highly connected in the original feature space and large otherwise. Diffusion distance is a robust, nonlinear measure that preserves the intrinsic structure of the data, which makes it an ideal choice for clustering problems involving related but highly polymorphic DNA sequences. Then the Kmeans algorithm is applied and the reads clustered in homogeneous groups. In the second step the consensus sequence for each cluster is established by majority rule at each position. The genotype is called by comparing the consensus sequence with that of previously known *HLA* allele sequences. The third step is performed for ensuring classification accuracy. To this end bagging [99], bootstrap aggregating, is used. Bagging is a machine learning technique that generates multiple versions of the same predictor, taking an "average" as the final result. In the present example it consists of sampling a fraction of reads from the total number of reads available, while performing steps 1 and 2 in an iterative fashion. The process is repeated $N$ times, and the final genotype is declared by majority rule.

Today, however, practically all the companies producing and selling next generation DNA sequencing equipment have consistently reduced all the cost involved in generating the data and have developed their own analyzing software allowing individual investigators to approach their use: a use before only permissible to major genome centers. Next-generation DNA sequencing "has the potential to dramatically accelerate biological and biomedical research" [100], eventually making histocompatibility typing by DNA sequencing a reliable and inexpensive routine.

## 8.3. Currently Most Used Methods for *HLA* Typing

### 8.3.1. Antibody Reactivity of the Patient Awaiting Transplant

The correct identification of the patient's *HLA* typing is just one role the HLA laboratory plays in the clinical transplant setting. The correct identification and subsequent characterization of anti-HLA antibodies in the sensitized patient are critical in achieving successful organ engraftment, and continued monitoring of those antibodies posttransplant is essential for long-term success. Advances in technology have allowed the characterization of anti-HLA antibodies to evolve from complement-dependent lymphocytotoxicity (CDC)-based methods to today's flow cytometry–based methods and solid-phase immunoassays (SPIs). The CDC method involves combining serum and lymphocytes, incubating for the appropriate amount of time and then adding complement (usually from rabbit serum). A vital dye is added and each individual well is visualized and scored based on the percentage of dead cells. The CDC crossmatch (XM) developed by Terasaki and colleagues [101,102] (described earlier) is the basis for serologic HLA typing and was the method used for HLA-specific antibody screening. The screening of patient sera pretransplant to identify and characterize HLA-specific antibody involved testing sera against a panel of cells that were representative of the HLA types of a given population. The results of this type of testing were expressed as a panel reactive antibody (PRA) where the presence and specificity of any preexisting antibody could ideally be identified. The extent to which a patient was presensitized, as a result of a previous transplant, transfusion, or pregnancy, could be measured, and potential "unacceptable antigens" could be identified and avoided in future donors. The use of the CDC-based method to identify donor-specific antibodies (DSAs) was beneficial, as the antibodies identified were complement binding (as per definition of the assay used to test them). However, the drawbacks of the assay are

that it is not very sensitive and it requires maintaining a large panel of viable cells. It could also yield positive results due to non-HLA antibodies. It is a difficult test to standardize, and the identification of HLA specificities can be skewed based on panel composition, which is usually somewhat limited based on the HLA types locally available. The other option was commercially available frozen cell trays where

results could be obtained in 1 day, which led to the name of "quick PRA" trays. Ultimately, CDC-based antibody screening fell short in identifying all specificities in highly sensitized patients who might have very complex antibody profiles [103]. Today, antibody identification and characterization are done using a combination of CDC-based methods and SPIs. Antibody identification refers to both the screening of patient sera to identify the presence of anti-HLA antibodies and the pretransplant XM done using the patient's serum against the specific donor's lymphocytes. In contrast to SPIs, the term "cell-based assay" refers to the use of actual donor cells to assess the reactivity of a patient's serum; the cells can be used as part of a screening panel as mentioned earlier, or they can be actual donor cells used for a pretransplant XM, for either a cadaveric or living donor transplant. A recent consensus report [103] has recommended that for antibody identification, laboratories should use at least one SPI to characterize HLA class I and II–specific antibodies and that both SPIs and cell-based assays should be used to assess donor-specific antibody status (i.e., donor crossmatching).

## 8.3.2. The CDC Crossmatch

Early work done by Patel and Terasaki [102] provided convincing evidence that pretransplant XMs were critical in detecting HLA antibodies. Sera from renal allograft recipients were retrospectively crossmatched and 80% (24 of 30) of the kidneys transplanted across a

positive XM were nonfunctional within 2 days, compared with only 4% (8 of 195) for XM-negative pairs (see summary [104]). It was then and still remains clear today that a positive XM is considered a

contraindication to transplantation and regarded as the single best predictor of short-term renal allograft survival.

The CDC XM, as developed in the 1960s [101,105], was and still is fairly straightforward in concept. Donor lymphocytes are isolated and combined with the sera of potential recipient in multiwell microtiter plate. After an incubation period, generally 30 min, complement is added and allowed to incubate for 1 h (or longer); incubations are all done at room temperature. A vital dye is added to the wells, and each well is visualized with a microscope and scored based on the percentage of dead cells in the well (Fig. 8.8). Negative control serum (commercially available human serum from healthy AB blood group donors) and positive control serum (known to strongly react to all HLA types) are always included in the assay to assess cell viability and the addition of functional complement, respectively. Over the years, modifications such as increased incubation times and the addition of wash steps before the addition of complement have increased the sensitivity of the assay. Another major step taken to increase assay sensitivity was the use of anti-human globulin (AHG). Adding AHG prior to the addition of complement enhances the assay by providing more immunoglobulin Fc regions capable of binding the C1q molecule of complement, thereby serving to bridge the gap that may exist if the existing HLA antibodies are too scarce and therefore not of the proper spatial arrangement necessary to bind the C1q molecule alone (Fig. 8.8, middle panel). Today, laboratories that rely solely on the CDC XM for pretransplant testing are strongly encouraged to increase the assay's sensitivity with the addition of AHG [103].

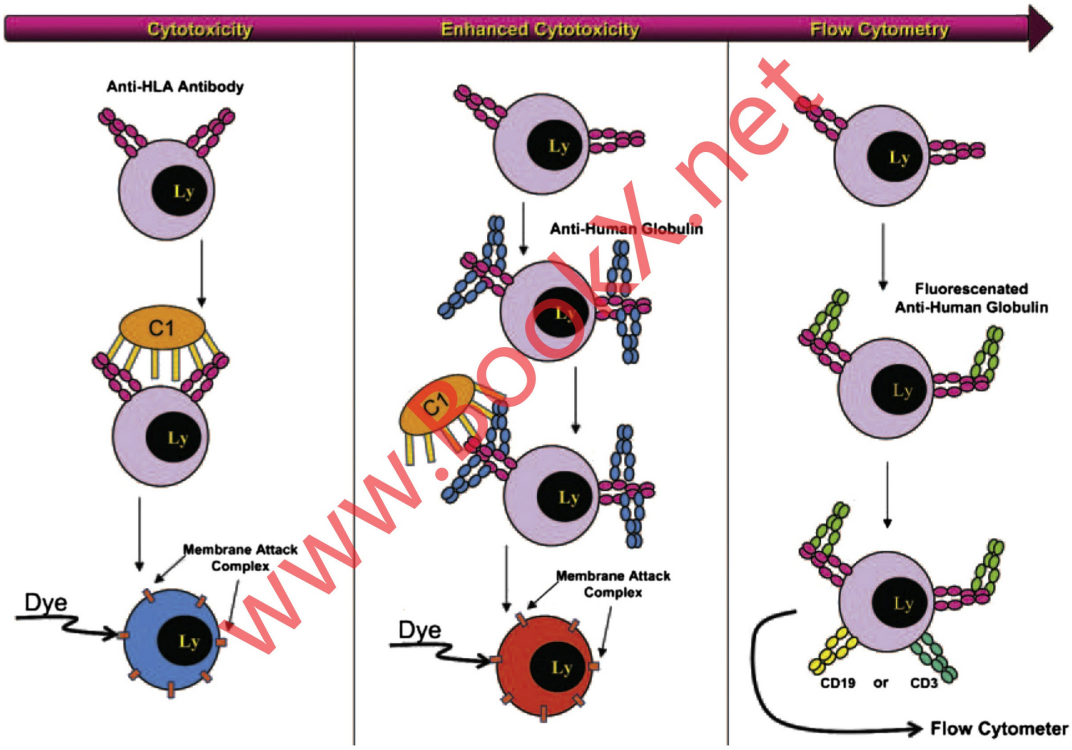



**FIGURE 8.8** Schematic representation of methods used to detect HLA antibodies. Complement-dependent cytotoxicity testing is represented in the first two panels. Antibody alone or via a secondary antibody (anti-human globulin) activates complement. The resulting damage to the lymphocyte membrane is detectable by the uptake of a vital dye. The third panel illustrates the flow cytometric crossmatch, a complement-independent assay that uses a fluorochrome-labeled secondary antibody to detect the presence of the primary anti-HLA antibody.
From Bray RA, Nickerson PW, Kerman RH, Gebel HM. Evolution of HLA antibody detection. Immunologic Research 2004;29(1–3):41–53.

www.book.net

As previously stated, early versions of the CDC XM used total lymphocytes obtained from the donor, and this presented a potential problem because the vast majority of those cells were T lymphocytes. T cells do express class I HLA antigens, however, they do not constitutively express class II antigens. Therefore, if a patient possessed antibody to class II antigens, they could very easily go undetected in the XM using only "total donor lymphocytes." Class II antigens are expressed on APCs such as B cells, monocytes/macrophages, and dendritic cells. Today, magnetic bead separation techniques are commonly used to isolate T cells and class II–expressing cells, which are mostly made up of B lymphocytes

(depending on the isolation bead used and the antibody used to deliver its specificity). Now, many laboratories (including ours) that perform CDC XMs do three versions for pretransplant testing: (1) T-cell XM, (2) T-cell XM using AHG, and (3) B-cell XM. The B-cell XM ensures that any class II antibodies that may exist in the patients' serum will be detected (Fig. 8.8).

The advantages of the CDC XM are that it detects HLA antigens expressed in their natural configuration on the surface of cells and it has the ability to detect antibodies (complement-binding) that can lead to a hyperacute rejection [102]. The major drawback is that the assay is not very sensitive (although the addition of AHG does help). It can also yield false-positive results due to the presence of non-HLA antibodies in the serum, and it does rely heavily on the availability of viable donor lymphocytes. While there are laboratories today that rely solely on the CDC XM for pretransplant screening, many have decided to move to a more sensitive assay to meet this need.

## 8.3.3. The Flow Cytometry Crossmatch

The flow cytometry crossmatch (FCXM), also considered a cell-based assay, uses donor-derived lymphocytes as the target for detecting anti-HLA antibodies. The assay, initially described in 1983 [106], uses the same key ingredients as the CDC XM: patient sera and donor lymphocytes. Patient serum is incubated with donor lymphocytes to allow for any DSAs to bind. The cells are washed, and a fluorescein-labeled anti-human IgG antibody is added, which will detect the presence of any DSAs. Analysis using the flow cytometer allows for the detection and the quantification of any DSAs present in the patient's serum (Fig. 8.8). The amount of DSAs present is measured in terms of fluorescence intensity, and how that level compares with the control is expressed as the channel shift; the greater the channel shift above the negative control, the greater is the amount of DSAs present in the serum sample. The assay was originally developed using a single color, but today it exists in a three-color configuration. One color (FITC) is used to detect the anti-HLA human IgG, and two additional colors re used for specific staining of T and B lymphocytes

[107,108]. This allows for the simultaneous detection of DSAs and the specific target to which they are binding. The amount of anti–class I (binding to T cells and B cells) and anti–class II antibody (binding only to B cells) can be determined. Modifications of the assay include using a different detection antibody that will allow for distinguishing between immunoglobulin classes and subclasses: are the DSAs of the IgG or IgM isotype and then, specifically, to which subtype of IgG do they belong? This will determine whether the DSAs will be likely to bind complement.

As with the CDC XM, the FCXM will also detect reactions caused by non-HLA antibodies, but overall it is markedly more sensitive than the CDC XM and it has shown its usefulness in identifying patients with weak DSAs who have an increased risk of antibody-mediated rejection (AMR) and graft rejection [109]. The FCXM and, in particular, the B-cell XM are susceptible to background antibody binding, which can lead to a false-positive result. This can be reduced greatly by incubating the donor lymphocytes with Pronase [110]. However, treating cells with Pronase can possibly lead to a false-positive T- cell XM due to its effect on HLA expression [111].

All flow cytometry assays can be difficult to standardize due to differences in instruments, antibodies, and fluorochromes and, as summarized by Tait et al. [103], each center must validate their FCXM result with respect to the clinical risk. The interpretation of a positive FCXM result is controversial; it provides a vital piece of information that must be considered in the context of each patient's current level of sensitivity, but many believe that it represents only a risk, not necessarily a clear contraindication to transplantation.

## 8.3.4. The Virtual Crossmatch

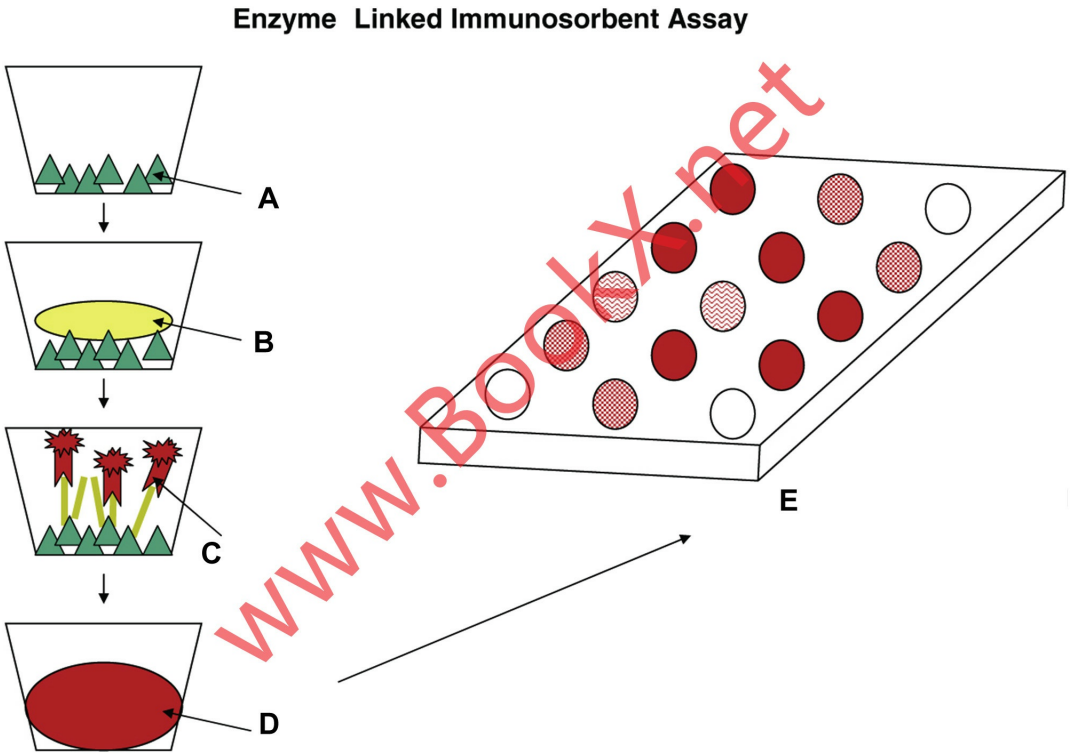
The virtual XM (VXM) is done when the unacceptable antigens identified in the patient are compared with the known HLA type of a potential donor. Thus, the test relies heavily on the laboratory's ability to identify correctly unacceptable antigens as part of the patient's pretransplant assessment. Characterizing antibody reactivity to HLA antigens and the subsequent identification of unacceptable antigens is

a task that has become more critical in recent years. A donor can be eliminated from consideration for a specific patient based solely on the unacceptable antigens and the VXM. As more unacceptable antigens are identified for a patient, the less likely it is that an organ will be offered and more likely that a patient can die on the waitlist before being transplanted. Over the years, the identification of a patient's unacceptable antigens has become a greater factor in determining whether a specific donor is used for transplant; therefore, the correct identification of these antigens is critical.

### 8.3.5. Solid-Phase Immunoassays

The ability to successfully identify and correctly characterize HLA antibodies in the patients' sera is due in large part to the development of SPIs. As mentioned previously, SPIs represent the most sensitive of the techniques for identifying HLA antibodies, and it is strongly recommended that at least one SPI be used to detect and characterize HLA class I and II–specific antibodies [103].

An SPI consists of solubilized HLA molecules bound to a solid matrix. The matrix can be either a microtiter plate (enzyme-linked immunosorbent assay [ELISA]) or polystyrene bead (flow beads or Luminex beads). The ELISA, introduced for HLA antibody screening in the early 1990s (see summary [112]), preceded the bead-based assays. HLA molecules purified from platelet pools (class I specificities) or lymphoblastoid cell lines (class II specificities) are bound to wells of an ELISA plate. Patient serum is added to the wells and any HLA antibody present will bind to the specific HLA molecule. An enzyme-conjugated anti-human Ig antibody is added, followed by the enzyme substrate resulting in a color change that can be measured by an optical density plate reader (Fig. 8.9). It is an objective, semiquantitative assay, normally used to detect IgG antibodies, but it can be adjusted to detect antibodies of a different isotype. It is more sensitive than the CDC method, and it can also detect non–complement fixing HLA antibodies that can be relevant to transplant outcome [112].




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**FIGURE 8.9** Enzyme-linked immunosorbent assay. (A) The wells of a microtiter tray are coated with purified HLA molecules. (B) Serum is added and incubated to allow antibody to bind to the HLA molecules. (C) After washing, an enzyme-conjugated anti-human immunoglobulin is added. (D) After further incubation and washing, the enzyme substrate is added. If antibody from the serum is bound on to the HLA molecules, then that will cause binding of the enzyme conjugated antibody and the presence of the enzyme will cause the substrate to change color. (E) The color change is recorded as optical density using an ELISA reader.
From Fuggle SV, Martin S. Tools for human leukocyte antigen antibody detection and their application to transplanting sensitized patients. Transplantation 2008;86:384–90.

Bead-based assays use polystyrene beads that have been coated with purified class I or class II HLA antigens. The beads are also impregnated with different ratios of two fluorescent dyes, providing a signature for each bead that can theoretically yield up to 500 distinguishable bead populations. Patient serum samples are combined with the beads, and the amount of bound antibody is measured using a PE-labeled anti-human IgG antibody (reporter signal). Using a dual laser instrument, each bead is interrogated with one laser used to identify the specific bead population while the

second laser excites the PE-labeled antibody. The bead array assay is analyzed on the Luminex platform and is semiquantitative. The amount of HLA antibody bound to each bead is expressed as the mean fluorescence intensity of the reporter signal [103] (Fig. 8.10).

Bead panels are available in three different formats, based on the composition of HLA antigens on their surface: (1) screening panels coated with affinity purified HLA class I or class II molecules obtained from multiple individual cell lines, (2) phenotype panels where each bead population is coated with either class I or class II proteins of a cell line derived from a single individual, and (3) single-antigen beads (SABs) where each bead population is coated with a recombinant HLA protein derived from a single cloned allelic class I or class II antigen. Screening panels are less expensive and they are used to identify the presence or absence of antibody to a particular HLA class, but they do not provide accurate specificity nor do they necessarily cover all possible antigens. SAB panels are the most sensitive and specific, providing the highest degree of antibody resolution. This allows for the identification of antibody specificities in highly sensitized patients [113] and for the detection and characterization of DSAs posttransplantation [114]. The SAB assay has technical limitations including positive results caused by antibodies binding to epitopes expressed on denatured HLA molecules, high background binding requiring repeat testing, and false-negative or inaccurate low assessment of antibody levels due to blocking factors that may be present in the sera [103].

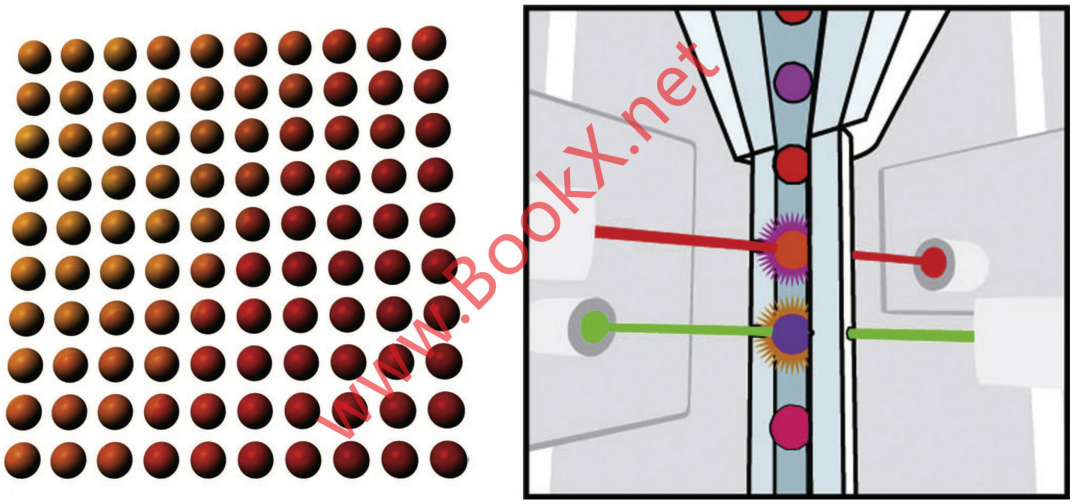


**FIGURE 8.10** Image represents bead regions dyed with 10 concentrations of each of two classification flurophores – to produce 100 distinct bead regions. Color-coding enables each microsphere set to be classified individually and to be multiplexed with other microsphere sets. Once inside the instrument, fluidics cause the microspheres to line up single file as they pass by two lasers – a red laser to classify each color-coded microsphere to determine which assay is carried on that particle, and the green laser to measure the assay result on its surface. If a reaction has occurred, it can be precisely quantified by the presence and abundance of the reporter tag.
From Fuggle SV, Martin S. Tools for human leukocyte antigen antibody detection and their application to transplanting sensitized patients. Transplantation 2008;86:384–90.

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Modifications to the SAB assay can be made to assess the functionality of the HLA antibodies detected. One such example is the C1q assay, designed to distinguish complement fixing from noncomplement fixing antibodies; it does not require complement activation, only the ability to bind to the C1q molecule [115]. The assay is highly sensitive, detecting more IgG antibodies than those detected by CDC, but it will also detect complement fixing IgM antibodies.

Standardization of reagents, existing technical limitations, and variability in result interpretation represent some of the obstacles preventing SPIs from becoming the gold standard for antibody characterization. However, with the recent progress made in the development of these assays, and the call for future collaboration between laboratories on test methods and results, the SPI and, in

particular, the bead-based assays represent the best hope for the most accurate characterization of HLA antibodies.

## 8.4. Clinical Significance of HLA Molecular Typing

A possible criticism of DNA typing is that it gives "too much" information. It has been hypothesized that even one nucleotide difference, and hence one amino acid disparity, can result in increased risk of alloreactivity in vivo [116], even if many nucleotide substitutions do not cause amino acid changes. Given the huge number of detectable alleles at the various loci, it would be important to determine which mismatches are allowable and which ones are instead nonpermissive for a positive clinical outcome.

A number of studies have been done to determine the clinical impact of HLA matching in unrelated BMTs. Many reports have shown that the incidences of both acute GVHD and rejection are significantly higher among patients transplanted with phenotypically matched bone marrow cells from unrelated donors compared with cells from genotypically identical siblings [117,118], in particular when only serologic techniques were used for *HLA* typing [119]. Further, the incidence and severity tend to increase with increasing HLA mismatch, except in very young patients, in whom a one–*HLA* locus mismatch can be tolerated.

Patients with acute myeloid leukemia can be favorably treated by BMT when paired with mismatched killer Ig-like receptors (KIRs) and one *HLA* class I allele [120–122]. Occurring in haploidentical BMT, the phenomena is believed to result from a failure to suppress NK cell killing when KIR–HLA class I mismatches occur [122]. Reduced incidence of leukemia relapse results from NK cell cytokine–induced killing of recipient patient tumor cells and is regulated by the reduced KIR–HLA class I interaction between NK cells and mismatched target cells. In these cases, only one of the donor HLA–carrying chromosomes is matched with the recipient, resulting from situations in which either parents or siblings matched at only one chromosome

provided donor marrow. Under these conditions, a KIR–HLA class I mismatch will present and a fraction of donor NK cell will express KIRs not able to engage the *HLA* class I alleles of the recipient, thus having the potential to kill the patient's leukemic cells [122,123]. Mismatched NK cells do not cause GVHD, possibly because nonhematopoietic tissues may not express NK receptor activating ligands.

Bone marrow transplant is a case apart in general tissue transplantation because drastic immunosuppressive regimens cannot be applied as they are for any other type of organ to be transplanted. Immunosuppressive therapy is necessary to prevent graft rejection even when donor and recipient are HLA-identical siblings. It is the effectiveness of the immunosuppressive therapy that permits the survival, often for many years, of allogeneic grafts, many of which are poorly HLA matched. There is an abundance of data that show little or no difference between well-matched and poorly matched grafts as measured by 1-year survival [124]. These considerations, added to the need for transplantation in a relatively short amount of time for the most severely diseased patients and to the limited number of donor organs available, have led many to propose that organ allocation should be governed primarily by criteria other than HLA matching [125]. This discussion was particularly contentious in those days [126,127]. However, over a longer time of transplanted organ survival, HLA matching of donors and recipients may actually have a relevant effect. In 1992, Terasaki and colleagues [128] developed logarithmic plots predicting 10-year rates of graft survival and rates of loss in organ half-life. They compared these predictions with actual graft survival of transplants done from 1975 to 1980. Their half-life actuarial estimate of 16,320 first cadaveric kidney grafts from the United Network for Organ Sharing (UNOS) registry was 10.3 years. From the 1975–80 actual 10-year survival data, they calculated a half-life of 24.0 years when the donor was an HLA-identical sibling, 11.8 with a parental donor, and 7.9 with a cadaveric donor. When the cadaveric

donor grafts were evaluated by HLA matching, the half-life was 19.7 for no *-A, -B, -DR* mismatch (or six-antigen match) and 7.5 years for all mismatches. The estimated half-life was similar though for one, two, three, four, and five mismatches. All other variables analyzed did not make any difference, including race, sex, original disease, and other nonhistocompatibility factors. Based on their conclusion that "HLA matching is the single most important factor that can improve nationwide 10-year graft survival rates," the equitable system of organ allocation approved by UNOS was the following: the priority system adopted for nonrenal patients included the length of time a patient has been on the waitlist, the severity of his or her illness, and the proximity of the available organ to the patient. For kidney allocation, the degree of HLA matching and the degree of HLA presensitization (i.e., PRA values or presence of DSAs) were added to the above criteria [129]. UNOS has also mandated national sharing of perfect HLA matches, irrespective of other criteria. The probability for a kidney patient to find a perfect match anywhere is, however, no greater than 5%.

The cumulative results from various European transplant centers seem to confirm the appropriateness of these criteria [130,131]. Starzl, Terasaki, and collaborators [132], using updated results obtained from UNOS registry, also proved the importance of HLA perfect matching for kidney transplants, although the negative effect of mismatches seems to be the same for more than one or all the six alleles and limited to a small percentage, even in a 10-year survival analysis (Fig. 8.11). This is the reason why all kidney patients are still typed for HLA and ABO blood groups prior to entry on the waitlist and their sera are screened for anti-HLA antibodies.

With the advent of DNA methodologies for HLA typing, unrelated donors who appeared HLA identical by serology were indeed not matched at the DNA level and truly matched pairs survived much longer. Studies using DNA methods for both class I and class II [133] typing have shown that molecular matching may reduce the risks of acute GVHD and patient mortality.

### 8.4.1. Epitope-Based Matching

The results of higher-resolution molecular typing, done to the allele level, can also be beneficial when used in an epitope-based matching system. One of the earliest examples of this type of matching was developed by Duquesnoy [134]. The HLA Matchmaker program considers the structural basis of epitopes on class I HLA antigens. The original program was based on two principles: each HLA antigen represents a distinct array of polymorphic amino acid triplets as potential immunogenic epitopes, and sensitized patients cannot produce alloantibodies specific for triplet-encoded determinants as they are expressed on their own HLA molecules [135]. It assumes that these self-triplets are safe, and it determines for each mismatched HLA antigen which triplets in corresponding sequence positions are different, and thus vulnerable to alloantibody assault. The number of amino acid triplet differences between the patient and donor correlates with the emergence of anti-HLA antibody triggered by a sensitizing event, such as pregnancy or kidney transplant [136]. The original program considered polymorphic triplets only in sequence positions that would be accessible by antibody (citing 132 polymorphic triplets in 40 sequence positions contained in the serologically defined class I antigens) [137]. Later versions of the program use the term "eplet," in place of "triplet," referring to a determinant that is spatially defined as opposed to being limited to a linear sequence. In contrast to a triplet, an eplet can contain amino acids that are not continuous in sequence but are clustered together as a result of the protein conformation [138] (Fig. 8.12). Today, antibodies are defined by the epitopes to which they bind, and these epitopes can be further defined by the distinct eplets they possess. The binding of antibodies to HLA antigens is based on their reactivity to the unique set of epitopes contained in that specific antigen [138]. Just as every HLA antigen contains a unique set of epitopes, it is also clear that many of these epitopes can be shared with other HLA antigens. This explains the cross-reactive nature of many HLA antibodies and how a patient can become sensitized to many HLA antigens after being exposed to only one foreign antigen. Sera from high PRA patients do

not necessarily react with a large number of epitopes; instead they may react with a limited number of high-frequency epitopes, present on a large number of HLA antigens [139]. Simply put, a program such as HLA Matchmaker makes its predictions based on the structural similarities of the epitopes contained in the HLA antigens of the patient and potential donor(s). It calculates to what degree potential donor antigens will or will not stimulate an alloantibody response based on their epitope repertoire.

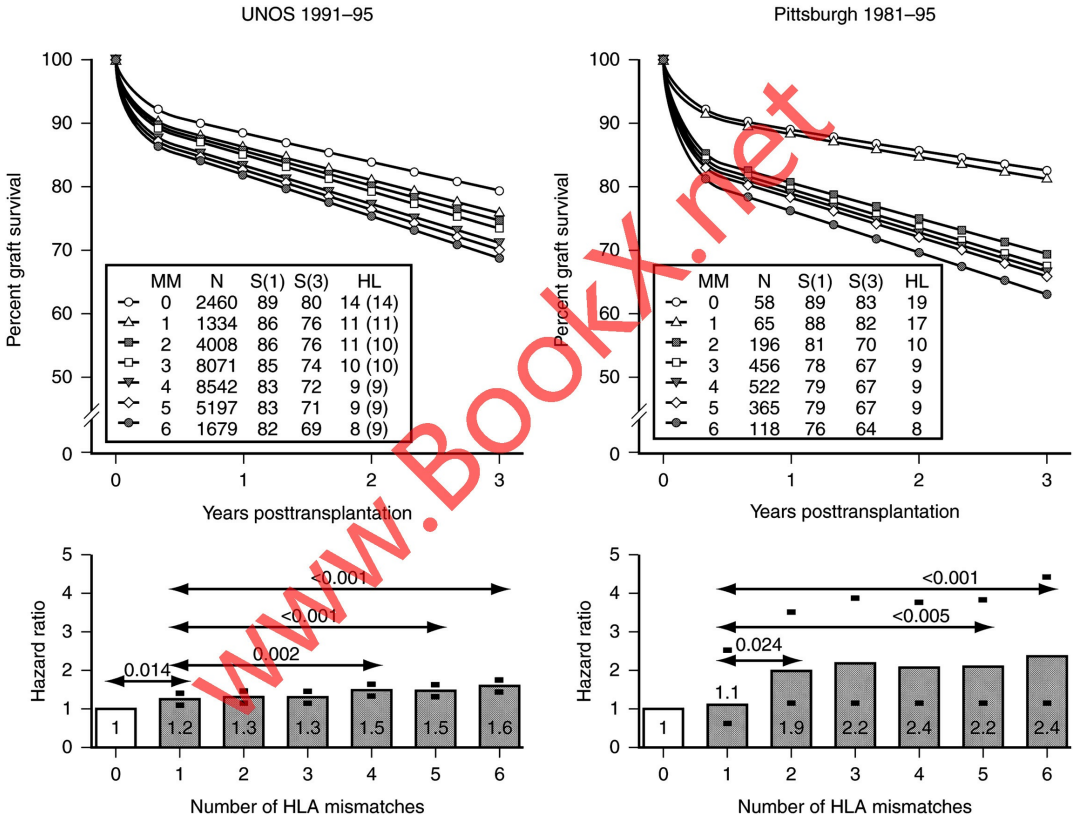



**FIGURE 8.11** Survival percentages of kidney transplants subdivided by 0–6 HLA antigen mismatches (MM).
From Starzl TE, Eliasziw M, Gjertson D, et al. HLA and cross-reactive antigen group (CREG) matching for cadaver kidney allocation. Transplantation 1997;64:983–87.

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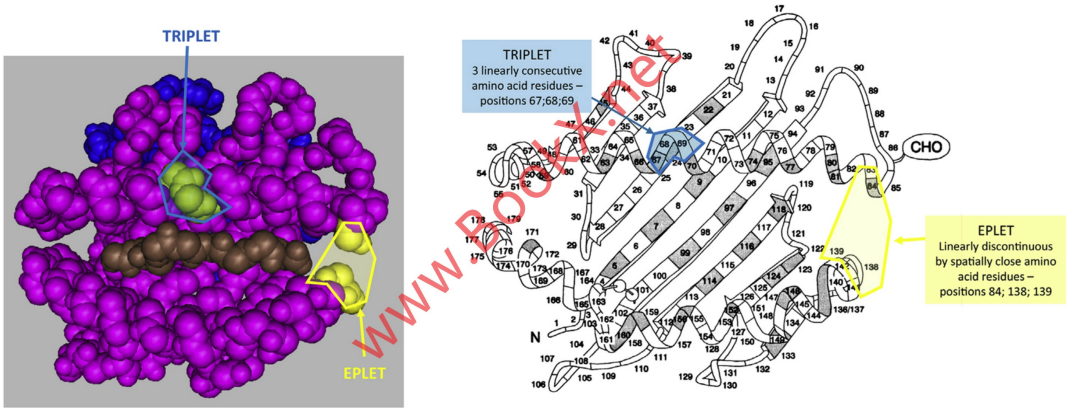



**FIGURE 8.12** Depiction of the principle difference between a triplet and an eplet.
From Tambur AR, Claas FHJ. HLA epitopes as viewed by antibodies: what is it all about? American Journal of Transplantation 2015;15:1148–54.

Discussions regarding the clinical utility of this type of program can be found in multiple reviews [104,138,139], and in Duquesnoy review [140], he highlights two points that can be taken from these studies. First, for each transplant, the eplet load (eplet mismatches) can be determined easily using the special matching programs that can be found on the HLA Matchmaker website (www.HLAmatchmaker.net). The level of mismatched epitope load can be considered a risk factor during posttransplant monitoring for AMR. Second, eplet loads can be useful in developing new donor selection strategies for nonsensitized recipients, especially younger patients. HLA mismatches with low eplet loads can be expected to improve transplant outcome, and even if the first allograft is rejected, retransplant candidates may be less highly sensitized, thus making it easier to find acceptable mismatches going forward.

In order to maximize the effectiveness of this type of predictive matching, high-resolution typing done at the allele level is required. Because multiple alleles of the same HLA antigen will contain different epitope repertories, high-resolution typing is needed to identify these alleles that simply cannot be distinguished when typing is done at the low-resolution antigen level. These differences can have many implications [141], but one example illustrates a simple point. A patient typed at the allele level as *A*24:03* may possess antibodies that

will bind to the "166DG" epitope, which is contained in the *A*24:02* allele. If low resolution typing at the antigen level is done, A24 would be incorrectly considered a match but not so if high-resolution typing is used.

As our populations become more ethnically and racially diverse, the number of different alleles seen within each antigen will increase. Thus the traditional approach of antigen-based matching may suffice for transplant programs that allocate organs from donors in racially and ethnically similar pools, as it seems more likely that a given HLA antigen is equivalent to one allele. However, that assumption becomes more risky as our population diversifies and high-resolution allele-based typing becomes even more crucial for successful transplant outcomes in both highly sensitized and nonsensitized patients.

The likelihood of finding a matched donor in the registries also depends on whether the patient has a common or rare HLA genotype. Not only are some alleles more common or rare within a particular ethnic or racial group, but because of linkage disequilibrium, certain alleles can give rise to different haplotypes within different populations. The NMDP is making an extraordinary effort to enroll in its registry minority groups with different HLA molecular types so to increase the likelihood that minority patients requiring any type of transplant, particularly kidney or bone marrow, can find a more suitable HLA-matched donor.

Even considering all the possible genotypically identical siblings as donors and the large number of potential donors enrolled in the NMDP registry, a substantial number of patients still will not be able to find an HLA-matched donor. Also, as the discovery of new loci and alleles that potentially must be matched increases, the odds of finding a complete match will decreases even further. Therefore, the most important charge for those involved in transplantation is a more precise definition of HLA mismatches that are clinically permissible versus those that are functionally significant with respect to rejection, GVHD, and overall patient mortality. This would be analogous to the epitope-matching protocols described earlier.

The transplantation of organs other than bone marrow or kidney

offers, however, a completely different scenario inasmuch as the patients are routinely transplanted on the basis of their serious clinical conditions that impose this type of intervention to be performed as soon as an organ becomes available. The HLA typing is generally performed a posteriori, and the presence of DSAs in the recipient is used only to modify the immunosuppressive regimen. To successfully transplant organs like the liver across the histocompatibility barriers seems due to the intrinsic characteristics of such a large human gland [142]. First, the liver is able to absorb a large quantity of antibodies without having its function impaired; second, the quantity of immunocompetent cells present in the liver at the moment of the transplant is considered, by some, to be the possible reason for the liver's tolerogenic power. Under the protective umbrella of immunosuppression, immunocompetent stem cells present in the liver are able to migrate into the recipient body, where they are not strongly confronted by a drug-impaired immune system of the recipient. In this peculiar context, the immune system of the recipient may become "accustomed" to the presence of immunocompetent cells of the donor so that both systems harmoniously survive in the transplanted individual. This exceptional situation known as "immunologic chimerism" can offer the invaluable advantage of generating a donor-specific type of tolerance in the recipient that ipso facto becomes able to host the foreign tissue without the need of a massive and, in general, particularly dangerous immunosuppressive regimen. These types of considerations were actually supported by the finding of immunocompetent cells of donor origin in the tissues (e.g., skin) or in the blood of patients who received a liver transplant

30 years earlier [124,143,144]. In different proportions, a certain degree of immunologic chimerism, or a "microchimerism," was also found in kidney and heart transplant patients years after transplantation [145] (Fig. 8.13). The enthusiasm produced by these incontrovertible findings promoted the implementation of therapeutic protocols aimed at favoring the establishment of immunologic chimerism. One protocol suggested the augmentation of

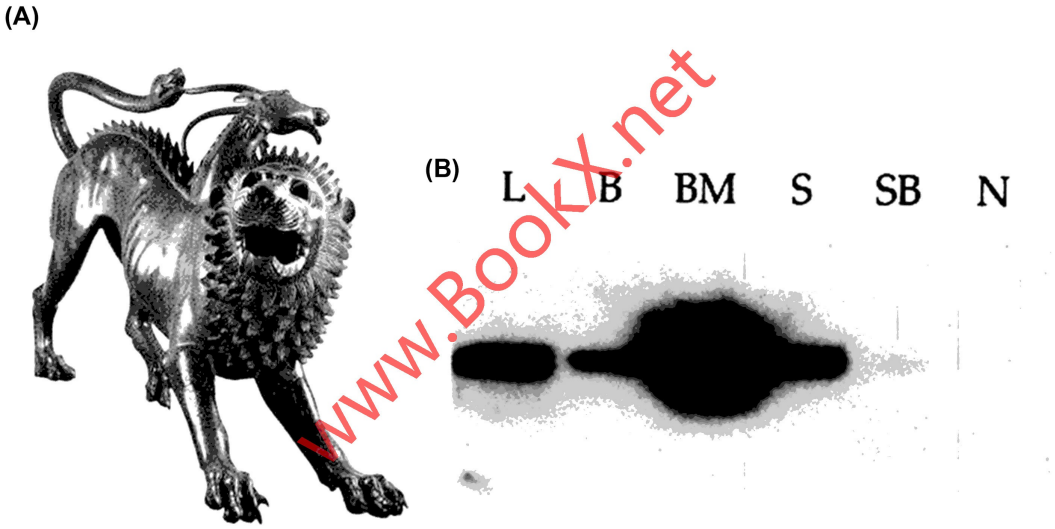
immunocompetent cells obtainable by cotransplanting the bone marrow cells from the same donor with a solid organ not generally rich in these cells (e.g., the heart or the kidney) [146]. Although this therapeutic intervention did not cause any appreciable harm to the recipient, the benefits associated with the procedure were not easy to define in terms of graft survival. Quality of cells, quantity of cells, time of administration, and prolongation of chimeric status were all considered variables that supported the impression of improved results but did not offer any unquestionable and tangible parameters to quantify this sense of improvement [147–151].

## 8.5. Stem Cells and Transplantation

Although all of these approaches are designed to bypass the limitations imposed by different genetic profiles, a rather new and somewhat revolutionary alternative has been offered: Stem cells can be derived from an embryo or found in different tissues of an individual and are able to replace differentiated cells of different organs.

A stem cell is, by definition, the one cell capable of duplicating itself and resuming its undifferentiated status, while also originating progeny that can differentiate into one or more final products that are physiologically defined by their specific functions [152,153]. Proceeding through the differentiation pathway, stem cells can be categorized as totipotent, pluripotent, multipotent, oligopotent, and unipotent, depending on all their possibly reversible, progressively acquired characteristics [154]. Embryonic stem (ES) cells are pluripotent cell lines derived from the inner cell mass of blastocyst-stage embryos. ES cells differentiating in culture may reproduce features characteristic of early embryonic development. The ability to clone human embryos and to derive from them human ES cell lines is a reality [155–158]. Even imagining that tomorrow's scientists could derive, by cloning, cell lines able to be differentiated toward cells with a desired phenotype and in sufficient number to satisfy the needs of the transplant recipient, to use them for clinical purposes they should be carrying the diploid genome of somatic cell nuclei derived from

individuals unrelated to the oocyte donor (i.e., the recipients). Even so, we would still need to isolate the broadest diversity of HLA polymorphisms, to more easily find a match between donor and recipient so to avoid the serious problem of allorejection. In the case in which we opt to avoid all this demanding preparative effort for matching donor and recipient, it would be necessary to reduce the likelihood of allorejection by other means [152,153]. However, in the case of ES cells, it may be easier to achieve this goal because the already studied human ES cell lines seem to be able to down-regulate the expression of MHC antigens at their surface [159,160]. Rapidly dividing cells with this unique phenotype have to spontaneously stop growing once a specific, predetermined, total population cell mass has been reached, as they seem not even normally controlled by NK cells. In the case in which the ES cells do not stop proliferating, we will transplant cancer precursors into our patients. Furthermore, even assuming that we could overcome all these challenges, the use of human ES cell lines tailored ad personam would constitute an extremely demanding and expensive proposition [153,161]. If ethical considerations will not block this type of experimentation, the stem cell avenue seems to be the most promising and exciting avenue for autologous, as well as allogeneic, transplantations.



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**FIGURE 8.13** Artistic representation of a chimera from Greek mythology and evidence of an immunologic chimera in a patient with

Gaucher disease. In (A), Khimaira, also spelled in the Latin “Chimera,” was a powerful beast with the head of a male lion, the body of a goat, and, as a tail, a serpent. The legend states that she was slain by Bellerophon with a golden arrow, while he rode on the back of Pegasus, a horse with wings. The Chimera of Arezzo is conserved at the Archaeologic Museum in Florence, Italy. In (B), genomic DNA was extracted from the recipient’s tissues and amplified with *HLA-DR* β-chain “generic” oligonucleotide primers to determine the subgroup of the donor’s alleles. “Specific” primers were then used to amplify the alleles selectively. The alleles were identified by hybridizing the amplified DNA to radiolabeled allele-specific probes. *HLA-DR1* was present only in the donor. After *HLA-DR1*–specific amplification of DNA from the liver (L), blood (B), bone marrow (BM), skin (S), and small bowel (SB) of the recipient, the DNA was separated by electrophoresis on an agarose gel and then analyzed by Southern blotting. The denatured DNA present on the nylon membrane was hybridized to a labeled *HLA-DR1* (donor)-specific oligonucleotide probe. For liver DNA, the quantity analyzed was reduced to 1% of the other samples. Although lower in intensity, the signal from donor DNA in the small bowel was clearly positive in the original film, although this can be seen here only faintly. The negative control was a reaction run without DNA

(last lane; N = negative control).

From Starzl TE, Demetris AJ, Trucco M, et al. Chimerism after liver transplantation for type IV glycogen storage disease and type I Gaucher’s disease. N Engl J Med 1993;328:745–49.

The use of “adult” stem cells, that is, precursor cells present in the tissue of already completely formed individuals, may be more acceptable ethically. Years ago, Mulligan and colleagues [162] were able to show how stem cells isolated from the bone marrow were able to replace malfunctioning myocytes in a mouse model of Duchenne muscular dystrophy. This approach envisions isolating in vitro adult pluripotent stem cells from any manageable source (e.g., the bone marrow) and physically introducing these cells into the already existing, appropriate environment of living recipients. This assumes that the signals sent via host-secreted factors or via cell-to-cell contacts are powerful enough to guide the transplanted precursors to differentiate into the same type of cells surrounding them, even across different lineages. By receiving spatially and temporally restricted signals from the environment, the precursors may differentiate into the cells constitutive of that specific, target tissue. Like hematopoietic

stem cells (HSCs), once transduced with a retrovirus to express bone morphogenetic protein 4 (BMP4), muscle-derived cell (MDC) precursors dramatically improve the healing of a spontaneously irreparable bone fracture [163].

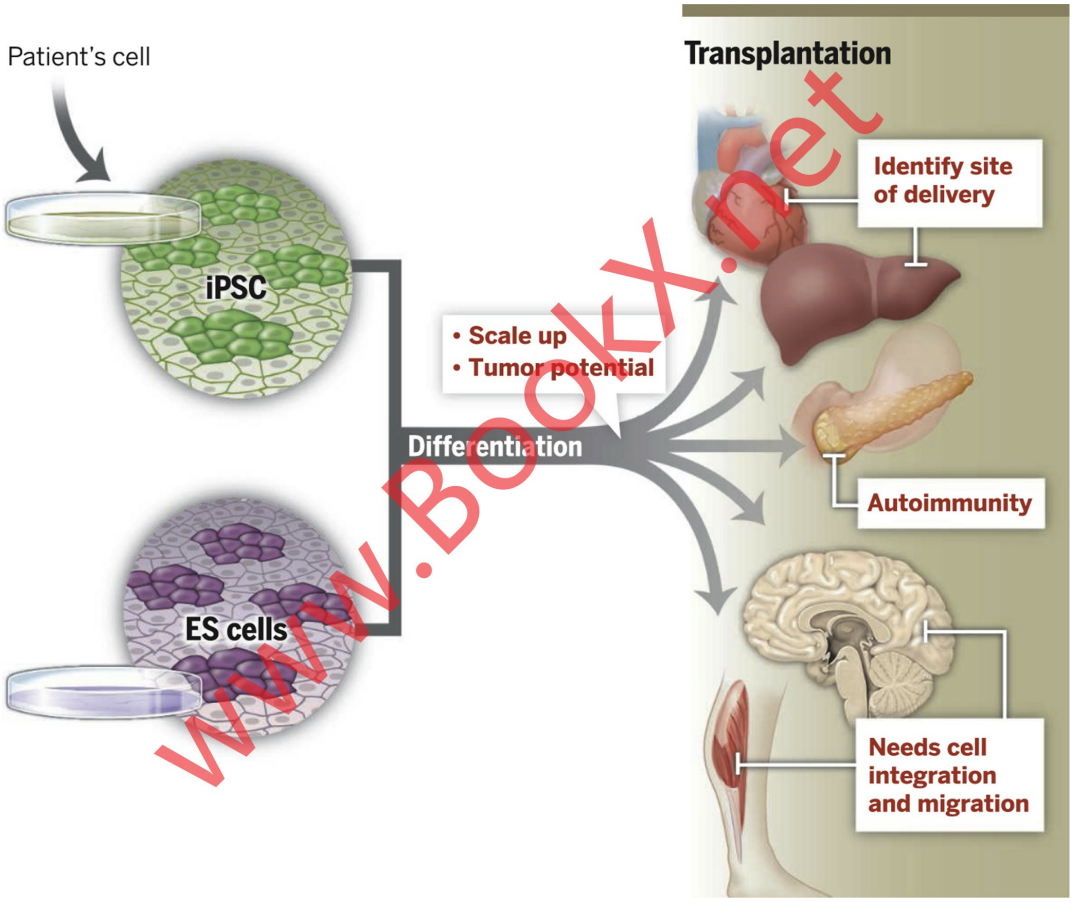
Taken together, these research reports suggest an abundance of promising opportunities for BMT in the treatment of certain chronic diseases. However, for the moment the only successfully tested clinical exploitation of adult stem cells is the one taking advantage of CD34+ cells of the bone marrow. In the most recent years, different groups established that 1%–3% of cells in the bone marrow are capable of long-term multilineage hematopoietic reconstitution after myeloablation [164]. These cells can be mobilized from the marrow to the peripheral circulation in clinically usable numbers, by the use of recombinant hematopoietic cytokines, like the granulocyte colony-stimulating factor. Once mobilized, these cells can be isolated from the peripheral blood by cell sorting using anti-CD34 antibodies. CD34+ cells isolated from the peripheral blood have a number of advantages compared with whole bone marrow for transplantation: they are potentially less tumor contaminated than autologous marrow when used for autologous transplantation; their time for engraftment is shorter; the isolated CD34+ cells are more amenable to ex vivo manipulations such as gene therapy; and even when used in the allogeneic setting, they eliminate acute GVHD and significantly reduced the incidence and severity of chronic GVHD. Unfortunately, these advantages are counterbalanced by the missing positive roles played by graft T cells able to facilitate engraftment, eliminate residual leukemic cells, and transfer of donor immune memory [165].

In 2006, the creation of induced pluripotent stem (iPS) cells was described [166]. Derived from mouse adult somatic cells by the ectopic expression of the transcription factors Oct3/4 (Pou5f1), Sox2, Klf4, and c-Myc using viral as well as plasmid-based vectors, they are sufficient to direct the reprograming of adult somatic cells. During the following year, iPS cells were generated from human somatic cells [167] as a result of the combined expression of Oct3/4, Sox2, Klf4, and c-Myc and independently with the expression of Oct3/4, Soc2, Nanog, and Lin28.

The importance of iPS cells to transplantation medicine occurs as a result of the cells being derived entirely from the recipient. Thus, these cells bypass transplantation complications such as GVHD and immune rejection. A recognized danger associated with the method for preparing iPS cells is the risk of inducing the expression of oncogenes, such as *c-Myc*, as well as the possible incorporation of transcription factors into unfavorable sites within the genomic DNA with the potential to induce coexpression of neighboring oncogenes. In 2009, Rudolf Jaenisch and coworkers reported the development of protocols for removing oncogenes after the induction of pluripotency [168], providing a possible solution to this complication. Moreover, iPS cells could be induced without alteration of genomic DNA [169]. This was accomplished by repeated treatment with proteins Oct3/4, Sox2, Klf4, and c-Myc modified with poly-arginine anchors to generate what has been termed protein-induced pluripotent stem cells. In the not too distant future, the use of iPS cells may circumvent the problem of stem cell–derived allotransplants (e.g., limiting material and immune-mediated rejection) making them equivalent to autotransplants, while also minimizing the risk of exposing the recipient to an uncontrolled neoplastic transformation of the cells (Fig. 8.14).

## 8.6. Concluding Remarks

The data presented clearly show that HLA matching does not and cannot influence solid organ distribution significantly. However, these analyses support the wisdom of the UNOS board of directors to mandate national sharing of the zero serologically mismatched HLA cadaveric donor kidneys in the rare cases where they occur.




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**FIGURE 8.14** Unlimited population of differentiated pluripotent stem cells (PSCs) should facilitate blood therapies and hematopoietic stem cell transplantation, as well as the treatment of heart, pancreas, liver, muscle, and neurologic disorders. However, successful stem cell transplantation will require optimizing the best cell type and site for engraftment, overcoming limitations to cell migration and tissue integration, and possibly needing to control immunologic reactivity (challenges indicated in red). iPSC, induced PSC; ES cells, embryonic stem cells.
From Fox IJ, Daley GQ, Goldman, et al. Use of differentiated pluripotent stem cells in replacement therapy for treating disease. Science 2014;345:889.

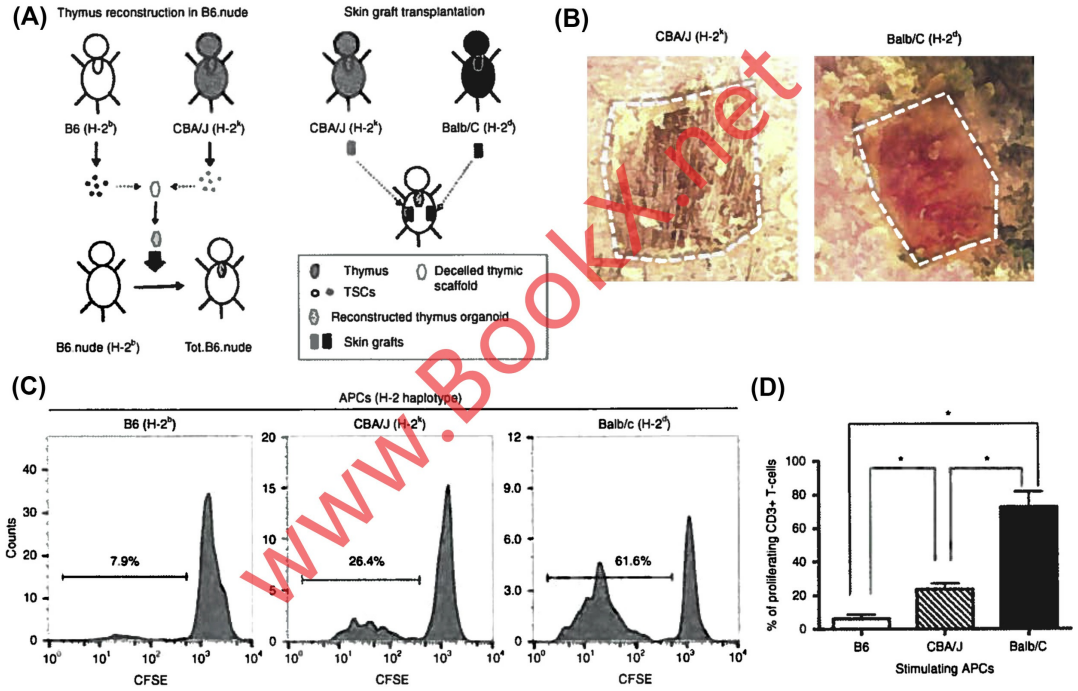
HLA matching for other solid organ grafts is even more problematic because the patients needing livers or thoracic organs are generally critically ill and cannot tolerate extensive waiting periods. The recent success in transplanting a single lobe of the organ from alive or cadaveric donors into one or more recipients, respectively, may expand these limited chances.

On average, only about 30% of candidates for BMT will have an

HLA-identical sibling or other suitable related donor. Leukemia is the most common diagnosis of BMT potential recipients. Survival and cure rates for acute nonlymphocytic leukemia patients are almost as great after autologous BMT as for HLA-identical sibling BMT. This is not the case for acute lymphocytic leukemia patients, however. Unfortunately, those patients with uncommon HLA phenotypes will not survive a lengthy search for an unrelated donor. At the present time, few chronic lymphocytic leukemia patients are considered candidates for BMT; therefore, the NMDP and other registries of unrelated donors benefit primarily patients with chronic myelogenous leukemia. Thus far, the outcome of unrelated transplants for chronic myelogenous leukemia has been poor because of HLA mismatching not detected by serologic and cellular assays. It is anticipated that improvement will be seen now that typing by DNA-based methods for class II are in place. Once this is accomplished, the next major effort for the future will be to identify which minor differences between variants can be tolerated with little or no risk of rejection or GVHD, or which tolerogenic protocol can offer safe but effective means to allow transplants across HLA boundaries or even across different species without the need for massive immunosuppression.

Although immunosuppressive drugs can prevent graft rejection to a certain degree, their efficacies are limited, transient, and associated with severe side effects. A possible solution could be the ability to establish immune tolerance of allografts. Induction of thymic central tolerance to allografts remains challenging, largely because of the difficulty of maintaining donor thymic epithelial cells in vitro to allow successful bioengineering. However, three-dimensional scaffolds generated from a decellularized thymus can support thymic epithelial cell survival in culture and maintain their unique molecular properties. When transplanted into athymic nude mice, the bioengineered thymus organoids effectively promoted homing of lymphocyte progenitors and supported thymopoiesis. Nude mice transplanted with thymus organoids promptly rejected skin allografts and were able to mount antigen-specific humoral responses against ovalbumin on immunization. Notably, tolerance to skin allografts was

achieved by transplanting thymus organoids constructed with either thymic epithelial cells coexpressing both syngeneic and allogenic MHCs, or mixtures of donor and recipient thymic epithelial cells (Fig. 8.15). These results demonstrate the technical feasibility of restoring thymic function with bioengineered thymus organoids and highlight the clinical implications of this thymus reconstruction technique in organ transplantation and regenerative medicine [170].




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**FIGURE 8.15** Induction of donor-specific immune tolerance with thymus organoids reconstructed of both donor and recipient thymic epithelial cells (TECs). (A) The schematic drawing shows the strategy of the experiment. TECs were isolated from B6 and CBA/J mice and mixed at 1:1 ratio. The thymus organoids were reconstructed with B6

Lin-bone marrow progenitors and TECs at 1:1 ratio ($2 \times 10^5$ each), and transplanted underneath the kidney capsules of B6.nude mice. Ten weeks postoperatively, skin grafts harvested from CBA/J and Balb/C (third-party allografts) mice were transplanted to the Tot.B6.nude mice

($n = 4$). Graft survival was monitored for up to 4 weeks. (B) Representative photographic images of skin grafts (outlined) on

Tot.B6.nude at 22 days postoperatively. While the third-party allograft (left panel, Balb/C) is largely rejected, the CBA/J allograft (right panel) is well tolerated. (C and D) Mixed lymphocyte reaction (MLR) experiments, showing the proliferation responses of CD3+ T cells of

the recipient Tot.B6.nude mice (n = 4) in the presence of syngeneic or allogeneic APCs. (C) Representative carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution histogram: Left panel, syngeneic B6 APCs; middle panel, CBA/J allogeneic APCs; right panel, third-party allogeneic APCs. (D) Percentages of proliferating CD3+ T cells.

$*P < .05$, nonparametric Mann–Whitney test.

From Fan Y, Tajima A, Goh SK, et al. Bioengineering thymus organoids to restore thymic function and induce donor-specific immune tolerance to allografts. Molecular Therapy 2015;23:1262–77.

If this approach were proven feasible in humans as well, immune tolerance induction might then become a real means to protect allotransplants even across HLA disparities. Thus, we like to end by quoting Thomas Starzl, who, concluding a famous talk in 1982, stated: "History tells us that procedures that were inconceivable yesterday, and are barely achievable today, often become routine tomorrow!"

## References


[1] Thorsby E. A short history of HLA. *Tissue Antigens*. 2009;74:101–116.

[2] Vandiedonck C, Knight J.C. The human Major Histocompatibility Complex as a paradigm in genomics research. *Brief Funct Genom Proteom*. 2009;8:379–394s.

[3] Grey H.M, Kubo R.T, Colon S.M, et al. The small subunit of HLA antigens is beta-2 microglobulin. *J Exp Med*. 1973;138:1608–1612.

[4] Bjorkman P.J, et al. Finding the groove. *Nat Immunol*. 2006;7:787–789.

[5] Mungall A.J, Palmer S.A, Sims S.K, et al. The DNA sequence and analysis of human chromosome 6. *Nature*. 2003;425:805–811.
[6] Robinson J., Mistry K, McWilliam H, et al. The IMGT/HLA database. *Nucleic Acids Res*. 2011;39:D1171–D1176.
[7] Maiers M., Gragert L, Klitz W. High-resolution HLA alleles and haplotypes in the United States population. *Hum Immunol*. 2007;68:779–788.
[8] Cao K., Hollenbach J., Shi X., et al. Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. *Hum Immunol*. 2001;62:1009–1030.
[9] Shiina T., Hosomichi K, Inoko H, Kulski J.K, et al. The HLA genomic loci map: expression, interaction, diversity and disease. *J Hum Genet*. 2009;54:15–39.
[10] Stewart C.A, Horton R.., Allcock R.J, et al. Complete MHC haplotype sequencing for common disease gene mapping. *Genome Res*. 2004;14:1176–1187.
[11] Ceppellini R, Curtoni E.S, Mattiuz P.L, et al. Genetics of leukocyte antigens: a family study of segregation and
linkage. In: Curtoni E.S, Mattiuz P.L, Tosi R, eds. *Hist testing*. Copenhagen: Munksgaard; 1967:149–187.
[12] Bodmer W.F. Population genetics of the HL-A system: Retrospect and
prospect. In: Dausset J, Colombani J, eds. *Histocompati testing*. Copenhagen: Munksgaard; 1972:611.

[13] Lewontin R.C, et al. The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics*. 1964;49:49–67.
[14] Pazmany L, Mandelboim O, Valés-Gómez M, et al. Protection from natural killer cell-mediated lysis by HLA-G expression on target cells. *Science*. 1996;274:792–795.
[15] Campbell R.D, Trowsdale J., et al. Map of the human MHC. *Immunol Today*. 1993;14:349–352.
[16] Fling S.P, Arp B., Pious D, et al. HLA-DMA and -DMB genes are both required for MHC class II/peptide complex formation in antigen-presenting cells. *Nature*. 1994;368:554–558.
[17] Morris P., Shaman J, Attaya M, et al. An essential role for HLA-DM in antigen presentation by class II major histocompatibility molecules. *Nature*. 1994;368:551–554.
[18] Peaper D.R, Cresswell P., et al. Regulation of MHC class I assembly and peptide binding. *Annu Rev Cell Dev Biol*. 2008;24:343–368.
[19] Smith-Garvin J.E, Koretzky G.A, Jordan M.S, et al. T cell activation. *Annu Rev Immunol*. 2009;27:591–619.
[20] Rossini A., Greiner D, Mordes J. Induction of immunologic tolerance for transplantation. *Physiol Rev*. 1999;79:99–141.
[21] Klein J., et al. *Natural history of the major histocompatibility complex*. New York: Wiley-Interscience; 1986.
[22] Silverstein A.M, et al. *A history of immunology*. San Diego: Academic Press; 1988 1-422146.
[23] Majno G. *The healing hand. Man and wound in the*

*ancient World*. Cambridge (MA): Harvard University Press; 1975:261–312.
[24] Wright J. *The nose and throat in medical history*. 1898 [Published privately].
[25] Gorer P.A, et al. The genetic and antigenic basis of tumor transplantation. *J Pathol Bacteriol*. 1937;44:691–697.
[26] Gorer P.A. The antigenic basis of tumor transplantation. *J Pathol Bacteriol Pathol*. 1938;47:231–252.
[27] Snell G.D, et al. Methods for the study of histocompatibility genes. *J Genet*. 1948;49:87–103.
[28] Gorer P.A, Lyman S., Snell G.D, et al. Studies of the genetic and antigenic basis of tumor transplantation: linkage between a histocompatibility gene and "fused'' in mice. *Proc R Soc Lond Ser B*. 1948;135:499–505.
[29] Medawar P.B, et al. The homograft reaction. *Proc R Soc Lond Ser B*. 1958;148:145–166.
[30] Medawar P.B, et al. The behavior and fate of skin autografts and skin homografts in rabbits. *J Anat*. 1944;78:176–280.
[31] Merrill J.P, Murray J.E, Harrison J.H, Guild W.R, et al. Successful homotransplantation of the human kidney between identical twins. *J Am Med Assoc*. 1956;160:277–282.
[32] Merrill J.P, et al. Transplantation immunology 1957-1975. *Ann Immunol*. 1978;129:347–352. .
[33] Dausset J. Iso-leuco-anticorps. *Acta Haematol*. 1958;20:156–166.
[34] Payne R, Rolfs M.R, et al. Fetomaternal leukocyte

incompatibility. *J Clin Investig*. 1958;37:1756–1763.
[35] Takasugi M, Sengar D.P, Terasaki P.I, et al. Microassays in transplantation immunology. *Am J Med Technol*. 1971;37:470–472.
[36] Payne R., Bodmer W.F, Troup G.M, Walford R.L. Sero activities and specificities of eleven human leukocyte antisera produced by planned immunization. *Transplantation*. 1967;5:597–605.
[37] Ceppellini R., Bonnard G.D, Coppo F, et al. Transplantation antigens: introductory symposium. Mixed leukocyte cultures and HL-A antigens. I. Reactivity of young fetuses, newborns and mothers at delivery. *Transplant Proc*. 1971;3:58–63.
[38] Trucco M, Stocker J.W, Ceppellini R. Monoclonal antibodies against human lymphocyte antigens. *Nature*. 1978;273:666–668.
[39] Bach F.H, Albertini R.J, Amos D.B, et al. Mixed leukocyte culture studies in families with known HL-A genotypes. *Transplant Proc*. 1969;1:339–341.
[40] Meo T, Vives J, Miggiano V, Shreffler D, et al. A major role for the Ir-1 region of the mouse H-2 complex in the mixed leukocyte reaction. *Transplant Proc*. 1973;5:377–381.
[41] Turco E, Fritsch R, Trucco M., et al. Use of immunologic techniques in gene analysis. In: 2nd ed. Herberman R, Mercer D.W, eds. *Immunodiagnosis of cancer*. vol. 53. New York: Marcel Dekker; 1990:205–222.
[42] Cascino I, Rosenshine S, Turco E., et al. Relationship

between DQ alpha and DQ beta RFLP and serologically defined class II HLA antigens. *J Immunogenet*. 1986;13:387–400.
[43] Carlsson B, Wallin J, Bohme J, Moller E. HLA-DR-DQ haplotypes defined by restriction fragment length analysis: correlation to serology. *Hum Immunol*. 1987;20:95–113.
[44] Trucco M, Ball E. RFLP analysis of DQ-beta chain gene: workshop report. In: *Histocompatibility testing 1987*. vol. 1. New York: Springer-Verlag; 1989:860.
[45] Trucco G, Fritsch R, Giorda R, Trucco M. Rapid detection of IDDM susceptibility, using amino acid 57 of the HLA-DQ beta chain as a marker. *Diabetes*. 1989;38:1617–1622.
[46] Hsia S, Tong J.Y, Parris G.L, et al. Molecular compatibility and renal graft survival: the HLA DRB1 genotyping. *Transplantation*. 1993;55:395–399.
[47] Tong J.Y, Hsia S, Parris G.L, et al. Molecular compatibility and renal graft survival: the HLA DQB1 genotyping. *Transplantation*. 1993;55:390–395.
[48] Mullis K.B, Faloona F.A. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. In: Diego R.W, ed. *Methods in enzymology*. San Diego: Academic Press; 1987:335.
[49] Saiki R.K, Gelfand D.H, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. 1988;239:487–491.
[50] Todd J.A, Bell J.I, McDevitt H.O. HLA-DQ-beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature*. 1987;329:559–

604.
[51] Morel P.A, Dorman J.S, Todd J.A, et al. Aspartic acid at position 57 of the HLA DQ-beta chain protects against type I diabetes: a family study. *Proc Natl Acad Sci USA*. 1988;85:8111–8115.
[52] Bodmer W.F. HLA
1991. In: Tsugi K, Aizawa M, Sasazuki T, eds. *HLA 1991: Proceedings of the Eleventh international histocompatibility workshop and conference*. UK: Oxford Science; 1992:7.
[53]
Saiki R.K, Walsh P.S, Levenson C.H, Erlich H.A. Gene analysis of amplified DNA with immobilized sequence-specific oligonulceotide probes. *Proc Natl Acad Sci USA*. 1989;86:6230–6234.
[54] Rudert W.A, Trucco M. DNA polymers of protein binding sequences generated by PCR. *Nucleic Acid Res*. 1990;18:6460.
[55] Rudert W.A, Trucco M. Rapid detection of sequence variations using polymers of specific oligonucleotides. *Nucleic Acid Res*. 1992;20:1146.
[56] Chia D, Terasaki P, Chan H, et al. Direct detection of PCR products for HLA class II typing. *Tissue Antigens*. 1993;42:146–149.
[57] Giorda R, Lampasona V, Kocova M, Trucco M. Non-radioisotopic typing of human leukocyte antigen class II genes on
microplates. *Biotechniques*. 1993;15:918–925.
[58] Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR

typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens*. 1992;39:225–235.
[59] Olerup O, Aldener A, Fogdell A. HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. *Tissue Antigens*. 1993;41:119–134.
[60] Browning M.J, Krausa P, Rowan A, et al. Tissue typing the HLA-A locus from genomic DNA by sequence-specific PCR: comparison of HLA genotype and surface expression on colorectal tumor cell lines. *Proc Natl Acad Sci USA*. 1993;90:2842–2845.
[61] Newton C.R, Graham A, Heptinstall L.E, et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res*. 1989;17:2503–2516.
[62] Krausa P, Bodmer J.G, Browning M.J. Defining the common subtypes of HLA A9, A10, A28 and A19 by use of ARMS/PCR. *Tissue Antigens*. 1993;42:91–99. .
[63] Sadler A.M, Petronzelli F, Krausa P, et al. Low-resolution DNA typing for HLA-B using sequence-specific primers in allele- or group-specific ARMS/PCR. *Tissue Antigens*. 1994;44:148–154.
[64] Bunce M, Welsh K.I. Rapid DNA typing for HLA-C using sequence-specific primers (PCR-SSP): identification of serological and non-serologically defined HLA-C alleles including several new alleles. *Tissue Antigens*. 1994;43:7–17.
[65] Guo Z, Hood L, Petersdorf E.W. Oligonucleotide arrays for high resolution HLA typing. *Rev Immunogenet*. 1999;1:220–230.

[66] Guo Z, Gatterman M.S, Hood L, et al. Oligonucleotide arrays for high-throughput SNPs detection in the MHC class I genes: HLA-B as a model system. *Genome Res*. 2002;12:447–457.
[67] de Bakker P.I, McVean G, Sabeti P.C, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet*. 2006;38:1166–1172.
[68] Leslie S, Donnelly P, McVean G. A statistical method for predicting classical HLA alleles from SNP data. *Am J Hum Genet*. 2008;82:48–56.
[69] International MHC, Autoimmunity Genetics Network, Rioux J.D, Goyette P, Vyse T.J, et al. Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. *Proc Natl Acad Sci USA*. 2009;106:18680–18685.
[70] Dunbar S.A. Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. *Clin Chim Acta*. 2006;363:71–82.
[71] Cai H, White P.S, Torney D, et al. Flow cytometry-based minisequencing: a new platform for high-throughput single-nucleotide polymorphism scoring. *Genomics*. 2000;66:135–143.
[72] Fulton R.J, McDade R.L, Smith P.L, et al. Advanced multiplexed analysis with the FlowMetrix system. *Clin Chem*. 1997;43:1749–1756.
[73] Ronaghi M, Karamohamed S, Pettersson B, et al. Real-time DNA sequencing using detection of pyrophosphate release. *Anal Biochem*. 1996;242:84–89.

[74] Ronaghi M, Uhlen M, Nyren P. A sequencing method based on real-time pyrophosphate. *Science*. 1998;281:363–365.
[75] Entz P, Toliat M.R, Hampe J, et al. New strategies for efficient typing of HLA class-II loci DQB1 and DRB1 by using Pyrosequencing. *Tissue Antigens*. 2005;65:67–80.
[76] Ramon D, Braden M, Adams S, et al. Pyrosequencing: a one-step method for high resolution HLA typing. *J Transl Med*. 2003;1:9.
[77] Ringquist S, Alexander A.M, Rudert W.A, et al. Pyrosequence based typing of alleles of the HLA-DQB1 gene. *Biotechniques*. 2002;33:166–175.
[78] Ringquist S, Alexander A.M, Styche A, et al. HLA class II DRB high resolution genotyping by pyrosequencing: comparison of group specific PCR and pyrosequencing primers. *Hum Immunol*. 2004;65:163–174.
[79] Alexander A.M, Pecoraro C, Styche A, et al. SOP3: a web-based tool for selection of oligonucleotide primers for single nucleotide polymorphism analysis by pyrosequencing. *Biotechniques*. 2005;38:87–94.
[80] Ringquist S, Styche A, Rudert W.A, Trucco M. Pyrose based strategies for improved allele typing of human leukocyte antigen loci. *Methods Mol Biol*. 2007;373:115–134.
[81] Garcia C.A, Ahmadian A, Gharizadeh B, et al. Mutation detection by pyrosequencing: sequencing of exons 5-8 of the p53 tumor suppressor gene. *Gene*. 2000;253:249–257.

[82] Gharizadeh B, Nordström T, Ahmadian A, et al. Long-read pyrosequencing using pure 2′-deoxyadenosine-5′-O′-(1-thiotriphosphate) Sp-isomer. *Anal Biochem*. 2002;301:82–90.
[83] Svantesson A, Westermark P.O, Kotaleski J.H, et al. A mathematical model of the pyrosequencing reaction system. *Biophys Chem*. 2004;110:129–145.
[84] Rothberg J.M, Leamon J.H. The development and impact of 454 sequencing. *Nat Biotechnol*. 2008;26:1117–1124.
[85] Bentley G, Higuchi R, Hoglund B, et al. High-resolution, high-throughput HLA genotyping by next-generation sequencing. *Tissue Antigens*. 2009;74:393–403.
[86] Gabriel C, Danzer M, Hackl C, et al. Rapid high-throughput human leukocyte antigen typing by massively parallel pyrosequencing for high-resolution allele identification. *Hum Immunol*. 2009;70:960–964.
[87] Lind C, Ferriola D, Mackiewicz K, et al. Next-generation sequencing: the solution for high-resolution, unambiguous human leukocyte antigen typing. *Hum Immunol*. 2010;71:1033–1042.
[88] Lank S.M, Wiseman R.W, Dudley D.M, O'Connor D.H novel single cDNA amplicon pyrosequencing method for high-throughput, cost-effective sequence-based HLA class I genotyping. *Hum Immunol*. 2010;71:1011–1017.
[89] Galan M, Guivier E, Caraux G, et al. A 454 multiplex sequencing method for rapid and reliable genotyping

of highly polymorphic genes in large-scale studies. *BMC Genom*. 2010;11:296.
[90] Flicek P, Birney E. Sense from sequence reads: methods for alignment and assembly. *Nat Methods*. 2009;6:S6–S12. .
[91] Erlich R.L, Jia X, Anderson S, Banks E, et al. Next-generation sequencing for HLA typing of class I loci. *BMC Genom*. 2011;18:12–42.
[92] Holcomb C.L, Höglund B, Anderson M.W, et al. A multi-site study using high-resolution HLA genotyping by next generation sequencing. *Tissue Antigens*. 2011;77:206–217.
[93] McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20:1297–1303.
[94] Gonzalez-Galarza F.F, Christmas S, Middleton D, Jones A.R. All frequency net: a database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acid Res*. 2011;39:D913–D919.
[95] Luca D, Ringquist S, Klei L, et al. On the use of general control samples for genome-wide association studies: genetic matching highlights causal variants. *Am J Human Genet*. 2008;82:453–463.
[96] Wu J, Devlin B, Ringquist S, Trucco M, Roeder K. Scre and clean: a tool for identifying interactions in genome-wide association studies. *Genet Epidemiol*. 2010;34:275–285.
[97] Ringquist S, Bellone G, Lu Y, et al. Clustering and

aligment of polymorphic sequences for HLA-DRB1 genotyping. *PLoS One*. 2013;8(3):e59835.
[98] Lafon S, Lee A.B. Diffusion maps and coarse-graining: a unified framework for dimensionality reduction, graph partitioning, and data set parameterization. *IEEE Trans Pattern Anal Mach Intell*. 2006;28:1393–1403.
[99] Breiman L. Bagging predictors. *Mach Learn*. 1996;24:123–140.
[100] Shendure J, Hanlee J. Next-generation DNA sequencing. *Nat Biotechnol*. 2008;26:1135–1145.
[101] Terasaki P.I, McClelland J.D. Microdroplet assay of human serum cytotoxins. *Nature*. 1964;204:998–1000.
[102] Patel R, Terasaki P.I. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med*. 1969;28:735–739.
[103] Tait B.D, Süsal C, Gebel H.M, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation*. 2013;96:19–47.
[104] Bray R, Nickerson P.W, et al. Evolution of HLA antibody detection. *Immunol Res*. 2004;29(1–3):41–53.
[105] Amos D.B, Bashir H, Boyle W, et al. A simple microtoxicity test. *Transplantation*. 1970;7:220–223.
[106] Garovoy M.R.R.M, Bigos M, Perkins H, et al. Flow cytometry analysis: a high technology crossmatch technique facilitating transplantation. *Transplant Proc*. 1983;XV(1939).
[107] Bray R.A, Lebeck L.K, Gebel H.M. The flow cytometric crossmatch. Dual-color analysis of T cell and B cell reactivities. *Transplantation*. 1989;48:834–

840.
[108] Bray R.A. Flow cytometry crossmatching for solid organ transplantation. *Methods Cell Biol*. 1994;41:103–119.
[109] Couzi L, Araujo C, Guidicelli G, et al. Interpretation of positive flow cytometric crossmatch in the era of the single-antigen bead assay. *Transplantation*. 2011;91:527–535.
[110] Lobo P.I, Spencer C.E, Stevenson W.C, et al. The use of pronase-digested human leukocytes to improve specificity of the flow cytometric crossmatch. *Transplant Int*. 1995;8:472–480.
[111] Hetrick S.J, Schillinger K.P, Zachary A.A, Jackson A.M of pronase on flow cytometric crossmatch outcome. *Hum Immunol*. 2011;72:330–336.
[112] Fuggle S.V, Martin S. Tools for human leukocyte antigen antibody detection and their application to transplanting sensitized patients. *Transplantation*. 2008;86:384–390.
[113] Vaidya S, Partlow D, Susskind B, Noor M, et al. Prediction of crossmatch outcome of highly sensitized patients by single and/or multiple antigen bead luminex assay. *Transplantation*. 2006;82:1524–1528.
[114] Zhang Q, Liang L.W, Gjertson D.W, et al. Development of posttransplant antidonor HLA antibodies is associated with acute humoral rejection and early graft dysfunction. *Transplantation*. 2005;79:591–598.
[115] Chen G, Sequeira F, Tyan D.B. Novel C1q assay

reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. *Hum Immnol*. 2011;72:849–858.
[116] Fleischhauer K, Kernan N.A, O'Reilly R.J, et al. Bone marrow-allograft rejection by T lymphocytes recognizing a single amino acid difference in HLA-B44. *N Engl J Med*. 1990;323:1818–1822.
[117] Anasetti C, Amos D, Beatty P.G, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *N Engl J Med*. 1989;320:197–204.
[118] Beatty P.G, Anasetti C, Hansen J.A, et al. Marrow transplantation from unrelated donors for treatment of hematologic malignancies: effect of mismatching for one HLA locus. *Blood*. 1993;81:249–253.
[119] Santamaria P, Reinsmoen N.L, Lindstrom A.L, et al. Frequent HLA class I and DP sequence mismatches in serologically (HLA-A, HLA-B, HLA-DR) and molecularly (HLA-DRB1, HLA-DQA1, HLA-DQB1) HLA-identical unrelated bone marrow transplant pairs. *Blood*. 1994;83:280–287. .
[120] Karre K. A perfect mismatch. *Science*. 2002;295:2029–2031.
[121] Moretta L, Bottino C, Pende D, et al. Different checkpoints in human NK-cell activation. *Trends Immunol*. 2004;25:670–676.
[122] Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivitiy in mismatch hematopoietic transplants. *Science*. 2002;295:2097–2100.

[123] Velardi A, Ruggeri L, Moretta A, Moretta L. NK cells: a lesson from mismatched hematopoietic transplantation. *Trends Immunol*. 2002;23:438–444.
[124] Starzl T.E, Demetris A.J, Trucco M, et al. Chimerism after liver transplantation for type IV glycogen storage disease and type I Gaucher's disease. *N Engl J Med*. 1993;328:745–749.
[125] Starzl T.E, Demetris A.J, Trucco M, et al. Cell migration and chimerism after whole organ transplantation: the basis of graft acceptance. *Hepatology*. 1993;17:1127–1152.
[126] Stolberg S.G. *Agreement on plan to revamp organ distribution*. New York Times; 1999.
[127] Stolberg S.G. *Iowa turf war over transplants mirrors feuds across the nation*. New York Times; 1999.
[128] Terasaki P.I, Cecka J.M, Gjertson D.W, et al. A ten-year prediction for kidney transplant survival. In: Terasaki P.I, Cecka J.M, eds. *Clinical transplants*. Los Angeles: UCLA Tissue Typing Laboratory; 1992:501–512.
[129] Wujciak T, Opelz G. Matchability as an important factor for kidney allocation according to the HLA match. *Transplant Proc*. 1997;29(1–2):1403–1405.
[130] Opelz G. HLA compatibility and kidney grafts from unrelated live donors. Collaborative Transplant Study. *Transplant Proc*. 1998;30:704–705.
[131] Opelz G. Impact of HLA compatibility on survival of kidney transplants from unrelated live donors. *Transplantation*. 1997;64:1473–1475.
[132] Starzl T.E, Eliasziw M, Gjertson D, et al. HLA and cross-reactive antigen group matching for cadaver

kidney allocation. *Transplantation*. 1997;64:983–991.
[133] Speiser D.E, Tiercy J.M, Rufer N, et al. High resolution HLA matching associated with decreased mortality after unrelated bone marrow transplantation. *Blood*. 1996;87:4455–4462.
[134] Duquesnoy R.J. HLAMatchmaker: a molecularly based donor selection algorithm for highly alloimmunized patients. *Transplant Proc*. 2001;33(1–2):493–497.
[135] Duquesnoy R.J. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. *Hum Immunol*. 2002;63:339–352.
[136] Dankers M.K, Witvliet M.D, Roelen D.L, et al. The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched human leukocyte antigens. *Transplantation*. 2004;77:1236–1239.
[137] Duquesnoy R.J, Mulder A, Askar M, et al. HLAMatchmaker-based analysis of human monoclonal antibody reactivity demonstrates the importance of an additional contact site for specific recognition of triplet-defined epitopes. *Hum Immunol*. 2005;66:749–761.
[138] Tambur A.R, Claas F.H.J. HLA epitopes as viewed by antibodies: what is it all about? *Am J Transplant*. 2015;15:1148–1154.
[139] Zeevi A, Girnita A, Duquesnoy R. HLA antibody analysis. *Immunol Res*. 2006;36(1–3):255–264.
[140] Duquesnoy R. Reflections on HLA epitope-based matching for transplantation. *Front*

*Immunol*. 2016;7:469 eCollection 2016.
[141] Duquesnoy R.J, Kamoun M, Baxter-Lowe L.A, et al. Should HLA mismatch acceptability for sensitized transplant candidates be determined at the high-resolution rather than the antigen level? *Am J Transplant*. 2015;15:923–930.
[142] Yagihashi A, Kobayashi M, Noguchi K, et al. HLA matching effect in liver transplantation. *Transplant Proc*. 1992;24:2432–2433.
[143] Starzl T.E, Demetris A.J, Murase N, et al. Cell migration, chimerism, and graft acceptance. *Lancet*. 1992;339:1579–1582.
[144] Starzl T.E, Demetris A.J, Trucco M, et al. Systemic chimerism in human female recipients of male livers. *Lancet*. 1992;340:876–877.
[145] Starzl T.E, Trucco M, Rao A, Demetris A. The role of HLA typing in clinical kidney transplants: 30 years later. The blind-folding of HLA matching (editorial). *Clin Transpl*. 1993;7:423–425.
[146] Fontes P, Rao A.S, Demetris A.J, et al. Bone marrow augmentation of donor-cell chimerism in kidney, liver, heart, and pancreas islet transplantation. *Lancet*. 1994;344:151–155.
[147] Hisanaga M, Hundrieser J, Böker K, et al. Development, stability and clinical correlations of allogeneic microchimerism after solid organ transplantation. *Transplantation*. 1996;61:40–45.
[148] Rudert W.A, Kocova M, Rao A, Trucco M. Fine quantitation by competitive PCR of circulating donor cells in post-transplant chimeric recipients. *Transplantation*. 1994;58:964–965.

[149] Schlitt H.J, Raddatz G, Steinhoff G, et al. Passenger lymphocytes in human liver allografts and their potential role after transplantation. *Transplantation*. 1993;56:951–955.
[150] Suberbielle C, Caillat-Zucman S, Legendre C, et al. Peripheral microchimerism in long-term cadaveric-kidney allograft recipients. *Lancet*. 1994;343:1468–1469.
[151] Wood K, Sachs D.H. Chimerism and transplantation tolerance: cause and effect. *Immunol Today*. 1996;17(12):584–588. .
[152] Trucco M. Regeneration of the β cell. *J Clin Investig*. 2005;115:5–12.
[153] Fox I.J, Daley G.Q, Goldman S.A, et al. Stem cell therapy. Use of differentiated pluripotent stem cells as replacement therapy for treating disease. *Science*. 2014;345(6199):1247391.
[154] Wagers A.J, Weissman I.L. Plasticity of adult stem cells. *Cell*. 2004;116:639–648.
[155] Cowan C.A, Klimanskaya I, McMahon J, et al. Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med*. 2004;350:1353–1356.
[156] Hwang W.S, Ryu Y.J, Park J.H, et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science*. 2004;303:1669–1674.
[157] Shamblott M.J, Axelman J, Wang S, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci USA*. 1998;95:13726–13731.
[158] Thomson J.A, Itskovitz-Eldor J, Shapiro S.S, et al. Embryonic stem cell lines derived from human

blastocysts. *Science*. 1998;282:1145–1147.
[159] Drukker M, Katz G, Urbach A, et al. Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc Natl Acad Sci USA*. 2002;99:9864–9869.
[160] Li L, Baroja M.L, Majumdar A, et al. Human embryonic stem cells possess immune-privileged properties. *Stem Cells*. 2004;22:448–456.
[161] Kennedy D. Stem cells, redux. *Science*. 2004;303:1581.
[162] Gussoni E, Soneoka Y, Strickland C.D, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature*. 1999;401:390–394.
[163] Rose T, Peng H, Shen H.C, et al. The role of cell type in bone healing mediated by ex vivo gene therapy. *Langenbecks Arch Surg*. 2003;388:347–355.
[164] Sutherland D.R, Keating A. The CD34 antigen: structure, biology and potential clinical applications. *J Hematother*. 1992;1:115.
[165] Donnenberg A.D. T cell depletion and allograft engineering. In: Lister J, Ball E.D, eds. *Hematopoietic stem cell therapy*. Philadelphia: Churchill Livingstone; 2000:335–344.
[166] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676.
[167] Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872.
[168] Soldner F, Hockemeyer D, Beard C, et

al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell*. 2009;136:964–977.
[169] Zhou H, Wu S, Joo J.Y, et al. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell*. 2009;4:381–384.
[170] Fan Y, Tajima A, Goh S.K, et al. Bioengineering thymus organoids to restore thymic function and induce donor-specific immune tolerance to allografts. *Mol Ther*. 2015;23:1262–1277.

9

# Genetic Evaluation for Common, Chronic Disorders of Adulthood

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## Abstract

Common, chronic diseases such as cardiovascular diseases, cancer, diabetes, chronic lung disease, and osteoporosis are due to genetic and nongenetic factors that include environmental exposures, infectious agents, and cultural and behavioral factors such as diet and exercise. Clinical genetic evaluation for common, chronic disorders should be considered for individuals with a strong family history or when a Mendelian disorder is suspected, although population-based genomic screening is emerging as a means to identify individuals with genetic predispositions. Genetic consultation usually consists of genetic risk assessment and diagnosis, which might include genetic testing, recommendations for management and prevention, and genetic counseling. Indications for genetic or genomic testing for common diseases can be characterized as diagnostic, prognostic, or predictive and results can inform decision-making by the patient, family, and clinician. Significant advances in genomic testing technologies have increased throughput and lowered the

cost of sequencing, promoting the integration of genetic information into clinical care. As a result of these advances, the number of genetic tests that can provide individualized, actionable clinical information is rapidly increasing, especially for adult-onset conditions. In addition, genetic testing has become more accessible and relevant to every medical specialty. There are two care models for genetic health care: the traditional model of coordinated services between clinical geneticists and other physicians, and the emerging model where genetic services are integrated within primary care and other specialties. The latter is more often the case with genetic services for adults. Given the technological and scientific advances since the sequencing of the human genome, the practice of clinical genetics and genetic evaluation is likely to grow not only in frequency but also in scope, especially as the population ages and common disease become an increasing burden to the healthcare system.

## Keywords

Common chronic disease; Genetic diagnosis; Genetic healthcare model; Genetic risk assessment; Genetic testing

## 9.1. Background

Common diseases such as cardiovascular diseases, cancer, diabetes, neuromuscular disorders, and osteoporosis are high-prevalence diseases that have considerable impact on public health. Common diseases are typically chronic conditions that develop over decades, usually occurring in adulthood due to the interaction of genetic and nongenetic risk factors (i.e., multifactorial etiology), including exposures, infectious agents, and cultural and behavioral factors, such as diet and exercise (Fig. 9.1). Rarely, a common chronic disease of adulthood can occur as the manifestation of a Mendelian disorder, and variable expressivity and reduced penetrance are typical [1]. Genetic heterogeneity is also characteristic of genes contributing to Mendelian forms of common diseases. For example, there are five

genes known to cause Lynch syndrome, the most common form of hereditary colon cancer [2], and at least 16 genes are known that cause familial hypertrophic cardiomyopathy [3]. Pleiotropic effects of genotypes contributing to Mendelian or multifactorial forms of common diseases can exist and must be considered when offering genetic testing.

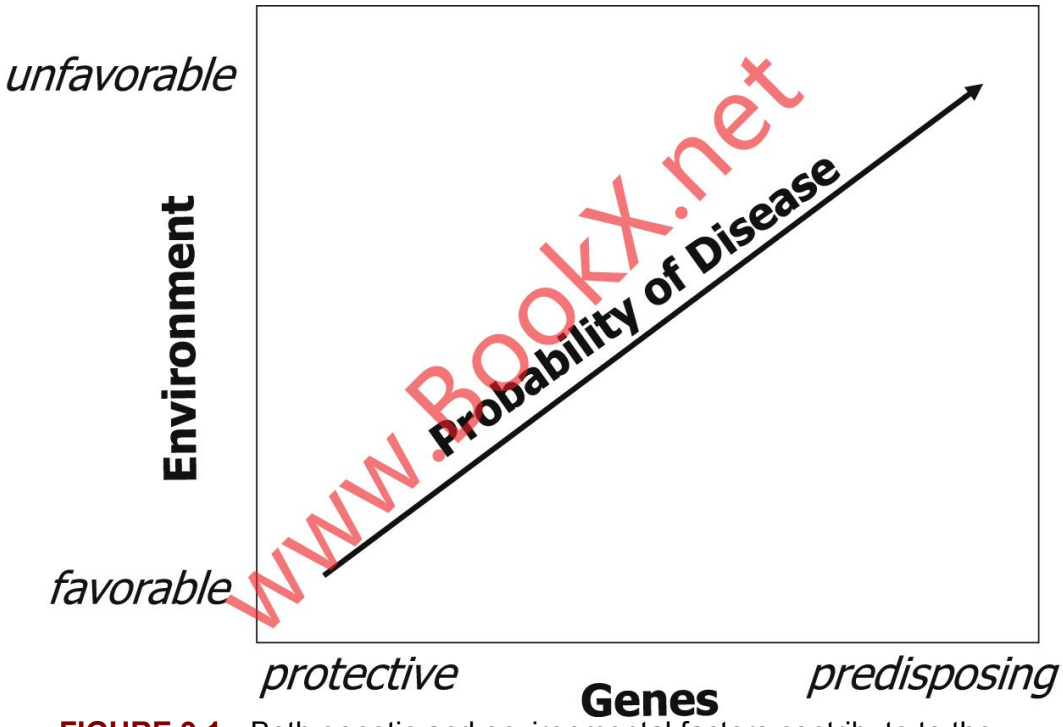
Advances in genomic testing technologies have increased throughput and substantially lowered the cost of sequencing [4]. Genetic tests are increasingly available for Mendelian forms of adult-onset common diseases [5–7]. Genetic test results have the potential to impact health outcomes by improving the ability to diagnose, treat, and prevent common diseases [8]. Other important outcomes relate to reproductive decisions, pychosocial implications of genetic diagnosis and risk assessment for patients and their family members [9–16]. Multiplex testing of low-risk alleles contributing to multifactorial common diseases has limited proven utility [17], and family history remains the best strategy for assessing genetic risk for these disorders [18]. Family history is also key to identifying individuals at risk for Mendelian forms of common diseases [1].

The process of genetic consultation informs the shared clinical decision-making process between healthcare providers and patients, as well as reproductive and personal decisions made by patients, such as decisions about career, finances, and marriage [19]. With the increasing availability of genetic information pertaining to common, chronic diseases of adulthood, the demand for genetic services has increased and integration of genetic services is occurring within primary care and other specialties [20].

## 9.2. Outcomes of Genetic Services

Outcomes resulting from genetic healthcare services, including genetic conslutation and genetic testing are shown in Table 9.1 [9–16]. These outcomes are mapped to five categories: diagnostic/prognostic, medical management, reproductive decisions, psychosocial and behavioral implications for patients, and implications for family members. In a nationwide survey of 363 clinical leaders from the

Department of Veterans Affairs on the topic of value for genetic healthcare outcomes, the frequency of high-value ratings was greatest for outcomes that inform disease management (58.6%), followed by disease prevention (56.4%), reproductive options (50.1%), life planning (43.9%), and confirming a suspected (39.9%) or established (32.3%) diagnosis [21]. Factors positively associated with high-value ratings included having an organizational culture that fosters adoption of genomics, genetic tests available on laboratory menus, availability of genetic testing guidelines, clinicians knowing when to request genetics referrals, and availability of genetics professionals [21].



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**FIGURE 9.1** Both genetic and environmental factors contribute to the development of common diseases such as coronary heart disease, diabetes and cancers. Disease is least likely for individuals who have genotypes that are protective and who have favorable environmental exposures. Conversely, disease is most likely for individuals who have predisposing genotypes and unfavorable environmental risk factors. In industrialized cultures, unfavorable environmental factors such as inactivity, excess calories, processed foods, tobacco, alcohol, radiation and pollution are common with nearly everyone encountering them. Therefore, individuals who develop common chronic diseases,

especially at young ages, are generally those who have the greatest genetic predisposition.

A survey of family practice physicians found that they expect their patients to be more likely to get screened for cancer and make lifestyle and behavioral changes if genetic testing identified them to be at increased risk [22]. However, studies assessing behavioral responses to genetic information among individuals at risk for cancer have found mixed results, with some studies showing no change in behavioral outcomes, while others reported increased participation in cancer screening [23,24]. A few studies have documented lifestyle changes in patients after receiving genetic test results. Increases in attempts to quit smoking and reduction in smoking were observed (though results were short-lived) after receiving genetic test results about disease susceptibility related to smoking [25–28], and a significant increase in dietary supplement use was observed in apolipoprotein E4 (*ApoE4*) allele carriers at risk for Alzheimer disease compared with noncarriers despite the absence of scientific support for dietary supplements in preventing Alzheimer disease [29,30].

## Table 9.1

### Outcomes of Genetic Healthcare Services

**Diagnostic/Prognostic**

1. Confirming a diagnosis of a hereditary disorder in a patient who meets established clinical criteria for the diagnosis.
2. Confirming a diagnosis of a hereditary disorder in a patient who does NOT meet established clinical criteria for the diagnosis.
3. Confirming a predisposition to a hereditary disorder in an asymptomatic individual with a family history of the disorder.
4. Ruling out a predisposition to a genetic disorder in an asymptomatic individual with a family history of the disorder (i.e., by excluding a familial mutation).
5. Ending the diagnostic odyssey for an individual with an unknown

disorder that may or may not be hereditary.

6. Providing prognostic information for a hereditary disorder (e.g., information about related conditions, severity of symptoms, or age of onset).
7. Providing risk of cancer recurrence (i.e., through testing of tumor tissue for prognostic markers).

**Medical Management**

8. Treatment with a drug or medical procedure that has proven health benefits
9. Treatment with a drug or medical procedure that has unproven health benefits.
10. Surveillance for early detection of disease due to a hereditary disorder using a strategy that has proven health benefits.
11. Surveillance for early detection of disease due to a hereditary disorder using a strategy that has unproven health benefits.
12. Use of medication that has proven health benefits for prevention of disease due to a hereditary disorder.
13. Use of medication that has unproven health benefits for prevention of disease due to a hereditary disorder.
14. Surgery to remove or repair at-risk tissue that has proven health benefits in prevention of disease due to a hereditary disorder.
15. Surgery to remove or repair at-risk tissue that has unproven health benefits in prevention of disease due to a hereditary disorder.

Table Continued

**Diagnostic/Prognostic**

16. Improve transitions in care, such as to adult healthcare, long-term care, or palliative care.
17. Decreases in a patient's need for urgent or emergency care.

18. Improvement in a patient's physical health.

19. Improvements in a patient's daily functioning.

20. Improvements in a patient's diet and nutrition.

21. Establish a patient's eligibility for a clinical trial.

**Reproductive Decisions**

22. Inform a patient's reproductive decisions (e.g., having children, adoption, egg or sperm donation, prenatal diagnosis).

23. Prenatal diagnosis of a fetus at high risk for a hereditary disorder (e.g., due to advanced maternal age, abnormal fetal ultrasound findings, positive maternal screening test) through a procedure like amniocentesis.

24. Prenatal diagnosis of a fetus at low risk for a hereditary disorder through a procedure like amniocentesis.

25. Detect a chromosomal imbalance (i.e., Down syndrome, trisomy 18, trisomy 13) in a fetus at high risk through noninvasive screening in a maternal blood sample. (NOTE: A positive screen would be an indication for an invasive prenatal procedure, like amniocentesis.)

26. Detect a chromosomal imbalance (i.e., Down syndrome, trisomy 18, trisomy 13) in a fetus at low risk through noninvasive screening in a maternal blood sample. (NOTE: A positive screen would be an indication for an invasive prenatal procedure, like amniocentesis.)

27. Identify a genetic factor contributing to infertility (e.g., multiple miscarriage, premature menopause, or low sperm count).

**Psychosocial and Behavioral Implications – Patient**

28. Improve a patient's understanding of the effectiveness of treatment, management, or prevention options.

29. Promote a patient's uptake of a clinical intervention with proven health benefits.

30. Discourage a patient's uptake of a clinical intervention with unproven health benefits.

31. Promote a patient's uptake of lifestyle choices with proven health benefits.

32. Discourage a patient's uptake of lifestyle choices with unproven health benefits.

33. Help a patient cope with grief, stress, and emotional challenges of having a genetic disorder.

34. End a patient's uncertainty about a diagnosis of a hereditary disorder.

35. Inform a patient's life planning decisions (e.g., decisions about career, marriage, finances).

36. Decreases in a patient's unhealthy behaviors (e.g., decrease in use of tobacco products, excessive alcohol intake, inactivity).

37. Determine a patient's ancestry without a particular hereditary disorder in mind.

38. Establish parental consanguinity for a patient (i.e., do the parents share a common ancestor) without a particular hereditary disorder in mind.

39. Establish the biological sex of a transgender patient.

**Psychosocial and Behavioral Implications — Family**

40. Promote communication in the family about a hereditary disorder.

41. Help family members cope with grief, stress, and emotional challenges of having a relative with a genetic disorder.

42. Provide genetic information to an at-risk family member to inform their chances of having a hereditary diagnosis or genetic risk.

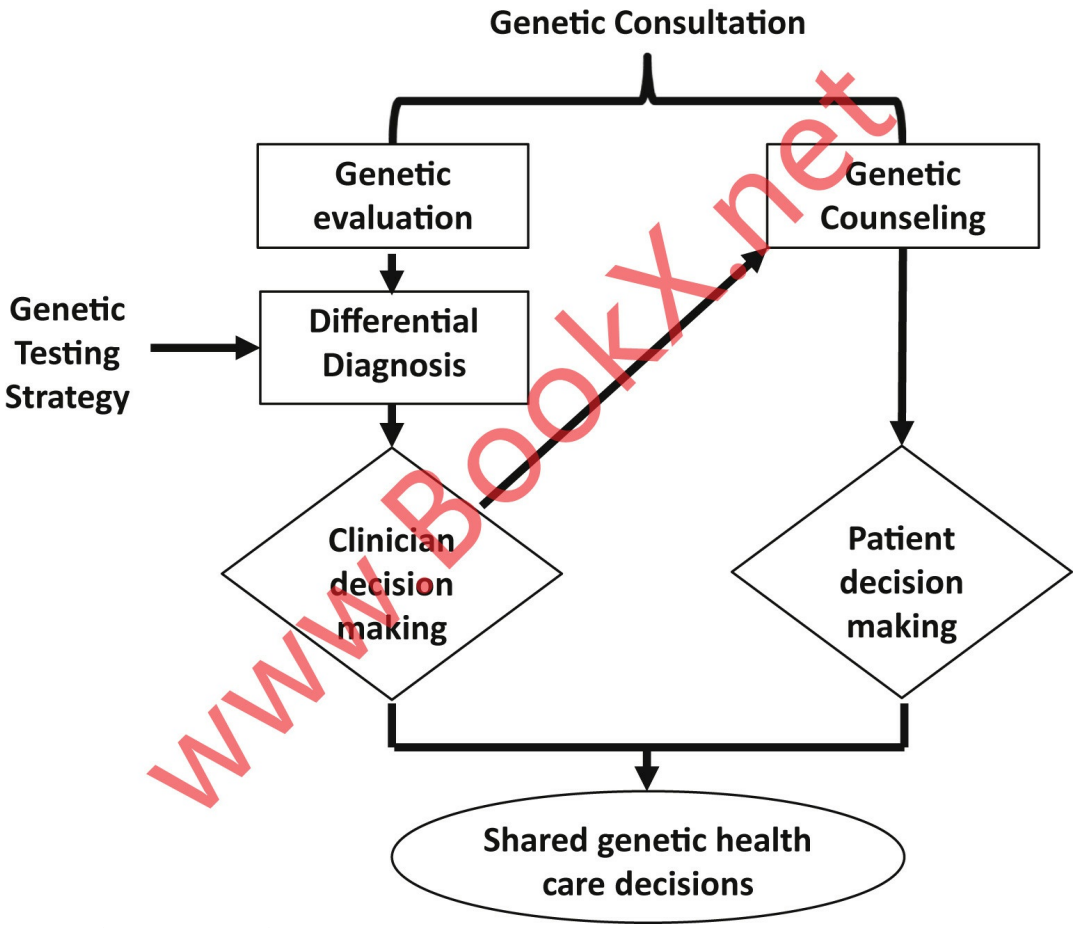
43. Establish paternity for a patient or their offspring.

44. Promote uptake of lifestyle choices by family members that can result in health benefits for those family members.

Robust evidence for clinical outcomes is emerging, such as reduced incidence of disease after a recommended intervention, especially for

hereditary cancer syndromes. For example, a prospective, multicenter cohort study of 2482 women with *BRCA1* or *BRCA2* mutations found that risk-reducing mastectomy was associated with a lower risk of breast cancer and risk-reducing salpingo-oophorectomy was associated with a lower risk of ovarian cancer, first diagnosis of breast cancer, all-cause mortality, breast cancer–specific mortality, and ovarian cancer–specific mortality [31]. Retrospective analysis of 55 women with Lynch syndrome who underwent combined screening of colonoscopy and endometrial biopsy every 1–2 years demonstrated that such interventions were effective in finding and removing precancerous adenomatous polyps, early endometrial cancer, and premalignant hyperplasia in more than half of the women [32].

Generally, psychological and cognitive outcomes related to genetic counseling and testing for common diseases are favorable. Many studies have shown that genetic counseling for inherited susceptibility to breast cancer among women affected with breast cancer and those at risk due to family history did not increase psychological distress and instead resulted in reduction in worry, anxiety, and depression [33,34], and studies assessing psychological or affective outcomes before and after genetic counseling in patients at risk for hereditary colon cancer reported improvements in satisfaction, knowledge, and reductions in worry [35,36]. A randomized controlled trial on the disclosure of *APOE* genotype to participants with a parent diagnosed with Alzheimer disease found no difference in depression, anxiety, or distress in those who received genotype information compared to those who did not [37]. Generally, even 1 year after *APOE* genotype disclosure, participants in the study considered that the benefits of genetic testing outweighed the risks [38]. A randomized controlled trial in adult nondiabetic offspring of diabetic parents reported that education and personalized prevention recommendations increased the accuracy of risk perception for diabetes without increasing worry [39].




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**FIGURE 9.2** Genetic consultation is a shared decision-making process between the clinician and patient. The genetic evaluation by the clinician consists of obtaining and documenting the medical, family and social history, physical examination, and review of pertinent laboratory and pathology results, imaging, procedures and specialty consultations. The genetic evaluation informs the differential diagnosis, which often will include the results of genetic testing. Clinician decision-making is based on the differential diagnosis in the context of an individual patient's clinical needs. Genetic counseling considers patient preferences, values, family dynamics, educational level and cultural norms that inform the patient's perception and knowledge of their genetic diagnosis or risk, and self-efficacy to cope with this information.

## 9.3. The Process of Genetic Consultation for Common, Chronic Diseases of Adulthood

Genetic consultation for common diseases should be considered for individuals with a strong familial risk or when a Mendelian disorder

is suspected. Genetic consultation for hereditary conditions, either Mendelian or multifactorial, is comprised of evaluation and counseling processes (Fig. 9.2). Genetic evaluation for a hereditary condition is a process informed by comprehensive review and synthesis of medical history, social history, family history, physical examination, and results from laboratory tests, imaging, procedures, and other specialty consultations [19]. Genetic evaluation results in a differential diagnosis, including both genetic and nongenetic diagnoses. If indicated, a genetic testing strategy is recommended to further inform the differential diagnosis. These genetic tests may include molecular, chromosomal, or biochemical tests. Clinical decision-making based on the genetic diagnosis may include recommendations for additional diagnostic evaluation, disease management and prevention, reproductive risk options, lifestyle changes, and longitudinal follow-up, including surveillance for early disease detection and anticipatory guidance.

Genetic counseling is a communication process that facilitates patient decision-making surrounding the genetic evaluation [40]. Patient preferences, values, family dynamics, educational level, and cultural norms are considered and inform the patient's perception and knowledge of their genetic diagnosis or risk and self-efficacy to cope with this information. A physician from almost any specialty, including primary care, may perform some or all aspects of a genetic consultation. Clinical geneticists are board-certified physicians with special training in medical genetics; they are trained to perform all aspects of a genetic consultation across the clinical spectrum and lifespan of a patient [19]. Genetic counselors/nurses receive specialized training in genetic counseling [40]; though other providers may also perform genetic counseling.

## 9.3.1. Clinical Assessment

Assessment of signs and symptoms of the disease of concern should be performed to more accurately assess risk for the patient. For example, when evaluating a genetic risk for heart disease, medical history should include questions regarding angina, shortness of

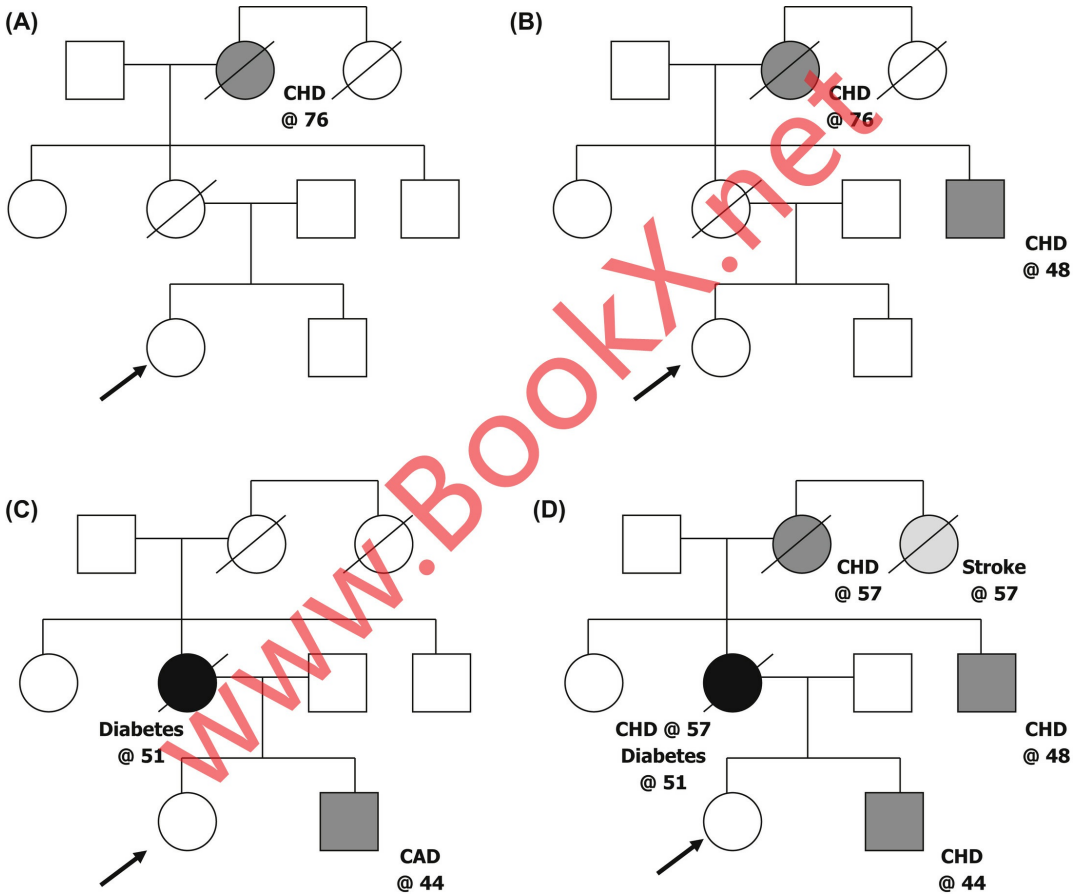
breath, dyspnea on exertion, paroxysmal nocturnal dyspnea, pedal edema, palpitations, claudication, and exercise tolerance. In the case of risk assessment for hereditary colorectal cancer, questions should be asked regarding number of polyps, pathology of polyps, age of diagnosis of cancer, and previous therapies. Additional investigations can be carried out to determine extent of disease or help with diagnosis.

Physical examination should be performed to identify signs of the disease of concern as well as characteristic manifestations of Mendelian forms of a disease. For example, besides the basic evaluation for cardiovascular risk that includes auscultation of the heart, lungs, and major vessels in the neck, abdomen, and groin and palpation of the aorta and distal pulses, one should also look for thin, translucent skin; hyperextensibility of the joints and skin; and abnormal facial features to assess for conditions like Ehlers–Danlos syndrome and Loeys–Dietz syndrome. Blood pressure in the upper and lower extremities can identify hypertension, and these measurements can be used to calculate the ankle/brachial blood pressure index (ABI). Values <0.9 are correlated with atherosclerosis. Blood pressure differences between the upper and lower extremities can suggest a diagnosis of coarctation of the aorta, which, for example, occurs in 12.6% of girls with Turner syndrome [41]. Weight and height should be obtained, and body mass index calculated to identify overweight and obese patients; follow-up measurements can help monitor diet and exercise interventions. Unusually tall or short stature can indicate a diagnosis of a connective tissue disorder or a skeletal dysplasia, respectively. Waist circumference should be obtained, as increased values are associated with the metabolic syndrome, a common cause of cardiovascular disease [42]. Evaluation of possible lipid disorders should include examination of the eyes, assessing corneal arcus and lipemia retinalis, and examination of the skin for xanthelasma and tendinous xanthomas.

## 9.3.2. Family History Assessment

Currently, family history collection and interpretation represents the

most practical strategy for identifying individuals with a genetic susceptibility to many common chronic diseases [18]. Family history represents complex interactions of genetic and nongenetic factors (e.g., exposures, diet, behaviors) shared by family members. For many common diseases, a positive family history is quantitatively significant with relative risks ranging from 2 to 5 times those of the general population, and this risk generally increases with an increasing number of affected relatives and earlier ages of disease onset [43–51]. Family history characteristics that suggest a possible Mendelian disorder include early age at diagnosis, two or more closely related relatives affected with the same or a related disorder, a single family member with two or more related diagnoses, multifocal or bilateral disease, and occurrence of disease in the less often affected sex (e.g., male breast cancer). By recognizing the magnitude of risk associated with these familial characteristics and patterns of disease consistent with known Mendelian disorders, stratification into different familial risk groups (e.g., weak, moderate, and strong) is possible (Fig. 9.3), which can guide risk-specific recommendations for management and prevention. Referral for genetic evaluation by a geneticist or other specialist is appropriate for individuals with strong familial risk or a suspected Mendelian disorder.




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**FIGURE 9.3** Each of the pedigrees are "positive" for a family history of coronary heart disease (CHD). However, the level of familial risk is different for each. In pedigree (A), the consultant reports a maternal grandmother with CHD at age 76; her family history is a weak risk factor. In pedigree (B), there are two second-degree maternal relatives affected with CHD: one diagnosed at an early age (48) and the other at a later age. This family history is more significant but is likely only a moderate risk factor. Pedigree (C) is more concerning and consistent with a strong familial risk since a first-degree relative is affected with CHD at a young age. The report of diabetes in a first-degree relative in pedigree (C) may be related and is suggestive of the metabolic syndrome. The highest familial risk is seen with pedigree (D), which has three generations of early-onset CHD.

An important step in the assessment of family history is creation of the pedigree structure. This usually includes all first- and second-degree relatives and spans three or four generations. Demographic information for each family member is documented, which typically includes each relative's current age or age at death. Medical history is documented for each family member including age at diagnosis, cause

of death if deceased, and known interventions or procedures, which can help clarify a diagnosis. For example, questioning regarding coronary artery bypass surgery, angioplasty, heart transplant, or pacemaker placement may help clarify a relative's diagnosis of heart disease. Information is also collected regarding important risk factors for a disease, such as use of hormone replacement therapy in the case of endometrial cancer, chest irradiation in the case of breast cancer, and smoking, asbestos exposure, and coal mining in the case of lung cancer. Medical records including genetic test reports are reviewed when possible to verify the medical history of family members who are critical to the genetic risk assessment and diagnosis. The family history should include ancestry and country of origin of grandparents, as certain conditions might be more prevalent in certain ancestral groups. For example, the prevalence of insulin resistance is high among individuals of Native American admixture and Asian Indian origin [52,53], and there are common pathogenic *BRCA* gene founder variants in Ashkenazi Jewish families with breast and ovarian cancer [54].

Once family history is collected, the most likely mode of inheritance (i.e., Mendelian vs. multifactorial) and the risk of disease to the patient and to unaffected relatives is determined based on their position in the pedigree. When a Mendelian disorder is suspected, this analysis also helps to elucidate a differential diagnosis through pattern recognition [1]. For example, when considering an inherited form of breast cancer, there are at least four different Mendelian disorders to consider, including hereditary breast–ovarian cancer syndrome, Cowden syndrome, Peutz–Jeghers syndrome, and Li–Fraumeni syndrome. The types of cancers and other conditions reported in the family help to distinguish each of these syndromes (Fig. 9.4). Pathogenic variants in different genes underlie the genetic susceptibility in these syndromes, and genetic testing can help to confirm a suspected diagnosis. For certain hereditary cancers, models are available to help predict the probability of an inherited gene mutation [55–57]. For pedigrees that lack convincing evidence of Mendelian inheritance and are more consistent with multifactorial

inheritance, quantitative risk information can be provided for some conditions through use of mathematical models or published estimates that consider the family history [58–63].

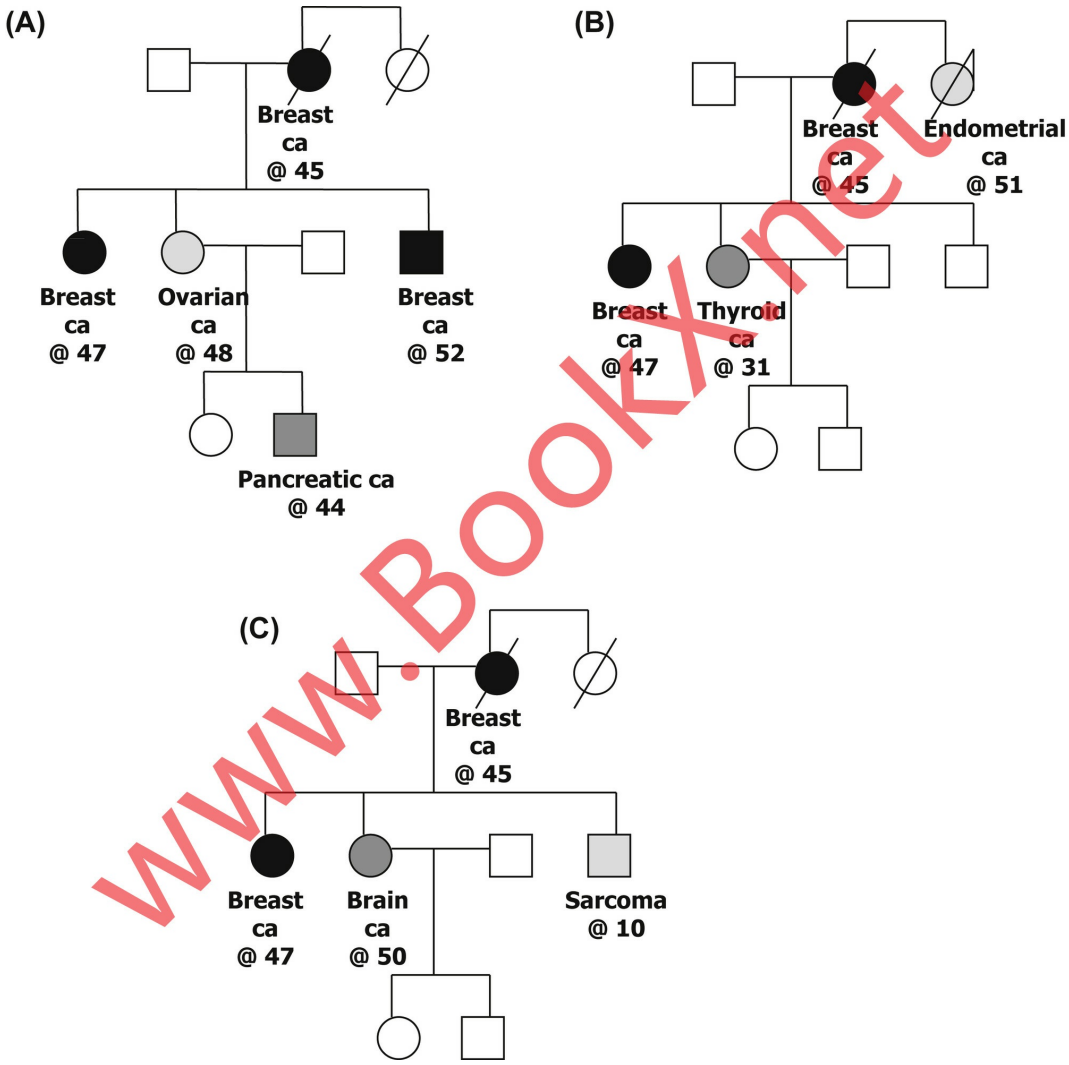
Accuracy of the family history data is paramount, particularly if clinical decisions will depend on the information. Many studies have shown that self-reports of family health history are relatively accurate for many common chronic conditions, such as coronary heart disease, stroke, diabetes, and many forms of cancer. Most positive predictive values for self-reports of a positive family history of these conditions in a first-degree relative range from 70% to 90%, and negative predictive values are usually 90% or greater [64–66]. These values depend on the type of disease, age of the historian, and distance of the relative from the person of interest. Therefore, before clinical decisions are based on such information, confirmation of family health histories is advisable. Generally, individuals in the genetics clinic request and review medical records, pathology reports, and death certificates or autopsy reports of family members to verify self-reports. This can be time-consuming and costly [67].

### 9.3.3. Genetic Testing

Germline genetic testing for common diseases in clinical practice is generally limited to Mendelian disorders. Indications for germline genetic testing of Mendelian disorders that feature common chronic diseases can be characterized as diagnostic, prognostic, and predictive of disease risk and therapeutic response (i.e., pharmacogenetics/pharmacogenomic testing). A particular indication for genetic testing can correspond to multiple reasons for testing (Table 9.2). For example, diagnostic testing in a patient with signs and symptoms of disease may be performed primarily for medical decision-making, yet test results may also inform a patient's reproductive or life planning decisions. Exome and genome sequencing is increasingly available for healthy individuals to provide predictive information about the risk of developing a genetic disorder, carrier status for recessive conditions, and response to drugs [68].

Diagnostic germline genetic tests are typically performed to confirm

a diagnosis suspected on the basis of signs or symptoms of a particular disorder. Diagnostic tests can also include those used for prenatal and preimplantation diagnoses. Testing can sometimes provide prognostic information on the basis of known genotype–phentoype correlations for Mendelian disorders. For example, *SOD1* gene testing can reveal information about disease progression in familial amyotrophic lateral sclerosis [69], and certain pathogenic *APC* gene variants are associated with increased colon polyp number and extracolonic manifestations in familial adenomatous polyposis [70]. Testing for somatic gene variants in tumor tissue is another type of prognostic genetic test; results can inform risk for recurrence and treatment options [71,72]. Predictive genetic testing for common diseases is performed in asymptomatic individuals often at risk due to family history of Mendelian disorders of high penetrance (presymptomatic testing) or reduced penetrance (predisposition testing). Pharmacogenetic testing is another type of predictive genetic test that helps to predict an individual's response to drugs including adverse reactions. Predictive testing for multifactorial conditions consisting of multiplex testing of low-penetrance alleles (susceptibility testing) is generally provided through direct-to-consumer marketing [73]. Little is understood regarding the interactions between genetic and nongenetic risk factors underlying multifactorial diseases; attempts to build genetic risk models have failed to show that genetic information can substantially improve the prediction of risk for common diseases such as breast cancer, prostate cancer, diabetes, or cardiovascular disease [74], and evidence for improved health outcomes is lacking [75].




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**FIGURE 9.4** Each pedigree depicts a strong familial risk for breast cancer. However, by recognition of the patterns of cancer in the family, a more accurate diagnosis can be made. Pedigree (A) features early-onset breast and ovarian cancer and is most consistent with hereditary breast-ovarian cancer syndrome, which is mostly due to a pathogenic variant in the *BRCA1* or *BRCA2* genes. In this case, a *BRCA2* gene variant is likely given the family history of male breast cancer and pancreatic cancer. Pedigree (B) features early-onset breast, thyroid, and endometrial cancer and is most consistent with Cowden syndrome due to a pathogenic variant in the *PTEN* gene. The early-onset breast cancer, brain tumor, and childhood sarcoma in pedigree (C) is most consistent with Li–Fraumeni syndrome due to a pathogenic *TP53* gene variant. Multiple primary cancers are common among individuals with Li–Fraumeni syndrome.

## Table 9.2

## Intended Uses of Genetic Testing Performed for Various Indications

| | Medical Decision-Making | Reproductive Decision-Making | Personal Decision-Making |
|---|---|---|---|
| **Diagnostic (confirmation)** | X | X | X |
| **Presymptomatic/predisposition** | X | X | X |
| **Susceptibility** | X | | |
| **Pharmacogenetic** | X | | |
| **Prognostic** | X | | X |
| **Carrier testing** | | X | |
| **Prenatal** | X | X | |
| **Preimplantation** | | X | |

There may be multiple intended uses of genetic testing performed for various indications, including medical, reproductive, and personal decision-making. Medical decision-making includes decisions regarding disease management (in fetus, too) and screening for early detection or prevention. Reproductive decision-making includes decisions regarding conception/timing of conception, adoption, gamete donor, and pregnancy termination. Personal decision-making includes decisions regarding life, career, and financial planning (does not meet “medical necessity” criteria of most insurers).

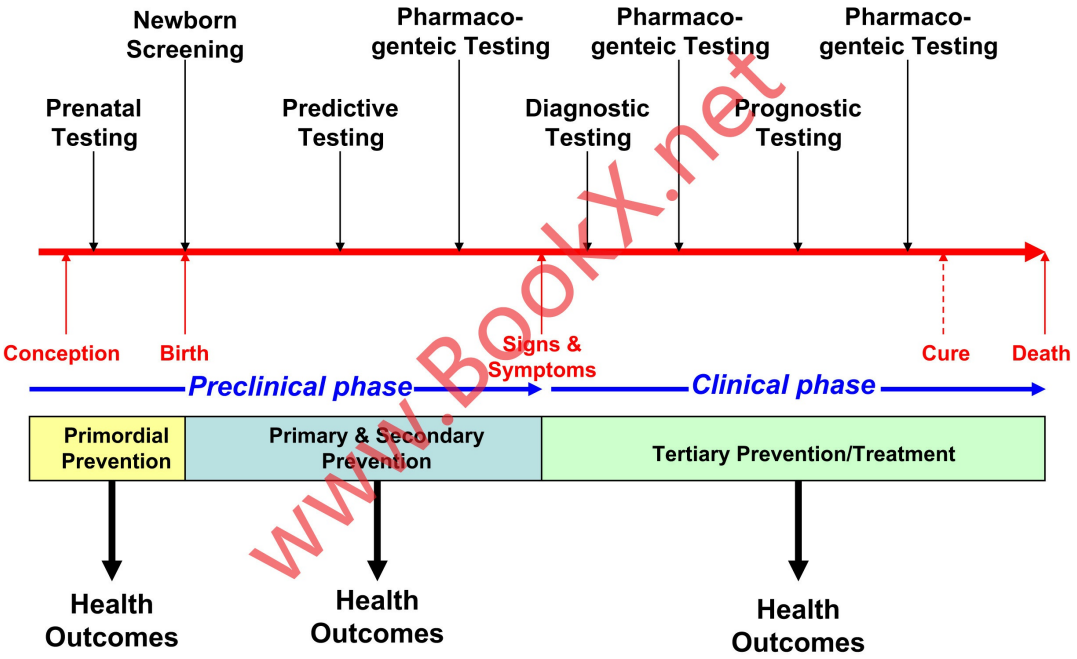
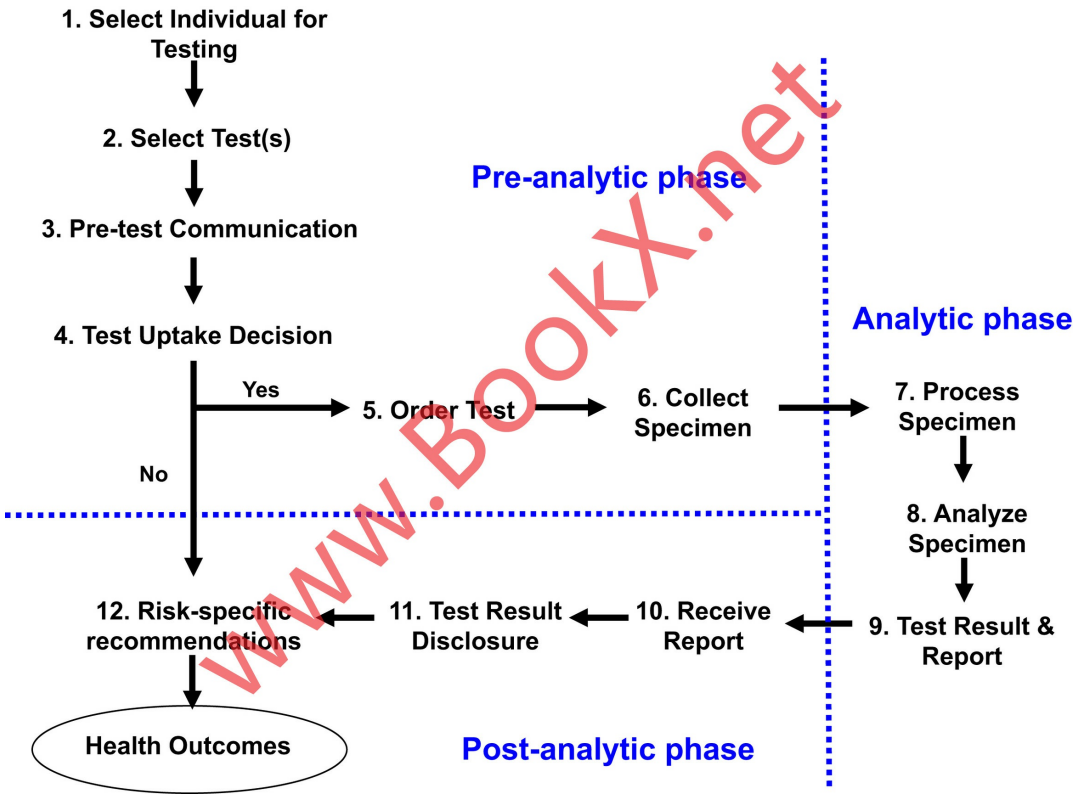



**FIGURE 9.5** Currently, genetic testing for common diseases of adulthood has a role throughout an individual's lifespan, including testing that can impact primordial, primary, secondary, and tertiary disease prevention. Predictive testing includes presymptomatic testing for highly penetrant Mendelian disorders, predisposition testing for Mendelian disorders with reduced penetrance, and susceptibility testing for common diseases due to multifactorial inheritance.

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Aside from newborn screening, genetic testing for common diseases of adulthood has a role throughout the lifespan of an individual (Fig. 9.5). However, most individuals undergo genetic testing for common diseases to diagnose or learn of a risk for Mendelian disorders. Prenatal and preimplantation genetic diagnoses are less often performed for Mendelian disorders that feature common, chronic diseases, mostly because of reduced penetrance, variable expressivity, adult onset, and the opportunities for prevention. Pharmacogenetic testing may be performed before or after signs and symptoms of disease occur.




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**FIGURE 9.6** A framework depicting the delivery of genetic tests according to the preanalytic, analytic, and postanalytic phases of the genetic testing process is shown. The specific actions of each step will vary, depending in large part on the indication for genetic testing. Yet in general, all steps should occur for any given clinical scenario.

The ACCE (Analytic validity, Clinical validity, Clinical utility and Ethical, legal and social issues) framework is commonly used to evaluate genetic tests for common diseases [76]. Analytic validity refers to how accurately and reliably the test measures the genotype of interest; clinical validity measures how consistently and accurately the test detects or predicts the intermediate or final outcomes of interest related to a genotype, clinical utility is a measure of how likely the test result will influence health outcomes; and the ethical, legal, and social implications that should be addressed are those that may arise as a result of genetic testing, such as loss of privacy, confidentiality, stigmatization, and discrimination. The disorder, setting, and clinical scenario are important considerations when applying the ACCE framework. The clinical scenario should include the purpose and indication for testing.

A framework depicting the delivery of genetic tests according to the preanalytic, analytic, and postanalytic phases of the genetic testing process is shown in Fig. 9.6. While the actions performed for each step involved in delivery of genetic testing services will vary, depending on the genetic testing indication, in general, all steps should occur for any clinical scenario.

Typically, germline genetic testing begins with patient selection (Step 1), which will depend on the indication or reason for testing. Personal and family history characteristics are usually collected before genetic testing is offered but may be ascertained after genetic test results are available to inform the differential diagnosis and the mode of inheritance. Thus, use of family history is central to interpreting genetic test results, and it is likely that the paradigm of familial risk assessment will inform future genetic testing of less-penetrant susceptibility alleles. Therefore, family history will likely remain a relevant genetic tool for many years to come.

A new approach to obtaining germline genetic test results comes with tumor sequencing that is performed primarily to inform options for cancer therapies. Although sequence variants found in a patient's cancer will often be somatic variants acquired in the course of tumor development, tumor sequencing will also identify germline sequence variants. If tumor sequencing is limited to cancer-related genes, most of the pathogenic germline variants found will be in genes that cause hereditary cancer syndromes, although there are some exceptions (e.g., germline variants in *SMAD3* or *TGFBR1/2* cause Loeys–Dietz syndrome, an autosomal-dominant connective tissue disorder that increases the risk of aortic aneurysm and other features). At this time, experts support informing patients of the possibility of diagnosing a hereditary disorder and ascertaining patient preferences regarding the receipt of germline information when tumor testing is performed [77]. Yet, there are significant gaps in our understanding of how to best provide pretest education, support patient preferences, and deliver posttest information about secondary germline test results when somatic tumor testing is performed, especially when performed in the laboratory as a reflexive test.

Step 2 in the genetic testing process consists of identification of appropriate tests that may be helpful in confirming a genetic diagnosis and refining genetic risk. When considering genetic testing, clinicians should choose the testing strategy that would be most informative for a patient and the family members. Ideally, genetic testing should begin in an affected family member to identify the specific genetic determinant(s) of disease. If an abnormality or abnormalities are identified, then at-risk relatives can be tested for those familial factors. Excluding a familial mutation in an at-risk relative can provide reassurance regarding disease risk due to the family history; however, there will always be a background or population risk for disease development. It is not always possible to test an affected family member. In this case, unaffected family members may participate in testing. A normal test result can exclude the genetic risk factors that have been tested but not the possibility of an inherited susceptibility. In such a case, medical management would typically depend on the empirical risks associated with the personal and family histories. To further clarify the situation, testing additional family members, both affected and unaffected, may be helpful.

Pretest communication about testing follows in Step 3 and often includes an informed consent process that describes the potential benefits, risks and limitations of genetic testing, as well as the alternatives to testing (see section on genetic counseling). In Step 4, a shared decision-making process between patient and provider, a decision is made regarding genetic testing. The provider then identifies a laboratory that can provide the testing and orders the test in Step 5, providing necessary information to the laboratory on the test requisition to facilitate selection of the appropriate test method and interpretation of results. Most genetic tests are performed in reference laboratories; therefore, sample processing and shipping are often necessary (Step 6). Clinicians ordering genetic tests typically help facilitate the billing process (providing the most appropriate diagnostic codes and documenting medical necessity for testing). Issues to consider when choosing a laboratory include methodology,

analytic sensitivity and specificity, technical support, cost, and turnaround time. Understanding which methodology is used is important because standards for many genetic tests currently do not exist. There is great variability in testing procedures for many conditions. As a result, clinicians need to be familiar with a given laboratory's protocol and the test limitations.

The analytic phase includes Steps 7–9. Genetic tests for assessing common diseases are typically DNA-based tests. Most commercial laboratories performing DNA-based testing use next-generation sequencing (also known as massively parallel sequencing). The major strength of this technology is the ability to detect gene variants across the entire exome, using smaller amounts of DNA than traditional approaches, at lower costs and faster turnaround times.

When the genetic test results are available, the laboratory provides the ordering clinician with a test report (Step 10). A posttest consultation typically follows to review the implications of the test result for both the patient and family members (Step 11). With multigene panels and exome sequencing, a greater number of variants of uncertain significance are identified, which can complicate test result disclosure. The genetic test results should be interpreted within the context of the individual's personal and family histories and the indication and purpose for testing, and recommendations for disease management and prevention should be specific to the risk assessment and diagnosis (Step 12).

## 9.3.4. Management and Prevention Strategies

Few clinical guidelines for diagnosis, treatment, and prevention of common chronic diseases incorporate recommendations based on family history or genetic information. Therefore, strict adherence to clinical guidelines may deny appropriate access to care for those patients who may benefit from alternative treatments or preventive strategies informed by a genetic test result or diagnosis. Knowledge of genetic susceptibility to common diseases may identify important biologic differences that could lead to better disease management and prevention through use of targeted therapies and enhanced screening

and prevention strategies. Prevention strategies for common chronic diseases include targeted lifestyle changes, screening at earlier ages nd more frequently and with more intensive methods than used for average-risk individuals, use of chemoprevention, and, for those at highest risk, prophylactic procedures and surgeries. For many Mendelian disorders that feature common chronic conditions, specific guidelines for initial evaluation, management, and follow-up for affected persons and surveillance for at-risk relatives are available. However, most of these guidelines are based on clinical observation and expert opinion, and outcomes research is needed that assesses their clinical utility. In the absence of clinical guidelines for common chronic diseases associated with Mendelian disorders, clinicians can suggest management and prevention strategies that have been proven effective for the general population.

Recommendations for management and prevention options specific to an individual's genetic risk should be communicated in writing so the patient and referring clinician can incorporate these recommendations into a plan for future health management. Family members who might benefit from risk assessment and genetic testing should be identified, and the clinician should facilitate communication between the index case and at-risk relatives and provide referrals to genetics professionals or other specialists for family members. Plans for follow-up with a genetics professional may be appropriate to review the individualized plan for management of genetic risk and to update personal and family history information with revision of the management and prevention plan as indicated. New information and technology available for genetic risk assessment or management and prevention of disease can also be discussed.

## 9.3.5. Genetic Counseling and Education

Genetic counseling is a communication process that facilitates patient decision-making by considering patient preferences, values, family dynamics, educational level and cultural norms that inform the patient's perception and knowledge of their genetic diagnosis or risk, and self-efficacy to cope with this information [40]. Genetic counseling

is critical for delineating a patient's motivation for genetic evaluation and likely responses to learning of a genetic risk or diagnosis. An important goal of genetic evaluation for common chronic diseases is the development of individualized management and preventive strategies based on the genetic risk assessment and the patient's personal medical history, lifestyle, and preferences. Through genetic counseling, patients will be educated about the role of genetic and nongenetic risk factors for disease; basic concepts of genetics, such as inheritance patterns, penetrance and variable expressivity; and the options for treatment, prevention or risk factor modification tailored to the genetic risk or diagnosis.

How genetic risk factor information may influence behavior is complex and may depend on the condition of concern, the contribution of the genetic factor to risk or severity of disease, and personal beliefs about the role of genetics [78]. A literature review of 25 studies found a positive relationship between a reported family history of cardiovascular disease and perceived risk. However, the relationship between a family history of cardiovascular disease and health-related behavior change and perceived risk and behavior change was inconsistent [79]. Among low-income, rural African American women who had not had recent mammogram, knowledge of family history of breast cancer was not associated with perceived risk or screening [80]. These results suggest that consumers may not appreciate how genetic and familial risk factors relate to their disease risk, or they may not be aware that despite a genetic predisposition there are actions they can take to lower their risk. Genetic counseling and education may be necessary to intervene actively in people with a genetic susceptibility to common chronic diseases.

Genetic counseling also ensures the opportunity to provide informed consent, including discussion of the potential benefits, risks and limitations of testing, and the alternative of not testing. A benefit in knowing of a genetic predisposition through genetic testing is the potential to improve diagnosis, management, and prevention efforts for an individual and their family, and there may be psychological and cognitive benefits as well. Family issues may arise with genetic

testing for common, chronic diseases [81]. Family members may experience loss of privacy when asked to share their medical history and medical records, and if labeled as having a genetic predisposition to disease, family, friends, or society may stigmatize them. Family dynamics may change from knowledge of a genetic risk for disease. For example, a parent may feel guilt about passing on a disease predisposition to a child, or a sibling may experience survivor guilt if a genetic susceptibility is excluded for them but identified in another sibling. There is also the concern that third parties such as employers, educators, and insurers could use genetic information to exclude individuals from employment or education opportunities or from obtaining health, life, or long-term care or disability insurance, and this concern has had a negative impact on utilization of genetic services despite the lack of evidence of genetic discrimination against otherwise healthy individuals [82–84]. The Genetic Information Nondiscrimination Act of 2008 provides legal protections from genetic discrimination by employers and insurers in the group and individual health insurance market [85].

Traditional pretest and posttest counseling may not be necessary or feasible when the intent of germline genetic testing is to inform therapeutic options, an emerging purpose for germline testing as targeted cancer therapies have been discovered for individuals with pathogenic variants in gene that cause hereditary cancer syndromes [86,87]. However, professional societies advise that patient preferences regarding receipt of germline findings should be honored, the relevance and potential benefits of this information for patients and their relatives should be discussed, and the limitations and potential harms of receiving germline information need to be addressed [77]. Population-based approaches to genetic testing – such as newborn screening or ethnicity-based screening – provide examples for patient education and informed consent on a large scale that may help to inform care processes when germline genetic test results are obtained to inform therapeutic options. Previous studies of population screening of pathogenic variants in the *BRCA1* and *BRCA2* genes among Ashkenazi Jewish individuals used written information

provided prior to testing and posttest counseling was offered only to individuals with pathogenic variants or a significant family history, and not those with normal genetic test results [88–90]. Participants reported high levels of satisfaction and there was no evidence of significant distress. However, these studies were performed in self-referred individuals. Such favorable outcomes may not be observed for individuals in a clinical setting.

## 9.4. Genetic Healthcare Models

Two care models for genetic services were described in a systematic review of genetic healthcare: the traditional model of coordinated services between clinical geneticists and other physicians, and the emerging model where genetic services are integrated within primary care and other specialties [20]. The traditional model is generally organized as a regional genetic center, usually affiliated with an academic medical center, offering special clinics for various patient care needs (e.g., cancer, prenatal). The emerging model increasingly involves decision support tools in the electronic health record assisting the nongenetic health professional in performing genetic risk assessment and identifying indications for genetic testing [91,92]. Genetic counselors or genetic nurses can provide support with both care models [93]. The lack of clinical geneticists has been recognized as a serious problem compromising the traditional model [94], and the lack of knowledge and awareness of genetics among nongenetic healthcare providers is a challenge for the emerging model [14,95–100].

To remove access barriers to genetic professionals, several new delivery models have evolved, including: "telegenetic" services that use video-telecommunication and information technologies to provide genetic services to patients in remote clinical settings [101,102], telephone consultation that provides genetic services directly to patients [103,104], and electronic consultation (e-consult) using information technology that allows nongenetic healthcare providers to obtain input from a genetics professional without requiring the patient to visit with the genetics professional [105]. Having options for

genetic healthcare arrangements is beneficial but only if the care arrangements are matched to the needs and preferences of patients and the referring providers and if they result in safe, effective, and efficient care coordination.

## Summary

Genetic factors play an important role in the etiology, natural history, and response to therapy for many common, chronic diseases. An important goal of genetic evaluation for such diseases is the development of individualized management and preventive strategies based on the genetic risk assessment and the patient's personal medical history, lifestyle, and preferences. Genetic counseling is critical for delineating a patient's motivations for genetic evaluation of a common, chronic disease of adulthood and likely responses to learning of a genetic risk or diagnosis. Currently, the family history is the best initial approach for genetic risk assessment and diagnosis of most common chronic diseases. Genetic testing can be used to further refine the genetic risk assessment, diagnosis, and management decisions. Genetic healthcare is traditionally provided by clinical geneticists, although increasingly other specialists and primary care providers are incorporating genetic services for common, chronic diseases of adulthood into their practice. Nontraditional modes of delivery are increasingly utilized to improve access to genetic services, including consultation via video-teleconferencing, phone, and electronic chart review consultation.

## References

[1] Scheuner M.T, Yoon P.W, Khoury M.J. Contribution of Mendelian disorders to common chronic disease: opportunities for recognition, intervention, and prevention. *Am J Med Genet C Semin Med Genet*. 2004;125C(1):50–65.

[2] Kohlmann W, Gruber S.B. Lynch

syndrome. In: Pagon R.A, Adam M.P, Ardinger H.H, al., eds. *GeneReviews*®. Seattle (WA): University of Washington, Seattle; February 5, 2004:1993–2017 Available from: https://www.ncbi.nlm.nih.gov/books/NBK1211/.

[3] Cirino A.L, Ho C. Hypertrophic cardiomyopathy overview. In: Pagon R.A, Adam M.P, Ardinger H.H, e al., eds. *GeneReviews*®. Seattle (WA): University of Washington, Seattle; August 5, 2008:1993–2017 Available from: https://www.ncbi.nlm.nih.gov/books/NBK1768/.

[4] Hayden E.C. Technology: the $1,000 genome. *Nature*. 2014;507(7492):294–295.

[5] Eble T.N, Nagamani S.C, Franco L.M, Plon S.E, Blazo practice of adult genetics: a 7-year experience from a single center. *Am J Med Genet A*. January 2013;161A(1):89–93.

[6] Posey J.E, Rosenfeld J.A, James R.A, Bainbridge M, Ni diagnostic experience of whole-exome sequencing in adult patients. *Genet Med*. July 2016;18(7):678–685.

[7] Taylor M.R, Edwards J.G, Ku L. Lost in transition: challenges in the expanding field of adult genetics. *Am J Med Genet Part C Semin Med Genet*. 2006;142C:294–303.

[8] Ramos E.M, Din-Lovinescu C, Berg J.S, et al. Characterizing genetic variants for clinical action. *Am J Med Genet C Semin Med Genet*. 2014;166C(1):93–104.

[9] Armstrong J, Toscano M, Kotchko N, et

al. Utilization of outcomes of *BRCA* genetic testing and counseling in a national commercially insured population. The ABOUT Study. *JAMA Oncol*. 2015;1(9):1251–1260.

[10] Kasparian A, Wakefield C.E, Meiser B. Assessment of psychosocial outcomes in genetic counseling research: an overview of available measurement scales. *J Genet Counsel*. 2007;16:693–712.

[11] McAllister M, Dearing A. Patient reported outcomes and patient empowerment in clinical genetics services. *Clin Genet*. 2015;88(2):114–121.

[12] McAllister M, Wood A.M, Dunn G, Shiloh S, Todd C. genetic counseling outcome scale: a new patient-reported outcome measure for clinical genetics services. *Clin Genet*. 2011;79:413–424.

[13] Payne K, Nicholls S, McAllister M, Macleod R, Donna measurement in clinical genetics services: a systematic review of validated measures. *Value Health*. 2008;11(3):497–508.

[14] Scheuner M.T, Sieverding P, Shekelle P.G. Delivery of genomic medicine for common chronic adult diseases; a systematic review. *J Am Med Assoc*. 2008;299(11):1320–1334.

[15] Silvey K, Stock J, Hasegawa L.E. Mann Au S. Outcomes for genetic services: creating an inclusive definition and outcomes menu for public health and clinical genetics services. *Am J Med Genet Part C Semin Med Genet*. 2009;151C:207–213.

[16]

Zierhut H.A, Shannon K.M, Cragun D.L, Cohen S.A. I genetic counseling outcomes from the perspective of genetic counselors. *J Genet Counsel*. 2016 doi: 10.1007/s10897-015-9930-9.

[17] Khoury M.J, Yang Q, Gwinn M, Little J, Dana Flanders W. An epidemiologic assessment of genomic profiling for measuring susceptibility to common diseases and targeting interventions. *Genet Med*. 2004;6(1):38–47. .

[18] Valdez R, Yoon P.W, Qureshi N, Green R.F, Khoury M history in public health practice: a genomic tool for disease prevention and health promotion. *Annu Rev Public Health*. 2010;31:69–87.

[19] American College of Medical Genetics, Genomics Board of Directors. Scope of practice: a statement of the American College of medical genetics and genomics (ACMG). *Genet Med*. 2015;17(9):e3. doi: 10.1038/gim.2015.94 Epub 2015 Jul 23.

[20] Battista R.N, Blancquaert I, Laberge A.M, van Schendel N, Leduc N. Genetics in healthcare: an overview of current an emerging models. *Public Health Genom*. 2012;15:34–45.

[21] Lerner B, Marshall N, Oishi S, Lanto A, Lee M, Hamil value of genetic testing: beyond clinical utility. *Genet Med*. July 2017;19(7):763–771.

[22] Gramling R, Nash J, Siren K, Culpepper L. Predictive genetics in primary care: expectations for the motivational impact of genetic testing affects the

importance family physicians place on screening for familial cancer risk. *Genet Med*. 2003;5(3):172–175.
[23] Marteau T.M, Lerman C. Genetic risk and behavioural change. *BMJ*. 2001;322(7293):1056–1059.
[24]
Hermel D.J, Wooj M, Chun J, Rounds T, Sands M, Sch institutional evaluation of women at high risk of developing breast cancer. *Clin Breast Cancer*. April 14, 2017 doi: 10.1016/j.clbc.2017.04.005 pii: S1526–8209(16)30264-6. [Epub ahead of print].
[25] Hartz S.M, Olfson E, Culverhouse R, Cavazos-Rehg P, Chen L.S, DuBois J, Fisher S, Kaphingst K, Ka of individual genetic results in a high-risk sample: enthusiasm and positive behavioral change. *Genet Med*. 2015;17(5):374–379.
[26]
Olfson E, Hartz S, Carere D.A, Green R.C, Roberts J.S, Study Group. Implications of personal genomic testing for health behaviors: the case of smoking. *Nicotine Tob Res*. 2016;18(12):2273–2277.
[27]
McBride C.M, Koehly L.M, Sanderson S.C, Kaphingst behavioral response to personalized genetic information: will genetic risk profiles motivate individuals and families to choose more healthful behaviors? *Annu Rev Public Health*. 2010;31:89–103.
[28]
McBride C.M, Bepler G, Lipkus I.M, Lyna P, Samsa G, genetic susceptibility feedback into a smoking cessation program for African-American smokers with low income. *Cancer Epidemiol Biomark*

*Prev*. 2002;11(6):521–528.

[29] Vernarelli J.A, Roberts J.S, Hiraki S, Chen C.A, Cupple of Alzheimer disease genetic risk disclosure on dietary supplement use. *Am J Clin Nutr*. 2010;91(5):1402–1407.

[30] Dwyer J, Donoghue M.D. Is risk of Alzheimer disease a reason to use dietary supplements? *Am J Clin Nutr*. 2010;91(5):1155–1156.

[31] Domchek S.M, Friebel T.M, Singer C.F, Evans D.G, Ly t'veer L, Tung N, Weitzel J.N, Couch F.J, Rubinstein W of risk-reducing surgery in *BRCA1* or *BRCA2* mutation carriers with cancer risk and mortality. *J Am Med Assoc*. 2010;304(9):967–975.

[32] Nebgen D.R, Lu K.H, Rimes S, Keeler E, Broaddus R, colonoscopy and endometrial biopsy cancer screening results in women with Lynch syndrome. *Gynecol Oncol*. 2014;135(1):85–89.

[33] Butow P.N, Lobb E.A, Meiser B, Barratt A, Tucker K.M outcomes and risk perception after genetic testing and counselling in breast cancer: a systematic review. *Med J Aust*. 2003;178(2):77–81.

[34] Schlich-Bakker K.J, ten Kroode H.F, Ausems M.G. A literature review of the psychological impact of genetic testing on breast cancer patients. *Patient Educ Couns*. 2006;62(1):13–20.

[35] Collins V, Halliday J, Warren R, Williamson R. Cancer

worries, risk perceptions and associations with interest in DNA testing and clinic satisfaction in a familial colorectal cancer clinic. *Clin Genet*. 2000;58(6):460–468.

[36] Pieterse A.H, van Dulmen A.M, Beemer F.A, Bensing J.M, Ausems M.G. genetic counseling: communication and counselees' post-visit satisfaction, cognitions, anxiety, and needs fulfillment. *J Genet Couns*. 2007;16(1):85–96.

[37] Green R.C, Roberts J.S, Cupples L.A, Relkin N.R, Whi Deegan R, Farrer L.A. REVEAL Study Group.. Disclosure of *APOE* genotype for risk of Alzheimer's disease. *N Engl J Med*. 2009;361(3):245–254.

[38] Christensen K.D, Roberts J.S, Uhlmann W.R, Green R. to perceptions of the pros and cons of genetic susceptibility testing after *APOE* genotyping for Alzheimer disease risk. *Genet Med*. 2011;13(5):409–414.

[39] Pierce M, Ridout D, Harding D, Keen H, Bradley C. M good than harm: a randomised controlled trial of the effect of education about familial risk of diabetes on psychological outcomes. *Br J Gen Pract*. 2000;50(460):867–871.

[40] National Society of Genetic Counselors' Definition Task Force, Resta R, Biesecker B.B, Bennett R.L, et al. A new definition of genetic counseling: national society of genetic counselors' Task Force report. *J Genet Couns*. 2006;15(2):77–83. .

[41]
Eckhauser A, South S.T, Meyers L, Bleyl S.B, Botto L.I Syndrome in girls presenting with coarctation of the aorta. *J Pediatr*. 2015;167(5):1062–1066.
[42]
Park Y.W, Zhu S, Palaniappan L, Heshka S, Carnethoı metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Intern Med*. 2003;163(4):427–436.
[43] Flossmann E, Rothwell P.M. Family history of stroke in patients with transient ischemic attack in relation to hypertension and other intermediate phenotypes. *Stroke*. 2005;36(4):830–835.
[44]
Scheuner M.T, Whitworth W.C, McGruder H, Yoon P risk assessment for early-onset coronary heart disease. *Genet Med*. 2006;8(8):525–531.
[45]
Pandey A.K, Mj B, Sharma K, Rivera J, Budoff M.J, Bla Mallah M, Wong N.D, Shaw L, Carr J, O'Leary D, Lim history of coronary heart disease and the incidence and progression of coronary artery calcification: multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. February 2014;232(2):369–376.
[46]
Hariri S, Yoon P.W, Qureshi N, Valdez R, Scheuner M history of type 2 diabetes: a population-based screening tool for prevention? *Genet Med*. 2006;8(2):102–108.

[47] Permuth-Wey J, Egan K.M. Family history is a significant risk factor for pancreatic cancer: results from a systematic review and meta-analysis. *Fam Cancer*. 2009;8(2):109–117.
[48] Matakidou A, Eisen T, Houlston R.S. Systematic review of the relationship between family history and lung cancer risk. *Br J Cancer*. 2005;93(7):825–833.
[49] Butterworth A.S, Higgins J.P, Pharoah P. Relative and absolute risk of colorectal cancer for individuals with a family history: a meta-analysis. *Eur J Cancer*. 2006;42(2):216–227.
[50] Stratton J.F, Pharoah P, Smith S.K, Easton D, Ponder B systematic review and meta-analysis of family history and risk of ovarian cancer. *Br J Obstet Gynaecol*. 1998;105(5):493–499.
[51] Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. *Lancet*. 2001;358(9291):1389–1399.
[52] Aronoff S.L, Bennett P.H, Gorden P, Rushforth N, Mil hyperinsulinemia in normal and "prediabetic" Pima Indians compared with normal Caucasians. An example of racial differences in insulin secretion. *Diabetes*. 1977;26(9):827–840.
[53] Sharp P.S, Mohan V, Levy J.C, Mather H.M, Kohner E resistance in patients of Asian Indian and European

origin with non-insulin dependent diabetes. *Horm Metab Res*. 1987;19(2):84–85.

[54] Struewing J.P, Hartge P, Wacholder S, Baker S.M, Berl risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *N Engl J Med*. 1997;336(20):1401–1408.

[55] Chen S, Wang W, Lee S, Nafa K, Lee J, Romans K, Wa Cancer Family Registry. Prediction of germline mutations and cancer risk in the Lynch syndrome. *J Am Med Assoc*. 2006;296(12):1479–1487.

[56] Antoniou A.C, Pharoah P.P, Smith P, Easton D.F. The BOADICEA model of genetic susceptibility to breast and ovarian cancer. *Br J Cancer*. 2004;91(8):1580–1590.

[57] Berry D.A, Iversen Jr. E.S, Gudbjartsson D.F, Hiller E. validation, sensitivity of genetic testing of *BRCA1/BRCA2*, and prevalence of other breast cancer susceptibility genes. *J Clin Oncol*. 2002;20(11):2701–2712.

[58] Freedman A.N, Slattery M.L, Ballard-Barbash R, Willis G, Cann B.J, Pee D, Gail M.H, Pfeiffe cancer risk prediction tool for white men and women without known susceptibility. *J Clin Oncol*. 2009;27(5):686–693.

[59] *National Cancer Institute breast cancer risk assessment tool*. January 2011 [cited 2011 Aug 6, 2017]; Available from:. http://www.cancer.gov/bcrisktool/.

[60] Claus E.B, Risch N, Thompson W.D. The calculation

of breast cancer risk for women with a first degree family history of ovarian cancer. *Breast Cancer Res Treat*. 1993;28(2):115–120.

[61] Claus E.B, Risch N, Thompson W.D. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. *Cancer*. 1994;73(3):643–651.

[62] Wilson P.W, Meigs J.B, Sullivan L, Fox C.S, Nathan D. of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Arch Intern Med*. 2007;167(10):1068–1074.

[63] Parikh N.I, Pencina M.J, Wang T.J, Benjamin E.J, Lanie risk score for predicting near-term incidence of hypertension: the Framingham Heart Study. *Ann Intern Med*. 2008;148(2):102–110.

[64] Ziogas A, Anton-Culver H. Validation of family history data in cancer family registries. *Am J Prev Med*. 2003;24(2):190–198. .

[65] Murff H.J, Spigel D.R, Syngal S. Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. *J Am Med Assoc*. 2004;292(12):1480–1489.

[66] Murabito J.M, Nam B.H, D'Agostino Sr. R.B, Lloyd-Jones D.M, O'Donnel C.J, Wilson P.W. Accuracy of offspring reports of parental cardiovascular disease history: the Framingham Offspring Study. *Ann Intern Med*. 2004;140(6):434–440.

[67] McPherson E, Zaleski C, Benishek K, McCarty C.A, G

genetics provider real-time workflow study. *Genet Med*. 2008;10(9):699–706.

[68] Lindor N.M, Schahl K.A, Johnson K.J, Hunt K.S, Mens Johnson D.L, McAllister T.M, Farrugia G, McCormick exome sequencing of 10 scientists: evaluation of the process and outcomes. *Mayo Clin Proc*. 2015;90(10):1327–1337.

[69] Juneja T, Pericak-Vance M.A, Laing N.G, Dave S, Siddique T. Prognosis in familial amyotrophic lateral sclerosis: progression and survival in patients with glu100gly and ala4val mutations in Cu,Zn superoxide dismutase. *Neurology*. 1997;48:55–57.

[70] Nieuwenhuis M.H, Vasen H.F. Correlations between mutation site in *APC* and phenotype of familial adenomatous polyposis (FAP): a review of the literature. *Crit Rev Oncol Hematol*. 2007;61(2):153–161.

[71] Tang G, Shak S, Paik S, Anderson S.J, Costantino J.P, C of the prognostic and predictive utilities of the 21-gene Recurrence Score assay and Adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. *Breast Cancer Res Treat*. 2011;127(1):133–142.

[72] Lin A.Y, Buckley N.S, Lu A.T, Kouzminova N.B, Salpe of *KRAS* mutational status in advanced colorectal cancer on the outcomes of anti-epidermal growth factor receptor monoclonal antibody therapy: a systematic review and meta-analysis. *Clin Colorectal*

*Cancer*. 2011;10(1):63–69.
[73] McGuire A.L, Burke W. Health system implications of direct-to-consumer personal genome testing. *Public Health Genom*. 2011;14(1):53–58.
[74] Krier J, Barfield R, Green R.C, Kraft P. Reclassification of genetic-based risk predictions as GWAS data accumulate. *Genome Med*. 2016;8(1):20. doi: 10.1186/s13073-016-0272-5.
[75] Palomaki G.E, Melillo S, Neveux L, Douglas M.P, Dot of genomic profiling to assess risk for cardiovascular disease and identify individualized prevention strategies–a targeted evidence-based review. *Genet Med*. 2010;12(12):772–784.
[76] Haddow J.E, Palomaki G.E. ACCE: a model process for evaluating data on emerging genetic tests. In: Khoury M.J, Little J, Burke W, eds. *Human genome epidemiology: a scientific foundation for using genetic information to improve health and prevent disease*. New York (NY): Oxford University Press; 2003:217–233.
[77] Robson M.E, Bradbury A.R, Arun B, Domchek S.M, Fo society of clinical oncology policy statement update: genetic and genomic testing for cancer susceptibility. *J Clin Oncol*. 2015;33(31):3660–3667.
[78] Kaphingst K.A, Lachance C.R, Condit C.M. Beliefs about heritability of cancer and health information seeking and preventive behaviors. *J Cancer Educ*. 2009;24(4):351–356.

[79] Innes C.C, Lewis F.M. Family history of cardiovascular disease (CVD), perceived CVD risk, and health-related behavior: a review of the literature. *J Cardiovasc Nurs*. 2014;29(2):108–129.
[80] West D.S, Greene P.G, Kratt P.P, Pulley L, Weiss H.L, impact of a family history of breast cancer on screening practices and attitudes in low-income, rural, African American women. *J Womens Health (Larchmt)*. 2003;12(8):779–787.
[81] Smith J.A, Dancyger C, Wallace M, Jacobs C, Michie S development of a methodology for examining the process of family communication of genetic test results. *J Genet Couns*. 2011;20(1):23–34.
[82] Apse K.A, Biesecker B.B, Giardiello F.M, Fuller B.P, Be of genetic discrimination among at-risk relatives of colorectal cancer patients. *Genet Med*. 2004;6:510–516.
[83] Lapham E.V, Kozma C, Weiss J.O. Genetic discrimination: perspectives of consumers. *Science*. 1996;5287:621–624.
[84] Joly Y, Feze I.N, Simard J. Genetic discrimination and life insurance: a systematic review of the evidence. *BMC Med*. 2013;11:25.
[85] *Genetic Information Nondiscrimination Act, in Public Law 110-233*. May 21, 2008 Washington (DC).
[86] Meehan R.S, Chen A.P. New treatment option for ovarian cancer: PARP inhibitors. *Gynecol Oncol Res Pract*. 2016;3:3. .
[87]

Le D.T, Uram J.N, Wang H, Bartlett B.R, Kemberling H
la
Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban
1 blockade in tumors with mismatch-repair
deficiency. *N Engl J Med*. 2015;372:2509–2520.
[88]
Metcalfe K.A, Poll A, Royer R, Llacuachaqui M, Tulm
for founder mutations in *BRCA1* and *BRCA2* in
unselected Jewish women. *J Clin Oncol*. 2010;28:387–
391.
[89]
Gronwald J, Huzarski T, Byrski T, Debniak T, Metcalf
to-patient BRCA1 testing: the Twoj Styl
experience. *Breast Cancer Res Treat*. 2006;100:239–245.
[90] Metcalfe K.A, Mian N, Enmore M, et al. Long-term
follow-up of Jewish women with a *BRCA1* and
*BRCA2* mutation who underwent population genetic
screening. *Breast Cancer Res Treat*. 2012;133:735–740.
[91]
Scheuner M.T, Hamilton A.B, Peredo J, Sale T.J, Austi
cancer genetics toolkit improves access to genetic
services through documentation and use of the
family history by primary-care clinicians. *Genet
Med*. 2014;16(1):60–69.
[92] Emery J. The GRAIDS Trial: the development and
evaluation of computer decision support for cancer
genetic risk assessment in primary care. *Ann Hum
Biol*. 2005;32(2):218–227.
[93]
Scheuner M.T, Marshall N, Lanto A, Hamilton A.B, O
of clinical genetic consultative services in the

Veterans Health Administration. *Genet Med*. 2014;16(8):609–619.

[94] Cooksey J.A, Forte G, Benkendorf J, Blitzer M.G. The state of the medical geneticist workforce: findings of the 2003 survey of American Board of Medical Genetics certified geneticists. *Genet Med*. 2005;7(6):439–443.

[95] Martin G, Currie G, Finn R. Bringing genetics into primary care: findings from a national evaluation of pilots in England. *J Health Serv Res Policy*. 2009;14:204–211.

[96] Burke W, Culver J, Pinsky L, Hall S, Reynolds S.E, Yas assessment of breast cancer risk in primary care practice. *Am J Med Genet A*. 2009;149A(3):349–356.

[97] Brierley K.L, Campfield D, Ducaine W, Dohany L, Do Et Errors in delivery of cancer genetics services: implications for practice. *Conn Med*. 2010;74(7):413–423.

[98] Dhar S.U, Cooper H.P, Wang T, Parks B, Staggs S.A, H differences among physician specialists in management recommendations of BRCA1 mutation carriers. *Breast Cancer Res Treat*. 2011;129(1):221–227.

[99] Plon S.E, Cooper H.P, Parks B, Dhar S.U, Kelly P.A, W testing and cancer risk management recommendations by physicians for at-risk relatives. *Genet Med*. 2011;13(2):148–154.

[100] Bensend T.A, Veach P.M, Niendorf K.B. What's the

harm? Genetic counselor perceptions of adverse effects of genetics service provision by non-genetics professionals. *J Genet Couns*. 2014;23(1):48–63.

[101] Zillacus E.M, Meiser B, Lobb E.A, Kelly P.J, Barlow-Stewart K, Kirk J.A, Spigelman A.D, Warwick L.J, Tuc videoconferenced consultations as effective as face-to-face consultations for hereditary breast and ovarian cancer genetic counseling? *Genet Med*. 2011;13(11):933–941.

[102] Hilgart J.S, Hayward J.A, Coles B, Iredale R. Telegene a systematic review of telemedicine in genetics services. *Genet Med*. 2012;14(9):765–776.

[103] Platten U, Rantala J, Lindblom A, Brandberg Y, Lindg use of telephone in genetic counseling versus in-person counseling: a randomized study on counselees' outcome. *Fam Cancer*. 2012;11(3):371–379.

[104] Cohen S.A, Marvin M.L, Riley B.D, Vig H.S, Rousseau of genetic counseling service delivery models in practice: a report from the NSGC Service Delivery Model Task Force. *J Genet Couns*. 2013;22(4):411–421.

[105] Liddy C, Maranger J, Afkham A, Keely E. Ten steps to establishing an e-consultation service to improve access to specialist care. *Telemedicine e-Health*. 2013;19(12):982–990.

10

# Carrier Screening and Heterozygote Testing

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## Abstract

The implementation of carrier screening programs must rely on education and standardization. Both patients and healthcare providers need education about genetic screening programs. This is amply illustrated by the cumulative experiences of the Tay–Sachs disease, β-thalassemia, and cystic fibrosis carrier screening programs. Even if the individual tested receives a negative screening result, it will still be appropriate for the physician to order a diagnostic test if warranted by the clinical circumstances. Subpopulations affected by targeted carrier screening programs also need to be reliably informed about the mutation detection rates and overall clinical utility to ensure that genetic counseling is effective. Literature about the clinical utility of expanded carrier screening panels is growing, and this approach will challenge variant interpretation and genetic counseling strategies.

## Keywords

Benign allele; Carrier screening; Expanded carrier screening; Genetic counseling; Genetic heterogeneity; Heterozygote testing; Probands

# Glossary

**Benign allele** Different alleles produce variations in inherited characteristics that can be benign or disease-causing. An example of benign allele variations are those that cause different eye colors or blood types.

**Carrier screening** Systematic identification of (usually) unaffected individuals who carry one copy of a gene for a disease that requires two copies for the disease to be expressed.

**Ethnicity-based carrier screening** Screening that is targeted at populations known to be at increased risk of particular conditions, such as screening individuals of Eastern European (Ashkenazi) Jewish descent for Tay–Sachs disease.

**Expanded carrier screening (ECS)** Screening that evaluates an individual's carrier state for multiple conditions at once and regardless of ethnicity or family history.

**Genetic heterogeneity** Genetic heterogeneity can be defined as mutations at two or more genetic loci that produce the same or similar phenotypes (either biochemical or clinical). This results in the production of the same or similar phenotype by different genetic mechanisms.

**Heterozygote testing** Use of specific assays to determine the genetic status of individuals already suspected to be at higher risk for an inherited disorder because of family history or clinical symptoms. Typically, heterozygote testing is conducted by more accurate diagnostic tests than may be typically used for population-based carrier screening purposes.

**Pan-ethnic screening** Screening that evaluates an individual's carrier state regardless of their ethnic background or family history, such as the recommendation to screen every pregnancy women for cystic fibrosis during her lifetime.

**Proband** Affected family member through whom a family with a genetic disorder is ascertained. Also called the propositus or index case. The proband may or may not be the consultant (the

individual presenting for genetic counseling).

**Pseudodeficiency allele** Clinically benign allele that exhibits reduction in functional activity as detected in vitro assays but that has sufficient activity in vivo to prevent haploinsufficiency.

## 10.1. Introduction

The specific biochemical or genetic abnormality in nearly 5000 Mendelian diseases have been determined [1]. Although individually uncommon, these Mendelian diseases collectively account for nearly 20% of infant mortality and 10% of pediatric hospitalizations [2]. The ability to identify the underlying molecular etiology in affected individuals (probands) permits the rapid and accurate diagnosis of such disorders, minimizing the time to diagnosis and reduces the overall cost of the diagnostic odyssey. More importantly, the delineation of the underlying molecular etiology has enabled investigators to develop rational and effective therapies for a variety of these disorders [3–6]. In addition, to the diagnostic and possible therapeutic implications of such discoveries, comparable methodologies have been employed for the detection of heterozygous carriers of many of the recessive traits involved. Accordingly, not only is a striking deficiency of enzymatic activity, gene dosage, or metabolic dysfunction evident in the diagnosis of the homozygous or hemizygous state (the affected male with an X-linked recessive disorder), but a distinct quantitative decrease of the same function can be demonstrated in the otherwise normal individual carrying the recessive allele. This capability to quantify gene dosage, and thereby to identify carriers of recessive traits, has been reported for many of the inborn errors of metabolism where the primary defect is known.

Advances in the applications of molecular genetic techniques have made it possible to identify heterozygous carriers of an increasing number of mutant alleles. In some instances, this may involve direct detection of the mutation (e.g., sickle cell trait [SCT] or carriers of mutations for β-thalassemia, cystic fibrosis [CF], Tay–Sachs disease [TSD], fragile X syndrome).

It is not our intent to review all known disorders and their current status regarding carrier detection. Rather, specific disorders will be cited as illustrative examples of the issue being discussed. Other chapters in this text more fully elaborate the fundamental genetic changes associated with such disorders, or deal specifically with issues of prenatal and neonatal screening as well as heterozygote detection. It is also important to make the distinction between individual heterozygote testing and carrier screening. Heterozygote testing is the use of specific assays to determine the genetic status of individuals already suspected to be at higher risk for an inherited disorder because of a family history or clinical symptoms [7]. In contrast, population-based carrier screening is the use of genetic tests to evaluate specific populations or groups of individuals independent of a family history of a disorder, and without clinical symptoms. While population-based carrier screening has traditionally targeted populations known to be at increased risk of particular conditions, such as screening individuals of Eastern European (Ashkenazi) Jewish descent for TSD, the approach of offering carrier screening for multiple conditions regardless of ethnicity or family history (expanded carrier screening) is becoming more mainstream.

## 10.2. Carrier Screening in Clinical Practice

Heterozygote testing and carrier screening have been a part of clinical practice for several decades, and professional guidelines for population carrier screening have been available for longer than

15 years. Heterozygote testing for individuals identified to be at high risk because of family history or clinical symptoms has been available longer than population carrier screening testing and has been fueled by patient desire for more accurate risk assessment than can be provided based on family history and traditional genetic counseling risk assessment models such as Bayes theorem. As you will learn within this chapter, both heterozygote testing and carrier screening are dependent on accurate and reproducible methods of carrier detection, much of which is now enabled by next-generation

sequencing (NGS) technologies.

### 10.2.1. Reproductive Screening and Testing Options

Carrier screening identifies couples at increased risk of having a child with a genetic disease and provides them with information to optimize pregnancy outcomes based on their personal preferences and values. Carrier identification allows for preconception planning and the option to choose alternative reproductive methods. If both prospective parents are found to be carriers, then comprehensive genetic counseling with a complete discussion of all available options can be initiated including prenatal diagnosis, gamete donation (e.g., sperm or ovum from an established noncarrier donor), preimplantation genetic diagnosis, and adoption. Identifying at-risk couples enables them to reduce or prevent the risk of transmitting the genetic disease to subsequent generations. It is becoming increasingly common for assisted reproductive technology to be considered by a couple as an alternative reproductive strategy to avoid the birth of a child affected with an autosomal recessive or X-linked disorder, either through gamete donation or preimplantation genetic diagnosis. When proceeding with gamete donation, it is critical that potential sperm or ovum donors are evaluated by appropriate carrier screening tests to ensure that genetic risks are appropriately accounted for. A recent survey of public sperm donor banks across the United States showed significant variation in the education, informed consent process, and genetic testing practices of sperm donors [8]. While all sperm banks reported that they follow carrier screening guideline of at least one professional organization, the adherence to these guidelines remains voluntary, contributing to the variability of genetic screening of sperm donors across the United States. This variability in donor screening regulation and practice is concerning given the potential risks that it poses for both the intended parents and the donor's future offspring.

Preconception carrier screening can also be used to inform marital decisions. Because of religious, moral, or other concerns related to

pregnancy termination, individuals may choose to use information about their carrier status in their decision regarding mate selection. This is the practice in certain Hassidic Jewish communities where strong religious proscriptions against abortion exist [9]. Because marriages are sometimes arranged (often in childhood) and require rabbinical approval, some rabbis have encouraged TSD carrier testing among the children or adolescents of their community [10]. Approval for marriage is withheld by the rabbi (to whom solely the results of carrier testing are conveyed) if two carriers are "matched." Alternative matches may then be made without specifying the reason, thus avoiding family or individual stigmatization.

Those who do not make alternative reproductive decisions based on their carrier status may still use this knowledge to prepare for the birth of an affected child and potentially facilitate early intervention. For example, knowing prenatally about an increased risk for certain inheritable metabolic disorders such as phenylketonuria can inform parents and physicians about the need for a rapid diagnosis upon birth and available treatment plans [11,12]. Regardless of how parents choose to use carrier screening information, testing should be offered during the preconception period so patients can benefit from the greatest number of testing and screening options. This is further elaborated in the section on the timing of carrier screening.

### 10.2.2. Cascade Testing

Carrier screening not only identifies couples at increased risk of having a child with a genetic disease but also enables other at-risk family members to pursue carrier testing for the identified familial mutation, triggering cascade genetic testing. These individuals are not likely to be identified another way or as early as when cascade testing is offered. Cascade testing, is then defined as the prioritized testing of individuals on the basis of degree of relatedness to a known carrier and is highly dependent on family communication and personal interest. The "cascade" refers to the process of testing family members for the identified familial mutation. In the event of a positive result, descendants are tested; descendants of an individual who receive a

negative test are not tested [13]. Because most Mendelian disorders are individually rare and require both prospective parents to be carriers, it is relatively unlikely that other family members will be at risk for affected offspring. In the cases where family members are found to be at risk for having an affected child, this information can facilitate informed reproductive decision-making.

### 10.2.3. Reduced Disease Incidence

When discussing the benefits of carrier screening, it is important to highlight the public health benefits. For example, population-based carrier screening outcomes studies have demonstrated reduction of disease incidence within a variety of tested populations. Reduced disease incidence has been reported for TSD, hemoglobinopathies, and CF after the implementation of carrier screening programs. Decreased birth prevalence of CF has been well documented within the United States as well as the United Kingdom and Italy [14]. In the United States, a study in Massachusetts demonstrated a gradual decrease in CF incidence following the adoption of the 2001 screening recommendations by the American College of Obstetrics and Gynecology (ACOG), the American College of Medical Genetics and Genomics (ACMG), and the National Institutes of Health to offer general population screening [15]. Because population carrier screening has resulted in a reduction within specific diseases of interest, it is reasonable to assume that large-scale implementation of expanded carrier screening will result in further reduction of disease incidence for rare Mendelian disorders.

## 10.3. Carrier Screening in Individuals of Defined Subpopulation Groups

Professional carrier screening guidelines have been available for longer than 15 years and have historically targeted specific diseases that occur at increased frequencies in defined ethnic populations. The

fact that select genetic diseases occur predominantly with certain ethnic, religious, or racial groups is not surprising when one considers the impact of genetic drift in combination with both historical and social factors, such as the relatively high degree of consanguinity seen in some defined subpopulations [16,17]. This is often the result of generations of cultural influences that encourage intracommunity or interfamilial marriage. An example of increased autosomal recessive disorders due to high rates of consanguinity is seen within the Pakistani Muslim community, where cousin marriages are viewed as a valued cultural tradition [18]. In addition, selective environmental factors may have existed at some point in history that provided a biological (reproductive) advantage to carriers of the recessive gene (e.g., relative resistance to malaria in individuals who are heterozygous for sickle cell hemoglobin, β-thalassemia, or G6PD deficiency). Because of this selective effect, the gene becomes enriched in subsequent generations for that population.

## Table 10.1

### Frequency and Incidence for Selected Autosomal Recessive Disorders in Defined Ethnic Groups in the United States

| Disease | Ethnic Group | Allele | Carrier | "At-Risk" Couple | Disea Incid |
|---|---|---|---|---|---|
| | | Frequency | Frequency | Frequency[a] | in Newb |
| **Sickle cell anemia** | African Americans | 0.040 | 0.080 | 1:150 | 1:600 |
| **Tay–Sachs disease** | Ashkenazi Jews | 0.016 | 0.032 | 1:900 | 1:360( |
| **β-Thalassemia** | Greeks, Italians | 0.016 | 0.032 | 1:900 | 1:360( |
| **α-Thalassemia** | Southeast Asians | 0.020 | 0.040 | 1:625 | 1:250( |

| | | | | | |
|---|---|---|---|---|---|
| | and Chinese | | | | |
| **Cystic fibrosis** | Northern Europeans | 0.020 | 0.040 | 1:625 | 1:250( |
| **Spinal muscular atrophy** | Asian | 0.010 | 0.020 | 1:2500 | 1:10,( |
| **Phenylketonuria** | Northern Europeans | 0.008 | 0.016 | 1:4000 | 1:16,( |

[a] Likelihood that both members of a couple are heterozygous for the same recessive allele (assuming nonconsanguinity and that both are of the same ethnic group).

A list of relatively "common" autosomal recessive disorders (Table 10.1) seen in defined subpopulations in the United States is presented with data indicating the respective carrier frequency and newborn disease incidence in those ethnic groups. Gene frequencies and incidence of the disorder may vary considerably in the same ethnic groups in other parts of the world. It should be emphasized that while many of these diseases are relatively rare, the overall frequency of carriers can be quite high because of the inheritance pattern of the disease. For example, the disease incidence of CF among Caucasians of Northern European ancestry is approximately one in 2500 newborns, yet nearly 1:25 such individuals is a carrier of a CF mutation. Similarly, the disease incidence for TSD among Ashkenazi Jewish newborns is about 1:3600, while the carrier rate in this population is approximately 1:30. For those relatively "common" autosomal recessive disorders that occur within defined ethnic groups, there is often no known prior history of the disease in either side of family [19]. Such a positive history may be present in only 20% of instances where a child with such a disorder is diagnosed.

Because of these population distributions and the availability of relatively simple, accurate, and inexpensive carrier detection methods, testing guidelines have historically focused on assessing a relatively small number of diseases selected based on similar characteristics: high frequency in a certain subpopulation and association with severe

morbidity or mortality. These approaches have proved to be successful when targeting specific subpopulations, and clinicians should offer carrier screening for all individuals in these subpopulation groups in accordance with professional carrier screening guidelines [20–22]. See Table 10.2 for a list of ethnic-specific screening panels recommended by either the ACMG or the ACOG. Both of their lists are similar but differ in relation to the number conditions to be screened in the Ashkenazi Jewish population and regarding pan-ethnic hemoglobinopathy screening. Specifically, ACOG does not recommend screening for Fanconi anemia group C, Niemann–Pick disease type A, Bloom syndrome, mucolipidosis IV, and Gaucher disease in the Ashkenazi Jewish population. While both ACOG and ACMG recommend pan-ethnic screening for CF and spinal muscular atrophy, only ACOG recommends that all patients be screened with a complete blood count to assess the risk of hemoglobinopathy. Please note that these recommendations may change with time.

## Table 10.2

### Ethnic-Specific Carrier Screening Panels Recommended by the American College of Medical Genetics and Genomics or American College of Obstetricians and Gynecologists

| Disease | Ashkenazi Jewish | Caucasian | African or African American | Asian |
|---|---|---|---|---|
| **Cystic fibrosis** | ✓ | ✓ | ✓ | ✓ |
| **Hemoglobinopathies** | ✓ | ✓ | ✓ | ✓ |
| **Tay–Sachs** | ✓ | *(some)* | | |
| **Canavan** | ✓ | | | |
| **Familial dysautonomia** | ✓ | | | |
| **Fanconi anemia C** | ✓ | | | |
| **Niemann–Pick A** | ✓ | | | |

| | | | | |
|---|---|---|---|---|
| **Niemann–Pick A** | ✓ | | | |
| **Mucolipidosis IV** | ✓ | | | |
| **Bloom syndrome** | ✓ | | | |
| **Gaucher disease** | ✓ | | | |
| **Spinal muscular atrophy** | ✓ | ✓ | ✓ | ✓ |

The manner in which carrier screening programs are structured varies greatly among countries, including whether the programs are mandatory or voluntary, the type of education and counseling provided, and whether screening is offered prepregnancy or antenatally. Guidelines and recommendations about the conduct of carrier screening programs have been developed by organizations such as the World Health Organization and the Nuffield Council on Bioethics as well as multiple national and international societies [23–26]. While no single strategy can meet the needs of every population, programs can learn from both the challenges and successes of carrier screening implementation and outcomes. As countries continue to experience demographic and cultural shifts, carrier screening programs and guidelines will need to be monitored to evaluate progress and ensure that programs are equitable and effective for a growing population.

## 10.3.1. Tay–Sachs Disease

Perhaps the most successful example of carrier screening in a defined subpopulation has been the experience with TSD carrier screening and prenatal diagnosis within individuals of Ashkenazi Jewish ancestry [27–29]. TSD is an autosomal recessive neurodegenerative disorder caused by a deficiency of β-hexosaminidase A (HexA), resulting in lysosomal accumulation of GM2 ganglioside. Individuals with Ashkenazi Jewish ancestry have a significantly increased carrier frequency of approximately 1:30. Low-risk populations are estimated to have a carrier frequency of approximately 1:300 [10]. From 1970 (when serum carrier detection methods were first described) to the

present day, community-based TSD education/screening/counseling programs have been initiated in Jewish communities throughout North America, as well as in Israel, South Africa, Europe, South America, and Australia. More than 1.4 million adults have been screened voluntarily and more than 51,000 carriers detected. More critically, nearly 1400 couples – none of whom had an affected child previously – have been identified as being at risk for this fatal disorder in their offspring. Diagnostic testing was therefore available for more than 3200 pregnancies discovered to be at risk for TSD by amniocentesis or chorionic villus sampling, and the births of more than 625 affected infants have been prevented. More importantly, this approach has been associated with the birth of more than 2550 unaffected infants who were otherwise at risk [30]. Overall, these efforts have contributed to a more than a 90% decline in the incidence of this disorder in Jewish infants throughout North America since community screening began [9,28,30]. Today, the majority of children born with TSD have non-Jewish parents [22]. HexA enzyme analysis is considered the gold standard method of population screening for carrier detection, detecting approximately 98% of carriers from all ethnic backgrounds. Alternatively, targeted mutation analysis is used to screen Ashkenazi Jewish individuals for the three most common disease-associated mutations (and two pseudodeficiency alleles) in the *HEXA* gene, which account for between 92% and 98% of heterozygotes in this population. Recent studies have suggested that combining traditional enzyme analysis with full gene sequencing leads to higher sensitivity given the ability to identify rare and novel variants among individuals of all ethnic backgrounds [31,32].

As summarized in Table 10.2, the ACMG currently recommends that individuals of Ashkenazi Jewish ancestry be offered carrier screening for CF, Canavan disease, mucolipidosis IV, Niemann–Pick disease type A, Fanconi anemia group C, Bloom syndrome, Gaucher disease, and familial dysautonomia, in addition to TSD [22]. The experience within the Ashkenazi Jewish community highlights how education, engagement, and acceptance of carrier screening programs can be successful if performed with sensitivity and respect for cultural

and religious differences.

## 10.3.2. Hemoglobinopathies

Hemoglobinopathies, which include thalassemia syndromes and structural hemoglobin (Hb) variants such as sickle cell disease (SCD), are the most common group of single gene disorders in the world. The first hemoglobinopathy screening programs were aimed at detecting thalassemia and were developed in the 1970s in several Mediterranean countries [33]. Carrier screening programs for the detection of SCD developed shortly thereafter.

## 10.3.3. β-Thalassemia

The thalassemias represent a wide spectrum of hematologic disorders that are characterized by a reduced synthesis of globin chains, which results in microcytic anemia. Thalassemias are classified according to the globin chain affected, with the most common types being $\alpha$-thalassemia and β-thalassemia. β-thalassemia is characterized by reduced or absent β-globin chain synthesis and is most frequently found in southeastern and southern Asia, the Middle East, the Mediterranean countries, and North and Central Africa [34]. The highest incidences are reported in Cyprus (14%) and Sardinia (12%). Cyprus has one of the highest carrier rates of β-thalassemia in the world, estimated at one in seven individuals (14%). Population migration has resulted in an increase in β-thalassemias in most other countries, including the United States, Canada, and Northern Europe. Population screening efforts directed at the prevention of β-thalassemia through education, carrier screening, and prenatal diagnosis have been initiated in several Mediterranean countries and certain areas of North America. These screening programs have dramatically reduced the newborn incidence of β-thalassemia in several Mediterranean countries [33,35]. In Sardinia, for example, these preventive measures have reduced the incidence of disease by more than 94% of the prescreening level [36]. Reductions in the incidence of β-thalassemia has also been documented in Taiwan,

China, and the United Kingdom [37,38]. In contrast, the incidence of β-thalassemia has changed little in Saudi Arabia, even though it is mandatory for couples to participate in the premarital β-thalassemia carrier screening program. Almost 90% of the high-risk couples identified via screening married each other within 2 years and prenatal diagnosis and pregnancy termination are not widely practiced within the country; therefore, the birth rate of affected children has not changed [39]. However, as preimplantation genetic diagnosis becomes a popular alternative, the incidence of thalassemia within Saudi Arabia may decrease.

### 10.3.4. Sickle Cell Disease

SCD is due to structural hemoglobin variants in the *HBB* gene that are inherited in an autosomal recessive fashion. These atypical hemoglobin molecules replace at least one of the normal β-globin subunits and are referred to as hemoglobin S (Hb S) based on the characteristic "sickling" shape of the resulting red blood cell. Numerous other β-globin gene variants are also known, some of which may be encountered in combination with Hb S (Hb C, E, D) [40]. Similar to β-thalassemia screening, premarital, antenatal, and newborn screening programs for SCD have been established in several parts of the world, including the Middle East and the United States. The United States has considerable experience with population screening for SCT given the high carrier frequency seen within the African American population (1:12 African Americans has SCT). The first report of population-based screening for SCT was in the 1970s, but the majority of experience stems from incidental identification of sickle cell carriers on newborn screening after screening for SCD and other hemoglobinopathies was recommended by the NIH in 1987 [41,42]. Newborn screening has not only allowed for early identification of those with SCD, greatly improving morbidity and mortality but also enabled early carrier status identification, resulting in cascade testing for at-risk family members. Within the United States, all 50 states identify and disclose sickle cell carrier status

through newborn screening. Significant variation in program policies and procedure exist surrounding disclosure of SCT and there are no current guidance on the best approach. California is one of the states that commonly notify several key stakeholders of SCT, including the primary care provider, family, and hospital [43]. Summary results of the newborn samples screened in California during an 8-year period revealed Hb S trait identified in 1:121 California births. Collectively, heterozygous globin chain variants (Hb S, C, E, D, and rare variants) were identified in 1:75 California births [44]. While screening programs aim to encourage education and testing before pregnancy to maximize the reproductive options available for at-risk couples, it is important to remember that education and outreach on carrier status should extend beyond the prenatal period, especially when carrier status is being disclosed on newborn screening.

## 10.3.5. Cystic Fibrosis

CF is the most common life-threatening, autosomal recessive condition in the non-Hispanic white population. The disease incidence is 1:2500 individuals in the non-Hispanic white population and carrier frequencies within this population can be as high as 1:27 [45]. Population carrier screening for this condition was first considered 1997 when a consensus panel convened by the National Institutes of Health recommended that CF carrier testing be offered to adults with a family history, couples that are planning a pregnancy and to couples that are asking for prenatal testing once an approved infrastructure for education and counseling was in place [46]. After a period of pilot testing the ACMG and the ACOG published guidelines in 2001 for the screening of CF in the general population using a pan-ethnic panel of 25 *CFTR* mutations and several polymorphisms [47,48]. These specific mutations were included in the panel because they had a >0.1% frequency in patients with CF. Another round of data review initiated in 2002 revealed that some of the mutations initially included on this panel were either less frequent than originally thought (c.1078delT) or were a benign polymorphic variant (p.I148T) when not coupled with the very rare disease-causing

mutation (c.3199del6) [49,50].

Because of these initial shortcomings in the CF carrier screening panel, the ACMG updated the recommended panel in 2004 [51], highlighting the value in designing and continually monitoring a large-scale carrier screening program from the inception. In 2005, in recognition of increasing population ethnic admixture, the ACOG Committee on Genetics stated it was reasonable to offer CF screening to Ashkenazi Jewish and non-Hispanic white women and make available to other patients who requested testing. In 2011, the ACOG Committee on Genetics updated their original 2001 screening recommendation stating that as a routine part of obstetric care and regardless of ethnicity, all women of reproductive age should be offered preconception and prenatal CF carrier screening [52]. The statement acknowledges that while CF is more common among the non-Hispanic white population compared with other racial and ethnic populations, it is becoming increasingly difficult to assign a single ethnicity to affected individuals, and therefore testing should be offered to all women of reproductive age. Recently, Strom et al. reviewed the performance of the ACMG/ACOG 23 *CFTR* mutation panel in a clinical database of approximately 3 million carrier screen results and the data from more than 2000 CFTR gene sequencing analyses and suggest this panel is performing as expected [45]. These data show detection of approximately 90% of non-Hispanic white CF carriers and 77% of CF carriers in a US pan-ethnic population (reported ethnicities consisted of 64% non-Hispanic whites, 16% Hispanic Americans, 14% African Americans, 5% Asian Americans, and 1% Ashkenazi Jews). As pan-ethnic screening for CF continues to be more widely adopted, there is a need to consistently revisit the composition of mutations on the panels to achieve higher test sensitivity and specificity among all patient populations, and improve access to patient and provider education and resources.

## 10.3.6. Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is the most common fatal hereditary disease among newborns and infants worldwide and affects about

1:10,000 live births [53]. SMA is caused by mutations in the survival motor neuron 1 (*SMN1*) gene, and in most cases, affected patients demonstrate the homozygous deletion of exon 7 in the *SMN1* gene, resulting in decreased *SMN1* copy dosage [54]. Increases in *SMN2* copy number often modify the phenotype. Carrier testing is often performed through SMN1 dosage analysis. Individuals who are not carriers usually have two whole copies of *SMN1*, though they can also have three or more. Carrier frequency averages 1:40 to 1:60 with the highest carrier frequency seen in Caucasians (1:35) and the lowest seen in Hispanic populations (1:117) [55]. In the US pan-ethnic population, the calculated a priori carrier frequency is 1:54 with a detection rate of 91%. Therefore, an individual from this pan-ethnic population with normal *SMN1* dosage testing would have a ~1:500 residual risk of being a carrier [56]. When ethnicity is known, it may be further possible to refine the carrier frequency, detection rate, and estimates of residual risk following identification of a two- or three-copy *SMN1* result. For example, a Caucasian individual with a two-copy result following carrier screening would have a ~1:800 residual risk to be a carrier, whereas an individual of African American descent with the same result would have a ~1:130 risk to be a carrier [56]. Universal carrier screening for SMA was recommended in 2008 by the ACMG [53]; however, screening is still not performed as routinely as CF testing, in part due to a conflicting ACOG statement that SMA carrier testing should be restricted only to individuals with a family history of SMA [57]. Despite the slower adoption rate, pilot studies of general population carrier screening studies within and outside the US support patient interest in the availability of SMA carrier testing and laboratories offering screening have demonstrated feasibility of high throughput carrier testing in pan-ethnic population [54,58]. These data suggest that the adoption of general population SMA carrier screening will increase, especially as professional organization recommendations expand.

## 10.3.7. Expanded Carrier Screening

Expanded carrier screening (ECS) is screening that evaluates an

individual's carrier state for multiple conditions at once and regardless of a specific ethnicity or family history, a departure from traditional methods of offering testing based on a patient's reported ethnicity or family history. ECS has been enabled by the rapid advancement of NGS technologies, allowing individuals to be screened for dozens to hundreds of diseases, some with lower frequency or clinical severity grade, at a relatively reasonable cost. ECS was first made available in late 2009 and adoption has been steady, despite some conflicting professional guideline statements [59]. One rationale for ECS focuses on the difficulty in assigning reliable ethnic identification to patients through traditional ethnicity based guidelines, resulting in increased likelihood of disease occurring in nontargeted groups. Recent demographic changes in the United States have also compromised the application of the ethnicity-based approach. The 2010 U.S. census data revealed sharp increases in individuals reporting mixed racial ancestry, particularly among younger populations approaching reproductive age [60]. In addition, there continues to be hesitation among certain populations over the use of racial and ethnic categorization in medicine due to longstanding histories of social injustice and discrimination. These and other factors have led to increased likelihood of disease occurrence within nontargeted groups. For example, over the last decade, the State of California has witnessed an increased prevalence of clinically significant hemoglobin disorders, mostly attributed to increased immigration from Asia and Latin America. This has resulted in increased numbers of infants diagnosed a hemoglobinopathy disorder that were outside of the specific ethnic groups included in ACOG's carrier screening guideline [44,61].

Another rationale focuses on the expansion of conditions tested for, especially as Mendelian diseases account for the nearly 20% of infant mortality and 10% of infant hospitalizations in the United States [2]. By testing for an increased number of conditions, large-scale implementation of ECS would likely impact a larger portion of related mortality and morbidity associated with inherited genetic disorders. The ECS approach of a larger disease panel coupled with a pan-ethnic

approach is quickly being positioned as an effective and equitable model for an evolving population.

A 2013 review of the first commercial ECS offering highlights clinical data from 23,453 patients of mixed ethnicities; 24.0% of individuals in this cohort were identified as carriers for at least one of 96 serious recessive conditions [59]. Further data from another large diverse ECS population demonstrated that the overall risk of having an affected child is 1:280 based on the reported carrier identification frequency of a collective group of 89 diseases, which is higher than the risk of having a child with a neural tube defect, for which screening is universal [62]. These studies contributed to the ACMG, the ACOG, the National Society of Genetic Counselors (NSGC), the Perinatal Quality Foundation, and the Society for Maternal-Fetal Medicine to issue a joint statement on ECS, suggesting a need for more data on "the frequency of variants in previously untested racial and ethnic groups" to facilitate general pan-ethnic testing [11].

## Table 10.3

### Recent Policy Statements Regarding Expanded Carrier Screening

| Date | Policy Statement or Publication | Summary |
|---|---|---|
| **March 2017** | ACOG: Committee Opinion Number 690 | Acknowledges that ethnic-specific, pan-ethnic, and expanded carrier screening are all acceptable strategies and urges healthcare providers to "establish a standard approach" they consistently offer to their patients. |
| **March 2016** | ESHG Policy Statement: Responsible Implementation of expanded carrier | Provides recommendations for healthcare policy and professionals implementing ECS. |

| | | |
|---|---|---|
| | screening | |
| **March 2015** | Joint Statement from ACMG, ACOG, NSGC, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine: Expanded Carrier Screening in Reproductive Medicine—Points to Consider | Highlights points to consider for clinical providers and laboratories implementing ECS. |
| **April 2013** | ACMG Policy Statement: ACMG position statement on prenatal/preconception expanded carrier screening. | First professional society position statement on ECS. Focused on the proper selection of appropriate disease-causing targets for general population-based carrier screening using clear, transparent selection criteria. |

There are two primary approaches for ECS: targeted genotyping for specific mutations and sequence analysis via NGS methodologies [63]. While sequencing significantly increases the number of carriers identified and the test sensitivity compared with genotyping, it also detects a broader array of variants, including novel variants that may have unknown pathogenic effects. This can challenge variant interpretation and counseling strategies. Literature regarding the clinical utility of ECS is beginning to emerge although further evidence regarding clinical decision outcomes and reproductive decisions are needed before universal guidelines for ECS are established [64,65]. Additional research should focus on cost-effectiveness of ECS in comparison to traditional carrier screening and

education, implementation, and counseling strategies. Guidelines regarding ECS test development and implementation are available from several professional organizations (see Table 10.3) [11,66–68].

### 10.3.7.1. Methods and Tissues

A variety of methodologies have been used for both carrier screening and heterozygote testing. Historically, these approaches have ranged from physiological studies to direct mutation analyses using nucleic acids (DNA or RNA). For example, somatic cell methods assessing enzymatic activity (HPRTase) or physiological ($^{3}$H-hypoxanthine incorporation) attributes of clones of fibroblasts were once useful for carrier detection in Lesch–Nyhan syndrome. Similarly, enzymatic testing of HexA levels for carrier screening and heterozygote testing for TSD is still common [69]. However, DNA-based (and some RNA-based) methods have become routine for many carrier screening and heterozygote detection methods in use at present. Some representative DNA/RNA–based testing methods are listed in Table 10.4. Increasingly, sequencing methods, including NGS methods, are being used for both carrier screening and heterozygote detection.

A wide variety of tissues were once used for carrier detection in representative disorders inherited in an autosomal recessive fashion. Tissues used have included serum, plasma, peripheral blood, leukocytes, hair follicles, tears, teeth, and liver and muscle specimens. Increasingly though, peripheral blood, saliva and buccal cells are now some of the most common tissues used for most routine testing purposes. Exceptions, however, do exist. For example, carrier screening for TSD still relies on both sequence-based analysis of the *HEXA* gene and HexA enzymatic activity testing of serum or leukocytes [70]. The combined approach of initial enzymatic testing followed by molecular testing can detect 98% of all carriers in the Ashkenazi Jewish population [71].

**Table 10.4**

**Representative Carrier Screening and Heterozygote Testing Modalities**

| Test Type | Representative Methods | Disease or Clinical Example |
|---|---|---|
| **Single gene** | PCR, Sanger sequencing | Cystic fibrosis, spinal muscular atrophy, Tay–Sachs disease |
| **Gene panels** | PCR, Sanger sequencing and next-generation sequencing | Muscular dystrophies, cardiomyopathies, hereditary cancer syndromes |
| **Whole exome or whole genome sequencing** | Next-generation sequencing | Autism, intellectual disability or selected populations at higher risk for inheritance of recessive disorders |

## 10.4. Therapeutic Implications for Heterozygotes

Although in most instances heterozygosity for a recessive trait has no known adverse health consequence to the individual, there are conditions where the heterozygous state may impart certain health implications. Heterozygotes of X-linked disorders may additionally, have therapeutic issues depending on the disorder and the extent of Lyonization. Accordingly, for these individuals, knowledge of their carrier status may have therapeutic or preventive health implications for them. For example, persons with SCT should be aware of the possible hazards of exposure to reduced ambient oxygen concentrations (e.g., mountain climbing at high altitude, flying in unpressurized aircraft above 8000 feet), specifically splenic infarcts. While most splenic infarcts are mild some have resulted in splenic rupture, necessitating emergency splenectomy [72]. Also, if a patient with SCT requires gaseous anesthesia, the anesthesiologist should be alerted to avert inadvertent hypoxia, which might be particularly hazardous. In a similar fashion, persons heterozygous for the Z allele

of $\alpha_1$-antitrypsin deficiency (*MZ*) may be predisposed to emphysema in early adulthood [73]. Avoidance of tobacco smoke and other noxious inhalants may reduce this risk. On identifying an individual as a carrier for this condition, it might be of great practical value for this person to be counseled and to be guided into appropriate job selection and/or environmentally safe areas, and informed of the particular importance of not smoking.

While the majority of international guidelines and policy statements recommend against carrier testing in children and adolescents, this rationale is, of course, altered when carrier status does convey health implications. For example, the National Collegiate Athletic Association now requires SCT screening of all Division I athletes, proof of prior testing, or a written waiver from the athlete before participation in its programs [74]. The justification for this policy is that adolescents and young adults with SCT have been shown to be at increased risk of exercise-related splenic infarct or fatal exertional rhabdomyolysis [72].

## 10.5. Sensitivity and Specificity

The ability to identify all true carriers (sensitivity) is reflected in the false-negative rates of the test used. The identification of only true carriers (specificity), and not those persons with a false-positive test result, is also of importance,. The greater overlap in the distribution of enzymatic values between a carrier and a noncarrier, the greater is likelihood for either or both types of misclassification. The advantage of mutation-specific DNA-based testing is that extremely high levels of specificity (few false-positive results) are usually achieved. For example, DNA testing for the diagnosis of spinal muscular atrophy in affected individuals shows 100% specificity [71].

Because polymerase chain reaction–based genotyping methods only identify the specific mutations examined, a concomitant decrease in sensitivity is inherent because other mutations in the same gene will not be identified. Carrier test sensitivities are usually the highest when the gene product analysis is the testing method used (e.g., enzyme,

protein, or mRNA transcript). Ideally, carrier screening should be done initially with a gene product assay if available with its expected high sensitivity. For example, in Egypt the prevalence of β-thalassemia carriers is quite high and the use of high-performance liquid chromatography alone to quantitate variant HbA2 levels has shown a sensitivity of 100% when HbA2 cutoff levels in this study were >3.5% [75]. High-specificity DNA-based methods can then be used to confirm abnormal results, to rule out pseudo deficient results, and to identify specific mutations that may have obvious implications for genetic counseling or diagnosis. Tiered testing initially with gene product testing and then moving on to DNA-based methods has been routinely used for carrier screening of TSD [71] and hemoglobinopathies [76].

However, with the advent of clinical applications of targeted NGS methods, several recent studies have shown that NGS methods can achieve both high sensitivities and high specificities. This has resulted in some rethinking of classic tiered carrier screening approaches and the recent recommendation of selective examples of NGS-based approaches in certain settings. For example, NGS has been recommended as the primary screening modality for the non-Ashkenazi Jewish population [69] as detection rates by NGS in this population are equivalent or better than those for enzymatic analyses. Similarly, NGS-based targeted thalassemia carrier screening has been recommended for the Dai ethnic minority population in China following a study where NGS screening found a carrier rate of 49.5% in contrast to 22% found using traditional hematologic screening methods. This study demonstrates that traditional hematologic screening methods are not optimal in populations with both a high carrier frequency and high degree of genetic heterogeneity [77].

## 10.6. Cost and Feasibility

Most health plans currently cover most or all of the financial burden of carrier screening, especially where professional society guidelines exist. However, where cost would be involved in performing carrier detection testing, these issues should be considered and discussed

with the individual before proceeding. Aside from individual patient costs, the cost-effectiveness of population carrier screening programs from a public health perspective is also important to consider. Screening programs must balance costs and benefits against the value that the public and families place on available screening. A framework for the development of criteria for comprehensive carrier screening can be inferred from an ACMG report on expansion of newborn screening for inherited conditions [78]. The criteria specifically called for an overall analytical cost of <$1 per test per condition for a condition to be considered for addition to a newborn screening program. Cost was based on the personnel, reagents, and other costs associated with the testing alone. While analytical test costs will decrease as NGS throughput improves, test interpretation, reporting, and genetic counseling will confer additional costs as carrier testing programs expand the number of conditions tested. Due to both cost and technical barriers, traditional genotyping methods have remained the most common type of carrier screening assay. While sequencing significantly increases the number of carriers identified and the test sensitivity compared with genotyping, it is often more costly because of the increased interpretation and reporting time. While no empirical data are available at this time, carrier screening by NGS was shown by recent simulation studies to provide greater benefit in healthcare outcomes and lower total costs compared with traditional genotyping methods [79]. This study modeled a population of one million couples that was representative of the United States and demonstrated that sequencing for a group of 14 conditions recommended for carrier screening by one or more professional societies averted 21 additional affected births, and reduced overall lifetime costs by approximately USD$13 million compared with traditional genotyping. As ECS becomes more widely accepted, there will be continued debate surrounding the cost-effectiveness of NGS compared with traditional genotyping methods when applied to a larger number of conditions than is currently recommended by professional societies.

## 10.7. Genetic Counseling and Informed

## Consent

Informed decision-making will remain a critical component of the carrier screening process as testing guidelines and physician experiences expand from ethnicity-based to pan-ethnic screening approaches. While traditional face-to-face genetic counseling sessions with lengthy pretest and posttest discussions have been favored in the past, limitations in access to genetic counselors and the increased numbers of conditions tested for with expanded carrier panels have forced informed consent and the genetic counseling process to evolve. Alternative service delivery models such as telemedicine and online results delivery and education hold significant promise. Regardless of what healthcare professional is performing the counseling or how extensive the informed consent process is, critical components of the genetic counseling session should remain. Core components of a genetic counseling session include equal access, patient education, voluntary utilization of services, and complete disclosure of information, including benefits, limitations, and alternatives of current testing technology [80]. It has been suggested that physicians will play a larger role in carrier screening results interpretation and case management, while the genetic counselor will continue to serve as a key resource for complicated cases that go beyond a physician's scope of practice [12].

## 10.7.1. Timing of Carrier Screening

Carrier testing should ideally be offered during the preconception period to provide patients the adequate information to guide pregnancy planning. In 2017, ACOG released a statement on carrier screening in the genomic era, supporting carrier screening in the prepregnancy period. This statement indicated that ethnic-specific, pan-ethnic, and expanded carrier screening are all acceptable strategies for prepregnancy and prenatal carrier screening but, ideally, carrier screening should be offered before pregnancy [66].

## 10.7.2. Age for Carrier Screening

The majority of international guidelines and policy statements recommend against carrier testing in children and adolescents when carrier status has no medical relevance in childhood. These recommendations have been rooted in perceived ethical concerns such as removal of the child's right to make an autonomous decision, possible harms to the child from testing such as adverse impacts on social, psychological, and emotional well-being, and potential stigmatization and discrimination based on carrier status. However, recent studies have highlighted the possible benefits of early carrier screening in childhood including potentially greater acceptance and integration of carrier status into future life plans and improved social support opportunities through relationships with friends and family.

The American Academy of Pediatricians and the ACMG published a joint position statement in 2013 stating that they do not support routine carrier testing for screening for recessive conditions when carrier status has no health implication in childhood [81]. The policy does recommend that carrier testing should be offered as clinically indicated for pregnant adolescents or adolescents considering reproduction. Clinicians should be aware that the legal authority for pregnant adolescents to make reproductive healthcare decisions independent of their parents or guardians varies from state to state and should check with their state regulations before offering carrier testing [81]. Most recently, the 2015 American Society of Human Genetics (ASHG) position statement on genetic testing in children and adolescents states that on the basis of evidence indicating potential benefits and a low risk of harm, the ASHG neither recommends nor discourages offering carrier testing to adolescents who desire such testing in the setting of a positive family history [82]. In the case that carrier testing is performed, adolescent assent and parental consent should be obtained and genetic counseling should be considered.

Advances in molecular technology and population wide screening programs will continue to magnify the debate of carrier testing in childhood, forcing existing guidelines and policy statements to further evolve as research and clinical experiences shape our understanding

of the appropriateness of carrier screening in both children and adolescents.

## 10.8. Conclusions

From the outset, a number of complex, important social and ethical issues have been identified with genetic screening [7,83]. Issues such as possible personal, familial, or even more general stigmatization of the identified carrier – maintenance of utmost confidentiality of test results, rigorous protection of individual privacy, informed consent of the tested individual – are but a few of the more important concerns raised. Clearly, the clinician must consider all of these matters in their interactions with the patient and families where possible genetic testing is anticipated.

The implementation of carrier screening programs must rely upon education and standardization. Both the at-risk population and healthcare providers need education about genetic screening programs. This is amply illustrated by the cumulative experiences of the TSD, β-thalassemia, and CF carrier screening programs. Even if an individual tested receives a negative screening result, it may still be appropriate for the physician to order a diagnostic test if warranted by the clinical circumstances. Subpopulations affected by targeted carrier screening programs also need to be reliably informed about the mutation detection rates and overall clinical utility to ensure that genetic counseling is effective [7,29]. Standardization plays an increasingly important role as carrier screening programs expand and new ones develop to ensure that screening programs are both effective and equitable. Some have suggested that technologies could be standardized to just a few analytical testing platforms [52].

In addition, carrier screening programs can borrow from the successes of newborn screening programs that exist in the United States and those around the world. Expanded carrier screening will likely become more common as more cost-effectiveness studies are published and professional society guidelines evolve.

Heterozygote detection in the future will continue to rely heavily on DNA-based methods including NGS methods and microarray. There

are currently more than 24,000 entries in the Online Mendelian Inheritance in Man (OMIM) database and the molecular basis is known for just over 5000 single-gene disorders [1]. We expect that novel methods will be developed that may involve the analysis of metabolite (e.g., peptide fragments) and gene products (e.g., enzyme). Eventually screening may involve the simultaneous analysis of multiple disease loci by whole exome or whole genome methods. With appropriate technical, medical, and educational expertise, the expanded use of these new approaches in the future will serve to further reduce the individual, familial, and societal burdens associated with many severe, and currently untreatable, hereditary disorders [84].

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## References

[1] *Online Mendelian Inheritance in Man, OMIM ®*. Baltimore, MD: Johns Hopkins University; 2017. http://www.ncbi.nlm.nih.gov/omim/

[2] Kingsmore S. Comprehensive Carrier screening and molecular diagnostic testing for recessive childhood diseases. *PLoS Curr*. 2012;4 e4f9877ab8ffa9.

[3] Selkirk S.M. Review Gene therapy in clinical medicine. *Postgrad Med J*. 2004;80:560–570.

[4] Ware R.E, de Montalembaert M, Tshilolo L, Abboud M.R. Sickle cell disease. *Lancet*. 2017;390:311–323.

[5] Bharucha-Goebel D, Kaufmann P. Treatment advances in spinal muscular atrophy. *Curr Neurol Neurosci Rep*. 2017;17(91) 7pp..

[6] Andersson H.C, Charrow J, Kaplan P, Mistry P, Pasto Cheng A, Rosenbloom B.E, Scott C.R, Wappner R.S, W of long-term enzyme replacement therapy for Gaucher disease. *Genet Med*. 2005;7(2):105–110.

[7] McCabe L.L, McCabe E.R.B. Genetic screening: carriers and affected individuals. *Annu Rev Genom Hum Genet*. 2004;5:57–69.

[8] Isley L, Callum P. Genetic evaluation procedures at sperm banks in the United States. *J Urol*. 2014;191(1):182. doi: 10.1016/j.juro.2013.10.014.

[9] Zlotogora J. Population programs for the detection of couples at risk for severe monogenic genetic diseases. *Hum Genet*. 2009;126:247–253.

[10] Kaback M, Lim-Steele J, Dabholkar D, Brown D, Levy N, Zeiger K. Ta Sachs disease – carrier screening, prenatal diagnosis and the molecular era. *J Am Med Assoc*. 1993;270(19):2307–2315.

[11] Edwards J.G, Feldman G, Goldberg J, Gregg A.R, Nor Carrier screening in reproductive medicine—points to consider. *Obstet Gynecol*. 2015;125(3):653–662.

[12] Nazareth S.B, Lazarin G.A, Goldberg J.D. Changing trends in Carrier screening for genetic disease in the United States. *Prenat Diagn*. 2015;35:931–935.

[13] McClaren B.J, Aitken M, Massie J, Amor D, Ukoumun carrier testing after a child is diagnosed with cystic fibrosis through newborn screening: investigating why most relatives do not have testing. *Genet*

*Med*. 2013;15(7):533–540.
[14] Castellani C, Picci L, Tridello G, Casati E, Tamanini A CF Lab Network. Cystic fibrosis carrier screening effects on birth prevalence and newborn screening. *Genet Med*. 2016;18(2):145–151.
[15] Hale J.E, Parad R.B, Comeau A.M. Newborn screening showing decreasing incidence of cystic fibrosis. *N Engl J Med*. 2008;358(9):973–974.
[16] McKusick V.A. *Mendelian inheritance in man*. 12th ed. Baltimore: The Johns Hopkins University Press; 1998.
[17] Bittles A.H, Black M.L. Consanguinity, human evolution, and complex diseases. *Proc Natl Acad Sci USA*. 2010;107(Suppl. 1):1779–1786.
[18] Mushtaq A. Communicating with clients from the Pakistani Muslim community. In: Wiggins J, Middleton A, eds. *Getting the message across: communication with diverse populations in clinical genetics*. New York: Oxford University Press; 2013:90–111.
[19] Williamson R. Universal community Carrier screening for cystic fibrosis? *Nat Genet*. 2013;3:195–201. .
[20] ACOG (American College of Obstetricians and Gynecologists). ACOG Committee Opinion No 442. Preconception and prenatal carrier screening for genetic diseases in individuals of Eastern European Jewish descent. *Obstet Gynecol*. 2009;114:950–953.
[21] ACOG (American College of Obstetricians and Gynecologists). ACOG Committee Opinion No. 691.

Carrier screening for genetic conditions. *Obstet Gynecol*. 2017;129:e41–55.
[22] Gross S.J, Pletcher B.A, Monaghan K.G. Carrier screening in individuals of Ashkenazi Jewish descent for the professional practice and guidelines committee. *Genet Med*. 2008;10(1):54–56.
[23] Wilson J.M.G. *Principles and practice of screening for diseases*. Geneva: World Health Organization; 1968.
[24] UK National Screening Committee. Programme appraisal criteria: criteria for appraising the viability, effectiveness and appropriateness of a screening programme. 2017. http://www.screening.nhs.uk/criter
[25] Godard B, ten Kate L, Evers-Kiebooms G, Aymé S. Population genetic screening programmes: principles, techniques, practices, and policies. *Eur J Hum Genet*. 2003;11(Suppl. 2):S49–S87.
[26] Nuffield Council on Bioethics. Genetic screening: a supplement to the 1993 report by the Nuffield Council on Bioethics. 2006. http://nuffieldbioethics.org/project/ge screening/2006-supplement.
[27] Kaplan F. Tay-Sachs disease Carrier screening: a model for prevention of genetic disease. *Genet Testing*. 1998;2(4):271–292.
[28] Kaback M.M. Screening and prevention, in Tay-Sachs disease: Origins, update, and impact. Chapter 20. In: R.J D, Kaback M.M, eds. *Tay-sachs disease, advances in genetics*. vol. 44. San Diego (CA): Academic Press; 2001:253–265.
[29] Kaback M, Lopatequi J, Portuges A.R, Quindipan C, F

Davido N, Rimoin D.L. Genetic screening in the Persian Jewish community: a pilot study. *Genet Med*. 2010;12(10):628–633.

[30] Kaback M.M. Population-based genetic screening for reproductive counseling: the Tay-Sachs disease model. *Eur J Pediatr*. 2000;159(Suppl. 3):S192–S195.

[31] Hoffman J.D, Greger V, Strovel E.T, Blitzer M.G, Umb generation DNA sequencing of HEXA: a step in the right direction for Carrier screening. *Mol Genet Genom Med*. 2013;1(4):260–268.

[32] Strom C.M, Park N.J, Morgan C, Lobo R, Crossley B, S Guerrero R, Salazar D. Tay-Sachs carrier screening in the genomics age: gene sequencing versus enzyme analysis in non-Jewish individuals. *Open J Genet*. 2013;3(1):61–66.

[33] Cao A. Carrier screening and genetic counseling in beta-thalassemia. *Int J Hematol*. 2002;76(Suppl. 2):105–113.

[34] Origa R. β-Thalassemia. *Genet Med*. 2017;19(6):609–619.

[35] Old J.M. Screening and genetic diagnosis of hemoglobin disorders. *Blood Rev*. 2003;17(1):43–53.

[36] Cao A, Saba L, Galanello R, Rosatelli M.C. Molecular diagnosis and Carrier screening for β-thalassemia. *J Am Med Assoc*. 1997;278:1273–1277.

[37] Chern J.P, Lin K.H, Su Y.N, Lu M.Y, Jou S.T, Lin D.T, of a national beta-thalassemia Carrier screening program on the birth rate of thalassaemia

major. *Pediatr Blood Canc*. 2006;46:72–76.
[38]
Liao C, Mo Q.H, Li J, Li L.Y, Huang Y.N, Hua L, Li Q. screening for a-and b-thalassemia in pregnancy: the results of an 11-year prospective program in Guangshou Maternal and Neonatal hospital. *Prenat Diagn*. 2005;25:163–171.
[39]
Alhamdan N.A, Almazrou Y.Y, Alswaidi F.M, Choud screening for thalassemia and sickle cell disease in Saudi Arabia. *Genet Med*. 2007:9,372–377.
[40] Piel F.B, Steinberg M.H, Rees D.C. Sickle cell disease. *New Engl J Med*. 2017;376:1561–1573.
[41] Neal-Cooper F, Scott R.B. Genetic counseling in sickle cell anemia: experiences with couples at risk. *Public Heal Rep Washington DC*. 1974;1988(103):174–178.
[42] NIH Consensus Statement. https://consensus.nih.gov/1987/1987screeningsicklehe
[43]
Kavanagh P.L, Wang C.J, Therrell B.L, Sprinz P.G, Ba of positive newborn screening results for sickle cell disease and sickle cell trait: variation across states. *Am J Med Genet Part C Semin Med Genet*. 2008;148C:15–22.
[44]
Michlitsch J, Azimi M, Hoppe C, Walters M.C, Lubin screening for hemoglobinopathies in California. *Pediatr Blood Cancer*. 2009;52(4):486–490.
[45] Strom C.M, Crossley B, Buller-Buerkle A, Jarvis M, Quan F, Peng M, Muralidharan K

fibrosis testing 8 years on: lessons learned from Carrier screening and sequencing analysis. *Genet Med*. 2011;13(2):166–172.

[46] Genetic Testing for Cystic Fibrosis. NIH Consensus Statement Online 1997 Apr 14-16 [2017, November, 1];15(4):1–37.

[47] Grody W.W, Cutting G.R, Klinger K.W, Richards C.S, standards and guidelines for population based cystic fibrosis Carrier screening. *Genet Med*. 2001;3:149–154. .

[48] American College of Obstetricians and Gynecologists, and American College of Medical Genetics. *Preconception and prenatal Carrier screening for cystic fibrosis*. Washington (DC): Clinical and Laboratory Guidelines, American College of Obstetricians and Gynecologists; 2001.

[49] Strom C.M, Crossley B, Redman J.B, Buller A, Quan F, fibrosis screening: lessons learned from the first 320,000 patients. *Genet Med*. 2004;6(3):136–140.

[50] Rohlfs E.M, Zhou Z, Sugarman E.A, Heim R.A, Pace I I148T allele occurs on multiple haplotypes: a complex allele is associated with cystic fibrosis. *Genet Med*. 2002;4(5):319–323.

[51] Watson M.S, Cutting G.R, Desnick R.J, Driscoll D.A, K fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet Med*. 2004;6(5):387–391.

[52] ACOG. Committee Opinion No. 486: update on carrier screening for cystic fibrosis. *Obstet Gynecol*. 2011;117(4):1028–1031.
[53] Prior T.W. Carrier screening for spinal muscular atrophy, ACMG practice Guideline. *Genet Med*. 2008;10:840–842.
[54] Prior T.W, Snyder P.J, Rink B.D, Pearl D.K, Pyatt R.E, and Carrier screening for spinal muscular atrophy. *Am J Med Genet Part A*. 2010;152A:1608–1616.
[55] Hendrickson B.C, Donohoe C, Akmaev V.R, Sugarma in SMN1 allele frequencies among ethnic groups within North America. *J Med Genet*. 2009;46:641–644.
[56] Sugarman E.A, Nagan N, Zhu H, Akmaev V.R, Zhou Osadsa D.A, Allitto B.A. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72400 specimens. *Eur J Hum Genet*. 2012;20(1):27–32.
[57] American College of Obstetricians and Gynecologists. ACOG committee opinion, No. 432: spinal muscular atrophy. *Obstet Gynecol*. 2009;113:1194–1196.
[58] Ben-Shachar S, Orr-Urtreger A, Bardugo E, Shomrat R, Yaron Y. Large-scale population screening for spinal muscular atrophy: clinical implications. *Genet Med*. 2011;13(2):110–114.
[59]

Lazarin G.A, Haque I.S, Nazareth S, Iori K, Patterson empirical estimate of carrier frequencies for 400+ causal Mendelian variants: results from an ethnically diverse clinical sample of 23,453 individuals. *Genet Med*. 2013;15(3):178–186.

[60] *Statistical abstract of the United States: 2011*. 130th ed. Washington (DC): U.S. Census Bureau; 2010.

[61] Shafer F.E, Lorey F, Cunningham G.C, Klumpp C, Vic screening for sickle cell disease: 4 years of experience from California's newborn screening program. *J Pedatr Hematol Oncol*. 1996;18(1):36–41.

[62] Haque I.S, Lazarin G.A, Raia M, Bellerose H, Evans E. carrier screening of 322,484 individuals: the case for going beyond cystic fibrosis. *Eur J Hum Genet*. 2015;23:S1.

[63] Lazarin G.A, Haque I.S. Expanded Carrier screening: a review of early implementation and literature. *Semin Perinatol*. 2016;40:29–34.

[64] Franasiak J.M, Olcha M, Bergh P.A, Hong K.H, Werne Carrier screening in an infertile population: how often is clinical decision making affected? *Genet Med*. 2016;18(11):1097–1101.

[65] Ghiossi C.E, Goldberg J.D, Haque I.S, Lazarin G.A, W utility of expanded Carrier screening: reproductive behaviors of at-risk couples. *J Genet Couns*. 2017 doi: 10.1007/s10897-017-0160-1.

[66] Romero S, Rink B, Biggio J.R, Saller D.N. Committee

opinion No. 690. *Obstet Gynecol*. 2017;129(3):e35–e40.
[67] Henneman L, Borry P, Chokoshvili D, Cornel M.C, va El C.G, Forzano F, Hall A, Howard H.C, Janssens S, K Wert G, Dondorp W.J, Peterlin B. Responsible implementation of expanded carrier screening. *Eur J Hum Genet*. 2016;24(6):e1–e12.
[68] Grody W.W, Thompson B.H, Gregg A.R, Bean L.H, M position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013;15(6):482–483.
[69] Mehta N, Lazarin G.A, Spiegel E, Berentsen K, Brenna sachs Carrier screening by enzyme and molecular analyses in the New York city minority population. *Genet Test Mol Biomark*. 2016;20(9):504–509.
[70] Kaback M.M, Desnick R.J. Hexosaminidase a deficiency. In: Adam M.P, Ardinger H.H, Pagon R.A, al., eds. *GeneReviews*®. Seattle (WA): University of Washington, Seattle; 2011:1993–2017 Available from: https://www.ncbi.nlm.nih.gov/books/NBK1218/.
[71] Yao R, Goetzinger K.R. Genetic Carrier screening in the twenty-first century. *Clin Lab Med*. 2016;36(2):277–288.
[72] Tsara G, Owusu-Ansah A, Boateng F.O, Amoeteng-Adjepong Y. Complications associated with sickle cell trait: a brief narrative review. *Am J Med*. 2009;122:507–512.
[73] Al Ashry H.S, Strange C. COPD in individuals with

the PiMZ alpha-1 antitrypsin genotype. *Eur Respir Rev*. 2017;26(146):170068.
[74] National Collegiate Athletic Association. *Sports medicine handbook*. 20th ed. Indianapolis (IN): National Collegiate Athletic Association; 2009:2009–2010.
[75] Abdel-Messih I.Y, Youssef S.R, Mokhtar G.M, Elmogy M.I, M to molecular screening paradigm for B-Thalassemia carriers. *Hemoglobin*. 2015;39(4):240–246.
[76] Barrett A.N, Saminathan R, Choolani M. Thalassaemia screening and confirmation of carriers in parents. *Best Pract Res Clin Obstet Gynaecol*. 2017;39:27–40.
[77] He J, Song W, Yang J, Lu S, Yuan Y, Guo J, Zhang J, Y generation sequencing improves thalassemia Carrier screening among premarital adults in a high prevalence population: the Dai nationality, China. *Genet Med*. 2017;19:1022–1031.
[78] Watson M.S, Lloyd-Puryear M.A, Mann M.Y, Rinaldo P, Howell R.R. Mai report. *Genet Med*. 2006;8(5):12S–252S.
[79] Azimi M, Schmaus K, Greger V, Neitzel D, Rochelle R screening by next generation sequencing: health benefits and cost effectiveness. *Mol Genet Genom Med*. 2016;4(3):292–302.
[80] Walker A. The practice of genetic counseling. In: Uhlmann W.R, Schuette J.L, Yashar B.N

*guide to genetic counseling*. 2nd ed. New Jersey: John Wiley & Sons, Inc; 2009:8–14.

[81] Ross L, Saal H, Anderson R, David K. Ethical and policy issues in genetic testing and screening of children. *Pediatrics*. 2013;131(3):620–622.

[82] Botkin J.R, Belmont J.W, Berg J.S, Berkman B.E, Bomb POSITION STATEMENT points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. *Am J Hum Genet*. 2015;97(1):6–21.

[83] Clayton E.W. Ethical, legal and social Implications of genomic medicine Review article. *Genom Med N Engl J Med*. 2003;349:562–569.

[84] Beaudet A.L. Global genetic Carrier testing: a vision for the future. *Genome Med*. 2015;7(79) 3pp.

11

# Circadian Rhythms and Disease

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## Abstract

Life evolved with the 24-h rotation of the Earth. As a result, organisms have evolved ways to measure daily time intrinsically with circadian clocks, which allow for anticipation of events. Many changes to physical, mental, and behavioral state are governed by endogenous circadian clocks. As a result, disruptions of clocks, which can because by misalignment between the environment and internal clocks or dyssynchrony of clocks within an organism, can have pathologic consequences. We review here the impact of clocks on overall physiology and the relevance of circadian disruption to disease, with a focus on metabolic and neurologic disorders.

## Keywords

Chronotherapy; Circadian disruption; Circadian rhythms; Disorders; Dyssynchrony; Endogenous clocks; Metabolic disorders; Neurologic

## 11.1. Introduction

The importance of intrinsic circadian clocks to fitness is underscored by the pervasiveness of such clocks, expressed in species from single-

celled cyanobacteria to mammals. External signals, most prominently light and food, entrain organismal clocks to ensure synchrony of body functions to our environment, so while clocks allow for precise timing of daily activities, they are also plastic to cope with a changing environment. The most obvious circadian output is our 24-h sleep-wake cycle, which, like other circadian rhythms, persists indefinitely in the absence of external cues. Circadian clocks regulate the timing of sleep, but the relationship to sleep duration and sleep need is unclear. In addition, many available reviews cover the relationship between the quality and amount of sleep and disease [1–5]. For those reasons, the focus of this chapter will be on circadian biology and diseases rather than sleep amount. First, we will give a brief overview of the circadian clock under normal physiologic conditions including the molecular mechanisms of the core clock and the hierarchy of clocks. Then, we will examine extreme sleep-wake cycles that result in an inability to conform to the 24-h environment. We will cover how disruptions in circadian rhythms, which can arise in many ways, result in pathologic effects including metabolic and cardiovascular disorders and psychologic/neurologic disorders. Finally, we will discuss possible methods for altering or exploiting the circadian clock to serve our therapeutic needs (Fig. 11.1).

## 11.2. Molecular Mechanisms

The principle behind circadian rhythms is the genetically encoded "molecular clock," comprised of transcriptional-translational feedback loops, first identified in *Drosophila* [6]. In mammals, the core molecular feedback loop consists of the core clock genes *Period* (*Per*) and *Cryptochrome* (*Cry*), which are activated by the transcription factors, BMAL1 and CLOCK/*NPAS2*, through upstream E-boxes and E′-boxes (5′CANNTG-3′ and 5′-CACGTT-3′ respectively). PER and CRY proteins dimerize and translocate into the nucleus to inhibit the BMAL1/CLOCK heterodimer. Other components of this ~24-h feedback loop include kinases that phosphorylate clock proteins to control their stability and/or activity and E3 ubiquitin-ligases that ensure timely turnover of PER and CRY. Mutations in any of these

components can affect circadian period through their effects on different aspects of the feedback loop. This major loop interlocks with another in which rhythmic expression of *BMAL1* is maintained through competing activity of two nuclear receptors, an activator, ROR-$\alpha$, and a repressor, REV-ERB$\alpha$. The nuclear receptors appear to be especially important for linking the clock to metabolic activity, particularly in the liver. Although the circadian clock in cyanobacteria can be reconstituted as a cycle of protein phosphorylation in vitro [7], transcription-translation feedback loops provide the basis of all eukaryotic clocks. Details of the molecular mechanisms of circadian clocks are described in many reviews and so are not further elaborated here [8,9].

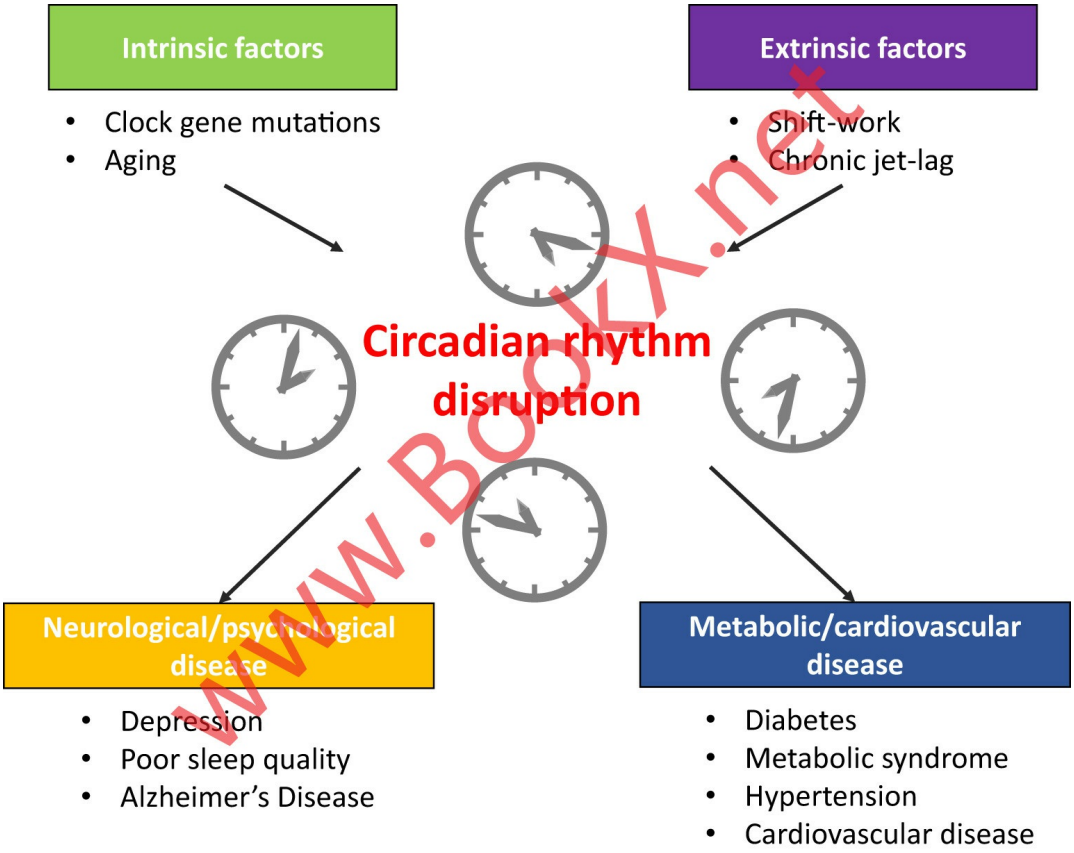


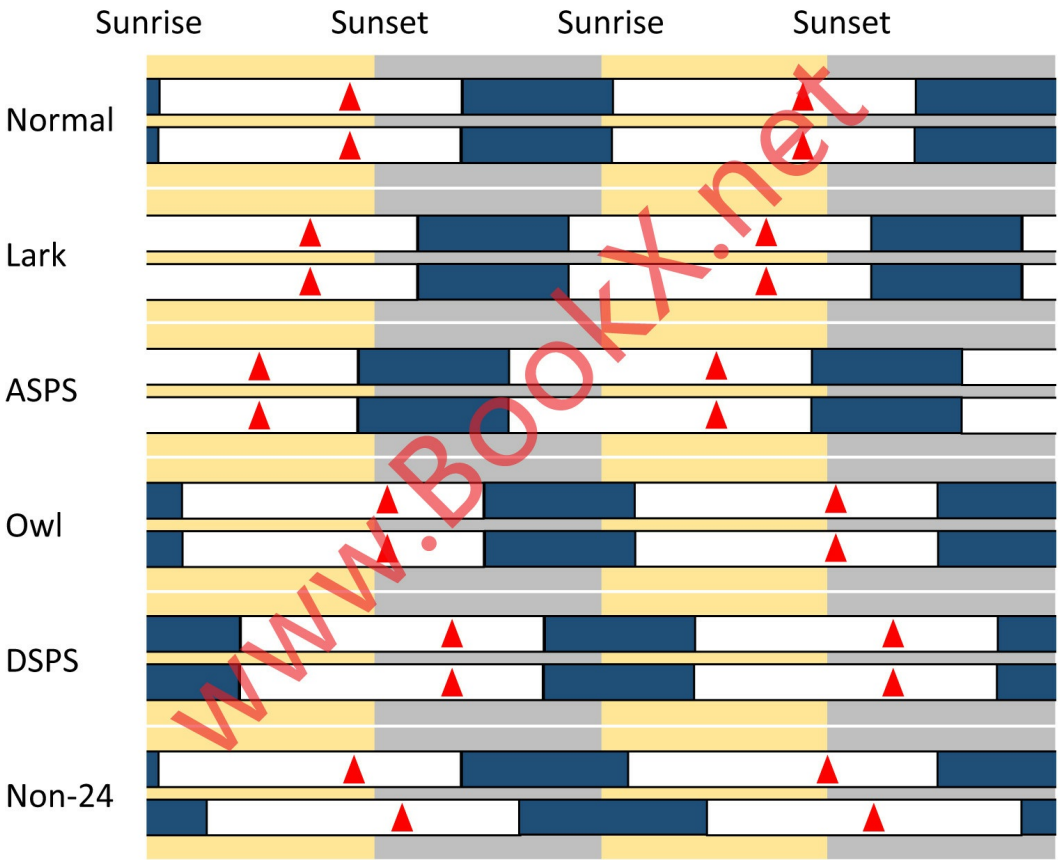

**FIGURE 11.1** Circadian rhythm disruptions result in pathologic effects.

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## 11.3. Central and Peripheral Clocks

In mammals, circadian clocks are hierarchical, with the suprachiasmatic nucleus (SCN) within the hypothalamus of the brain acting as a "master" clock. The SCN is both necessary and sufficient for behavioral free-running rhythms as demonstrated by classical hamster lesion and transplantation experiments [10–12]. Clocks in the SCN are self-sustaining and required to entrain behavioral rhythms as well as peripheral clocks to light-dark cycles. Peripheral clocks reside in nearly every cell in the body; however, phases of clocks in different organs vary [13]. While the major input pathway to the SCN is light from the retina, the peripheral clocks are light insensitive, but can be entrained by other extrinsic factors such as food intake. Synchronization of oscillators within the organism involves SCN inputs through either humoral or neural mechanisms [14].

Up to 20% of the mammalian transcriptome in a given peripheral tissue is regulated by a circadian clock under normal conditions, although the precise numbers are unclear due to variations in methodology and evaluation of significance [15]. Other than core clock components, the oscillating genes across tissues are generally nonoverlapping, allowing for cells to temporally control relevant cellular physiology for a particular tissue [16]. A growing body of evidence suggests tissue specificity of circadian control relies on epigenetic regulation, and the combination of the molecular clock components and tissue-specific transcription networks dictate the transcriptome of a given tissue [17]. Tissue-specific peripheral clocks are found in many other organisms including insects, birds, and plants, suggesting the importance of local clocks in temporal management of physiology [18].




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**FIGURE 11.2** Comparison of sleep profiles of circadian rhythm disorders.Each line is a 48 h period. Sleep is represented by *dark blue and the red triangles* denote melatonin onset.

## 11.4. Circadian Diseases

### 11.4.1. Intrinsic Circadian Disorders

Individual performance peaks at specific times of day with a wide variance across a population. In humans, our temporal preferences in behavior are termed "chronotype" and can be assessed by questionnaires [19–21]. Individuals whose peak alertness is in the morning are colloquially known as "larks," and those whose alertness peaks later in the day are "owls," with most people in between. Core body temperature and melatonin, two strong outputs of the circadian clock, have different phases between early and late chronotypes (Fig. 11.2) [22–24]. Chronotype is primarily a genetic trait, with genetics

accounting for 54% of the variance in morningness and eveningness in human subjects [25,26]. Temporal preference is not limited to humans; a study of fruit flies found different strains have early and late chronotypes [27]. These studies have identified candidate genes and polymorphisms that may determine chronotype. Polymorphisms in *Per1* and the 5′-UTR of *Per2* have been associated with morning preference [28,29]. A nongenetic factor influencing chronotype is age, with older adults phase shifted toward morningness and adolescents favoring eveningness [30].

On either end of the morningness-eveningness spectrum are "extreme larks" and "extreme owls," individuals who have advanced sleep phase syndrome (ASPS) or delayed sleep phase syndrome (DSPS) respectively. ASPS and DSPS are intrinsic circadian disorders in which individuals' internal clocks are out of phase with the standard 24-h day [31,32]. Large families with autosomal dominant ASPS, known as familial ASPS, have been identified and studied to find underlying genes causing ASPS. Linkage analysis of a large ASPS family found a missense mutation in the *Per2* gene [33]. Transgenic mice carrying the mutant human *Per2* gene exhibited shorter period and a 4–6 h phase advance [34]. The mutation of hPER2 inhibits PER phosphorylation by CKIδ/ε, resulting in hypophosphorylated PER and reduced and phase advanced levels of *Per* transcription [33,34]. A second mutation underlying familial ASPS was identified as a missense mutation in CKIδ causing decreased enzymatic activity [35]. Transgenic mice carrying the mutant CKIδ displayed slightly shorter periods; however, the same transgene in fruit flies lengthened periods [35]. A missense mutation in *Cry2* has also been identified in another family with ASPS, and the same mutation expressed in transgenic mice results in earlier phase and shorter periods [36].

ASPS is a very rare disorder, while DSPS is present in the population with higher frequency, approximately one out of 1000 people [37]. Phase delay and DSPS have also been associated with clock genes. A polymorphism of *CLOCK* (3111C to T) located in the 3′-UTR of *CLOCK* was associated with evening preference and DSPS

[38,39], although other studies did not find this association [40,41]. Studies of *CLOCK* mutations in mice demonstrated phase delays in activity and body temperature, supporting the idea that *CLOCK* polymorphisms are linked to DSPS [42,43]. Single nucleotide polymorphisms as well as variable tandem repeat regions in *Per3* have been associated with delayed phase in humans [44,45]. Recent work has found a variant of *Cry1* in humans that increases nuclear localization of CRY1, reducing expression of transcriptional targets and lengthening the period to cause DSPS [46]. Nevertheless, DSPS has generally been more difficult to dissect genetically, perhaps because the phenotypes tend to be smaller and less consistent. Combinations of polymorphisms of clock genes may drive circadian phenotypes, and DSPS in particular, unlike familial ASPS, may represent a polygenic trait that usually only manifests with multiple polymorphisms [47].

At an even more extreme end of circadian disorders, some individuals suffer from a non-24-h circadian period, which is known as non-24-h sleep-wake disorder (non-24SWD). Even normal humans have a slightly longer than 24-h period when allowed to free-run in constant conditions [48]; however, environmental cues entrain the SCN to a 24-h cycle. Due to the slightly longer than 24-h period, individuals must phase advance each day to maintain the same temporal relationship with the environment. Non-24SWD individuals are unable to shift every day, so their intrinsic circadian clock becomes out of synchrony with the regular schedule, resulting in periods of nighttime insomnia and daytime sleepiness. Non-24SWD is a disorder stemming from failure to entrain, and since light is the most potent zeitgeber in humans, it follows that the prevalence of non-24SWD is high in totally blind individuals, while rare in sighted individuals [49]. Although non-24SWD individuals are able to entrain to nonphotic cues, these are very weak synchronizers compared to light [50]. It has been hypothesized that DSPS and NON-24SWD are due to a decreased capacity to phase advance in response to morning light and an increased sensitivity to evening light [51,52].

A final intrinsic factor that determines circadian rhythms in humans

is aging. Although there are many confounds in the cohorts used to measure circadian rhythms in aging, the consensus in longitudinal studies is building that healthy older individuals have a shifted rhythm favoring morningness, as mentioned earlier, but also reduced amplitude of rhythms of melatonin and cortisol [53,54]. Disruption of rhythms may contribute to the increased prevalence of prodromal progressive neurodegeneration with age (the idea will be explored in-depth subsequently); on the other hand, it is unclear whether "healthy" aging would also produce disrupted rhythms [54], as peripheral clock dysfunction also may be a result of comorbidities associated with aging such as obesity, type 2 diabetes, and metabolic syndrome (more discussion following) that increase in prevalence with age. However, it is worth noting that circadian dysfunction in peripheral tissues has also been observed with age in mice and fruit flies. Behavioral rhythms also dampen with age in these models, as they do in humans, although the underlying circadian mechanisms are unknown given that brain clocks are robust in older animals [55]. Nevertheless, even a robust SCN clock is unable to promote robust rhythms in dampened peripheral tissue [56,57].

### 11.4.2. Extrinsic Circadian Disorders

Misalignment in circadian rhythms and external environment also occurs due to extrinsic factors such as jet lag during traveling and shift work. While jet lag is an acute disorder and likely has a more limited effect on individuals, the chronic misalignment of shift work can produce lasting health effects. Our society is dependent on shift workers; in the United States, approximately 15% workers work outside a standard work schedule [58]. Normally, humans sleep during a period of rising melatonin and decreasing body temperature; however, shift workers have to sleep at inappropriate times to the circadian clock. Night-shift workers are presumably exposed to more light at night compared to day-shift workers, dampening and shifting their circadian clock [59]. Night-shift workers are at risk of insomnia during daytime, decreased alertness and cognitive performance at night, and increased rate of accidents compared to day-shift workers

[60,61]. In laboratory studies of blood showed that 95% of metabolites and 73% of transcripts of peripheral blood mononuclear cells that normally have 24 hr rhythmicity were disrupted (reversed, phase-shifted, or absent) after simulated 3- or 4-day night shifts suggesting that the external environment heavily influences daily fluctuations [62,63]. Epidemiologic data suggests, although is inconclusive, that shift work is associated with a multitude of health problems including increased incidences of heart disease, diabetes, obesity, and cancer [64]. It is unclear whether a subset of shift workers is more susceptible to these diseases or whether the risks of circadian dysfunction apply to all shift workers.

## 11.5. Metabolic and Cardiovascular Disorders

The interconnection between circadian rhythm and metabolism manifests on many levels within the organism. There are bidirectional interactions between the molecular clock network and cellular metabolic processes. At a systems level, SCN outputs regulate synchrony of metabolism, while food intake and energy stores directly impact peripheral clocks and also signal to the brain to modify behavior. We will discuss a condensed overview of these relationships; detailed mechanisms have been covered previously in several in-depth reviews [65,66].

### 11.5.1. Molecular Interactions Between the Clock Network and Metabolic Processes on a Cellular Level

The positive arm of the molecular clock, BMAL1/CLOCK or BMAL1/NPAS heterodimers, activates the transcription of many oscillating genes including the ligand-activated transcription factors REV-ERBs, RORs, and PPARs [67]. These transcription factors are regulators of the molecular clock, but they have also been shown to regulate metabolic processes. REV-ERBs repress expression of *BMAL1* through binding its promoter element RORE, while RORs compete for

RORE sites to induce expression of *BMAL1* [68]. PPARα directly regulates the transcription of *BMAL1* and *Rev-erbα* through binding of their promoters [69]. REV-ERBα is expressed in liver, adipose tissue, and muscle and is a key regulator of hepatic gluconeogenesis, adipocyte differentiation, and lipid metabolism [70]. RORα promotes expression of genes involved in lipid homeostasis, and mice with mutant RORα are resistant to diet-induced obesity [71,72]. PPARα is activated by endogenous fatty acids and regulates genes involved in lipid homeostasis [73]. Recent data suggests that RORa regulation of lipid metabolism is through the inhibition of *PPAR* transcription. Other known clock output genes involved in key aspects of metabolism are *Dbp* (D-box binding PAR bZIP transcription factor) [74] and *Pgc-1α* (PPARγ coactivator 1α) [75]. DBP regulates expression of key genes involved in detoxification and metabolism [76]. PGC-1α is a coactivator that induces mitochondrial biogenesis and activates expression of metabolic genes [77]. Given the extensive role of the transcription factor network in regulating both metabolism and circadian rhythms, we hypothesize that these genes were originally metabolic genes that were coopted by the molecular clock network to appropriately time metabolic functions.

Aside from clock-controlled genes that feedback to regulate circadian rhythms, cellular sensors also influence circadian rhythms. The redox state of nicotinamide adenine dinucleotide (NAD(H)) cofactor, which is heavily influenced by nutrient intake, determines the activity of BMAL1/CLOCK and BMAL1/NPAS heterodimers [78]. Oxidized $NAD^+$ and NAMPT (nicotinamide phosphoribosyltransferase), the rate-limiting enzyme of the $NAD^+$ salvage pathway, oscillate and are regulated by BMAL1/CLOCK [76,77]. The $NAD^+$-dependent sirtuin SIRT1, induced by fasting or caloric restriction [78,79], regulates circadian rhythms by acting as a histone deacetylase affecting core clock genes [80]. Another indicator of cellular state, AMPK (AMP-activated protein kinase), which senses AMP/ATP ratio, has been shown to phosphorylate CRY1, destabilizing and targeting it for subsequent degradation [83]. In cultures of mouse embryonic fibroblasts, the absence of AMPK

resulted in altered circadian rhythmicity [83]. These effects of sensors that respond to starvation or energy deprivation on circadian rhythms suggest that the urgent requirements of starving or fatigued cells are likely to supersede and hijack time-of-day mechanisms.

### 11.5.2. Interactions Between the Circadian System and Energy Homeostasis on a Systems Level

Ultimately, interactions between the central circadian clock and peripheral metabolism together regulate feeding and energy homeostasis. At a circuit level, the SCN neurons project to the many cell bodies within the hypothalamus and the brain stem; many of the peptidergic cells reciprocate connections with the SCN [65]. These neurons entrain hormonal, behavioral, and autonomic nervous system pathways to regulate peripheral organs involved in metabolism [84]. For example, the central clock is required for a rhythm of insulin sensitivity and resistance, which informs glucose uptake by peripheral tissue and glucose production by the liver [85], whereas the peripheral clock in pancreatic beta islets is required for a rhythm of insulin secretion and effects glucose tolerance [86]. Metabolic state can also feedback onto circadian rhythms. Exposure of mice to a high-fat diet (HFD) disrupted behavior and molecular rhythms as well as their ability to entrain to light [87,88]. These changes are likely mediated by peripheral effects of HFD on neurons downstream of the SCN [89], and are perhaps secondary to the overall effect of HFD on peripheral rhythms. Metabolomics profiling of different mouse tissues, including SCN, liver, and adipose tissue, shows strong temporal coordination; however, the temporal relationships are greatly weakened under conditions of HFD [90].

Interestingly, some peripheral clocks can be entrained by food and uncoupled from the SCN. Time-restricted feeding (tRF), i.e., feeding restricted to a specific time of day, alters the phase of peripheral tissues, notably, the liver, while the SCN phase is entrained to the light-dark cycle [91,92]. Under the tRF paradigm, the SCN does not

contribute to the entrainment of the liver clock [93]. In fact, oscillations of some genes can be restored in clockless animals by temporally restricted feeding [94]. Taken together, these data suggest that nutrient/caloric availability and SCN control converge on peripheral tissues; however, food availability is a stronger zeitgeber for peripheral metabolic clocks, so feeding restricted to the wrong time of day (nighttime for diurnal animals) desynchronizes body clocks and has physiologic consequences, which include obesity in rats and decreased reproductive fitness in flies [95,96].

### 11.5.3. Circadian Rhythm Disruption and Metabolic Disorders at an Organism Level

Many of the animal models of circadian rhythm ablation described subsequently make use of BMAL1 deficiency because knockouts of this single gene do not have a functional circadian clock, whereas other single clock gene mutants remain rhythmic due to compensation of other genes. However, BMAL1 is a transcription factor that regulates the expression of many genes. Therefore, it is possible that the gene expression level in the absence of BMAL1 causes pathology and is not reflective of cycling disruption per se. It is critical to differentiate the two in understanding circadian components to human disease, as they are not likely caused by BMAL1 deficiency. Considering those caveats, a link between circadian rhythms and metabolism has been observed in knockout mice: BMAL1-deficient mice have impaired glucose homeostasis [97] and adipocyte-specific Bmal1-deletion results in obesity [98]; Clock$^{\Delta 19}$-mutant mice develop symptoms of metabolic syndrome and obesity [99]. However, the conditional adult *BMAL1* knockout mice do not have the same metabolic problems, suggesting that many of these problems have a developmental origin [100]. Importantly, as BMAL1 and CLOCK are transcriptional activators, phenotypes of knockouts may be caused by reduced expression of key metabolic genes rather than loss of the clock.

Complete absence of circadian rhythms may be less detrimental

than misaligned rhythms. There is an association between circadian rhythm disruption in shift workers and metabolic syndrome and diabetes, although the increase in sleep deprivation in these populations is also a contributing factor [101–104]. Mouse models of shift work show an altered daily rhythmic liver transcriptome, increased lipogenesis, and increased blood levels of leptin [105]. *Per1*/2-deficient mice do not develop as severe of a metabolic response, suggesting that misaligned rhythms exacerbated the effect of sleep loss [106]. Metabolic syndrome is hypothesized to be due to circadian misalignment of peripheral clocks with the central clock, induced by night eating or light at night [107,108]. In mice, tRF (aligned with the light cycle) restores peripheral oscillations of genes and has a protective effect against metabolic disorders without an effect on caloric intake [109]. Time-restricted eating may be an effective method for shift workers to prevent the development of metabolic disorders.

## 11.5.4. Circadian Clock and Cardiovascular Disorders

Approximately 6% and 4% of protein coding transcripts in mouse heart and aorta show circadian rhythmicity, respectively [110]. Cyclic expression of molecular clock genes has also been found in human hearts [111]. Variations in the function and efficiency of the heart and aorta of mice ex vivo are time-of-day dependent [112], and some of these parameters have been linked to specific underlying mechanisms. For instance, clock-control of ion channel transcription, through the transcription factor Kruppel-like factor 15 (Klf15), appears to regulate cardiac electrical stability [113]. Loss of clock genes results in a range of phenotypes, depending perhaps on the effects of the clock factor on overall expression levels of target genes: tissue-specific *BMAL1*-deletion of cardiomyocytes results in reduced heart rate, abnormal electrocardiography, and increased incidence of arrhythmia [114]; cardiomyocytes of dominant-negative CLOCK mutants have a disrupted response to fatty acids [115]; oscillations of blood pressure

are absent in mice with endothelial cell-specific or vascular smooth muscle cells-specific deletion of *BMAL1* [116,117]; mice with vascular-specific PPARγ ablation have reduced blood pressure rhythms and heart rate [118]; and *Cry1/2*-null mice have increased salt-sensitive hypertension [119]. Taken together, these studies suggest that circadian regulation of blood pressure and cardiac function is important for maintaining a healthy cardiovascular system [112].

The relationship between circadian rhythms and risk for cardiovascular disease manifests as both acute and chronic diseases. In humans, circadian variation in blood pressure produces a sharp increase in blood pressure in the morning, resulting in an increase in acute cardiovascular events such as cardiac death, stroke, and myocardial infarction [120]. In fact, even the 1-h shift (to and from daylight savings time) is associated with increased myocardial infarction [121], and individuals subjected to circadian misalignment (a 28-h schedule or an inverted nighttime schedule) in the laboratory showed increases in blood pressure [122,123]. Not surprisingly, epidemiologic studies have found that misaligned circadian rhythms of shift workers constitute a risk factor for hypertension and cardiovascular disease [124]. Misaligned circadian rhythms may also be increasing cardiovascular disease risk indirectly through disrupting metabolic processes and increasing the susceptibility to obesity and diabetes.

## 11.6. Cancer

Despite evidence for molecular clock genes regulating transcription of cell cycle checkpoint genes [125], whether the cell cycle is gated by the circadian clock remains controversial [126,127]. The disruptions of circadian genes, however, have been associated with cancer. Of the core clock genes, *Period* genes have been found to act as tumor suppressors in mice [128]. Human cancer cell lines with ectopic expression of PER1 also decreased cell proliferation. Support for circadian effects on tumor growth is also supported by nongenetic studies. Disruption of circadian rhythms through constant light exposure or through pinealectomy was found to increase

carcinogenesis and spontaneous mammary tumors [129]. Human studies have shown an increased risk of breast cancer in shift workers [130–132]. Loss of the SCN increased tumor progression in mice [133]. Aggressive breast cancers in humans show an association with lack of clock gene expression [134]. Despite a lack of evidence in circadian involvement in the initiation of tumors, disruption in clock genes clearly exacerbates progression.

## 11.7. Psychologic and Neurologic Diseases and Circadian Rhythms

### 11.7.1. Alzheimer Disease

Alzheimer disease (AD) is a neurodegenerative disease characterized by progressive cognitive decline. Circadian and sleep disturbances are a common symptom of AD, affecting nearly half of patients; indeed, even patients with mild AD show a disruption of daily rest-activity rhythms and increased sleep fragmentation [135–141]. Increasing severity of dementia correlates with increased circadian disruption [142,143]. In addition, to impaired sleep-wake cycles, core body temperatures of AD patients show a delayed circadian phase [144,145], and expression of core clock proteins is desynchronized in human AD brains [146]. Severity of "sundowning," the exacerbation of behavioral symptoms of AD in the afternoon and early evening, exhibited by many AD patients, is thought to be related to circadian disturbances and associated with shifts in the circadian phase [147]. Animal models for AD have recapitulated aspects of disease progression and symptoms [148,149], with several showing loss or dampening of circadian rhythms concurrently with the accumulation of plaques [150–156].

Although the exact cause of changes in circadian rhythms in AD is unknown, it is perhaps unsurprising that AD would result in sleep and circadian dysfunction as the accumulation of misfolded protein plaques triggers neurodegeneration, which then affects visual system input, the central pacemaker, and/or circadian output neurons. In

*Drosophila* overexpressing β-amyloid, the decline in rhythms is likely due to degeneration of circadian output neurons and not the central pacemaker [157,158]. In humans, however, evidence suggests that AD degeneration affects the retina and SCN neurons directly [159–162]. In a mouse model of AD ($ApoE^{-/-}$), plaque accumulation and degenerative morphologic changes of synapses and mitochondria were observed in the retina and SCN [155,162]. The neurodegeneration in AD is primarily attributed to extracellular β-amyloid peptide plaques; however, AD is also characterized by intracellular neurofibrillary tangles of the microtubule binding protein tau [163]. In a model of tauopathy (Tg4510), the cyclic expression of *Per2* is disrupted in the hypothalamus, which houses the SCN, and also in the hippocampus [164]. In addition, degeneration of the pineal gland, which produces melatonin, a hormonal regulator of sleep and circadian rhythms, may facilitate loss of rhythms. Melatonin loses its rhythm and is greatly reduced in AD, further exacerbating the decline that occurs with normal aging [140,165–170]. These results suggest that the pathologic consequences of AD result in nonspecific neuronal degeneration that either directly or indirectly ablates rhythms.

While the progression of AD clearly results in circadian disruption, whether circadian disruption contributes to AD ontology or pathogenesis is less clear. In an epidemiologic study, circadian rhythm impairment was found to be a risk factor for AD, suggesting that circadian dysfunction may precede neurodegeneration [171]. In addition, a triple transgenic mouse model of AD (3xTg-AD) showed disruptions in behavioral rhythms and phase delays in Per1 and Per2 in the SCN prior to the onset of obvious brain pathologies [177]. Rhythmic DNA methylation of *BMAL1* is altered in individuals with early AD [173]. *BMAL1* deletion in the mouse cortex and hippocampus without altering the SCN resulted in cortical astrogliosis and synaptic degeneration, while behavior and sleep-wake cycles were maintained [174]. Deletion of *CLOCK* in combination with *NPAS* also induce similar degenerative phenotypes; however, brains of *Per1*/*Per2* dual deletion appeared normal. Similarly, *period* null *Drosophila* mutants with transgenic human-derived β-amyloid peptide

showed no differences in brain neurodegeneration over WT [157]. Together, these experiments suggest a model in which the circadian clock transcriptional activators regulate transcription of genes that are also integral to protection against AD, and the loss of these genes rather than a disruption of their cycling results in exacerbation of disease. Mechanistically, the level of β-amyloid protein has been shown to display diurnal oscillations that have been tied to sleep-wake state in normal brains of both mice and humans [156,175,176]. It is tempting to think that circadian rhythms regulate β-amyloid levels and dysfunction of rhythms then results in accumulation of plaques; however, there is insufficient evidence to support this argument currently. Nevertheless, loss of rhythms during AD progression may contribute to severity of the disease.

## 11.7.2. Parkinson Disease

Another neurodegenerative disease that has been associated with circadian rhythms is Parkinson disease (PD). PD is characterized by progressive deterioration of motor function due to the loss of dopaminergic neurons [177]. It has been well-established that sleep disturbances are associated with PD; 80% of patients report sleep disorders [178]. The association of PD with circadian dysfunction is less clear; however, normal humans reporting excessive daytime sleepiness had three times the risk for developing PD [180]. In addition, the presence of the CLOCK 3111T/C polymorphism, which has been associated with DSPS, correlates with increased severity of Parkinson's disease [179]. Patients with PD display fragmented activity and dampened melatonin and peripheral *BMAL1* expression [181–183]. In human studies, a confound in circadian measurements is the treatment of patients with L-Dopa (levodopa) for management of PD. Shifts in melatonin phase were only found in patients treated with L-Dopa [184,185], and dopamine has been found to regulate expression of *Per2* and accelerate circadian rhythm dysfunction in rats [186,187]. Unlike in AD, the pathologic hallmarks of PD, including Lewy bodies and synuclein deposition, have not been reported in the SCN in the early stages, suggesting that circadian dysfunction is likely

not due to pathology of the master clock [188,189]. It remains unclear whether peripheral circadian dysfunction contributes to pathology of the disease. In a mouse model of PD (MPTP neurotoxin treatment) in which all animals develop symptoms of PD, those with disrupted circadian rhythms have higher levels of neuroinflammation and an exacerbated motor deficit [190]. It may be that the circadian disruption results in exacerbation of symptoms rather than affecting pathology.

### 11.7.3. Mood Disorders

Abnormal sleep-wake behavior has long been associated with mood disorders such as major depressive disorder and bipolar disorder [191]. Circadian irregularities reported in a large subset of patients include phase shifts and dampened rhythms, as measured by body temperature, activity, and hormones [192]. On a molecular level, monoamine neurotransmitters that are aberrant in depression including serotonin, dopamine, and norepinephrine are all under circadian regulation [193]. Genetic variations in core clock genes *NPAS2* (471 Leu/Ser) and *CLOCK* (T3111C) have been associated with specific mood disorders [194,195]. Circadian rhythms impact not only the causes, but also treatments for several mood disorders, with selective serotonin reuptake inhibitors for depression and lithium for bipolar disorder linked to changes in circadian period [191].

In clinical studies, light therapy to reset the circadian phase alleviated depressive symptoms, leading researchers to hypothesize that depression results from a phase shift of circadian rhythms [196]. In line with this hypothesis, SCN-specific *BMAL1* knockdown in mice resulted in depression-like phenotypes and the period was lengthened [197]. *Rev-erbα* knock out and $CLOCK^{\Delta 19}$ mutant mice both exhibit manic-like behaviors; although, interestingly, the effects on period lengths are in opposite directions, perhaps suggesting that phase misalignment in either direction might result in mood dysregulation [198,199]. *Cry1*- or *Cry1*/2-deficient mice also exhibit changes in mood 200]. There is likely also a role for non-SCN brain clocks in mood disorders, particularly the mood-regulating neurons within the lateral

habenula, ventral tegmentum, and hippocampus [201]. While the precise relationship between circadian rhythms and mood disorders is unclear because the cellular and molecular mechanisms regulating mood and mood disorders are poorly understood, neuropsychiatric consequences arguably represent the best-documented neural pathologies of circadian disruption.

## 11.8. Chronotherapy

The goal of chronotherapy is to optimize medical treatments by taking into account the circadian rhythms of the body. Chronotherapy is referenced and practiced in two ways: 1) to alter the patients sleep-wake rhythms to improve pathologies, and 2) to take into account the circadian rhythms of the patients to improve therapeutics.

### 11.8.1. Sleep-Phase Therapy

For patients with DSPS desiring a normal daily schedule, chronotherapy refers to gradual adjustments of their sleep-wake cycle until it coincides with their desired schedule. Using the phase-delay technique, the patient is instructed to sleep 3 h later each night over the course of weeks until the desired sleep-wake schedule is achieved [51]. The new schedule must be strictly maintained or else the patient will revert to a delayed sleep schedule. Chronotherapy makes use of the human phase response to light or melatonin. Bright light exposure (>1000 lux) in the morning or melatonin administration in the evening produces phase advances, while light exposure in the evening or melatonin in the morning produces phase delays [51,202]. The use of bright light in the morning and restricted light exposure in the evenings have been shown to successfully phase advance the rhythm of core body temperature of patients with DSPS [203]. The American Academy of Sleep Medicine has recommended chronotherapy for the treatment of circadian rhythm and sleep disorders [204]; however, there have been a few reported cases of chronotherapy resulting in lengthening of intrinsic rhythms of DSPS patients, so they suffered

non-24-h sleep-wake disorder that persisted over many years [205,206]. Bright light treatment and melatonin have also been used for treatment of ASPS; however, since ASPS is less common and less obstructive in a "lark" society, there has been limited clinical evidence regarding the efficacy of either chronotherapy [207].

Due to the link between mood and neurological disorders and circadian rhythm disruption (as discussed above), chronotherapy may be an effective treatment for these diseases [208,209]. In patients with AD or dementia, bright light therapy has improved symptoms including sleep, agitation, and aggression [210]. The use of timed melatonin administration in AD patients resulted in improved sleep quality and reduced sundowning [211]. Although it is unlikely that chronotherapy is treating the underlying disease, it is clear that stabilizing and promoting strong sleep-wake rhythms can improve symptoms of AD. For treatment of mood disorders, bright light therapy, particularly as part of triple chronotherapy (including bright light, phase advance, and sleep deprivation), has been used successfully to rapidly improve depressive symptoms with effect sizes similar to those of antidepressant trials [212,213]. Strengthening of dampened neurotransmitter rhythms via chronotherapy may underlie the improvement.

### 11.8.2. Pharmaceutical Treatment Scheduling Therapy

Because bodily functions vary so much throughout the day, the timing of drug administration can maximize therapeutic efficacy and/or minimize side effects. Chronotherapeutic dosing strategies take advantage of drugs that can be given as a rapid pulse rather than sustained release, which allow for temporal targeting of diseases that display circadian or ultradian oscillations. There are some considerations for chronotherapeutic drug release including the phase of the target tissue, the timing of absorption of drug and delivery to the target tissue, and side effects in other tissues [214]. Drug delivery systems have been developed to temporally deliver drugs to target

tissue; these include time-controlled administration, stimuli-induced delivery (change in temperature or chemical changes in the body), and externally regulated delivery (magnetism or ultrasound) [215]. Chronotherapy has been suggested to improve treatments for many diseases such as asthma, cardiovascular diseases, rheumatoid arthritis, epilepsy, and cancers [216].

## 11.9. Concluding Remarks

Circadian rhythms are ubiquitous, present in nearly all mammalian tissues and regulate many cellular functions. More than an absence of rhythms, temporal misalignment between peripheral tissues and the central clock or the intrinsic clock and temporal environmental cues manifests as disease. Indeed, the known disease-associated polymorphisms in clock genes in humans are generally period altering rather than loss of functions.

## References


[1] Mullington J.M, Haack M, Toth M, Serrador J.M, Meie Ewert H.K. Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation. *Prog Cardiovasc Dis*. 2009;51:294–302.

[2] Knutson K.L, Spiegel K, Penev P, Van Cauter E. The metabolic consequences of sleep deprivation. *Sleep Med Rev*. 2007;11:163–178.

[3] Pal P.K, Calne S, Samii A, Fleming J.A. A review of normal sleep and its disturbances in Parkinson's disease. *Parkinsonism Relat Disord*. 1999;5:1–17.

[4] Bencal R.M, et al. Sleep and mood disorders. *Sleep Med Rev*. 1997;1:45–56.

[5] Bubu O.M, et al. Sleep, cognitive impairment, and Alzheimer's disease: a systematic review and meta-

analysis. *Sleep*. 2017;40.
[6] Dubowy C, Sehgal A. Circadian rhythms and sleep in *Drosophila melanogaster*. *Genetics*. 2017;205:1373–1397.
[7] Rust M.J, Markson J.S, Lane W.S, Fisher D.S, O'Shea E phosphorylation governs oscillation of a three-protein circadian clock. *Science*. 2007;318:809–812.
[8] Partch C.L, Green C.B, Takahashi J.S. Molecular architecture of the mammalian circadian clock. *Trends Cell Biol*. 2014;24:90–99.
[9] Buhr E.D, Takahashi J.S. Molecular components of the Mammalian circadian clock. *Handb Exp Pharmacol*. 2013:3–27. doi: 10.1007/978-3-642-25950-0_1.
[10] Stephan F.K, Zucker I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci USA*. 1972;69:1583–1586.
[11] Drucker-Colín R, Aguilar-Roblero R, García-Hernández F, Fernández-Cancino F, Bermudez Rattoni F. Fetal suprachiasmatic nucleus transplants: diurnal rhythm recovery of lesioned rats. *Brain Res*. 1984;311:353–357. .
[12] Ralph M.R, Foster R.G, Davis F.C, Menaker M. Transp suprachiasmatic nucleus determines circadian period. *Science*. 1990;247:975–978.
[13] Yoo S.-H, et al. PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral

tissues. *Proc Natl Acad Sci USA*. 2004;101:5339–5346.
[14] Guo H, Brewer J.M, Champhekar A, Harris R.B.S, Bitt control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. *Proc Natl Acad Sci USA*. 2005;102:3111–3116.
[15] Mohawk J.A, Green C.B, Takahashi J.S. Central and peripheral circadian clocks in mammals. *Annu Rev Neurosci*. 2012;35:445–462.
[16] Yan J, Wang H, Liu Y, Shao C. Analysis of gene regulatory networks in the mammalian circadian rhythm. *PLoS Comput Biol*. 2008;4:e1000193.
[17] Masri S, Sassone-Corsi P. Plasticity and specificity of the circadian epigenome. *Nat Neurosci*. 2010;13:1324–1329.
[18] Bell-Pedersen D, et al. Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nat Rev Genet*. 2005;6:544–556.
[19] Horne J.A, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol*. 1976;4:97–110.
[20] Zavada A, Gordijn M.C.M, Beersma D.G.M, Daan S, F of the Munich chronotype questionnaire with the Horne-Ostberg's morningness-eveningness score. *Chronobiol Int*. 2005;22:267–278.
[21] Smith C.S, Reilly C, Midkiff K. Evaluation of three circadian rhythm questionnaires with suggestions for an improved measure of morningness. *J Appl Psychol*. 1989;74:728–738.

[22] Gibertini M, Graham C, Cook M.R. Self-report of circadian type reflects the phase of the melatonin rhythm. *Biol Psychol*. 1999;50:19–33.
[23] Bailey S.L, Heitkemper M.M. Circadian rhythmicity of cortisol and body temperature: morningness-eveningness effects. *Chronobiol Int*. 2001;18:249–261.
[24] Lack L, Bailey M, Lovato N, Wright H. Chronotype differences in circadian rhythms of temperature, melatonin, and sleepiness as measured in a modified constant routine protocol. *Nat Sci Sleep*. 2009;1:1–8.
[25] Hur Jr. Y.-M, T. J. B, Lykken D.T. Genetic and environmental influence on morningness–eveningness. *Pers Indiv Differ*. 1998;25:917–925.
[26] Kalmbach D.A, et al. Genetic basis of chronotype in humans: insights from three landmark GWAS. *Sleep*. 2017;40.
[27] Pegoraro M, et al. Gene expression associated with early and late chronotypes in *Drosophila melanogaster*. *Front Neurol*. 2015;6:100.
[28] Carpen J.D, Schantz M, Smits M, Skene D.J, Archer S.N silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. *J Hum Genet*. 2006;51:1122–1125.
[29] Carpen J.D, Archer S.N, Skene D.J, Smits M, Schantz M single-nucleotide polymorphism in the 5'-untranslated region of the hPER2 gene is associated with diurnal preference. *J Sleep Res*. 2005;14:293–297.
[30] Fischer D, Lombardi D.A, Marucci-Wellman H, Roenneberg T. Chronotypes in the US -

influence of age and sex. *PLoS One*. 2017;12:e0178782.
[31] Ptček L.J, et al. Familial advanced sleep-phase syndrome: a short-period circadian rhythmvariant in humans. *Nat Med*. 1999;5:1062–1065.
[32] Weitzman E.D, et al. Delayed sleep phase syndrome. *Arch Gen Psychiatry*. 1981;38:737.
[33] Toh K.L, et al. An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science*. 2001;291:1040–1043.
[34] Xu Y, et al. Modeling of a human circadian mutation yields insights into clock regulation by PER2. *Cell*. 2007;128:59–70.
[35] Xu Y, et al. Functional consequences of a CKIδ mutation causing familial advanced sleep phase syndrome. *Nature*. 2005;434:640–644.
[36] Hirano A, et al. A Cryptochrome 2 mutation yields advanced sleep phase in humans. *Elife*. 2016;5:e16695.
[37] Schrader, Bovim, Sand. The prevalence of delayed and advanced sleep phase syndromes. *J Sleep Res*. 1993;2:51–55.
[38] Katzenberg D, et al. A CLOCK polymorphism associated with human diurnal preference. *Sleep*. 1998;21:569–576.
[39] Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y. 3111T/C polymorphism ofhClock is associated with evening preference and delayed sleep timing in a Japanese population sample. *Am J Med Genet Part B Neuropsychiatr Genet*. 2005;133B:101–104.
[40] Robilliard D.L, et al. The 3111 Clock gene polymorphism is not associated with sleep and

circadian rhythmicity in phenotypically characterized human subjects. *J Sleep Res*. 2002;11:305–312.
[41] Pedrazzoli M, et al. *Clock* polymorphisms and circadian rhythms phenotypes in a sample of the Brazilian population. *Chronobiol Int*. 2007;24:1–8.
[42] Sei H, Oishi K, Morita Y, Ishida N. Mouse model for morningness/eveningness. *Neuroreport*. 2001;12:1461–1464.
[43] Wakatsuki Y, Kudo T, Shibata S. Constant light housing during nursing causes human DSPS (delayed sleep phase syndrome) behaviour in Clock-mutant mice. *Eur J Neurosci*. 2007;25:2413–2424. .
[44] Ebisawa T, et al. Association of structural polymorphisms in the human *period3* gene with delayed sleep phase syndrome. *EMBO Rep*. 2001;2:342–346.
[45] Archer S.N, et al. A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep*. 2003;26:413–415.
[46] Patke A, et al. Mutation of the human circadian clock gene CRY1 in familial delayed sleep phase disorder. *Cell*. 2017;169:203–215 e13.
[47] Pedrazzoli M, et al. Interactions of polymorphisms in different clock genes associated with circadian phenotypes in humans. *Genet Mol Biol*. 2010;33:627–632.
[48] Czeisler C.A, et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science*. 1999;284:2177–2181.

[49] Okawa M, Uchiyama M. Circadian rhythm sleep disorders: characteristics and entrainment pathology in delayed sleep phase and non-24 sleep–wake syndrome. *Sleep Med Rev*. 2007;11:485–496.
[50] Klerman E.B, et al. Nonphotic entrainment of the human circadian pacemaker. *Am. J. Physiol. Integr. Comp. Am. J Physiol Integr Comp Physiol*. 1998;274:R991–R996.
[51] Czeisler C.A, et al. Chronotherapy: resetting the circadian clocks of patients with delayed sleep phase insomnia. *Sleep*. 1981;4:1–21.
[52] Aoki H, Ozeki Y, Yamada N. Hypersensitivity OF melatonin suppression IN response to light IN patients with delayed sleep phase syndrome*. *Chronobiol Int*. 2001;18:263–271.
[53] Weitzman E.D, Moline M.L, Czeisler C.A, Zimmerma of aging: temperature, sleep-wake rhythms and entrainment. *Neurobiol Aging*. 1982;3:299–309.
[54] Hood S, Amir S. The aging clock: circadian rhythms and later life. *J Clin Invest*. 2017;127:437–446.
[55] Nakamura T.J, et al. Age-related decline in circadian output. *J Neurosci*. 2011;31:10201–10205.
[56] Yamazaki S, et al. Effects of aging on central and peripheral mammalian clocks. *Proc Natl Acad Sci USA*. 2002;99:10801–10806.
[57] Luo W, et al. Old flies have a robust central oscillator but weaker behavioral rhythms that can be improved by genetic and environmental manipulations. *Aging Cell*. 2012;11:428–438.
[58] Bureau of Labor Statistics. Workers ON flexible and

shift schedules IN may 2004.
[59] Zubidat A.E, Haim A. Artificial light-at-night – a novel lifestyle risk factor for metabolic disorder and cancer morbidity. *J Basic Clin Physiol Pharmacol*. 2017;28:295–313.
[60] Åkerstedt T. Work hours, sleepiness and the underlying mechanisms. *J Sleep Res*. 1995;4:15–22.
[61] Smith L, Folkard S, Poole C.J. Increased injuries on night shift. *Lancet*. 1994;344:1137–1139.
[62] Skene D.J, et al. Separation of circadian- and behavior-driven metabolite rhythms in humans provides a window on peripheral oscillators and metabolism. *Proc Natl Acad Sci U S A*. 2018;115:7825–7830.
[63] Kervezee L, Cuesta M, Cermakian N, Boivin D.B. Sim night shift work induces circadian misalignment of the human peripheral blood mononuclear cell transcriptome. *Proc Natl Acad Sci U S A*. 2018;115:5540–5545.
[64] Wang X.-S, Armstrong M.E.G, Cairns B.J, Key T.J, Travis R.C. S work and chronic disease: the epidemiological evidence. *Occup Med (Lond)*. 2011;61:78–89.
[65] Huang W, Ramsey K.M, Marcheva B, Bass J. Circadia rhythms, sleep, and metabolism. *J Clin Invest*. 2011;121:2133–2141.
[66] Eckel-Mahan K, Sassone-Corsi P. Metabolism and the circadian clock converge. *Physiol Rev*. 2013;93:107–135.

[67] Yang X, et al. Nuclear receptor expression links the circadian clock to metabolism. *Cell*. 2006;126:801–810.
[68] Guillaumond F, Dardente H, Giguère V, Cermakian N control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. *J Biol Rhythm*. 2005;20:391–403.
[69] Canaple L, et al. Reciprocal regulation of brain and muscle Arnt-like protein 1 and peroxisome proliferator-activated receptor $\alpha$ defines a novel positive feedback loop in the rodent liver circadian clock. *Mol Endocrinol*. 2006;20:1715–1727.
[70] Yin L, Wu N, Lazar M.A. Nuclear receptor Rev-erbalpha: a heme receptor that coordinates circadian rhythm and metabolism. *Nucl Recept Signal*. 2010;8:e001.
[71] Lau P, Nixon S.J, Parton R.G, Muscat G.E.O. ROR$\alpha$ regulates the expression of genes involved in lipid homeostasis in skeletal muscle cells. *J Biol Chem*. 2004;279:36828–36840.
[72] Lau P, et al. The orphan nuclear receptor, RORalpha, regulates gene expression that controls lipid metabolism: staggerer (SG/SG) mice are resistant to diet-induced obesity. *J Biol Chem*. 2008;283:18411–18421.
[73] Varga T, Czimmerer Z, Nagy L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim Biophys Acta*. 2011;1812:1007–1022.
[74] Lopez-Molina L, Conquet F, Dubois-

Dauphin M, Schibler U. The DBP gene is expressed according to a circadian rhythm in the suprachiasmatic nucleus and influences circadian behavior. *EMBO J*. 1997;16:6762–6771.

[75] Liu C, Li S, Liu T, Borjigin J, Lin J.D. Transcriptional coactivator PGC-1$\alpha$ integrates the mammalian clock and energy metabolism. *Nature*. 2007;447:477–481. .

[76] Gachon F, Olela F.F, Schaad O, Descombes P, Schibler circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. *Cell Metab*. 2006;4:25–36.

[77] Liang H, Ward W.F. PGC-1$\alpha$: a key regulator of energy metabolism. *Adv Physiol Educ*. 2006;30:145–151.

[78] Rutter J, Reick M, Wu L.C, McKnight S.L. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science*. 2001;293:510–514.

[79] Ramsey K.M, et al. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. *Science.* 2009;324:651–654.

[80] Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone Corsi P. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. *Science*. 2009;324:654–657.

[81] Nemoto S, Fergusson M.M, Finkel T. Nutrient availability regulates SIRT1 through a forkhead-dependent pathway. *Science.* 2004;306:2105–2108.

[82] Cohen H.Y, et al. Calorie restriction promotes

mammalian cell survival by inducing the SIRT1 deacetylase. *Science.* 2004;305:390–392.
[83] Lamia K.A, et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science*. 2009;326:437–440.
[84] Kalsbeek A, et al. SCN outputs and the hypothalamic balance of life. *J Biol Rhythm*. 2006;21:458–469.
[85] Kitazawa M. Circadian rhythms, metabolism, and insulin sensitivity: transcriptional networks in animal models. *Curr Diabetes Rep*. 2013;13:223–228.
[86] Marcheva B, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature*. 2010;466:627–631.
[87] Kohsaka A, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab*. 2007;6:414–421.
[88] Mendoza J, Pévet P, Challet E. High-fat feeding alters the clock synchronization to light. *J Physiol*. 2008;586:5901–5910.
[89] Pendergast J.S, et al. High-fat diet acutely affects circadian organisation and eating behavior. *Eur J Neurosci*. 2013;37:1350–1356.
[90] Dyar K.A, et al. Atlas of Circadian Metabolism Reveals System-wide Coordination and Communication between Clocks. *Cell*. 2018;174:1571–1585.
[91] Damiola F, et al. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev*. 2000;14:2950–2961.

[92] Stokkan K.-A. Entrainment of the circadian clock in the liver by feeding. *Science.* 2001;291:490–493.
[93] Hara R, et al. Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Gene Cells*. 2001;6:269–278.
[94] Vollmers C, et al. Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc Natl Acad Sci USA*. 2009;106:21453–21458.
[95] Arble D.M, Bass J, Laposky A.D, Vitaterna M.H, Turek timing of food intake contributes to weight gain. *Obesity*. 2009;17:2100–2102.
[96] Xu K, Zheng X, Sehgal A. Regulation of feeding and metabolism by neuronal and peripheral clocks in Drosophila. *Cell Metab*. 2008;8:289–300.
[97] Rudic R.D, et al. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biol*. 2004;2:e377.
[98] Paschos G.K, et al. Obesity in mice with adipocyte-specific deletion of clock component Arntl. *Nat Med*. 2012;18:1768–1777.
[99] Turek F.W, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*. 2005;308:1043–1045.
[100] Yang G, et al. Timing of expression of the core clock gene *Bmal1* influences its effects on aging and survival. *Sci Transl Med*. 2016;8 324ra16-324ra16.
[101] Guo Y, et al. Shift work and the relationship with metabolic syndrome in Chinese aged workers. *PLoS One*. 2015;10:e0120632.

[102] Canuto R, Garcez A.S, Olinto M.T.A. Metabolic syndrome and shift work: a systematic review. *Sleep Med Rev*. 2013;17:425–431.
[103] Pan A, Schernhammer E.S, Sun Q, Hu F.B. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. *PLoS Med*. 2011;8:e1001141.
[104] Karlsson B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27 485 people. *Occup Environ Med*. 2001;58:747–752.
[105] Barclay J.L, et al. Circadian desynchrony promotes metabolic disruption in a mouse model of shiftwork. *PLoS One*. 2012;7:e37150.
[106] Husse J, Hintze S.C, Eichele G, Lehnert H, Oster H. Ci clock genes Per1 and Per2 regulate the response of metabolism-associated transcripts to sleep disruption. *PLoS One*. 2012;7:e52983.
[107] Fonken L.K, et al. Light at night increases body mass by shifting the time of food intake. *Proc Natl Acad Sci USA*. 2010;107:18664–18669.
[108] Mukherji A, et al. Shifting eating to the circadian rest phase misaligns the peripheral clocks with the master SCN clock and leads to a metabolic syndrome. *Proc Natl Acad Sci USA*. 2015;112:E6691–E6698. .
[109] Hatori M, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab*. 2012;15:848–860.

[110] Zhang R, Lahens N.F, Ballance H.I, Hughes M.E, Hog circadian gene expression atlas in mammals: implications for biology and medicine. *Proc Natl Acad Sci USA*. 2014;111:16219–16224.
[111] Leibetseder V, et al. Clock genes display rhythmic expression in human hearts. *Chronobiol Int*. 2009;26:621–636.
[112] Durgan D.J, Young M.E. The cardiomyocyte circadian clock: emerging roles in health and disease. *Circ Res*. 2010;106:647–658.
[113] Jeyaraj D, et al. Circadian rhythms govern cardiac repolarization and arrhythmogenesis. *Nature*. 2012;483:96–99.
[114] Bray M.S, et al. Disruption of the circadian clock within the cardiomyocyte influences myocardial contractile function, metabolism, and gene expression. *Am J Physiol Cell Physiol*. 2008;294:H1036–H1047.
[115] Durgan D.J, et al. The circadian clock within the cardiomyocyte is essential for responsiveness of the heart to fatty acids. *J Biol Chem*. 2006;281:24254–24269.
[116] Takeda N, et al. Thrombomodulin is a clock-controlled gene in vascular endothelial cells. *J Biol Chem*. 2007;282:32561–32567.
[117] Xie Z, et al. Smooth-muscle BMAL1 participates in blood pressure circadian rhythm regulation. *J Clin Invest*. 2015;125:324–336.
[118] Wang N, et al. Vascular PPARγ controls circadian variation in blood pressure and heart rate through

Bmal1. *Cell Metab*. 2008;8:482–491.
[119] Doi M, et al. Salt-sensitive hypertension in circadian clock–deficient Cry-null mice involves dysregulated adrenal Hsd3b6. *Nat Med*. 2010;16:67–74.
[120] Muller J.E, Tofler G.H, Stone P.H. Circadian variation and triggers of onset of acute cardiovascular disease. *Circulation*. 1989;79:733–743.
[121] Janszky I, Ljung R. Shifts to and from daylight saving time and incidence of myocardial infarction. *N Engl J Med*. 2008;359:1966–1968.
[122] Scheer F.A.J.L, Hilton M.F, Mantzoros C.S, Shea S.A. A metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci USA*. 2009;106:4453–4458.
[123] Morris C.J, Purvis T.E, Hu K, Scheer F.A.J.L. Circadian misalignment increases cardiovascular disease risk factors in humans. *Proc Natl Acad Sci USA*. 2016;113:E1402–E1411.
[124] Vyas M.V, et al. Shift work and vascular events: systematic review and meta-analysis. *BMJ*. 2012;345:e4800.
[125] Feillet C, van der Horst G.T.J, Levi F, Rand D.A, Delaunay F. Coupling between the circadian clock and cell cycle oscillators: implication for healthy cells and Malignant growth. *Front Neurol*. 2015;6:96.
[126] Nagoshi E, et al. Circadian gene expression in individual fibroblasts. *Cell*. 2004;119:693–705.
[127]

Yeom M, Pendergast J.S, Ohmiya Y, Yamazaki S. Circa independent cell mitosis in immortalized fibroblasts. *Proc Natl Acad Sci USA*. 2010;107:9665–9670.
[128] Fu L, Pelicano H, Liu J, Huang P, Lee C.C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell*. 2002;111:41–50.
[129] Fu L, Lee C.C. The circadian clock: pacemaker and tumour suppressor. *Nat Rev Cancer*. 2003;3:350–361.
[130] Hansen J. Increased breast cancer risk among women who work predominantly at night. *Epidemiology*. 2001;12:74–77.
[131] Schernhammer E.S, et al. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J Natl Cancer Inst*. 2001;93:1563–1568.
[132] Davis S, Mirick D.K, Stevens R.G. Night shift work, light at night, and risk of breast cancer. *J Natl Cancer Inst*. 2001;93:1557–1562.
[133] Filipski E, et al. Host circadian clock as a control point in tumor progression. *J Natl Cancer Inst*. 2002;94:690–697.
[134] Cadenas C, et al. Loss of circadian clock gene expression is associated with tumor progression in breast cancer. *Cell Cycle*. 2014;13:3282–3291.
[135] Vitiello M.V, Prinz P.N, Williams D.E, Frommlet M.S, disturbances in patients with mild-stage Alzheimer's disease. *J Gerontol*. 1990;45:M131–M138.
[136] Hatfield C.F, Herbert J, van

Someren E.J.W, Hodges J.R, Hastings M.H. Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer's dementia. *Brain*. 2004;127:1061–1074.

[137] Ancoli-
Israel S, Parker L, Sinaee R, Fell R.L, Kripke D.F. Sleep fragmentation in patients from a nursing home. *J Gerontol*. 1989;44:M18–M21.

[138] Okawa M, et al. Circadian rhythm disorders in sleep-waking and body temperature in elderly patients with dementia and their treatment. *Sleep*. 1991;14:478–485.

[139]
Witting W, Kwa I.H, Eikelenboom P, Mirmiran M, Sw in the circadian rest-activity rhythm in aging and Alzheimer's disease. *Biol Psychiatry*. 1990;27:563–572. .

[140] Mirmiran M, et al. Circadian rhythms and the suprachiasmatic nucleus in perinatal development, aging and Alzheimer's disease. *Prog Brain Res*. 1992;93:151–162 3.

[141]
Bliwise D.L, Hughes M, McMahon P.M, Kutner N. Ob sleep/wakefulness and severity of dementia in an Alzheimer's disease special care unit. *J Gerontol A Biol Sci Med Sci*. 1995;50:M303–M306.

[142] Mishima K, et al. Different manifestations of circadian rhythms in senile dementia of Alzheimer's type and multi-infarct dementia. *Neurobiol Aging*. 1997;18:105–109.

[143] Harper D.G, et al. Dementia severity and Lewy

bodies affect circadian rhythms in Alzheimer disease. *Neurobiol Aging*. 2004;25:771–781.
[144] Satlin A, Volicer L, Stopa EG, Harper D. Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease. Neurobiol Aging 1995;16:765–71.
[145] Harper D.G, et al. Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. *Am J Geriatr Psychiatry*. 2005;13:359–368.
[146] Cermakian N, Waddington Lamont E, Boudreau P, Boivin D.B. Circadian clock gene expression in brain regions of Alzheimer 's disease patients and control subjects. *J Biol Rhythm*. 2011;26:160–170.
[147] Volicer L, Harper D.G, Manning B.C, Goldstein R, Sat and circadian rhythms in Alzheimer's disease. *Am J Psychiatry*. 2001;158:704–711.
[148] Webster S.J, Bachstetter A.D, Nelson P.T, Schmitt F.A, Eldik L.J. Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. *Front Genet*. 2014;5:88.
[149] Elder G.A, Gama Sosa M.A, De Gasperi R. Transgenic mouse models of Alzheimer's disease. *Mt Sinai J Med*. 2010;77:69–81.
[150] Characterization of the 3xTg-AD mouse model of Alzheimer's disease: Part 1. Circadian changes. *Brain Res*. 2010;1348:139–148.
[151] Wisor J.P, et al. Sleep and circadian abnormalities in

a transgenic mouse model of Alzheimer's disease: a role for cholinergic
transmission. *Neuroscience*. 2005;131:375–385.
[152] Vloeberghs E, et al. Altered circadian locomotor activity in APP23 mice: a model for BPSD disturbances. *Eur J Neurosci*. 2004;20:2757–2766.
[153]
Bedrosian T.A, Herring K.L, Weil Z.M, Nelson R.J. Al temporal patterns of anxiety in aged and amyloid precursor protein (APP) transgenic mice. *Proc Natl Acad Sci USA*. 2011;108:11686–11691.
[154] Huitrón-Reséndiz S, et al. Age-independent and age-related deficits in visuospatial learning, sleep-wake states, thermoregulation and motor activity in PDAPP mice. *Brain Res*. 2002;928:126–137.
[155] Zhou L, et al. Degeneration and energy shortage in the suprachiasmatic nucleus underlies the circadian rhythm disturbance in ApoE−/− mice: implications for Alzheimer's disease. *Sci Rep*. 2016;6:36335.
[156] Roh J.H, et al. Disruption of the sleep-wake cycle and diurnal fluctuation of β-amyloid in mice with Alzheimer's disease pathology. *Sci Transl Med*. 2012;4 150ra122.
[157] Long D.M, et al. Relationships between the circadian system and Alzheimer's disease-like symptoms in Drosophila. *PLoS One*. 2014;9:e106068.
[158] Chen K.-
F, Possidente B, Lomas D.A, Crowther D.C. The central molecular clock is robust in the face of behavioural arrhythmia in a Drosophila model of Alzheimer's disease. *Dis Model Mech*. 2014;7:445–458.

[159] La Morgia C, et al. Melanopsin retinal ganglion cell loss in Alzheimer disease. *Ann Neurol*. 2016;79:90–109.
[160] Swaab D.F, Fliers E, Partiman T.S. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res*. 1985;342:37–44.
[161] Zhou JN, Hofman MA, Swaab DF. VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. Neurobiol Aging 1995;16:571–76.
[162] Stopa E.G, et al. Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. *J Neuropathol Exp Neurol*. 1999;58:29–39.
[163] Bloom G.S. Amyloid-β and tau. *JAMA Neurol*. 2014;71:505.
[164] Stevanovic K, et al. Disruption of normal circadian clock function in a mouse model of tauopathy. *Exp Neurol*. 2017;294:58–67.
[165] Liu R.-Y, Zhou J.-N, van Heerikhuize J, Hofman M.A, Swaab D.F. Decreased melatonin levels in postmortem cerebrospinal fluid in relation to aging, Alzheimer's disease, and Apolipoprotein E-ε4/4 genotype [1]. *J Clin Endocrinol Metab*. 1999;84:323–327.
[166] Uchida K, Okamoto N, Ohara K, Morita Y. Daily rhythm of serum melatonin in patients with dementia of the degenerate type. *Brain Res*. 1996;717:154–159.
[167] Skene D.J, et al. Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human

pineal gland: effect of age and Alzheimer's disease. *Brain Res*. 1990;528:170–174.
[168] Ohashi Y, et al. Daily rhythm of serum melatonin levels and effect of light exposure in patients with dementia of the Alzheimer's type. *Biol Psychiatry*. 1999;45:1646–1652. .
[169] Wu Y.-H, et al. Pineal clock gene oscillation is disturbed in Alzheimer's disease, due to functional disconnection from the "master clock". *Faseb J*. 2006;20:1874–1876.
[170] Mahlberg R, et al. Pineal calcification in Alzheimer's disease: an in vivo study using computed tomography. *Neurobiol Aging*. 2008;29:203–209.
[171] Tranah G.J, et al. Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. *Ann Neurol*. 2011;70:722–732.
[172] Wu M, et al. Abnormal circadian locomotor rhythms and Per gene expression in six-month-old triple transgenic mice model of Alzheimer's disease. *Neurosci Lett*. 2018;676:13–18.
[173] Cronin P, et al. Circadian alterations during early stages of Alzheimer's disease are associated with aberrant cycles of DNA methylation in BMAL1. *Alzheimers Dementia*. 2017;13:689–700.
[174] Musiek E.S, et al. Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. *J Clin Invest*. 2013;123:5389–5400.
[175] Kang J.-E, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science*. 2009;326:1005–1007.
[176]

Bateman R.J, Wen G, Morris J.C, Holtzman D.M. Fluct of CSF amyloid-ss levels: implications for a diagnostic and therapeutic biomarker. *Neurology*. 2007;68:666–669.
[177] Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron*. 2003;39:889–909.
[178] Chahine L.M, Amara A.W, Videnovic A. A systematic review of the literature on disorders of sleep and wakefulness in Parkinson's disease from 2005 to 2015. *Sleep Med Rev*. 2017;35:33–50.
[179] Lou F, Li M, Luo X, Ren Y. CLOCK 3111T/C Variant Correlates with Motor Fluctuation and Sleep Disorders in Chinese Patients with Parkinson's Disease. *Parkinsons Dis*. 2018 4670380.
[180] Abbott R.D, et al. Excessive daytime sleepiness and subsequent development of Parkinson disease. *Neurology*. 2005;65:1442–1446.
[181] Breen D.P, et al. Sleep and circadian rhythm regulation in early Parkinson disease. *JAMA Neurol*. 2014;71:589.
[182] Videnovic A, et al. Circadian melatonin rhythm and excessive daytime sleepiness in Parkinson disease. *JAMA Neurol*. 2014;71:463.
[183] Cai Y, Liu S, Sothern R.B, Xu S, Chan P. Expression of clock genes *Per1* and *Bmal1* in total leukocytes in health and Parkinson's disease. *Eur J Neurol*. 2010;17:550–554.
[184] Fertl E, Auff E, Doppelbauer A, Waldhauser F. Circad secretion pattern of melatonin in de novo parkinsonian patients: evidence for phase-shifting

properties of l-dopa. *J Neural Transm Park Dis Dement Sect*. 1993;5:227–234.
[185] Bolitho S.J, et al. Disturbances in melatonin secretion and circadian sleep–wake regulation in Parkinson disease. *Sleep Med*. 2014;15:342–347.
[186] Hood S, et al. Endogenous dopamine regulates the rhythm of expression of the clock protein PER2 in the rat dorsal striatum via daily activation of D2 dopamine receptors. *J Neurosci*. 2010;30:14046–14058.
[187] Li S.-Y, et al. Long-term levodopa treatment accelerates the circadian rhythm dysfunction in a 6-hydroxydopamine rat model of Parkinson's disease. *Chin Med J*. 2017;130:1085.
[188] Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res*. 2004;318:121–134.
[189] Langston J.W, Forno L.S. The hypothalamus in Parkinson disease. *Ann Neurol*. 1978;3:129–133.
[190] Lauretti E, Di Meco A, Merali S, Praticò D. Circadian rhythm dysfunction: a novel environmental risk factor for Parkinson's disease. *Mol Psychiatry*. 2017;22:280–286.
[191] Vadnie C.A, McClung C.A. Circadian rhythm disturbances in mood disorders: insights into the role of the suprachiasmatic nucleus. *Neural Plast*. 2017;2017:1–28.
[192] Hickie I.B, Naismith S.L, Robillard R, Scott E.M, Herm the sleep-wake cycle and circadian rhythms to improve clinical management of major

depression. *BMC Med*. 2013;11:79.
[193] Hampp G, et al. Regulation of monoamine Oxidase a by circadian-clock components implies clock influence on mood. *Curr Biol*. 2008;18:678–683.
[194] Johansson C, et al. Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. *Neuropsychopharmacology*. 2003;28:734–739.
[195] Benedetti F, et al. Influence ofCLOCK gene polymorphism on circadian mood fluctuation and illness recurrence in bipolar depression. *Am J Med Genet*. 2003;123B:23–26.
[196] Lewy A.J, Sack R.L, Singer C.M, Whate D.M, Hoban T depression and the phase-shift hypothesis for bright light's therapeutic effects: History, theory, and experimental evidence. *J Biol Rhythm*. 1988;3:121–134.
[197] Landgraf D, et al. Genetic disruption of circadian rhythms in the suprachiasmatic nucleus causes Helplessness, behavioral despair, and anxiety-like behavior in mice. Biol. *Psychiatry*. 2016;80:827–835. .
[198] Chung S, et al. Impact of circadian nuclear receptor REV-ERB$\alpha$ on Midbrain dopamine production and mood regulation. *Cell*. 2014;157:858–868.
[199] Roybal K, et al. Mania-like behavior induced by disruption of CLOCK. *Proc Natl Acad Sci USA*. 2007;104:6406–6411.
[200] Anand S.N, et al. Distinct and separable roles for endogenous CRY1 and CRY2 within the circadian molecular clockwork of the suprachiasmatic nucleus,

as revealed by the Fbxl3(Afh) mutation. *J Neurosci*. 2013;33:7145–7153.
[201] Mccarthy M.J, Welsh D.K. Cellular circadian clocks in mood disorders. *J Biol Rhythm*. 2012;27:339–352.
[202] Lewy A.J, Ahmed S, Jackson J.M, Sack R.L. Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiol Int*. 1992;9:380–392.
[203] Rosenthal N.E, et al. Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. *Sleep*. 1990;13:354–361.
[204] Morgenthaler T.I, et al. Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. An American Academy of Sleep Medicine report. *Sleep*. 2007;30:1445–1459.
[205] Oren D.A, Wehr T.A. Hypernyctohemeral syndrome after chronotherapy for delayed sleep phase syndrome. *N Engl J Med*. 1992;327 1762–1762.
[206] Eliott A.L, Mills J.N, Waterhouse J.M. A man with too long a day. *J Physiol*. 1971;212:30P–31P.
[207] Sack R.L, et al. Circadian rhythm sleep disorders: part II, advanced sleep phase disorder, delayed sleep phase disorder, free-running disorder, and irregular sleep-wake rhythm. An American Academy of Sleep Medicine review. *Sleep*. 2007;30:1484–1501.
[208] Hühne A, Welsh D.K, Landgraf D. Prospects for Circadian Treatment of Mood Disorders. *Ann Med*. 2018:1–35 2014 Dec 8;6:325.
[209] Landry G.J, Liu-Ambrose T T. Buying time: a rationale for examining the use of circadian rhythm and sleep interventions to delay progression of mild

cognitive impairment to Alzheimer's disease. *Front Aging Neurosci*. 2014;325:1–21.
[210] Hanford N, Figueiro M. Light therapy and Alzheimer's disease and related dementia: past, present, and future. *J Alzheimers Dis*. 2013;33:913–922.
[211] Cardinali D.P, Brusco L.I, Liberczuk C, Furio A.M. Th use of melatonin in Alzheimer's disease. *Neuroendocrinol Lett*. 2002;23(Suppl 1):20–23.
[212] Golden R.N, et al. The efficacy of light therapy in the treatment of mood disorders: a review and meta-analysis of the evidence. *Am J Psychiatry*. 2005;162:656–662.
[213] Dallaspezia S, Benedetti F. Chronobiological therapy for mood disorders. *Expert Rev Neurother*. 2011;11:961–970.
[214] Smolensky M.H, Peppas N.A. Chronobiology, drug delivery, and chronotherapeutics. *Adv Drug Deliv Rev*. 2007;59:828–851.
[215] Innominato P.F, Lévi F.A, Bjarnason G.A. Chronother and the molecular clock: clinical implications in oncology. *Adv Drug Deliv Rev*. 2010;62:979–1001.
[216] Lévi F, Okyar A, Dulong S, Innominato P.F, Clairamb timing in cancer treatments. *Annu Rev Pharmacol Toxicol*. 2010;50:377–421.

12

# The Genomic Health Record

## Current Status and Vision for the Future

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## Abstract

Genetics and genomics are emerging in clinical practice. The volume and complexity of the information exceed the capacity of even the best providers. This results in medical decisions that are not fully informed by this important information. Clinical information systems such as electronic health records have the potential to aid the provider as they synthesize data about the patient and translate that into medical decisions. Currently, electronic health record systems have significant deficiencies that limit their use for genetic and genomic information. The chapter examines the current state of the electronic health record, catalogs progress in achieving a genomic-enabled electronic health record system, and articulates a vision for the electronic health record in the era of genomic medicine.

## Keywords

Applications; Clinical decision support; Electronic health record; Genetics; Genomics; Infobutton; “Just-in-time” education; Knowledge representation

## 12.1. Introduction

### 12.1.1. The Genomic Medicine Age?

> *If it were not for the great variability among individuals, medicine might as well be a science, not an art.*
>
> *Sir William Osler, Physician 1892.*

If the nineteenth century was the Industrial Age and the twentieth was the Atomic Age, it is possible that the twenty-first century will be remembered as the Genomic Age. The rapid pace of discovery and, increasingly, clinical implementation in genetics and genomics (for the purposes of readability, the term "genomics" will be used in the chapter, of which "genetics" is a subset) is breathtaking. Supporting this contention and, perhaps, even more amazing considering the current climate of political polarity, the Precision Medicine Initiative (now All of Us) [1,2] garnered bipartisan support and initial funding of $200 million. As the understanding of our genome and its relation to health and disease increases, the opportunity to use this information to improve patient care increases concomitantly. With this opportunity comes the issue of how to prepare clinicians to use genomics along with all the other relevant information needed to care for patients.

Even if genomics is not considered, the problem of information overload is well recognized in medicine. Denson asserts, "It is estimated that the doubling time of medical knowledge in 1950 was 50 years; in 1980, 7 years; and in 2010, 3.5 years. In 2020 it is projected to be 0.2 years—just 73 days" [3]. This exceeds the ability of any conceivable combination of continuing medical education, journal (print, web, or podcasts), or maintenance of certification to distill this new information into clinically actionable nuggets that can substantively impact a clinician's practice. The sheer volume of information quickly outstrips the brain's ability to retain all but a

fraction (and it may not retain the needed fraction). The introduction of genomics into the mix will only magnify this problem. With the increasing emphasis on delivery of evidence-based medicine, the importance of incorporating up-to-date knowledge into practice is even more important.

One proposed solution to this problem is the intelligent use of fully functional electronic health records (EHRs). Simply creating electronic version of paper records is not sufficient to improve care. However, the incorporation of EHRs with advanced capabilities into the workflow of care delivery can improve the quality and reliability of care. To support genomic medicine, sophisticated EHRs must be created that can represent information about family history, newborn screening, or other genetic or genomic test results in such a way that computers can "read" them (what is called structured data) such that the information can be computed (that is analyzed, stored, combined with other information, etc.). This will be essential if the dream of "precision medicine" is to be realized. This work is being led by an emerging medical subspecialty—clinical informatics.

In this chapter, the necessary elements for a genomic electronic health record will be examined. The chapter will be organized around a seminal article from 2012 by Masys et al.: "Technical Desiderata for the Integration of Genomic Data Into Electronic Health Records." [4] The authors identify seven key elements needed to realize a genomic EHR (Table 12.1). The chapter will be organized around these seven elements. Each element will be defined, a rationale provided as to why the element is important, an assessment of the current state of EHRs with regards to the element will be given, and an analysis of what is needed to close the gap between the current and desired functionality will be provided.

**Table 12.1**

**Elements Necessary for the Integration of Genomic Data Into Electronic Health Records**

1. Maintain separation of primary molecular observations from the clinical interpretations of those data.
2. Support lossless data compression from primary molecular observations to clinically manageable subsets.
3. Maintain linkage of molecular observations to laboratory methods used to generate them.
4. Support compact representation of clinically actionable subsets for optimal performance.
5. Simultaneously support human-viewable formats and machine-readable formats in order to facilitate implementation of decision support rules.
6. Anticipate fundamental changes in the understanding of human molecular variation.
7. Support both individual clinical care and discovery science.

## 12.1.2. Element 1: Maintain Separation of Primary Molecular Observations From the Clinical Interpretations of Those Data

*What does this mean?* The full sequencing results must be filtered and interpreted and only the relevant clinical interpretation(s) at that point in time are presented to the clinician to guide clinical decision-making in the EHR. This is the approach currently used for imaging studies and other high-complexity data-rich interventions. The large primary datasets are maintained in a repository outside of the EHR (e.g., a picture archiving and communication system [PACS] used for radiology images), a manageable subset of the information is passed to the EHR (the interpretation in the form of a report), and an interface is available so that the clinician can review the image.

*Why is this important?* In a traditional laboratory test, such as a serum sodium level, the result is represented in the EHR with a test name and a result in a 1:1 relationship. This approach is not feasible for genomic results where next-generation sequencing generates thousands to millions of "results," only a few of which are relevant to

the care of the patient at a given point in time. One of the major differences between genomic data and other types of health care data is the persistence of the data over the patient's lifetime. The serum sodium result has relevance to patient care for a very limited period (in emergent situations sometimes on the timescale of minutes), while germline sequence results, with rare exceptions, are immutable for the life of the patient. This means that the data could be reinterpreted in the future based on emerging knowledge (element 6) and used for different clinical indications over the patient's life. Current approaches place a clinical interpretation in the EHR, usually as a text document while the genomic data work is held at the laboratory without a connection to the EHR, rendering it unavailable for future use. As Masys et al. note, this leads to three problems: (1) loss of most of the data from the genomic test to the clinician due to destruction or inaccessibility; (2) the report in the form of a document that is only readable by humans limiting the use of computer-aided clinical decision-making; and (3) a "point in time" interpretation that is limited by current knowledge in a field that is undergoing rapid expansion of understanding of genomic variation. These issues severely limit the value that can be realized from a genomic test.

*What is the current state?* At present, there are no implemented solutions to this issue in commercial EHRs. Systems that are using genomic information for clinical decision-making are using manual processes to convert genomic data into structured data available in the EHR environment. This is neither desirable nor scalable given the increasing number of exomes and genomes being obtained in both the research and clinical settings. Solutions have been proposed [5], but none are currently implemented. An interesting approach is when the laboratory generating the genomic data issues the report using a service layer that serves as an intermediary that creates standard interfaces between the laboratory and the health system [6]. This has been implemented in a few instances and has been successful in communicating updated interpretations from the laboratory to the health system [7]. At present this has been limited to communicating information pertaining to a single variant, but this approach

demonstrates the feasibility of establishing a connection between an EHR and an external data repository.

*What is needed?* Creation of an -omic ancillary system accessible by the EHR is essential. In contrast to a PACS system that is tethered to a specific EHR, the genomic solution would ideally be available to any compatible EHR system. The only constant actor in the health care delivery system is the patient, and patients move between different health care systems. Therefore, a genomic ancillary system tied to an institutional EHR limits the accessibility of the data when the patient is cared for in another system. If the data are not portable, it means that care will be suboptimal as genomic data will not be available for clinical decisions. In addition, it raises the possibility of duplication and waste, if each system must generate its own genomic data. Duplication and waste are already significant issue for the US healthcare system even if genomics is not considered. Proposed solutions include putting the data on portable media (a disk or flash drive) that the patient controls and can carry to healthcare encounters. While this is a viable solution, the time and technology involved to access, upload, and interpret the information for the clinical context coupled with variable capabilities between EHRs raise many barriers to implementation. A more desirable solution would be the creation of a central store of genomic data under the patient's control that can be accessed by EHRs through standard application interfaces. Creating the infrastructure outside the constraints of the EHR environment will support the development of innovative solutions that support best care for the patient while being sensitive to the issues of clinician workflow.

### 12.1.3. Element 2: Support Lossless Data Compression From Primary Molecular Observations to Clinically Manageable Subsets

*What does this mean?* Lossless compression means storage of data in

smaller volumes than the source raw data file with **no loss of fidelity** of the original data. Readers may be familiar with the use of .zip files, which allows the compression and reconstitution of files to facilitate transmission via e-mail.

*Why is this important?* Most data compression, such as the PACS approach noted in the prior section, results in some data from the original source being lost (that is the reduction in file size is associated with removal of some data), so that in reconstruction some original data are sacrificed. This is an acceptable compromise as the reconstructed image is sufficient for clinical decision-making (if you've ever listened to a CD or an MP3 file, you've experienced compression with loss of a portion of the original source data). This approach will not work in genomics as we do not know a priori which data could be sacrificed without harm to the patient. As Masys et al. note, "… changing even a single letter of the 'genetic alphabet' … may dramatically affect human physiology." At present, most of these are unknown. This means that the data compression approach needs to reproduce a complete and accurate copy of the original sequence.

*What is the current state?* The size of genomic data files (gigabytes to terabytes in raw form) exceeds the capacity of EHR storage and creates a prohibitive requirement for data storage capacity that is beyond the reach of all but a few healthcare systems. Presently, there are no implemented solutions.

*What is needed?* Compression requires the development of algorithms that can compress and reconstitute the original data file. This is an area of active research, and some tools are now available for use through open source access [8]. However, current tools must still be considered developmental and are not appropriate for use in the clinical setting.

### 12.1.4. Element 3: Maintain Linkage of Molecular Observations to Laboratory Methods Used to Generate Them

*What does this mean?* Current standards for genetic test reports require

the inclusion of a technical section that describes the testing methods used to generate the data. Inclusion of this information has contributed to the length and complexity of genetic test reports, which impacts the clinician's ability to use the test result [9].

*Why is this important?* It is important to understand what methods are used for most, if not all, laboratory and imaging studies. This information allows the clinician, at least in theory, to understand both the strengths and the limitations of a given test. The latter is important if the test result does not lead to a diagnosis as it contributes information necessary to decide on the next steps in the diagnostic odyssey. Next generation sequencing results are associated with a number of complexities that impact interpretation. Take exome sequencing as an example. Whole exome sequencing (WES) is increasingly used as a diagnostic test for a variety of indications. WES looks at about 1.5% of the genome with a focus on the regions that code for proteins as well as some flanking regions that contain regulatory elements. However, it might more appropriately characterized as hole exome sequencing (acknowledgment to Dr. Michael F. Murray, who, to my knowledge, first used this). The coverage of the exome is estimated to be between 85% and 95%, with most current-generation WES approaching the latter figure [10]. Technically, it performs well in calling single nucleotide variants and small insertions and deletions (indels). However, it has variable coverage for some regions of the genome and performs poorly in areas with repeated motifs. There are challenges identifying larger copy number variants (although algorithms to call these from exome data are beginning to emerge into practice). What this means is that the clinician must understand how the limitation of WES impacts its usefulness as a test. Take the example of a patient with a clinical suspicion of a spinocerebellar ataxia. If WES results were available, its usefulness in this setting is limited as it is unlikely to pick up the trinucleotide expansions that are responsible for many of these disorders. Similarly, if WES was used to diagnosis a patient with suspected Prader–Willi or Angelman syndrome, it would diagnosis only a small subset of the cases as WES is unlikely to detect large

deletions or heterodisomy (although it could identify isodisomy on the basis of large runs of homozygosity). It would likely identify a case of Angelman syndrome due to a pathogenic variant in the *UBE3A* gene. If these limitations are not appreciated it can lead to missed diagnostic opportunities resulting in lost opportunities for condition-specific interventions as well as inappropriate utilization with its attendant costs to the patient and healthcare system. In addition, to the test methodology, next generation sequencing also requires the use of informatic pipelines to filter out variants of poor quality or unlikely to be related to the condition. The performance characteristics of these pipelines (including information about the assembly and filtering algorithms used, the reference sequence used, quality scoring, variant annotation, etc.) must be available to interpret the results and, if a causal variant is not identified, to assess whether this could be due to the filtering approach used. In almost every instance of comparative evaluation, a higher yield of variants is obtained when multiple filtering methods are used.

*What is the current state?* At present, EHRs can represent this information only as text, limiting its usefulness. Manual processes have been established by some laboratories, healthcare systems, and even payers using personnel with genetic expertise (usually genetic counselors) to improve appropriate test ordering, utilization, and interpretation. These approaches have been demonstrated to improve testing outcomes and save money [11]. While effective, current practice is not a scalable solution given the anticipated increase in genetic testing and genomic analysis.

*What is needed?* If the methodology and technical aspects of the test can be represented as structured data elements, then computerized approaches could be developed to assess issues of importance, to patients and their providers. These would include optimizing test selection, facilitating exchange of clinical information with the laboratory to improve interpretation by providing clinical context (for example, consanguinity in the parents, which would necessitate the use of a different interpretive approach), assisting with clinician interpretation, and selecting subsequent testing approaches if

appropriate.

### 12.1.5. Element 4: Support Compact Representation of Clinically Actionable Subsets for Optimal Performance

*What does this mean?* One of the functions of an advanced EHR is to rapidly find, assemble and display clinical data needed for clinical decision-making at the point of care. An example is an alert that pops up when a clinician orders a medication to which the patient is allergic. The EHR uses the drug order to search the patient's allergy list and, if there is a match, sends a message to the clinician to choose another medication. Many of these also include suggestions for appropriate alternatives and one-click ordering to improve clinical workflow.

*Why is this important?* As noted in the introduction to this chapter, the volume and complexity of information needed to make clinical decisions exceed human cognitive capacity. The time pressures experienced in the clinical encounter limits the ability to find, retrieve, and synthesize data even were it cognitively possible to use all the data available to guide care. This has led to the development of clinical decision support (CDS) systems. A working definition of CDS has been proposed by Dr. Robert Hayward of the Centre for Health Evidence is, "Clinical Decision Support systems link health observations with health knowledge to influence health choices by clinicians for improved health care" [12]. This definition implies an active system to automatically and autonomously perform the desired linkages. Another definition by Osheroff and colleagues describes the process of CDS. Clinical decision support "… refers broadly to providing clinicians and/or patients with clinical knowledge and patient-related information, intelligently filtered, or presented at appropriate times, to enhance patient care" [13]. This active surveillance and linkage are capable of presenting actionable information to the clinician and the patient at the appropriate time in the workflow, assisting decision-making to increase the likelihood of

doing the right thing for the patient. Studies have shown that when these CDS systems are appropriately implemented, they can improve the safety and quality of care [14]. An argument can be made that this is the most important functionality of the genomic health record, although the other elements are essential to allow CDS to function.

Decision support can be grouped in three general categories: passive, asynchronous, and active. Passive decision support consists of nonmandatory resources available at the time of care. Access to electronic resources through the EHR is an example of passive decision support. Examples of electronic resources that can be accessed from the EHR include open access resources like PubMed and subscription resources like UpToDate or MicroMedex. Passive CDS requires clinicians to recognize that they have an information need for a clinical decision. Two other types of CDS, asynchronous and active, have the potential to address the issue of a clinician not being aware of an information need. Asynchronous CDS presents information to the clinician outside of the clinical workflow. This could include an inbox message at the beginning of the workday that lists preventive care interventions that are needed for the patients on the schedule. This dramatically reduces the time involved to identify care gaps that need to be addressed. Active CDS involves presenting information needed for a clinical decision **at the time the decision is being made.** The allergy example noted here earlier is an example of real-time active CDS.

*What is the current state?* CDS for genomic medicine is not implemented as a standard function for any commercial EHR. However, this has been an area of substantial scholarship during the past decade led by individual institutions and by networks such as the electronic Medical Records and Genomics (eMERGE) network [15]. Most of these efforts require a substantial amount of manual curation to make the genomic data available, although there are some early efforts that use standards currently available in commercial EHRs, raising the potential for more rapid dissemination and implementation. A comprehensive review of the current state is beyond the scope of this chapter, but examples illustrating each of the

three types of CDS will be presented.

**Passive CDS** As noted earlier, passive CDS involves accessing content collections from within the EHR. In most cases, the clinician clicks a link to go to a resource such as UptoDate and enters terms into the search bar to find the necessary content. This "google" approach is inefficient as the terms provided may not allow the clinician to find the answers to the clinical question requiring additional attempts, which may exceed the time available for searching. If the EHR could understand the location of the clinician in the EHR, it would be possible to use this context to presearch content collections. For example, if the clinician was in the medication order section of the EHR, one could surmise that the question would involve medication choice or dose. One approach that has been shown to improve the speed of access to the answer is the InfoButton. InfoButtons build and run queries against electronic resource collections based on patient data and clinical context to take the user to the most appropriate section(s) within a content collection with a minimum number of clicks. All certified EHR systems in the United States are required to have InfoButton capability, although many systems have chosen to suppress this functionality. Openinfobutton is an open source suite of Web services that enable Infobutton capabilities within EHR systems [16]. The Clinical Genome Resource [17], a National Human Genome Research Institute (NHGRI)-funded project, is using Openinfobutton to allow direct access of ClinGen resources from the EHR [18]. For systems with active InfoButtons in the EHR, a link to the ClinGen resources using Openinfobutton provides "one stop shopping" as the ClinGen site not only presents ClinGen resources but also aggregates external genomic resources (e.g., GeneReviews, Genetic Home Reference, Online Mendelian Inheritance in Man), putting the most used vetted genetic content collections at the clinician's fingertip with a single click.

**Asynchronous CDS** Asynchronous CDS involves the system monitoring behaviors, perhaps assembling data that were not completely available at the time of clinician–patient interaction and recognizing a potential risk or need for further consideration. The

output of asynchronous decision support can be an email to the inbox, a report summarizing patients that need a second look or other approaches. This could be used to monitor surveillance for carriers of pathogenic variants in *BRCA1* or *BRCA2* to ensure they are receiving mammograms and breast imaging at intervals recommended by practice guidelines. Because the interval for these studies if different than those for noncarriers, a system could develop a query that would identify all variant carriers and determine if a screening test were overdue. These "care gaps" could be messaged to the respective patient's primary care clinician so that contact could be initiated to get the tests scheduled. This takes place outside of scheduled clinical encounters, allowing for improved adherence to recommended care. These systems are already used for routine preventive care services and would be straightforward to implement if the genetic test result were available as structured data. This system has been implemented at the author's institution to track recommended follow-up for participants who have received an actionable result from the MyCode return of results program [19].

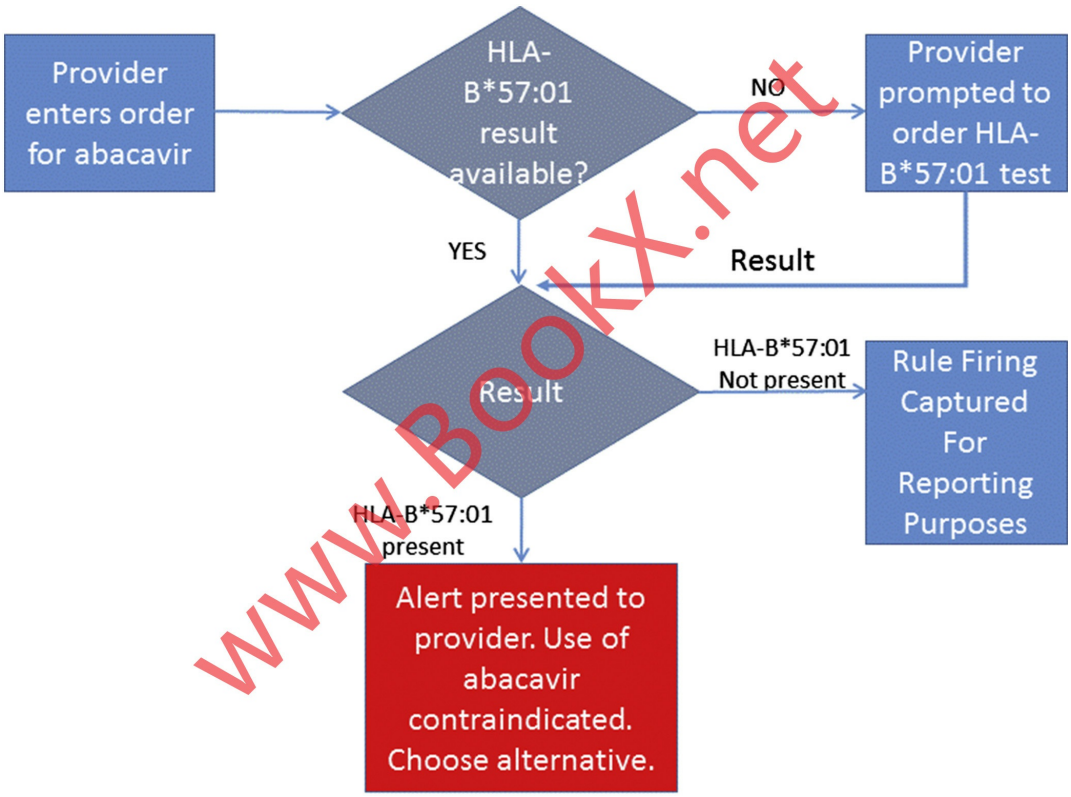
**Active CDS** Active (or synchronous) CDS generally defines a workflow in which a process, such as prescribing a medication, is monitored in real-time by a rules engine that employs logic to assemble and analyze data elements. The intent is to influence clinician behavior based on the logic embedded in a rule. Pharmacogenomic testing for the antiretroviral drug abacavir demonstrates how active CDS can work. It is known that patients who carry a specific HLA type (B*57:01) are at increased risk for severe cutaneous adverse reactions (which includes Stevens–Johnson syndrome and toxic epidermal necrolysis). The US Food and Drug Administration (FDA) black box warning recommends testing for HLA-B*57:01 prior to the use of abacavir. Active CDS would create a set of logic rules embedded within the medication order system (Fig. 12.1). When a clinician goes to order abacavir, the system checks to see if there is a result for HLA-B*57:01. If a result is not available, the clinician receives an alert indicating that this test is required for the use of abacavir. A functional alert would allow the clinician to enter a

result that had been obtained outside the system or, if testing had not been done, to order the test for the patient with one click. If the HLA-B*57:01 result is available, the rules logic determines if this HLA type is present or absent. If absent, the order proceeds, but if the patient carries HLA-B*57:01, the clinician receives an alert that includes the warning and a list of alternative medications that can be used (again with one-click ordering to reduce work). InfoButtons can be included in the alert, allowing the clinician to access additional information if he or she wishes. Systems like this have been implemented in several healthcare systems. The best-studied example supports the PREDICT project (a pharmacogenomic implementation project) at Vanderbilt University [20].

*What is needed?* In contrast to most of the other elements, EHRs as currently configured can support this element. The biggest implementation barrier is the lack of representation of genomic information as structured data. This is necessary for the CDS system to use the logic rules. Manual processes are used to create the structured data elements in systems using genomic CDS. The National Academy of Medicine's Displaying and Integrating Genetic Information Through the EHR [21] project has assembled a diverse group of stakeholders, including EHR vendors, and developed example genomic CDS implementations (including the abacavir example) that can be implemented in standard EHRs using published implementation guides [22]. In addition, several groups are depositing examples of genomic CDS rules in a public repository [23]. This element is thought by many to be the key to the successful implementation of genomic medicine. Building off the desiderata that are the focus of this chapter Welch et al. [24] identified seven desiderata for genomic clinical decision support. The author co-moderated a 1.5-day meeting sponsored by the NHGRI on genomic CDS that generated a list of recommendations, which have led to some of the efforts described earlier. The full content of the meeting is available at https://www.genome.gov/27558904/genomic-medicine-centers-meeting-vii/.

There is one external issue that needs to be discussed in the context

of this section. The underlying presumption for this element is the accessibility of well-validated and annotated knowledge repositories. The examples presented use resources that have free access or are available through a subscription service. However, there are databases that are treated as proprietary and are not publicly available [25]. If this becomes the norm, this could significantly impair the effective use of genomic information in clinical care. While this issue remains unresolved, there is strong support among patients and clinicians for free access to genomic variant annotations as exemplified by the Free the Data Movement (2017).




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**FIGURE 12.1** The Food and Drug Administration recommends testing for HLA-B*57:01 before to the use of the antiretroviral drug abacavir because individuals with this HLA type are at high risk for developing a severe cutaneous adverse reaction (Stevens–Johnson syndrome, toxic epidermal necrolysis). A rules-based clinical decision support system can be constructed and implemented to ensure that the testing is done and that abacavir is not prescribed inappropriately. When a provider orders abacavir, a rule is triggered that looks at the laboratory reports to see if the patient has had prior testing for HLA-B*57:01. If the testing

has been done and the result is available, the system proceeds to the next rule. If test results are not available, the provider is notified through a pop-up alert that a test for HLA-B*57:01 must be ordered prior to prescribing abacavir. Usually this alert will include the ability to order the test with a single click. Given the severe nature of the adverse reaction, this would be built as a "hard stop," so the clinician must order the test and will not be able to proceed with the abacavir order. Once a result is available, the decision support system triggers the second rule—is HLA-B*57:01 present? If the answer is no, the order for abacavir proceeds and the clinician does not receive an alert. The triggering of the rule is captured by the system as a quality check to make sure it is operating properly. If the patient carries HLA-B*57:01, an alert is fired and the provider is informed that this medication is contraindicated for the patient and an alternative medication should be used. The alert may include a link to additional information in case the provider is interested in learning why she received the alert. To improve provider workflow, the ability to reach a list of preselected alternative medications with a single click in the alert is usually included.

## 12.1.6. Element 5: Simultaneously Support Human-Viewable Formats and Machine-Readable Formats in Order to Facilitate Implementation of Decision Support Rules

*What does this mean?* A human readable document can embed structured data that can be read by a human but also can be parsed by a computer supporting functions in the EHR such as those described in the Element 4 section.

*Why is this important?* The preceding sections make the answer to this question self-evident. However, this element also includes the generation of documents in both human- and machine-readable form simultaneously, implying a reduction of resources that would be required to create one or the other form and have a second system to create the alternative version.

*What is the current state?* EHRs are currently unable to represent genomic information as structured data without some type of manual entry. However, there are solutions that have been designed for use in certified EHRs, such as clinical document architecture (CDA). CDA

defines the structure of a document through standardization of the location and content of elements of the document. The predefined nature of the document makes it easier for computers to locate information needed for CDS or other functions. Genomic test reports would lend themselves to this type of organization, but this approach has not been widely used. This approach was used in the creation of patient [26] and provider [27] facing genomic test interpretive reports that are being used to support the MyCode Return of Results program at the author's institution.

*What is needed?* While there are current solutions that could be used, none are emerging as a solution for this element. CDA has been endorsed by the Genomic Working Group of Health Level-7 (HL7), establishing it as the only EHR standard that supports this element. However, it seems the field is looking for another solution that may be less resource intensive and more scalable.

## 12.1.7. Element 6: Anticipate Fundamental Changes in the Understanding of Human Molecular Variation

*What does this mean?* If the Human Genome Project has taught us anything, it's how little we know about the genome and its relation to health and disease. The intent of this element is a reminder that systems must be designed with flexibility to rapidly incorporate new knowledge that has evidence for clinical utility.

*Why is this important?* Implementation of new knowledge into routine clinical practice takes a long time—up to 17 years as estimated by an Institute of Medicine (IOM [now the National Academy of Medicine]) study. The reasons for this would fill another book. The contribution of information systems as a cause of the delay has not been extensively studied, although information systems have been shown to accelerate implementation of evidence based guidelines. The early experience in the implementation of pharmacogenomics in several healthcare systems has confirmed the

key role played by genomically enabled EHR systems as a facilitator for use of this information in practice.

*What is the current state?/What is needed?* This element has no current state, so it makes sense to combine these two questions. The key for developers of genomically enabled information systems is to stay abreast of new developments in the field, particularly those that are showing early evidence of clinical utility. In this case a gap analysis of current capabilities against the anticipated need for the new information type is needed to inform the design of the next generation of genomic EHR. It is also clear that a multidisciplinary approach is needed to make sure the necessary perspectives are available to design the systems. Interdisciplinary training is also needed to develop the expertise to support development of flexible genomic EHR systems [28].

### 12.1.8. Element 7: Support Both Individual Clinical Care and Discovery Science

*What does this mean?* Use the same systems (which would include nomenclature, data formats, informatic standards, etc.) for clinical care and research.

*Why is this important?* Genetics has always recognized the importance of close collaboration between clinicians and researchers. Astute clinicians embracing the counsel of Garrod to "… treasure your exceptions" partnering with researchers have driven much of the genomic discovery that is increasingly impacting clinical practice. Clinical research in general is embracing pragmatic research methods that draw from quality improvement and the new field of implementation science with the hopes that this will result in a learning healthcare system. In 2015, the National Academy of Medicine defined a learning healthcare system as a system in which "science, informatics, incentives, and culture are aligned for continuous improvement and innovation, with best practices seamlessly embedded in the delivery process and new knowledge captured as an integral by-product of the delivery experience" [29]. To

fully appreciate the impact of genetic variation on human health and disease will require the free movement of information from the laboratory to the clinic and back again—a true virtuous cycle. The eMERGE network was established to explore this premise. In phase I the feasibility of such an approach was established. Phase II was focused mostly on discovery using clinical data extracted from EHR data, although a supplement to phase II, eMERGE-PGx, explored the implementation of preemptive pharmacogenomics in the clinic [30]. Phase III, which is ongoing, is focused on the return of results and measurement of outcomes from 25,000 participants sequenced on a next-generation chip that includes 109 genes (inclusive of the ACMG 56) and hundreds of single nucleotide variants that include many of the important pharmacogenomic variants. eMERGE has resulted in more than 500 publications, many of which provide evidence to inform realization of this element.

*What is the current state?* There are some early examples of the genomic learning healthcare system. The aforementioned PREDICT program at Vanderbilt, as well as other preemptive pharmacogenomic programs at the University of Florida and St. Jude's Children's Research Hospital, are generating new discoveries as well as demonstrating improved outcomes as a result of these implementations. At Geisinger Health System, the DiscovEHR project [19] is bridging the gap between research and the clinic. As of June 2017, more than 92,000 participants from Geisinger have completed exome sequencing. Geisinger is building a scalable clinical genomic return of results program using the model of a learning healthcare system [31]. This has resulted in significant scientific discovery, but, of relevance to this topic, the project is beginning to use the combination of the sequence and clinical data to identify significant care gaps in individuals that carry pathogenic variants in actionable genes [32]. Other examples in familial hypercholesterolemia [33] and arrhythmogenic right ventricular cardiomyopathy [34] demonstrate the feasibility of the approach. Indeed, the evidence that can lead to improved care for these conditions derived from these and other projects that are in progress with this cohort would not be possible

without the learning healthcare system framework.

However, as noted, the limitations of the infrastructure to allow genomic information to be represented in the EHR environment is the major barrier to a broad realization of this today.

*What is needed?* This question has been the subject of a workshop sponsored by the National Academy of Medicine [35]. It goes without saying that the prior elements are necessary to realize the vision of the genomics-enabled learning healthcare system. The workshop addressed each of issues over the course of the meeting. They also addressed the role of the consumer (or patient) in the design of such a system. Incorporating the patient perspective along with the rest of the clinical and genomic information is necessary to fully realize this system. However, this transformation cannot be realized by genomics in isolation. This will only be broadly realized if the healthcare system as a whole embraces the tenets of a learning healthcare system.

## 12.2. Conclusion

Genomic information, if intelligently combined with other information sources, has the potential to transform healthcare and usher in the era of precision medicine. To accomplish this, EHR systems must be able to represent and integrate genomic information to support clinical decision making. The elements discussed here are for the most part under development, although there are examples in use that provide support for the feasibility of the genomic EHR. However, the genomic EHR is simply a tool that must be implemented within the framework of the learning healthcare system if genomic information is to improve health outcomes.

## References

[1] All of Us. https://obamawhitehouse.archives.gov/precision-medicine.

[2] Collins F.S, Varmus H. A new initiative on precision

medicine. *N Engl J Med*. 2015;372:793–795.
[3] Densen P. Challenges and opportunities facing medical education. *Trans Am Clin Climatol Assoc*. 2011;122:48–58.
[4] Masys D.R, Jarvik G.P, Aberneth y N.F, Anderson N.R, Papanicolaou G.J, Paltoo D.N, Hc desiderata for the integration of genomic data into Electronic Health Records. *J Biomed Inform*. 2012;45:419–422.
[5] Starren J, Williams M.S, Bottinger E.P. Crossing the Omic Chasm: a time for Omic ancillary systems. *J Am Med Assoc*. 2013;309:1237–1238.
[6] Aronson S.J, Clark E.H, Babb L.J, Baxter S, Farwell L.M GeneInsight Suite: a platform to support laboratory and provider use of DNA-based genetic testing. *Hum Mutat*. 2011;32:532–536.
[7] Klinkenberg-Ramirez S, Neri P.M, Volk L.A, Samaha S.J, Newmark a qualitative pilot study of novel information technology infrastructure to communicate genetic variant updates. *Appl Clin Inform*. 2016;7:461–476.
[8] Nicolae M, Pathak S, Rajasekaran S. LFQC: a lossless compression algorithm for FASTQ files. *Bioinformatics*. 2015;31:3276–3281.
[9] Lubin I.M, McGovern M.M, Gibson Z, Gross S.J, Lyon Wilson J, Pass K. Clinician perspectives about molecular genetic testing for heritable conditions and development of a clinician-friendly laboratory report. *J Mol Diagn*. 2009;11:162–171.

[10] Xue Y, Ankala A, Wilcox W.R, Hegde M.R. Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing:single-gene, gene panel, or exome/genome sequencing. *Genet Med*. 2015;17:444–451. .

[11] Miller C.E, Krautscheid P, Baldwin E.E, Tvrdik T, Ope counselor review of genetic test orders in a reference laboratory reduces unnecessary testing. *Am J Med Genet A*. 2014;164A:1094–1101.

[12] CDS. http://www.infosources.org/what_is/Clinical_decisior

[13] Osheroff J.A, Teich J.M, Middleton B.F, Steen E.B, Wri roadmap for national action on clinical decision support. *J Am Med Inform Assoc*. 2007;14:141–145.

[14] Murphy E.V. Clinical decision support: effectiveness in improving quality processes and clinical outcomes and factors that may influence success. *Yale J Biol Med*. 2014;87:187–197.

[15] eMERGE. https://emerge.mc.vanderbilt.edu/.

[16] OpenIB. http://www.openinfobutton.org/.

[17] ClinGen https, ClinGen. https://www.clinicalgenome.org/.

[18] Heale B.S.E, Overby C.L, Del Fiol G, Rubinstein W.S, Maglott D.R, Nelson T.H, Mil genomic resources with electronic health records using the HL7 infobutton standard. *Appl Clin Inform*. 2016;3:817–831.

[19]

Dewey F.E, Murray M.F, Overton J.D, Habegger L, Le
Hout C.V, Staples J, Gonzaga-
Jauregui C, Metpally R, Pendergrass S.A, Giovanni M
Husn N.S, Hartzel D.N, Lavage D.R, Kost K.A, Packer
Tse C.A, Mason-
Suares H.M, Bruse S, Mellis S, Phillips R, Stahl N, Mu
and clinical impact of functional variants in 50,726 whole-exome sequences from the DiscovEHR study. *Science*. 2016;354(6319) pii: aaf6814.

[20] Pulley J.M, Denny J.C, Peterson J.F, Bernard G.R, Vne
Jones C.L, Ramirez A.H, Delaney J.T, Bowton E, Broth
implementation of prospective genotyping for personalized medicine: the design of the Vanderbilt PREDICT project. *Clin Pharmacol Ther*. 2012;92:87–95.

[21] DIGITizE. http://www.nationalacademies.org/hmd/Activities/Re
Collaboratives/EHR.aspx.

[22] Implementation Guide. http://www.nationalacademies.org/hmd/
~/media/Files/Activity%20Files/Research/GenomicBa
%20Final%201_0.pdf?la=en.

[23] CDSKB https, CDSKB. https://cdskb.org/.

[24] Welch B.M, Eilbeck K, Del
Fiol G, Meyer L.J, Kawamoto K. Technical desiderata for the integration of genomic data with clinical decision support. *J Biomed Inform*. 2014;51:3–7.

[25] Guerrini C.J, McGuire A.L, Majumder M.A. Myriad take two: can genomic databases remain secret? *Science*. 2017;356(6338):586–587.

[26]

Stuckey H, Williams J.L, Fan A.L, Rahm A.K, Green J, genomic laboratory reports from the patients' view: a qualitative analysis. *Am J Med Genet A*. 2015;167:2238–2243.

[27] Williams J.L, Rahm A.K, Stuckey H, Green J, Feldman genomic laboratory reports: a qualitative analysis of provider review. *Am J Med Genet A*. 2016;170:1134–1141.

[28] Williams M.S, Ritchie M.D, Payne P.R.O. Interdisciplin training to build an informatics workforce for Precision Medicine. *Appl Transl Genom*. 2015;6:28–30.

[29] Institute of Medicine. The learning healthcare system: workshop summary. Washington (DC): The National Academies Press; 2007. https://doi.org/10.17226/11903.

[30] Rasmussen-Torvik L.J, Stallings S.C, Gordon A.S, Almoguera B, B and anticipated outcomes of the eMERGE-PGx project: a multicenter pilot for preemptive pharmacogenomics in electronic health record systems. *Clin Pharmacol Ther*. 2014;96:482–489.

[31] Williams M.S, Buchanan A.H, Davis F.D, Faucett W.A Centered Precision Health In A Learning Health Care System: Geisinger's Genomic Medicine Experience. *Health Aff (Millwood)*. 2018;37:757–764. .

[32] Manickam K, Buchanan A.H, Schwartz M.L.B, Hallqu Suares H.M, Hoskinson D.C, Abul-

Husn N.S, Dewey F.E, Overton J.D, Reid J.G, Baras A, Underascertainment of BRCA1/2 Cancer Risk in 50,726 Unselected Adult Biobank Participants. *JAMA Network Open*. 2018;1(5):e182140. https://doi.org/1001/jamanetv

[33] Abul-Husn N.S, Manickam K, Jones L.K, Wright E.A, Hartz Jauregui C, O'Dushlaine C, Leader J.B, Kirchner H.L, identification of familial hypercholesterolemia within a single U.S. health care system. *Science*. 2016;354(6319) pii: aaf7000.

[34] Haggerty C.M, James C.A, Calkins H, Tichnell C, Lea Suares H, Austin-Tse C, Rehm H.L, Delisle B.P, Makowski D.J, Mehra V health record phenotype in subjects with genetic variants associated with arrhythmogenic right ventricular cardiomyopathy: a study of 30,716 subjects with exome sequencing. *Genet Med*. May 4, 2017 doi: 10.1038/gim.2017.40 [Epub ahead of print].

[35] Roundtable on Translating Genomic-Based Research for Health; Board on Health Sciences Policy; Institute of Medicine. Genomics-enabled learning health care systems: gathering and using genomic information to improve patient care and research: orkshop summary. Washington (DC): National Academies Press (US); July 8, 2015 doi: 10.17226/21707 Available from:. https://www.ncbi.nlm.nih.gov/books/NBK2941

## Further Reading

Free the Data. http://www.free-the-data.org/.

13

# Ethical and Social Issues in Clinical Genetics

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## Abstract

Many ethical and social issues arise within clinical genetic practice for practitioners and their clients. Patients confront questions about their possible obligations to generate personal genetic information or pass it to other family members, including their children, or whether to continue with a wanted pregnancy when the child (if born) would be affected by a serious disorder impacting on their health, abilities, and potential for human thriving.

Practitioners may have personal views on such matters, but their role is not to impose these but to help clients make their own decisions. They may guide their patients/clients to accept specific interventions when these are of clear health benefit and they may seek to persuade clients to act in the interests of others in the family, but they will usually support whatever considered decisions are made by their clients.

Practitioners may seek the best course of action by following a preferred set of rules, attempting to pursue the "optimal" outcomes for all parties, or seeking to behave with professional integrity.

As genomic information about patients is being produced faster than it can be interpreted, patients and practitioners may generate answers to questions they had not asked and will often have to deal with uncertainty instead of clarity; these difficulties appear likely to persist for many years.

## Keywords

Antenatal screening; Carrier screening; Confidentiality; Consent; Genetic counseling; Newborn screening; Nondirectiveness; Outcomes; Predictive genetic testing; Privacy

## 13.1. Introduction

Genetic services have always faced an unusually broad range of contentious ethical issues. Important areas include reproductive issues such as prenatal diagnosis and selective termination of pregnancies, proper communication of genetic information within families, genetic investigation of those who cannot consent (children or incompetent adults), and professional issues such as the role of the clinician and the scope of their potentially competing obligations to patients, patients' families, and society more broadly.

Two additional factors make any reflection upon ethical issues in the practice of clinical genetics particularly complex. First, it is often our clients, as individuals and families, who are confronted by the ethical difficulties. In most areas of medicine there are issues that challenge professionals and complex issues where society has to declare its view, but in genetics our role is often to formulate the ethical issues for our clients to consider.

Second, our discipline is characterized by the rapid development of technologies for generating genetic information, and with this the rapid growth of genetic knowledge. Since the sequence of the human genome was first reported in 2001, it has become feasible for an individual's genome to be sequenced to achieve a full diagnosis of his/her condition. The application of genomics in medical practice now also includes informing decisions about disease prevention,

surveillance for disease complications in those at risk from recognized familial disorders, assisting in reproductive decisions by either identifying conceptions (after implantation or before) that are affected by specific genetic disorders or identifying the healthy carriers of recessive disorders, and guidance on the choice of therapies, especially in the treatment of malignancy.

The challenge for practitioners has shifted from generation of the DNA sequence of important single genes to the interpretation of vast quantities of data—whole exome sequencing or even whole genome sequencing. This new situation of abundant data of which we can make only limited sense may well persist for a decade or two, or longer. These technical developments are changing the shape of the ethical and social issues confronting us in clinical genetics. The underlying principles and the issues at stake may not be new, but the contexts within which clinicians and clients/patients/families are confronting these difficulties are only slowly becoming familiar. We are gradually beginning to develop common habits of professional practice that allow all parties to feel comfortable and safe. Accordingly, the questions arising in practice require particular care. It is our aim here to sketch out the principal social and ethical issues that arise in our work in clinical genetics, considering in particular the impact of recent developments.

## 13.2. The Historical Context

The study of heredity and its influence on human variation, especially "natural ability", was developed in 19th-century Britain by Sir Francis Galton. His focus on quantitative traits was most productive in developing the field of statistics, and eventually merged with Mendelian genetics, but he applied this focus with enthusiasm to intelligence, social virtues (or lack thereof), and social achievement. He regarded these traits as primarily genetic and, like many before him, had the goal of improving the genetic constitution of future generations. He coined the term *eugenics* to describe "the study of agencies under social control that may improve or impair the racial qualities of future generations either physically or mentally". The

perennial concern about the moral decay of society, widespread among senior citizens and the wealthy in societies ancient and modern, latched on to theories of heredity in late Victorian Britain as offering solutions to the vices of alcoholism, pauperism, homelessness, and feeble-mindedness. The same set of ideas also reinforced assumptions about the relative evolutionary development of different populations, reassuring the citizens of the imperial powers that they were justified, indeed destined, to rule over their biologically inferior colonial subjects. Such ideas flourished in literature and the arts, as well as in the sciences, and were integral to the mentality of society at large in much of Europe and North America. Indeed, the belief or claim that one's nation is "superior" may lead directly to the need to identify other, less favored peoples over whom to rule, and hence to expansionist policies that justify the "rightful" exploitation of subject peoples, whether in Africa or Eastern Europe. Eugenics may in this way have played a part in fostering the mindset that led to the atrocities committed by the Nazis during the Second World War.

It is unhelpful for contemporary geneticists to be perpetually apologizing for the sins committed by their intellectual forebears, but neither can we afford to forget those horrific events that happened so very recently. As the scientific validity of contemporary genetics and its potential impact on society are now much greater than was the case 70 years ago, it is essential that we identify and make explicit our current taken-for-granted attitudes and our deeply held, and perhaps unexamined, convictions. How might these be leading us into errors that we or our descendants will only recognize too late, with hindsight, once great damage has been done to our profession, to our patients, or to society at large? This is the hard lesson we should learn from considering the past abuses of human genetics.

## 13.3. Genetic Counseling, Testing, and Screening

There is a critical distinction between clinical genetic services made

available in response to the preexisting concerns of particular individuals and families, and the screening programs offered proactively to large groups of the population, who have usually not sought the test that is being offered.

In the context of a specific family, genetics professionals will respond to their questions or concerns. For example, parents may seek an explanation for a serious developmental problem affecting their child, or an individual at risk of a familial neurodegenerative condition or malignancy may wish to know their chance of developing their family's disorder, or a couple may wish to know the chance of their future children being affected by an inherited condition that has previously affected others in the family. During such consultations, great sensitivity is often required to explore the client's understanding, to address their preexisting concerns, and to provide appropriate emotional support.

In the context of population screening, the encounter between professional and client is very different. The professional is actively promoting a specific course of action. The client may never have had any questions or concerns, so the professional's suggestion of the screening test may itself generate concerns rather than allaying them. In such circumstances the burden of an unsought decision is imposed, where one course of action (compliance with the offer of testing) is being strongly recommended by the very act of making the test available at all. Although there are some parallels, this differs from the commercial marketing of a genetic test in several ways; there is an implied professional approval in both settings, but this is stronger if no charge is made for screening provided through public mechanisms and the question of cost is not there to prompt the warning cry of *caveat emptor*.

There most certainly are circumstances in which it is thoroughly appropriate for health services to promote a screening test or other health intervention, such as the immunization of infants. In the sphere of reproduction and genetics, however, the promotion of screening can be regarded as especially contentious; in this context, individual choice is highly valued. Whereas newborn screening for the

remediable disorders phenylketonuria (PKU) and congenital hypothyroidism is universally applauded, there is dissent in relation to reproductive genetic screening—including antenatal screening for Down syndrome or congenital malformation and carrier screening for autosomal-recessive diseases.

It is unarguable that the context of proactive population screening for genetic disease is very different from that of genetic counseling as a response to preexisting family concerns. This has important ethical implications for professional practice in these areas.

## 13.3.1. Goals and Outcomes of Genetics Services

In the bad old eugenic past, the principal aim of reproductive genetics was to improve the biological quality of the next generation. Echoes of this movement are still heard in advanced Western societies from populist politicians intent on profiting from intercommunity strife.

In the contemporary rhetoric of clinical genetics and genetic counseling, however, our professional goals are quite different from former ideas of "the health of the people" (i.e., of the race). Respect for individual autonomy takes precedence over measures of the impact of genetic services on the population, such as the birth incidence of genetic disorders or the number of pregnancy terminations for specific conditions. To describe our goals in terms of such crude population outcomes would now be regarded as unacceptable, indeed repugnant.

However, there is a contemporary context within which comparable assumptions about the effectiveness of genetic services could be seen by some as natural and defensible. This is the economic frame, in which the primary goal of genetic services becomes the reduction of expenditure on caring for those with genetic disease or disability, achieved sometimes through a reduction in the birth incidence of conditions for which prenatal or carrier screening has been made available. From this perspective, those affected by Down syndrome or β-thalassemia (for example) would be regarded as a net economic burden for society to bear. Despite the contested nature of these

claims, over the last 30 years many studies of population screening, including prenatal screening for Down syndrome and carrier screening for cystic fibrosis (CF), have essentially argued for the implementation of such programs because screening and the termination of affected pregnancies are less costly than caring for affected individuals.

Although many clinicians may regard these arguments as ethically unacceptable, they are still employed *sotto voce*. Some who do so have no moral qualms; others may value the offer of reproductive choice per se and therefore support the existence of prenatal screening programs while rejecting the monetary, neo-eugenicist (or "consumer eugenics") arguments. The stance taken on this issue by different social groups, including political parties, religious communities, and ethnic groups, is heavily influenced by national political alignments.

Arguments based on crude costs are not generally used to justify the provision of family-based clinical genetic services. Such an analysis might show a net cost saving to society because genetic counseling does sometimes have the consequence of reducing the number of infants born with serious (costly) genetic disorders due to parental decisions not to have children or the selective termination of affected pregnancies, but clinical genetic professionals may and (we would argue) should refuse to use these arguments to justify their services. To adopt such arguments would concede that families deciding to have affected children are making bad decisions. Furthermore, such economic analyses depend on numerous assumptions about the future provision and cost of healthcare and social care, to be traded against current actual costs of providing the clinical genetics services. These analyses would be complex and contentious even within a purely economic frame.

How, then, can the outcomes of clinical genetics services be measured to permit a comparison with the expenditure on altogether different areas of health service activity? The following outcome measures have been proposed and employed in research studies, although it remains unclear whether any of them is suitable for

ongoing evaluation of a clinical service.

- The recall of risk information (e.g., the risk of recurrence of a disease in a future child) can be assessed after a defined time lapse. However, recall is the result of many psychological processes, including the tendency to polarize probabilities toward 0 or 1; that is, No (it will not happen = 0) or Yes (it is certain to happen = 1). Further, the significance attached to recurrence risks by clients is itself influenced by the way in which the information is framed in the counseling process [1].
- Reproductive plans can be assessed before and after genetic counseling. This approach prejudges reproduction as the primary concern of our clients, many of whom are not specifically seeking information about reproduction. Furthermore, such plans are merely hypothetical, so their expression will be heavily dependent on the context and framing of the issues by the professional. It is even more difficult, and more contentious, to assess the impact of genetic services on reproductive behavior.
- Client satisfaction with the service can be assessed—but may be heavily influenced by the environment in which a service is provided. Clients may not be aware of how the service they have experienced compares with other services, and their views are likely to be influenced by the information they are given in the clinic. For example, clients may be distressed if given "bad news" or resentful if even the most skillful diagnostic process has failed to achieve a diagnosis.
- For the same reasons, questionnaire-based measures of healthy physical and mental functioning are unsuitable outcome measures for genetic services, whereas they may be entirely appropriate for assessing care for osteoarthritis of the hip.
- A more client-focused measure, "perceived personal control", has been developed as a major improvement on other

outcome measures. This avoids many of the previously identified pitfalls and can contribute to the assessment of the quality of services provided [2]. A related outcome measure has emerged independently from clinical genetics services in the United Kingdom, encapsulated by the concept of "empowerment" [3]. Both approaches can be applied across many different contexts, and are not restricted to reproduction in clinical genetics.

The ethical importance of the choice of outcome measure is especially grave within a large population-based health service because professionals can be driven to comply with population-based or corporate goals, in contrast to a system of truly individual healthcare arranged between the physician and the patient. If a health service decided to reduce the birth incidence of Down syndrome, for example, and to judge the performance of health service staff according to their "achievements" in this respect (perhaps with implications for their rates of pay or job security) then it would be no surprise to see service goals systematically distorting the pattern of healthcare provided to large numbers of individual patients [4]. Professionals must watch to ensure that their responsibilities are not subverted, perhaps rather subtly, by the imposition of institutional (state or corporate) policies.

There is thus no utilitarian calculus that can determine the worth of clinical genetics services in a neutral, detached, or objective fashion. Despite these difficulties, there are ways of using research evidence to help decide what services should be made available, and ways of assessing the quality of the services provided. These decisions are inevitably the product of a political process reflecting both the value that society places upon autonomy in the sphere of reproduction and society's concern for the welfare of individuals with genetic disorders and their families. Once the decisions have been made about what genetics services to provide, their quality can be assessed using empowerment-centered measure of outcomes [5].

### 13.3.2. Nondirectiveness in Genetic Counseling

Respect for client autonomy is one of the core values of the genetic counseling profession. One manifestation of this is the idea that genetic counseling is, or at least should be, "nondirective": it should not lead the client to make specific choices predetermined by the counselor or the healthcare system. The claim that genetic counseling is nondirective allows a professional to distance herself or himself from emotional involvement in the decision. This may be very helpful to the professional, in that emotional overinvolvement can be damaging, and it reminds us that the legal responsibility for any decision lies firmly with the clients.

There are several other respects, however, in which the concept of "nondirectiveness" may be problematic and open to challenge. First, at least some elements of clinical genetic services may inevitably be "directive"—leading clients toward specific decisions and outcomes. This applies particularly to population genetic screening programs, especially in the context of reproductive screening, where the existence of a screening program may be taken as a recommendation to use it. This can be referred to as "structural" directiveness and does not imply that the individual professionals involved are actively recommending one course of action or another, although they may be doing so. The screening program may be so routine that neither professionals nor clients give active consideration to the issues involved and both parties merely accept screening as standard practice [6,7]. The challenge for professionals in these settings is to enable clients to appreciate that the decision really is one for them to make.

Second, there are contexts in clinical genetics where it is entirely proper for a professional to recommend a specific course of action to clients. This may involve the recommendation that a client at risk of a specific disorder should undergo surveillance for that disorder, e.g., colonoscopy for those at increased risk of bowel cancer. This in turn may involve recommending a predictive genetic test, so surveillance

can be targeted to those family members with a high risk, and those who are at population risk can avoid the burden, costs, and hazards of unnecessary investigations.

Genetic counseling professionals may also actively recommend to their clients that they share genetic information within their family. It could be relevant to the future health of the client's relatives or their reproductive decisions. In general, genetic counselors will promote open family communication about any relevant genetic disorder and will indicate the type of problems that can arise if communication is blocked. This is generally acknowledged to be good professional conduct—although it clearly conflicts with "nondirectiveness" if that is interpreted in a narrow and rigid manner.

There is another sense in which an exaggerated respect for nondirectiveness can be unhelpful: when compliance with "nondirectiveness" is taken to mean that genetic counselors accept without challenge every statement expressed by their clients, however poorly considered. Effective counseling helps the client to think through the consequences of any decision or course of action. This inevitably entails the use of authoritative counselor interventions, such as direct questioning to challenge the client, for example with the need to consider previously unanticipated potential consequences of planned action. Not to make use of such interventions would greatly limit the worth of the genetic counseling [7–10]. An inappropriately passive understanding of "nondirectiveness" will undermine the authority and effectiveness of the genetic counselor and reflects a shallow understanding of "counseling" in general. It is all too easy for such misunderstandings to arise unless professionals monitor their practice with regular supervision.

Finally, there is the danger that nondirectiveness in relation to a complex practical question with extensive implications may be felt as abandonment by the client. This has been particularly problematic for patients recently diagnosed with cancer, who are faced by choices between therapeutic options where the evidence is unclear and difficult to interpret. An impersonal and detached presentation of the options can then, understandably, be experienced as an emotional

withdrawal from the client.

Any claim that genetic counseling should always be "nondirective" would be unhelpful, and any claim that it is so would clearly be false. Respect for nondirectiveness is appropriate, but only when it is understood as a rich and complex concept that usefully sustains the tension within practitioners between respect for the client's autonomy and other competing priorities.

## 13.4. Diagnostic Genetic Testing

In healthcare, two key ethical issues are encountered in the context of pursing a diagnosis for a patient: the difficulty of handling diagnostic information about the patient when it has important implications for others, and the difficulties that may arise when investigations reveal information of uncertain significance.

### 13.4.1. Implications for Others

Navigating the complexities of these ethical issues with a patient is a fundamental element of genetic counseling, although not unique to clinical genetics as a specialty. Communicable disease, especially sexually transmitted disease, is another context in which a diagnosis may have important implications for others; health professionals will work with a patient in an attempt to pass on the relevant information to their sexual partner/contacts, and there may be legal sanctions when important information is withheld. While legal force is not usually applied to compel a patient to disclose sensitive personal information in the context of genetic conditions, or to compel a professional to do so if the patient does not, legal protection may sometimes be available to health professionals who disclose such information to the at-risk relatives of the reluctant patient.

When a physician is investigating the cause of a patient's symptoms, the possibility of an underlying genetic cause of the presenting problem may not be explored with the patient or family to any depth, unless circumstances make this rather likely. When should the clinician give this possibility some weight? Two examples may

assist reflection.

Firstly, when the diagnosis of Duchenne muscular dystrophy (DMD) is being seriously considered in a young boy with delay of motor and perhaps language skills, telling the parents that such a diagnosis is a possibility will cause distress but may help the parents prepare themselves and the wider family to deal with the implications if the suspected diagnosis is confirmed. It would usually be remiss not to have mentioned the possibility of an inherited disease before proceeding to either analysis of the dystrophin gene or a muscle biopsy.

Secondly, if an adult presents with dementia, the clinical picture will occasionally be the result of an underlying genetic condition, such as Huntington disease (HD) or one of the other less common inherited dementias. This is more likely if the onset of disease is at a younger age, if the patient has additional neurological manifestations, or if there is a relevant family history. Depending upon these factors, the physician will need to consider whether the possibility of an inherited disorder should be mentioned, and if so how, with what emphasis, and to whom. The possibility of an inherited basis would often be discussed with the patient (and perhaps their relatives) if a diagnosis of HD were strongly suspected, but perhaps not if HD was the least likely of a long list of possible diagnoses under consideration. Contextual factors, including the degree of family involvement, will often be decisive.

While the genetic nature of a condition remains uncertain, it may be difficult for the family to communicate effectively about it. This leaves open the question of how the professional strikes the right balance between backing away altogether from mentioning this possibility or creating needless anxiety, while of course always respecting patient confidentiality.

## 13.4.2. Information of Uncertain Significance

As laboratory techniques have progressed, both scientific knowledge and clinical experience have accumulated. However, genetic investigations have always carried the potential to generate more

information than can be interpreted with confidence. It has taken years for the medical implications of chromosomal anomalies to be appreciated, and there can still be real difficulty in predicting or interpreting the reproductive consequences of complex chromosomal rearrangements.

The early application of genetic linkage analysis gave physicians good practice in explaining the limitations of molecular diagnostics and estimating the frequency of misleading conclusions (e.g., double recombination events). As mutation testing became possible for some important loci, it seemed that the ease of interpretation was steadily increasing. Thus the distinction between DMD and Becker muscular dystrophy caused by different mutations in the same gene (*DMD*) could usually be accounted for by the frameshift hypothesis. However, the complexity of interpreting sequence information soon became evident, most prominently in some of the Mendelian cancer-predisposing genes such as *BRCA1* and *BRCA2*. How should a novel sequence variant in an individual with a strong family history of cancer be interpreted? "Variants of uncertain significance" continue to be a problem far beyond the realms of the cancer-predisposing genes, as discussed below.

Comparable difficulties were encountered in relation to autosomal-recessive disorders. For example, sequence changes in the CF locus cystic fibrosis transmembrane conductance regulator (CFTR) may:

i) be entirely innocent
ii) be weakly pathogenic when accompanied by a definitely pathogenic allele on the other chromosome (leading to a late-onset and rather mild predisposition to chest infection), or
iii) predispose to a mild phenotype, such as adult-onset lung disease or congenital bilateral absence of the vas deferens, leading to male infertility.

This highlights an important distinction. Having a mutation in both CFTR alleles is not necessarily equivalent to having the clinical disorder known as CF (this emphasizes the problems of talking about the "gene for" something).

At the same time as these complexities arose, powerful genome-wide association studies were developed that focused on single nucleotide polymorphisms (SNPs). These have proved useful in research applications, but their clinical utility in the common complex diseases of developed societies has not been established. Moreover, while many highly significant associations have been found between SNPs and disease, the relative risks involved have generally been rather small and the known genetic contribution to the risk of disease is generally only in the range of 10%–20%; the remaining 80%–90% is termed the "missing heritability" [11]. There are unresolved questions about how to avoid overlooking important gene–gene and gene–environment interactions, and account for the high levels of genetic polymorphism observed within human populations, which suggests there are strong interactions impacting on selection to maintain variation but concealed because the net effects are small.

If the standard karyotype was the first genomic (whole genome) technology, the second genomic technology to enter regular clinical practice was the chromosomal microarray for the direct detection of abnormalities via comparative genomic hybridization. This technology has not only delineated the contribution of specific deletions and duplications to disorders of physical and mental development and susceptibility to psychiatric disease, but also the high frequency of copy number variants (CNVs) that are compatible with normal development and functioning. Some CNVs may appear compatible with normal development but contribute to severe developmental problems in the presence of environmental triggers or other specific CNVs (the "two-hit" model of developmental disorders) [12]. Our ability to interpret such variants continues to be limited by the difficulty in recognizing the various other factors with which they may interact in contributing to disease.

The third genomic technology to affect genetic diagnostic services is next-generation sequencing, which enabled the switch from mutation searching in single Mendelian genes to searching simultaneously (in parallel) for mutations in many loci. This can be whole genome sequencing (WGS) or the sequencing of selected sequences, usually

transcribed sequences (whole exome sequencing) or a subset of protein coding sequences. Experience is being gained with the sequencing of exomes and large gene panels associated with specific disease phenotypes, and through research-led studies of both healthy individuals and those with overt disease. Large-scale human genome sequencing projects in many countries are revealing many rare variants, some of which are likely to make a substantial contribution to disease or risk of disease [13]. The generation of DNA sequence data is no longer the rate-limiting step in diagnostics; it is the interpretation that remains difficult and demanding. Computer modeling of the functional consequences of "mutations" can give helpful results but is not infallible, while functional RNA and structural protein studies remain essentially research endeavors. It will take years, perhaps decades, of data accumulation and analysis for the interpretation of most DNA sequence variants to be interpretable in a clinical context with reasonable confidence. In the meantime we have two major challenges: how do we organize high-throughput sequencing and the associated data storage and analysis to answer clinical questions while ensuring collective access to pooled information about variants, and what do we say to patients?

With respect to data storage and analysis, we may need to decide either to store the sequence data as part of an individual's medical record or to interpret the sequence as best we can and then store only the report. The latter option avoids imposing on health services the onerous obligation to store large quantities of data for each patient's lifetime. The costs of updating software and hardware over many decades would be great, while the clinical benefits might often be slight and the data stored would rapidly become obsolete. The obligation to reinterpret the WGS sequence data and issue updated interpretations, as in the first option1, would consume an ever greater proportion of a laboratory's efforts, while in the second storage option2 the occasional reanalysis of a fresh sample would cost much less.

Each patient needs to decide what information they want to be given from their sequence, and whether they want updated

interpretations in the future should these become available. Would they want only information relating to the reason for testing, or would they also want to be given "incidental" findings about other health issues? If they want incidental findings, would this be actionable findings only, or any important health-related information? Would information about recessive disease carrier risks and the risks of adult disease be reported in children, or only childhood-onset risks?

A long-standing debate on these issues was greatly enlivened by the 2013 proposals from the American College of Medical Genetics and Genomics that variants in some 56 genes (regarded, rather optimistically, as "actionable") should be actively sought and reported [14]. That position was later softened by the requirement for a prior opportunity to opt out of such reporting [15].

Decisions of this sort have implications not only for laboratory organization but also for clinical interactions, as the discussion about consent for an investigation will need to take account of what analyses the laboratory will perform. Awkward questions arise, such as whether the sequence information generated but not interpreted for the laboratory report of a gene panel investigation counts as part of the patient's medical records.

Various points along a policy spectrum can be imagined, from active searching for additional findings and regular reanalyses to very narrowly targeted testing followed by the discarding of data and samples after initial reporting (still permitting submission to large collaborative datasets of deidentified data on variants).

The second consideration is what to say to the patient or at-risk individual regarding identified variants. It will often be impossible to say nothing, especially if further family studies may be helpful to resolve diagnostic uncertainty. The diagnostic laboratory will need to make decisions as to what information about which variants to pass to the patient's clinical team, and then the clinical team will need to decide which of these to work on actively (e.g., sampling other relatives, pursuing further bioinformatic analyses, arranging functional studies), which to declare as being of uncertain significance but with the possibility that their interpretation may become clearer in

time, and which to file away without mention until and unless more indications emerge of their possible relevance.

## 13.5. Predictive Genetic Testing

Individuals at risk of developing an inherited disorder will sometimes wish to find out if they have inherited the relevant genetic alteration prior to the onset of symptoms. For some conditions, such as HD, highly accurate predictive testing is possible. In these circumstances, uncertainty as to whether the individual will develop the disease is replaced by uncertainty as to when (and how) it will manifest. In other circumstances, such as the autosomal-dominant familial cancer disorders, there are inevitable limitations to the predictive power of these tests because of the stochastic element in the pathogenesis. The presence of the relevant genetic mutation confers only a predisposition, for example to breast or colon cancer; some individuals with their family's gene mutation will escape the relevant cancer whereas others, without the relevant mutation, may nevertheless develop the cancer.

Our experience of predictive genetic testing in those recruited to research studies has revealed that in general the process of testing for those at risk of a familial disorder has been well tolerated, even when unfavorable results have been generated. Surprisingly, and perhaps counterintuitively, those given a favorable predictive test result can nevertheless experience distress and social dislocation. A favorable result can lead to feelings of guilt in those with siblings who are already affected or are shown to be at high risk of disease—a form of survivor guilt. Family ties, perhaps strengthened from sharing the impact of the disease, may weaken for those no longer directly involved with the condition. Those who have made important life decisions, perhaps about career, relationships, or reproduction, may wish they had acted differently when they find that the basis on which the decisions were made (their genetic risk) has been removed.

A further issue to consider in the family dimension of predictive tests is the potential for conflict of interest between family members. The genetic testing of individuals at 25% prior risk of an autosomal-

dominant disease provides clear examples, as when a young adult has a grandparent with HD. The grandchild with a one-in-four risk may want to know if s/he will develop the disease too, while the at-risk parent appears healthy and has made it clear to all that s/he does not want testing. If the grandchild is found to carry the HD mutation, then his parent must carry the mutation too. In most families a resolution can be found to the potential conflict if the professionals encourage discussion within the family. Occasionally, however, real conflict persists, and professionals have to decide whether to make testing available to one family member if doing so may have adverse consequences for another. The process of resolving such family differences can be particularly problematic and pressured in the context of a pregnancy if the couple is considering whether to perform a prenatal diagnosis.

Additional difficulties can arise in families with diseases that show marked anticipation, such as myotonic dystrophy. In this case a diagnostic test (e.g., of an infant with possible congenital myotonic dystrophy) may effectively be a predictive test when considered from the perspective of an apparently healthy mother who has not yet recognized her minor symptoms. Similarly, complex transgenerational issues arise in fragile X syndrome families, where testing the affected child's maternal grandparents is a form of both carrier testing and, for the possibly premutation-carrying grandfather, predictive testing for a late-onset disease, fragile X-associated tremor/ataxia syndrome.

In counseling for predictive tests, the professional is faced with the difficulty of how to challenge those going into tests by helping them to consider the impact on themselves and others of the various possible outcomes. This is a process with which not everyone will wish to engage: it can then be unclear how hard we should try to encourage them [16].

## 13.6. Confidentiality

Respect for confidentiality is one of the oldest principles of medical ethics—at least as old as Hippocrates, and certainly more ancient than the contemporary attachment to autonomy. Confidentiality in relation

to genetics, however, is subject to challenge on several counts. First, genetic information might be sought about individuals by third parties, such as employers, insurance companies, or the state. Second, as we share (almost) all our genes with other members of our families, it has been claimed that genetic information about one individual "really" belongs to his or her relatives, too—so respect for confidentiality (or "genetic privacy") need not apply so strongly within families. This raises the question of who "owns" genetic information.

### 13.6.1. Third Parties

Insurers have an interest in genetic information as part of the process of assessing risk for providing cover, e.g., for life or health insurance. Access to genetic information protects them from adverse selection, in which clients at high risk of serious problems take out more insurance while paying only standard premiums, so the company (and ultimately its other clients) loses out and higher premiums have to be charged. Insurance corporations have generally accepted that they should not require genetic testing before making life insurance available, in part because applicants may be deterred from undergoing testing should it be required that all test results are disclosed to insurance companies. However, they have been less ready to concede that prospective clients should be able to have genetic tests performed without passing on that information to the insurance company. If the individual is currently in good health, genetic information is essentially predictive or risk modifying and not at all like information about current clinical problems. Moreover, individuals at high risk of serious genetic disease will in any case have to declare their family history, and so the insurance corporation will be aware of some of their pretest risks.

Arguably, the number of adults at high risk of a serious genetic disorder but who are in good health when seeking life insurance is small and the number who are likely to die from the disorder during the term of insurance is smaller, so the industry is unlikely to suffer much from adverse selection in this context. The potential for adverse

selection is probably greater for health insurance, especially in the context of a predominantly commercial healthcare sector, as in the United States, although there is no evidence that such adverse selection has occurred to any great extent. Currently, the Association of British Insurers has agreed (until 2019) to a policy of not asking for the results of predictive genetic tests for standard life, health, and critical illness policies, except for HD testing for life insurance cover of more than £500,000.

Employers may wish to gain access to genetic information about current or potential employees for several reasons. Genetic information might be used to select job applicants or promote prospective staff less likely to develop serious disease before retirement, although the scope for genetic screening for such susceptibility is small as the tests currently available are of insufficient utility for this to be commercial. An employer may also wish to screen the workforce for susceptibility to specific occupational disorders. A case has been made for screening for $\alpha_1$-antitrypsin deficiency in industries where workers are exposed to smoke or dust, as in quarries, mines, and building sites, because this condition predisposes individuals to severe chronic chest disease after such exposure. An argument for caution in genetic testing of a workforce is that the exclusion of susceptible workers from hazardous sites could be used as a cheap but inappropriate substitute for proper environmental protection that would in fact benefit the whole workforce and the local environment.

Several countries in Europe and North America have developed national agreements to restrict the gathering and application of genetic information by insurance companies or introduced legislation to prevent discrimination in insurance or employment against those affected by, or at risk of, genetic disease. However, without proper legal protection there is a fear that individuals at above-average risk of specific disorders may become unable to find insurance cover; in some jurisdictions this will also make it harder for them to find employment if health insurance is provided through the employer. This could lead to the formation of an underclass of people who would be excluded

from full participation in society, as in George Orwell's 1984.

## 13.6.2. Privacy Within the Family

The primacy of respect for the confidentiality of individual patients, except in the most exceptional cases, has been challenged in the context of genetic disease. Information about one member of a family can be regarded as information about their relatives, too [17]. Harm could result, it is argued, if genetic information is not shared with relatives—an individual at risk of a disorder may develop complications that could have been avoided if they had been given foreknowledge of the risk, or a child may be born with a serious disorder when the pregnancy could have been terminated if the parents had been warned of this possibility [18]. To what extent these "harms" could be avoided and whether the birth of a child with a genetic condition can be regarded as a "harm" are complex issues. Most of us can at least agree that family members have a moral obligation to pass on potentially relevant genetic information to their relatives [19], even if we might dispute the legal force of this obligation or whether the obligation extends also to professionals.

How, then, can professionals best promote the appropriate transmission of information within family networks? Our patients may find it practically difficult or emotionally burdensome to contact some family members, while others would be distressed by receiving genetic information that would turn out not to be relevant to them: there may be no "right" time to transmit difficult (but necessary) information.

What should the professional do if an individual persists in refusing to transmit the information or breaks off contact with the genetics team?

There are those who argue that professionals should be prepared to pass on information about one family member to others without the first person's consent—although always informing her/him of this decision before implementing it. The circumstances in which such a breach of confidence is justified have been formulated by several learned bodies, although there may still be no consensus about what

to do in specific cases [20].

This could lead us to consider some of the various ethical frameworks within which such issues could be addressed. One approach would be to reflect on the competing principles at stake—with autonomy usually trumping the others in contemporary, individualistic, Western societies. Another approach, utilitarianism, would lead us to weigh, in a notional calculus, the likely outcomes of particular courses of action. A third approach is that of virtue ethics, cultivating a disposition that will lead to right action. A fourth is that of "common morality", which promotes respect for rationality and impartiality in decision-making within an open, public framework of moral rules. The final approach is that of narrative ethics, in which the professionals do not seek to abstract the issues of principle from the particulars of the case (the context) but to focus on the experience of their clients and encourage them to reflect on the future paths they can envisage taking. This approach draws together a virtue-based morality with the professional skills of humanistic counseling.

Some studies have examined how professionals say they respond in practice to situations in which they are aware that a client has failed to disclose important information to his/her relatives. It seems that professionals only rarely become seriously concerned and hardly ever breach confidentiality. While the obligation to inform relatives carries weight, empirical research has demonstrated that multiple barriers hinder communication with the family, while other factors promote it [21,22]. One approach to such problems, which can be adopted when there are clear health benefits at stake, is a very proactive professional orientation to passing on information to relatives. This normalizes the contacting of individuals directly by professionals with genetic information, as in some programs of cascade testing for familial hypercholesterolemia [23].

Some professionals fear that policies of breaching confidentiality and forcing the disclosure of genetic information will undermine professional respect for their patients' privacy and damage public trust in health professionals. Patients, aware that confidentiality could be jeopardized, may become less than frank about their disease or

their family structure from their very first contact with the clinical genetics team. Professionals could become less inclined to persist with alternative approaches to resolving these communication problems, although this might lead to an obligation to force disclosure that could itself become a serious burden for professionals.

## 13.7. Genetic Testing in Childhood

When is it appropriate to carry out genetic tests on children? There are circumstances in which all agree that genetic testing is entirely appropriate. If a child has an undiagnosed clinical disorder that is possibly of genetic origin, genetic testing may be the most appropriate investigation. For example, in a young boy with a clinical phenotype suggestive of DMD, molecular genetic investigation may well yield a diagnosis and thereby perhaps avoid the need for the invasive procedure of muscle biopsy.

### 13.7.1. Predictive Testing of Children

It can also be appropriate to offer predictive testing in childhood for a disorder with onset usually in childhood. Thus, in the family above, if the boy does have DMD it may be appropriate to test his younger, apparently healthy, brother. The family is likely to be highly anxious about his risk of developing the same condition, and it may take months or even years before either the diagnosis becomes apparent or the family is reassured that he is healthy. But there may be contexts where parents experience an unfavorable result as especially difficult to live with, as when a test indicates that the child has an increased risk of sudden death or psychotic illness. Decisions made to test children for hypertrophic cardiomyopathy can certainly be regretted [24].

It might also be reasonable for a child to have predictive testing for a genetic disorder, even for a condition where onset is usually in adulthood, when the child is at risk of complications for which surveillance can usefully begin in childhood (e.g., familial adenomatous polyposis coli). In contrast, predictive testing for

untreatable adult-onset disorders is another matter altogether. To test a child who is too young to participate in the decision prevents her/him from making her/his own decision later. This may be especially important where, as in HD, most at-risk adults choose not to be tested.

To carry out a test for an untreatable adult-onset disorder "on demand", at the request of the parents, without challenge, and principally to resolve their anxiety, would in our view be unprofessional. As well as abrogating the child's future autonomy, it breaches the principle of respect for confidentiality: if tested as an adult, a person has complete control over who else is told about the test result. In addition, there is the possibility that a child's upbringing could be damaged by the result of the genetic testing. Might altered parental expectations of the child's future career prospects, educational attainments, or personal relationships be damaging and perhaps self-fulfilling? There is no firm evidence on this point but, as it would be difficult to gather "evidence" that would resolve this, we have strong grounds for caution. Most professional bodies and family disease support groups share this perspective and would not be prepared to carry out predictive tests for HD or similar disorders on healthy children, at least in the United Kingdom [25] and Europe, and perhaps also in North America, although some policies in the United States are more oriented to accede to parental requests without serious challenge [26].

We should also consider the impact of disease, and information about the risk of disease, on both the child and the family when discussing the genetic testing of children. One major influence on children's responses to a risk of inherited disease is how they learn about the condition, and whether this happens gradually in childhood or abruptly in adult life. Making life decisions on the basis of genetic information is not a single event but a process.

## 13.7.2. Carrier Testing of Children

Carrier testing is perhaps the most hotly contested issue in relation to the genetic testing of children. Parents of children with autosomal-

recessive disorders may wish to know which of their healthy (unaffected) children are genetic carriers. Is there any reason why professionals should not comply with parental requests to test young children to determine their carrier status? One response is to point out that the children themselves will not benefit from the testing until adulthood—the results are not relevant to them until they come to make their own reproductive decisions in the future. Being identified as a carrier can have effects on an individual's sense of well-being, and we do not know how this could affect a child's developing sense of self. Carrier testing presents the same challenge to the ethical principles of autonomy and confidentiality as predictive testing. Although there will usually be less at stake because the child's own health is not in question, there is still the concern that parental expectations could exert an undue influence on future relationships and reproduction. There are therefore considerations that weigh in favor of deferring a test until the child can participate in the decision. Furthermore, performing a test in childhood does not ensure that the results will ever be passed to the child in a comprehensible and helpful manner. Several studies have found that genetic carrier test results are not always transmitted to the children tested even when they are adolescents or young adults, and there are often misunderstandings about the meaning of the test results and the inheritance of the disorder in the family. Carrier testing of a young child is thus not a complete solution to family concerns, and the offer of genetic counseling will often be helpful to an older child even when the test has been carried out years before.

However, not testing for a condition in childhood is not the same as not talking about it. If the family has very strong feelings about testing, when a child is too young to contribute to the discussion, the counselor can prompt a careful discussion of the issues, including the parents' expectations of testing. Although it may be appropriate to defer testing to allow some further reflection, a complete refusal to perform carrier tests in young children can lead to anger and resentment that may be very unhelpful in the future. It may be better to avoid rigid positions in such a sensitive area—to advocate caution,

but then leave the final decision with the parents.

One approach that can be helpful is to encourage openness within a family but defer testing until the child is older and able to participate in the decision, typically in adolescence or later. Several studies have suggested that openness about a family's inherited disorder is helpful to the children's future adjustment [27,28]. While an imposed solution may result in rejection and defiance, this may help these adolescents and young adults to arrive at reasonable and responsible decisions with which they can live more happily in the future.

These issues and guiding principles can work out rather differently in the setting of a child being considered for adoption [29]. On the one hand it may be important not to subject a child to genetic testing that would not take place if the child had been able to remain in his/her birth family. The idea of testing a child so that he/she can be adopted if the result is favorable but otherwise left in the care system seems repugnant; surely the best adoptive family would be one that is willing to adopt while being aware of the genetic issues and willing to face the possibility that the child may have inherited a genetic problem of some sort. On the other hand families adopting a child already face numerous uncertainties, and the possibility of resolving one of these uncertainties may seem very helpful. In practice it is usually possible to defer testing, and for a serious potential adopting family to meet with the clinical genetics team for discussion.

The final topic to be considered in this section is the handling of requests from a child or adolescent for a predictive or carrier test. Is the minor sufficiently mature to understand the considerations involved in making a decision about testing? Gaining sufficient experience of life to arrive at a wise decision must take time but, arguably, living through her/his family experiences may have given the child an intimate knowledge of the family's genetic disease and accelerated this process. Such experience may give a child the capacity to make decisions about carrier testing that would otherwise be inappropriate. It is important to recognize that a child can participate in the decision-making without necessarily making the decision alone.

The question as to when children can make their own decisions

about genetic testing without parental involvement or even knowledge is separate. An assessment approach has been found helpful in the context of requests from adolescents for predictive testing for HD [30], and may be helpful for young adults too [31].

## 13.8. Population Genetic Screening

Three ethical aspects to genetic screening programs need to be considered further here.

First, there is the issue of consent—or, as it is commonly known, informed consent. It is important that participation in a population screening program is conditional upon consent, although this may be difficult to ensure. As a minimum, potential participants need to recognize that they have been given a choice, and to understand that the choice has consequences, including potential advantages and disadvantages. Probably the most serious limitations to informed consent for genetic screening arise from the routinization of the offer of testing—its incorporation into standard clinical practice, perhaps with subtle discouragement of questioning, and the active promotion of testing by committed and enthusiastic staff.

The second issue to be considered is the decision-making process whereby any screening program is made available to, or provided for, a population. In a national healthcare system, this is decided by the health service at regional or national level. What is important from the perspective of ethics is how the decision is made: what evidence is taken into account and regarded as relevant to the decision? Which interest groups have a say in the decision? On what grounds is the decision made and justified? Are these judgments defensible in open public debate? This perspective amounts to a procedural ethics, appropriate to the democratic bureaucracies of the early 21st century.

Finally, there is the distinction between maximal and optimal rates of uptake of a screening test. There will often be a tension between the conventional goal of maximizing uptake for a screening test that is considered to be "a good thing" and the goal of good clinical practice. A high uptake may be seen as necessary to achieve maximal efficiency in both the clinic and the laboratory, but a very high uptake may be

regarded as presumptive evidence of routinization and hence a poor quality of consent. When screening for the health benefit of the individual it may be assumed that uptake has to be maximized but, especially in the context of reproductive decisions, it may be more helpful to recognize that screening will be valued by some individuals and not by others. It then becomes more appropriate to optimize the uptake of screening by offering it in such a way that those who are likely to find it helpful participate in screening, rather than maximizing the total number of participants.

## 13.8.1. Newborn Screening

The first population screening program to be introduced for genetic disease was newborn screening for PKU, which transformed the prognosis for affected infants. There is no doubt that this, and the subsequent introduction of screening for congenital hypothyroidism, has been a great success. But what ethical issues arise in these flagship programs—the "acceptable face" of genetic screening?

First, there is the issue of whether newborn screening for these treatable disorders should be mandatory, as it is in some countries and some states of the United States, or whether it should be voluntary, on the basis of assumed or explicit parental consent. There seems to be little reason to justify compulsion in Western countries, as the uptake of testing is universally high in the absence of compulsion and it may be acceptable to operate on a basis of assumed parental consent. If "real" informed consent is preferred, there is no basis for any attempt by professionals to persuade reluctant parents to agree to testing for their infants: the parents' views should be accepted. However, this program is not a setting where nondirective counseling is appropriate: responsible professionals will try to persuade parents to permit screening to go ahead, and regard parental refusal as indicating parental misunderstanding.

The other issues around newborn screening relate to disorders where the child is unlikely to benefit directly from any clinical intervention. There are reasoned arguments both in favor of and against newborn screening for DMD, for example, and it is not

possible to resolve this issue "in principle" (i.e., by reflection in the abstract) until clear benefits from early therapeutic interventions have been demonstrated.

There are two principal arguments for screening. First, many families with a boy affected by DMD notice a problem in the second or third year of life, but it often takes about two more years before a diagnosis is made. Parents in these families often struggle with this diagnostic delay and the impact it has on their son's treatment; they state that they would like to have known the diagnosis earlier. The second, related, argument is that in some of these families another affected child is born before the elder affected boy is diagnosed; the family then has two (or three) affected sons. If the diagnosis had been made earlier the parents could have been offered genetic counseling in advance of another pregnancy, perhaps thereby averting the birth of the second affected child. Although newborn screening would not have the goal of reducing the birth incidence of DMD, it could have a modest effect in this direction.

Arguments against the program include the distress caused to the family and the potential negative impact on the parent–child relationship without improving the outlook for the child's health. Practical experience of newborn screening for DMD in Wales, which ran between 1990 and 2011, revealed that most families were pleased to have an early diagnosis, despite the inevitable distress, and most used this knowledge in practical planning for the future, including in their reproductive decisions.

Newborn screening for many other diseases is now feasible: CF, sickle cell disease (SCD), and many inborn errors of metabolism can be detected through tandem mass spectrometry (TMS). However, which of these tests should be made available? The case for introducing newborn screening for CF is strong, in that it allows active management to commence earlier, and it is plausible to suggest that this may improve the long-term prognosis, although this remains unproven. Screening for SCD is also of clear benefit to affected infants, so a program is worthwhile where the disease incidence is more than minimal.

However, newborn screening for CF and SCD introduces another issue—that of detecting carriers incidentally but unavoidably through the methods used to detect affected infants. A very small proportion of CF carriers are detected, because a biochemical test is used to identify those with raised serum trypsin levels; molecular genetic testing performed on those samples then identifies the common disease-associated CFTR alleles. Those with raised immuno-reactive trypsin (IRT) and a single CF-causing allele have a sweat test performed to see whether they are affected. Roughly equal numbers of affected infants and carriers are identified. In contrast, population screening to identify infants affected by SCD identifies all carriers of SCD, not just a small proportion, because the usual laboratory test employed identifies all those with sickle hemoglobin, HbS, whether they are homozygous or heterozygous. This is useful, in that those compound heterozygotes with HbS and β-thalassemia are recognized, but it means that many more carriers than affected infants are detected. This complicates the process of seeking parental consent and may greatly increase the work of community health professionals to inform and support these carriers and perhaps refer them for genetic counseling. It can also bring to the fore questions about a child's paternity.

In relation to metabolic disorders that can be detected by TMS, an important question is whether consent should be obtained for testing each disease individually or consent should be given to "testing by TMS" as a single test (i.e., specific consent versus generic consent). Testing for PKU is now performed by TMS, but the simplified approach to consent fails to give any real choice about testing for many of the metabolic disorders for which early diagnosis leads to medicalization of the child but fails to confer substantial benefit, so parents might wish to decline screening for some disorders while accepting the test for PKU. Any process of evaluation of these screening programs must be broad-based, examining not only the clinical medical outcomes but also the social impact on the families caught up in screening and the costs imposed on both the families and the health services involved.

When we consider newborn screening from a broader perspective, it is important that all cases of genetic variation in the relevant gene are not brought under the umbrella of the clinical disease being screened for. Thus in screening for CF it is important to recall that the aim is to commence early treatment of infants who would otherwise be malnourished or suffer serious pulmonary infections before the diagnosis was made. The goal is not to identify all cases of disease associated with variants in CFTR, including late-onset respiratory problems or male infertility, where diagnosis in early infancy is most unlikely to be helpful. Those involved in making decisions about the implementation of such screening programs must be mindful of the influence of vested interest groups—enthusiastic professionals, the manufacturers of the technology, and medical administrators interested primarily in containing healthcare costs.

Furthermore, there is active debate, particularly in the United States, about broader issues that threaten public acceptance of screening, including the long-term retention of newborn blood spots and their potential forensic use by the state. There are also potential ethical hazards from the mounting of newborn screening programs to recruit infants to a therapeutic trial, as in the exemplar case of spinal muscular atrophy [32]. It would be easy for screening to be accepted by parents on the understanding that an affected infant would benefit from a proven treatment, but participation in a trial does not amount to that.

## 13.8.2. Antenatal Screening

The provision of antenatal screening is fraught with ethical issues that require further consideration. First there is the question of the goals of the screening. Then there are the unanticipated consequences of screening—especially the impact of antenatal screening on women and couples who become caught up in the clinical process but may not have really thought through the issues in advance. Finally there is the impact of antenatal screening on those individuals already affected by the conditions for which screening is made available.

### 13.8.2.1. Goals

Is the primary purpose of antenatal screening to reduce suffering, to provide choice, or to reduce the costs of healthcare? Perhaps the best way to address this question is to examine particular screening programs in detail and look at their rhetoric against their operation in practice. What is the impact on pregnant women and families of the screening? That is the type of empirical ethics research that is needed alongside cost–benefit analyses, which may drive decisions inappropriately if the only evidence drawn upon relates to the narrowly economic aspects of screening.

It is almost incontestably good to reduce "suffering"—but whose suffering do we mean? Until recently prenatal screening was primarily targeted at Down syndrome, other autosomal trisomies, and structural malformations of the fetus. Fetuses affected by (or at high risk of) structural malformations and chromosomal anomalies were detected through maternal serum screening and fetal ultrasound scans. Such screening can indeed reduce perinatal mortality, although most of this effect results from bringing forward inevitable neonatal deaths so they are no longer counted in either perinatal or neonatal mortality figures. However, this reclassification of inevitable deaths does not reflect any improvement in the clinical reality for the mother or family, because a midtrimester termination of pregnancy takes the place of a sad but inevitable neonatal death, which might have left the family with fewer regrets, less guilt, and greater emotional support.

Neural tube defects can certainly cause physical suffering for a child, but there is no consensus among adults with these defects about the rights and wrongs of selective termination. Many take a "permissive" view, arguing that screening and termination should not be prohibited, but often maintaining that their lives are worthwhile despite their physical problems and that they must be acknowledged and respected as full members of society. There is a tension in this position, discussed further below, between the freedom of pregnant women to make their own reproductive decisions on the one hand and the respect due to our fellow human beings, whatever their physical or intellectual capacities, on the other.

Although Down syndrome can cause serious physical and medical problems for affected individuals, it is the intellectual limitations and stigmatizing physical features that are usually thought of as its major difficulties. As such, the suffering to be avoided through selective termination of pregnancy is in large part a combination of the sadness often experienced by the parents and wider family and the social stigma experienced by the affected individual, rather than any direct physical suffering. The parents' feelings will be complicated—including sadness and disappointment at the child's physical health problems and the limitations imposed on him/her by their genetic constitution, and irrational but often powerful feelings of guilt, social stigmatization, and worry about the child's future happiness in society outside the home, especially when the parents are no longer able to care for the child. However, there will often be positive feelings, too. To say that antenatal screening is motivated by a desire to reduce "suffering" is therefore a complex claim, difficult to justify in any simple and unqualified sense. It is made more difficult to accept in practice by the reluctance of many practitioners to accept without challenge a mother's decision not to terminate a pregnancy affected by Down syndrome or a similar condition, so those who decline the offer of termination may need real strength to resist the pressure of professional expectations and continue with the pregnancy.

The offer of informed reproductive choice is also given as a possible goal of prenatal screening, but this begs many questions. Given that the making of decisions about testing and (dis)continuing wanted pregnancies can be burdensome, with its own social and emotional costs, what decisions is it appropriate to impose on every pregnant woman? It is not possible to sustain the view that a simple maximization of the number of decisions made is a valid end in itself. We must examine the context within which these decisions are suggested.

### 13.8.2.2. Decisions in Social Context

A rarely proposed approach to the termination of wanted pregnancies

"to avoid suffering", based on the result of prenatal screening, is to place the onus of change on society. Developed societies could respond to the challenge of Down syndrome, or intellectual disability more generally, by ensuring that affected children are welcomed by society and integrated effectively into schooling and work, that parents are provided with a high standard of social support, and that older individuals with Down syndrome are offered well-supported sheltered housing near the homes of their friends and families. The decisions made about prenatal screening and the termination of pregnancies might be different in at least two circumstances. First, if parents were confident that if their child were to have Down syndrome or a similar condition, neither they nor their child would experience overt hostility or more subtle forms of stigmatization. Second, if they could have a realistically optimistic view about the future for their child once they were no longer able to provide care and support.

This idyllic state of affairs would require major change in public attitudes and the provision of health and social services in most societies; however, without such developments, any promotion of prenatal screening can be understood as (at least implicitly) coercive. Some families may feel that they have little real choice about prenatal screening because the support they can expect to receive from society for a baby with special problems and needs would be inadequate. Altering the social context in this broad way, at the "macro" level, would also be likely to improve "micro" aspects of social functioning —the stigmatization and subtle hostility that can all too frequently be displayed toward individuals with disability and their parents.

In contemporary society the notion that "reproductive choice" is inherently "a good thing", and hence sufficient justification for the promotion of prenatal genetic screening, is therefore highly questionable. To consider ethical issues in the abstract, divorced from the social context of those confronting them, is to inhabit a fantasy [33]. This is not to argue that individual choice is "a bad thing" or that individuals should be prohibited from using prenatal screening, but the active promotion of screening on the grounds of respecting

individual choice is disingenuous if the social framework within which the choice is made remains unexamined. Such a policy deflects attention away from the processes through which society arrives at decisions about healthcare and social care. These decisions inevitably reflect the interests of particular social groups, and to present them as neutral and based solely on objective considerations is actively misleading—making it harder for those with little power and few resources to contribute their views in an effective manner that will be "heard" and acknowledged.

### 13.8.2.3. Entry to Antenatal Screening

Consent for entry to prenatal screening is often not based on carefully considered and well-informed decision-making [34]. It is perhaps the fetal anomaly scan where "consent" is most problematic, in that this is often approached as a happy social occasion rather than a serious search for fetal abnormality [35]. Indeed, the way in which the test is offered is often routinized, and this leads to further problems for those women and couples for whom the screening test indicates a possible problem. If they have complied with screening in the expectation that the test will "make sure the baby is all right" then they can find it very difficult when a possible or definite problem is identified. Such women can feel swept along on a diagnostic conveyor belt that often leads to amniocentesis and sometimes to a termination of pregnancy.

Such difficult experiences would be minimized if it were made very clear to all potential screening participants:

- that the primary purpose of screening is to identify pregnancies in which the fetus has a problem, not to provide photographs of the baby or "promote bonding";
- that participation is entirely voluntary;
- that professionals may raise the question of terminating the pregnancy if the tests do indicate a problem;
- that the reassurance gained through a normal screening test result is always incomplete;
- that some test results may be of uncertain significance, being

neither completely “normal” nor definitely “abnormal”, and these may require more time or further investigation and/or consideration before any decision could be made;
- that there are services available locally to support families who have a child with Down syndrome, spina bifida, or other conditions likely to be detected by the screening program.

It is emotionally difficult and demanding for both staff and pregnant women to confront these issues hypothetically in every pregnancy. The issues are therefore often evaded until there is a serious decision to be made in a hurry—and of course these are not the best circumstances in which to make such decisions.

As patients, professionals, and society we must ask ourselves “under what circumstances, and with what goals and ethos, are we prepared to make prenatal screening and diagnosis available?” Furthermore, if the system of prenatal screening within which we work is unsatisfactory, to what extent does our compliance with it serve to bolster it and perpetuate these problems?

Pregnant women must decide whether or not to participate in the prenatal screening programs on offer. Whereas some will want to welcome their child unconditionally into this world, others may wish to take any suffering upon themselves—experiencing the distress of terminating a wanted pregnancy so as to spare their child from physical suffering or life in a hostile society. Society has to decide how to support pregnant women and vulnerable children. Should it set out to welcome every infant unconditionally and generate confidence in the willingness of society to care lifelong for those with special needs? Or should it adopt a more hard-nosed, instrumentalist approach to its future citizenry by discouraging the birth of those likely to require more than the average from its health and social services?

Entry to antenatal screening may often be routinized in practice, but it is to be hoped that any further decisions (such as whether to accept the offer of an amniocentesis, or whether to terminate the pregnancy) will be made deliberately. Some accounts suggest that once a screening test gives a positive result, some women feel pressure to

take the next step, which becomes an inevitability. Research examining the decision-making process suggests that women often consider the interests of the fetus alongside those of the family overall [36], and that they usually make decisions in small steps, as they are obliged to, rather than as a grand strategic plan [37].

### 13.8.2.4. Disability Issues, Health Economics, and Sex Selection

Many individuals with disability take offence at the way their lives have been devalued by the system of medical screening. As discussed above, antenatal screening programs were often introduced with the goal of reducing the birth incidence of "costly" conditions, such as neural tube defects and Down syndrome, where the cost of care was found to exceed the cost of antenatal screening and the termination of affected pregnancies. The brazenly cost-driven nature of antenatal screening has since been softened, but the suspicion remains that the rhetoric of patient choice is merely a mask adopted with reluctance.

Such cost-saving arguments would be totally unacceptable in other medical contexts. Indeed, it is generally accepted that medical care costs money and society should be prepared to pay for it. The narrowly economic approach to prenatal screening reduces human value to a cash price and reflects an amoral and cynical instrumentalization. Such policies have helped to generate considerable hostility toward genetics within the disability movement. This is most unfortunate because genetics has a lot to offer society, including those with disease and disabilities, and a rejection of genetics could obstruct much useful progress. The simplistically utilitarian approach to screening fails to respect our fellow humans and acknowledge the duties we owe them, including our future selves when we become unwell or infirm; it fails to recognize the important virtue of human solidarity.

Those with disabilities may fear that genetics will be used by society to eradicate either their disabilities or the people themselves. It is likely that for many years it will remain technically much easier to eradicate people with inherited disabilities prenatally (sometimes,

rather inappropriately, regarded as prevention of the disease) than to prevent the loss of abilities in those with progressive disorders or restore them to someone who has lost them. Suspicion of genetics will therefore persist, but it is possible for the disabled and their health professionals to develop a dialogue that slowly softens and addresses these different perspectives, some of which arise from misunderstandings.

One particular disabled community that warrants specific mention is that of the deaf, especially the Deaf—those with prelingual deafness who use sign language. This group sees itself as a threatened cultural minority, and genetic research into deafness is perceived by some as a direct threat to their continued existence. The Deaf often do not see their deafness as a problem, in contrast to those with later-onset hearing impairments. Is such prelingual deafness, then, a disease? Or a disability? Or a condition—an innocent trait, like red hair? Or a marker of belonging to a specific cultural community? The Deaf can feel threatened by the twin assault of genetic technologies with the potential for prenatal diagnosis and the treatment of deafness by cochlear implants in infants diagnosed through newborn screening. The latter is much the more powerful factor, and may indeed impact on recruitment to the social world of the Deaf.

Mention must also be made of fetal sex selection. Many cultures have a preference for sons. The pressure to have sons has powerful socioeconomic roots, and relates to cultural practices such as the dowry and the need for sons to fight wars, work in the fields, join the family business, or inherit property, titles, and names. In some societies newborn daughters may be exposed or killed. Furthermore, women who have daughters are sometimes abused, assaulted, divorced, or murdered—so the women can be as eager as their menfolk to have a son. In such cultural settings, those who can afford prenatal diagnosis sometimes use it to ensure that a pregnancy does produce a son, especially if they already have a daughter [38]. Sex selection is so common in some areas that the sex ratio in parts of India and China has declined dramatically and is approaching 0.7 female infants per live born male. Indian immigrants to the United

States utilize the prenatal sex selection that is legal there, whereas it is illegal in India [39].

Compliance with requests for fetal sex determination to achieve a son can be regarded in the West as collusion with a patriarchal system that oppresses women and is hugely disrespectful to them. British medicine has decided not to permit prenatal diagnosis for such purposes—to do so would be lending support to an unacceptable system of discrimination. This raises two important but uncomfortable questions. First, if a women seeks fetal sex selection out of fear that she will be assaulted or killed if she has a daughter, then is that not at least as powerful a justification for prenatal diagnosis and pregnancy termination as many of the "social" terminations carried out every day? Second, if we Western professionals adopt this stance because we hold women in such high esteem, what message is conveyed by our collective willingness to promote prenatal screening for Down syndrome? If the disability movement had a higher profile, would we discontinue that type of prenatal selection, too?

### 13.8.2.5. The Future of Antenatal Screening and Reprogenetics

The analysis of cell-free fetal DNA in the maternal blood is likely to transform the practice of antenatal screening and prenatal diagnosis. This DNA is derived from the chorion, not the embryo or fetus itself, and its analysis often makes it possible to avoid or minimize the use of invasive tests on the fetus.

This is already in widespread use for three main purposes: to indicate pregnancies likely to be affected by an autosomal trisomy, to diagnose *de novo* anomalies identified by fetal scan, such as skeletal dysplasias, and to look for paternally derived alleles of interest (such as the Y chromosome for fetal sexing and the Rhesus antigen for assessing the risk of hemolytic disease) or paternally derived autosomal-dominant disorders for which the fetus is at risk. Additional applications, such as prenatal diagnosis for other disorders (autosomal-recessive or maternally derived dominant disorders), have also been developed and are becoming more widely available.

These methods allow noninvasive prenatal testing (NIPT) from ~8 weeks of gestation. In some contexts NIPT is diagnostic, but in others its findings need to be confirmed by invasive tests, especially when it is used to screen for aneuploidy. Thus the positive predictive value of an NIPT indication of Down syndrome can vary from 80% to 95% depending upon the prior risk of Down syndrome in that pregnancy. It is essential that patients understand the limitations of NIPT, as its technical performance varies greatly between contexts. To describe it as highly (>99%) accurate in the context of general population screening for Down syndrome is irresponsible and misleading: that level of accuracy can be achieved by giving a low-risk report to every woman without performing the test at all.

NIPT will shape women's experiences of pregnancy, as amniocentesis did when that was developed some decades ago. It will also impact on the place of disability in developed societies. It is therefore important that NIPT is introduced with care and restraint and without inflated claims for its performance [40].

One likely (although avoidable) problem is the generation of more information about the fetus than is required to determine the diagnosis of any disorder suspected on clinical grounds or from family history. Testing for a family's known disorder should use a more targeted test and thereby avoid these problems. The prenatal determination of fetal genome structure (i.e., CNVs) or fetal genome sequence may answer important questions, but will present serious challenges for families and professionals. Unlike the interpretation of WGS results for a live-born child or an adult, where there remains a substantial degree of uncertainty about the meaning of many of the variants identified, we will not have the phenotype of the child available for direct physical examination or developmental assessment and so the interpretation will be even more troublesome. Furthermore, there will be major concerns about access to the future child's genome sequence and its interpretation. Will the data and the interpretation be stored? Will the parents be given only the clear items of information about the specific questions being asked in the

pregnancy, but not about other health issues? Or will the "full" results (as far as that is an intelligible concept) be made available? Many of these same issues arise in the genetic testing of children.

### 13.8.3. Carrier Screening

Screening to identify unaffected, presumably healthy, carriers of autosomal-recessive diseases in the general population is another form of reproductive genetic screening, and it can be made available (ideally) to a couple contemplating reproduction or (when it may seem "most relevant") already in a pregnancy.

There are potential benefits from being identified as a carrier—especially the ability to make reproductive decisions, such as asking a partner to be tested. However, there are disadvantages, including the emotional impact of this unwelcome information, the burden of future reproductive decisions, possible lingering concerns about one's own health, and the potential for stigmatization and discrimination in personal relationships. Given that there will always be many more individuals identified as carriers than there will be carrier couples who could have an affected child, how does one weigh these minor burdens on many people with the more serious implications for carrier couples, who will need to make decisions about prenatal testing, and perhaps termination, in their pregnancies?

Pilot carrier screening programs for a number of disorders in the United Kingdom showed that the general public has little interest in carrier screening but will often comply if screening is actively offered by enthusiastic health professionals [41]. Indeed, some studies have shown that the rate of uptake is critically dependent on the way in which testing is made available—varying from <10% to >70%, with higher rates if testing is offered actively and is available immediately but much lower if a separate appointment is required. If screening is made available to women or couples in early pregnancy, the uptake is substantially higher again (>95%). How is this to be interpreted? Does it indicate a real interest in testing, or compliance with an actively promoted test because of the vulnerability of pregnant women to suggestions that they should have a test "to make sure baby is all

right"?

Further issues arise in antenatal carrier screening as to the precise offer to be made (testing just the pregnant woman, or the couple as individuals, or the couple as a unit), because the nature of the offer made reflects the goals of the program and will have implications for how these are perceived.

As the number of disorders for which screening is possible increases, the process of "information and consent" needs to develop so patients understand that they are giving a broad, generic consent to the process [42]. With the new sequencing technologies, it is becoming much simpler to screen for a broad range of disorders.

The social context within which carrier screening is made available can be a highly charged political issue. The variation in frequency of autosomal-recessive diseases between populations and ethnic groups means that the frequency of the carrier state varies widely. A carrier screening program that could be appropriate in one population may be inappropriate in another. Problems may arise where racial disharmony and discrimination exist in a mixed population, where the promotion of carrier screening could be used by one group to stigmatize and discriminate against another. This indeed occurred in the 1970s in the United States, when screening for sickle cell trait (carrier state) was actively promoted and exacerbated discrimination against Afro-Americans in employment.

When testing is actively promoted by the dominant social institutions (as with screening for β-thalassemia in Cyprus, promoted by the Orthodox Church, and comparable programs in many of the wealthier Islamic countries), it can be very difficult for outsiders to distinguish popular consent from institutional coercion. Societies certainly differ in the degree to which individuals are willing to comply with decisions made by authority figures within the family or society at large.

As has been appreciated in some Mediterranean and Middle Eastern countries, the survival of large numbers of children with diseases such as β-thalassemia, which can be treated successfully but at substantial cost, raises ethical challenges for a country's health

services. An exponentially increasing proportion of the resources available for healthcare would be needed, and could compromise a country's ability to provide services for many other conditions. Some decades ago, as mentioned above, Cyprus confronted this by establishing a carrier screening program with the support of the Orthodox Church; this led to a dramatic decline in the births of affected infants so that the country could afford to care for those individuals who are affected. This raises the question of how active the promotion of carrier screening should be when "too enthusiastic" a program could become coercive. Furthermore, should the program seek to influence the choice of partner (as with premarital screening in a country with arranged marriages) or to promote prenatal diagnosis and the selective termination of affected pregnancies?

## 13.9. Other Challenges in Genetic Counseling

Several other ethical issues arise in genetic counseling, to which we must refer before passing on to some broader topics.

### 13.9.1. Religion or Culture?

Religious beliefs may be given as an explanation by the client to the counselor in accounting for their decision not to make use of genetic testing, especially in the context of prenatal diagnosis and a possible pregnancy termination. Religious traditions, however, do not always give a clear ruling on the "correct" decision for an adherent to make; differences exist on such matters within many traditions, and it may be the cultural customs and assumptions surrounding a religion that influence client decisions and understandings more than the details of religious doctrine *per se*. A patient's appeal to "religion" may be used as a signal to indicate that s/he is unwilling to pursue the topic under consideration, having deep-seated objections to the whole process, although these objections need not necessarily be specifically theological. This signal is usually respected by professionals in a way that the mere expression of an opinion by the client may not be.

## 13.9.2. Decision-Making

Cross-cultural genetic counseling may also raise the question of different patterns of decision-making in families. Who would make the decision about predictive genetic testing for a young adult at risk of HD, for example? Would it be the individual at risk, or a patriarch or matriarch, or a wider group within the family? When a couple is discussing prenatal diagnosis in a pregnancy, who makes the decisions? (In the United Kingdom couples usually make a joint decision but, in the face of a disagreement, it is always the woman's voice that is decisive.) If parents request predictive or carrier testing for a young child, they may assume they have the right to do this without any need to explain their reasoning to professionals.

These "cultural" questions come down to issues of power. To what extent can we Western professionals use our power in the clinical setting to steer family decisions and communication practices (i.e., family power relations) along a path that we find acceptable?

## 13.9.3. Customary Consanguineous Marriage

Customary consanguineous marriage is especially prevalent in certain Asian and Middle Eastern groups, and may be more pronounced in these groups within Britain than is usual in their countries of origin because of the smaller number of potential partners in the relevant community in Britain. Although this does increase the risk of autosomal-recessive disorders appearing in children born into these communities [43], it would be all too easy for Western health professionals (or politicians) to attribute blame and responsibility for the disorders to the minority culture and its practices. Such provocative attributions of blame ignore the stabilizing social functions of consanguinity in many communities, and are not likely to promote useful mutual understanding or even change in customs. If not provoked by such moral assaults, communities that place social value on consanguineous marriages may be able to express concern about a high perinatal mortality or the frequency of serious genetic problems in young children. It then becomes possible to develop an

education program that would be acceptable to the community, and perhaps to incorporate genetic testing as part of an effort to counter the genetic risks of consanguinity [44]. High perinatal mortality may have multiple causes, including poverty, and should be tackled by attention to maternal nutrition and antenatal care as well as genetic issues.

## 13.9.4. The Naming of Syndromes

Many families arrive in the genetics clinic with the hope that the condition affecting their child will be diagnosed. The process of labeling, however, can have substantial repercussions for the family, which they may not always have foreseen [45].

Although a diagnostic label may facilitate access to support services, it may also provide an unwelcome insight into the likely future for the child that a family may not (yet) be prepared to confront. It may have adverse consequences if educational provision for the child is altered inappropriately—if the child is treated as the diagnosis and not primarily as an individual. There is also much scope for self-fulfilling prophecies of low expectations on the part of professionals. Conversely, when a diagnosis is not available, as frequently happens, the family may feel bitter and resentful, especially if the lack of a diagnostic label makes it more difficult for them to access appropriate social, educational, or health services for their child.

A very real problem for some individuals affected by genetic disorders and their families is the name given to their diagnosis, their syndrome. It can be difficult enough for a family to accept that one (or more) of them has a disorder manifest in their physical appearance, without insult being added to injury by the inconsiderate name given to their condition. For example, although the name "cri du chat" syndrome was coined in good faith as a clinically useful description, it can be unfortunate when it causes a family to flinch whenever they hear their child's cry (the cry having caused no concern before they were given the name of the condition). There are many other syndrome names, especially acronyms, which have surely been

devised for their verbal felicity with complete disregard for the impact of the name on those affected or their families. Examples include CATCH 22 syndrome, LEOPARD syndrome, DEFECT syndrome, and CRASH syndrome. The giving of a name to a syndrome can be as serious a matter as the giving of a diagnostic label to a child.

## 13.9.5. A Duty to Recontact?

When a family attends for genetic counseling, the clinician and counselor provide the best answers they can to the family's questions. When new information comes to light in the future, especially if it impacts on surveillance for disease manifestations or the possibility of treatments, is it reasonable to expect the clinician to recontact the family to pass on this information? If the family is still under review, no special problem arises—but what if the family has not attended for some years, or follow-up was never planned, or they have changed address? How far does the obligation to recontact such families extend?

Any obligation to recontact families must be feasible within the constraints of staffing and the resources available, but the *need* to recontact in response to changes in the interpretation of genetic variants in the light of new knowledge is increasing, as predicted by Pyeritz [46 ,47] , with the generation of progressively more sequence information. The recognition that a variant previously considered pathogenic is in fact benign can be at least as important as the attribution of pathogenicity to a variant of previously unknown significance [48].

Talk of shared responsibility in recontacting (putting an onus on patients to seek updated interpretations of genetic test results) appears superficially very plausible, but runs the serious danger of exacerbating inequity in access to healthcare. As expected from Tudor Hart's inverse care law, the articulate and healthy with good IT skills and equipment are likely to place demands on health services that prevent practitioners from attending to the greater needs of the multiply disadvantaged [49].

## 13.9.6. Pharmacogenetics, Commercialization, and the Common Complex Disorders

The promise of "the new genetics" is that an improved understanding of the genetic basis of diseases will give new insights into their pathogenesis and thus enable the provision of truly personalized healthcare. It will be possible (so goes the implicit promise) to calculate each individual's personal profile of disease susceptibilities and hence define a suitable set of lifestyle measures, nutritional recommendations, or preventive medications that will keep the individual in the best possible health. This goal sounds laudable, but as soon as it is examined closely numerous difficulties and objections appear.

First, although it has proved possible to define gene loci important in a small subset of individuals affected by common complex diseases, such as Alzheimer disease and the common cancers (the 5% or so of cases where there is a strong, effectively Mendelian predisposition to disease), there has been much less progress through genome-wide association studies toward defining the risk-modifying genetic factors of lesser effect. For most complex conditions, only 10%–20% of the genetic contribution to risk of disease has been assigned.

Second, where molecular variation associated causally with disease susceptibility is identified, it still might not be amenable to effective therapeutic intervention. In the context of genetic disorders, there has been a long lag period between improved understanding of pathogenesis and improved treatments for patients. It would be irresponsible to generate unrealistic public expectations.

Third, there has been a strong temptation for commercial laboratories to make available molecular genetic testing for disease susceptibility, no matter how poor the clinical utility. Indeed, several providers of SNP panels have achieved high visibility on the internet. The principal weakness is that the SNPs tested account for such a small proportion of the causal factors but the marketing of the tests often claims (misleadingly, but sometimes only implicitly) that they can be used to guide health-related decisions. Such claims are

especially worrying if someone with a significant family history of disease relies on these spurious tests instead of seeking a formal clinical genetics assessment and mutation testing in potentially relevant Mendelian genes. There have been discussions in European countries about the need for regulation of such "direct-to-consumer" marketing of genetic tests, and in the United States the Food and Drug Administration has intervened against some companies' more outrageous claims.

Another set of concerns about genetic testing for disease susceptibility relates to the behavioral responses to risk information. Although the rhetoric emphasizes the positive behavioral application of risk information, experience so far does not suggest that it works out that way [50]. Indeed, there is potential for paradoxically unhelpful responses to risk information, such as either fatalistic inevitability or inappropriate invulnerability, both of which paralyze helpful behaviors, and this may distract health services from established, worthwhile interventions.

However, the possible use of genetic tests to guide pharmacological therapy is more promising. What might become possible is selection of the most appropriate therapy for each individual, given the pattern of molecular genetic variation that has predisposed them to develop the disorder and/or influences their likely response to a range of different therapies, in terms of both ensuring efficacy and avoiding toxicity. This tailoring of the treatment to the individual represents the Holy Grail of therapeutics. We must not expect such developments to work out quickly [51], but it is reasonable to entertain the modest hope that progress in this direction will accumulate over the coming years.

## 13.9.7. Research in Human Genetics

The principal issues to be considered are consent, confidentiality, and control over the process and application of the research. We also consider questions relating to the study of human diversity and "race".

### 13.9.7.1. Consent and Feedback

At recruitment into a research project, it is important that participants appreciate that the genetic sample being obtained for study is going to be examined in a research laboratory—the analytic process may not be subjected to the same quality control measures as in a regular diagnostic laboratory, and the applicability of the results to any healthcare decisions may not have been established. It may be simplest and cause least confusion if the research studies are thoroughly separated from the clinical services being provided to the patient, although this is not always feasible. It is obligatory, however, to make clear to the participant who is also a patient how, if at all, the research results may alter their prognosis or treatment. If participation will make no difference to their condition or treatment, or if the results will not be revealed to the participant, this must be clear at the point of recruitment to the study.

The rules governing databases and biobanks (what information can be generated, how it may be stored and accessed, and how/whether it will be passed back to the individual) vary widely [52]. Information generated from biobanks has the potential to be complex and to increase uncertainty. It may or may not be necessary to return to the sample donor for consent to carry out new investigations. Fresh contact allows the donor to be given updated information about the research. If the donor has died, it might be appropriate to contact his/her family if information to be generated in the research could have implications for them. Such recommendations for the conduct of research may be feasible in relation to uncommon disorders, such as malformations or Mendelian diseases, where small numbers of patients or families are involved and the research team may have a continuing relationship with the participants. In the context of the common complex diseases, however, where data on large numbers of individuals is being examined, the imposition of these requirements may make it impractical for some types of research (e.g., genetic epidemiology) to proceed at all unless it is set up with great attention to the governance issues.

With banked tissue samples that were obtained for diagnostic

purposes (perhaps at surgery for the removal of a malignancy) and for which consent for research use was not obtained, should researchers have to contact the patient and gain their consent before carrying out the research? Surely yes, if the investigation might generate clinically important information about the patient's genetic constitution, but otherwise perhaps no, if the research is only intended to characterize the tumor tissue. If the patient has died, should the family be asked to give consent before the researchers proceed with their studies? Again yes, if the results could be relevant to other members of the family.

Obtaining consent is a complex social process, especially in a research context, not a simple binary switch. Nuanced conceptions of consent are available and should be accorded proper weight [53]. It is also important to distinguish between research investigations carried out on individuals or groups of individuals who have a specific genetic condition or disease, and population-based studies that seek to identify the genetic influences upon the more complex multifactorial disorders.

Prospective agreement to the principle of being given individual results at the end of a research project will usually be invalid, because the nature of the results will usually not be at all clear at recruitment. If feedback is left to the discretion of the researchers, the inevitable enthusiasm of the researcher could exaggerate the weight of the findings. It will often be more appropriate for researchers to feed the general results of the study back to participants but not their individual results. Interested participants could then seek appropriate counseling and testing (to validate any personally applicable findings) in a clinical environment.

In recruitment to open-ended research, the future applications of the sample or data will be unknown. In these circumstances it is important to provide assurances that future investigations will be subject to independent ethics review rather than requiring researchers to recontact participants to approve each proposed new analysis.

It has become progressively more difficult to guarantee complete privacy of research findings as greater quantities of genome sequence data are produced and released. The need is to focus instead on clarity

(transparency) about this, and ensuring independent ethics review of new projects and the prevention of genetic discrimination on the basis of information that is released or reconstituted inappropriately.

Research participants should also be aware that the research could provide information that would be relevant to third parties such as insurers or employers, as well as to the individuals and their families. This needs to be explained in counseling before recruitment unless either the samples and the relevant clinical information were anonymized or the results of the analysis were provided to participants in the form of general conclusions only. Full anonymization, not merely deidentification, prevents the addition of updated information about each participant over the coming years, which may undermine the purpose of the biobank.

There are additional ethical problems with obtaining consent for the recruitment of children or incompetent adults. Although parents can give valid consent for the recruitment of their children to research, research should in general not be performed on children where it could be conducted adequately on adults. Particular concerns might be raised by research into the predictive testing of children for later-onset (usually adult-onset) disease for which no useful health intervention is available in childhood. That being said, children are not "mini-adults" and, for diseases which primarily (or only) impact children, knowledge gained through research involving children is invaluable. In these circumstances, children should be involved in discussions about research to the extent that they can understand the issues and contribute their views. If children withdraw their assent for research, this must be respected, as it cannot be argued that participation against their wishes (even with parental consent) would be in their "best interests".

The important considerations relating to the recruitment of incompetent adults into research are not specific to genetics research and will be passed over here—suffice it to say that any decisions made must take the subject's best interests into account and must not be enforced against their will or at the expense of their dignity; at a minimum, the assent of the subject is required.

### 13.9.7.2. Confidentiality

In research, as in clinical practice, third parties should not be given personal genetic information about individuals without their specific consent. In studies where the samples and data are anonymized the protection of genetic privacy is arguably simpler, although it has been demonstrated that even "anonymized" and pooled genetic information can be used to identify individuals positively [54]. In population studies with continuing data collection, additional precautions (including encryption and tightly restricted access) are needed to protect the privacy of subjects.

There is growing pressure on the research community to obtain the maximum benefit from the data generated through research by making the raw data available at the end of a project for other researchers to analyze. This carries dangers, as it makes it more likely that the identity of individual research participants could become known and their very personal genome sequence data might in effect become public.

It is crucially important that participants' research results are kept distinct from the individual's regular medical records. Otherwise, a member of staff unaware of the background context could inadvertently pass on research results that are highly sensitive or of uncertain significance, or where the participant had chosen not to be told, or staff could act on the results in a clinically inappropriate fashion.

### 13.9.7.3. Control and Ownership

In most jurisdictions, patients who have donated a sample of blood or tissue for research (or as part of their medical care) thereby lose rights in that tissue. Ownership passes to the relevant clinical or research institution. However, the commercialization of biotechnology and biomedical research has partially undermined the former "gift relationship" which motivated participants to donate their samples, as potentially large sums are accrued by researchers and developers of molecular technologies. Although research subjects are expected to treat their genetic material as a part of their person, and therefore not

a commercial resource, the very same genetic material can be treated as property by researchers. This contrast is explicit in many of the research consent forms used in genetics research, in which patients contributing samples sign away any right to commercial exploitation or benefit with respect to the sample before it is accepted into research.

Patients and clinicians generally acknowledge that financial investment from biotechnology and pharmaceutical corporations is required for advancement in human molecular genetics through research that ultimately benefits individual patients. Many, however, feel revulsion at the way this is working in practice to the advantage of corporations and laboratories which have contributed only to the very last stage of an arduous struggle to identify the genetic basis of the relevant diseases. It offends the sense of justice of many individuals that patients and families with genetic disorders, who have contributed biological samples to research and often also supported the research by fundraising activities over many years, now find that they (or the health services that care for them) are being charged license fees for applying the discoveries that "their" research has led to.

The question of whether DNA sequences can be patented has roused similar passions. Patenting in general is conditional upon three criteria: novelty, an inventive step, and an industrial application. Discoveries should therefore be clearly distinct from inventions, and so naturally occurring DNA sequences, genes, and organisms would seem not to be patentable. Yet patents that give effective control over certain genetic sequences have been awarded. A landmark legal decision was the granting of a patent on a modified organism (an oil-eating bacterium) by the US Supreme Court in 1980, following which precedent further patents on organisms and DNA sequences were granted. This heralded, and perhaps enabled, the spate of investment in medical biotechnology over the past few decades. A rush for patents on sequences of human DNA led to competition between research groups eager to patent specific disease genes and a race between the public and private human genome sequencing projects.

The debates about patenting human gene sequences continue within the United States and Europe, although several recent court rulings have blocked further patenting of human gene sequences.

## 13.9.8. Genetics, Geneticization, and Society

Genetics has received increasing levels of public attention over the past three decades. Although gratifying to geneticists, there are problems with this focus on genetics—especially when society looks to genetics to explain, and even to "solve", problems that are essentially "social".

When the phrase "the gene for X" is translated into something like scientific language, the absurdity of some of the public expectations becomes clear. This phrase becomes "a site in the genome—not necessarily a gene—at which DNA sequence variation is associated with variation in the propensity to develop X as a problem, although this association need not be causal and either the variant or the association may only be found in one or a few population groups". The links mediating the social consequences of genetic variation are so complex that, even in principle, it could never be possible to predict these consequences from the molecular facts. The genetic sites at which molecular variation correlates with behavioral consequences, for example, will not be genes "for" the social problems. The brain is far too complex an organ for geneticists to be able to make detailed predictions about individual behavior, although certain statistical associations between molecular variation and the probabilities of specific behaviors may be established.

Genetic determinism is the attempt to reduce the whole of biology to the physical sciences, with the behavior of organisms being shaped largely by their genetic constitution. This is a powerful but destructive concept, and in reality is a doomed enterprise. Given the inevitable limitations of the physical sciences in dissecting the functions of the brain, and a fortiori our limitations in tackling societal characteristics and problems, how should clinical geneticists respond to the widespread misunderstandings about the relevance of genetics to social issues?

As a minimum, we should ensure that we do not inadvertently exacerbate these misunderstandings through the inappropriate use of pseudoscientific terminology ("genes for X") or by exaggerating the genetic contribution toward health problems. We should also be wise to dissociate ourselves from research that relies upon or promotes such misunderstandings, and publicly speak out against the misuse of genetics by those with damaging social or political motivations.

The tendency to attribute causation of health or social problems to genetic factors is an example of "geneticization", a term coined by Abby Lippman [55] to describe the more general practice of accounting for observed differences between individuals and populations in terms of underlying genetic differences. This process allows vested political interests to obscure the remediable environmental causes of social inequalities in health and individual attainment by focusing attention upon the fixed and unalterable genetic constitutions of individuals. This, of course, fits well with the conservative view of human nature as often propagated under the label of sociobiology or evolutionary psychology; from such a perspective, social problems are framed as biological inevitabilities determined by each individual's genetic constitution. This can lead those frustrated by the problems they see in society to advocate technological solutions when collective political action may be much more appropriate and effective. The process of geneticization therefore distracts energy and resources from the social and environmental strategies that could lead to real solutions.

One aspect of genetics research that is especially alarming (because of the ease with which it could be misapplied, especially in the context of "race" and human diversity) is the genetic dissection of nondisease traits, particularly intelligence and personality traits, and including variation in intelligence within the normal range. The apparent rationale for pursuing this research is that it may teach us about mild learning difficulties and fundamental brain processes involved in learning. Given the barriers to interpreting any associations that may be found between intelligence and molecular variation, however, we would argue that the short-term results of such research are more

likely to produce social conflict than a productive understanding of cognitive processes [56]. The results are likely to be distorted, deliberately, by those intent on mobilizing political support behind racist or nationalist campaigns.

## 13.9.9. Reproductive Technologies: "Reprogenetics"

Two developments in reproductive technologies are discussed here: preimplantation genetic diagnosis (PGD), and the use of carrier screening and NIPT to eliminate (almost) recessive disease and chromosomal aneuploidy.

PGD entails all the procedures of in vitro fertilization (IVF) used in the treatment of infertility. The zygotes are allowed to develop to the morula stage, when one or two cells are removed for analysis—employing either fluorescent in situ hybridization techniques to look for chromosome anomalies or microdeletions, or polymerase chain reaction-based methods to identify specific disease-associated mutations or the number of repeat units in a triplet repeat or a haplotype of SNP variants. Then an embryo (or perhaps two) without evidence of the family's genetic disorder is implanted into the woman's uterus.

PGD can appear to be an ideal solution for couples who are concerned about transmitting their genetic disorder to a child and wish to avoid prenatal diagnosis and, perhaps, pregnancy termination. The process of PGD, however, is expensive, inconvenient, and emotionally and physically draining.

Objections have been made to PGD because it is a technology that could be applied to choose between embryos on the basis of nondisease traits, including sex. Although there are good grounds for rejecting fetal sex selection in general, as discussed above, if there are six healthy embryos and two are to be implanted then it may seem reasonable to allow the couple to choose the sex of the two embryos. Should such an allowance be made for other nondisease traits for which testing might become possible—such as physical or even

mental characteristics? If so, should the same be permitted for couples with no fertility problem but who simply wish to select the characteristics of their child?

PGD can be seen as being located part of the way down the "slippery slope" to one type of "designer baby". Although the burdensome nature of IVF technology and its costliness make it unlikely that many fertile couples would wish to use it now, testing for nondisease traits may become available and the PGD technology is likely to improve and become less burdensome. It is worth resolving these issues before that occurs. There is no reason why a couple wishing to use such services would consult a clinical geneticist, so in that sense it is not a problem for "us"—but this area of "reprogenetics" lies so close to clinical genetics that we are bound to be affected by these developments.

One temporary resolution is to treat PGD for disease, for sex selection, and for selection on the basis of nondisease traits as distinct circumstances.

1. PGD for disease presents fewer ethical problems than prenatal diagnosis and selective pregnancy termination, and appears a reasonable option for those undergoing infertility treatment or with strong objections to pregnancy termination.
2. PGD for sex selection is disrespectful to women and should not be performed except as a way of avoiding sex-linked disease.
3. PGD for nondisease traits is not available at present because of technical limitations, and should probably not be practiced at all. Such a development would be widely understood as a step toward a brave new world of genetically manipulated children.

Finally, we turn to the use of carrier screening by next-generation sequencing and NIPT chromosome analysis to eliminate much genetic disease: virtually all recessive disease and most chromosomal aneuploidy (including small deletions and duplications). This raises profound questions about the type of society in which we wish to live, the extent to which we should regulate or control the biological

composition of our communities, and whom we are prepared to welcome among us as fellow citizens. In the past eugenics could be dismissed as a flawed science and eugenic policies could be seen as science fiction, but we now have the capacity to implement really effective "eugenic" policies. We must therefore be correspondingly more thoughtful about how and under what circumstances these developing technologies are permitted to enter practice.

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## References

[1] Shiloh S, Sagi M. Effect of framing on the perception of genetic risk recurrence risks. *Am J Med Genet*. 1989;33:130–135.

[2] Berkenstadt M, Shiloh S, Barkai G, et al. Perceived personal control (PPC): a new concept in measuring outcome of genetic counseling. *Am J Med Genet*. 1999;82:53–59.

[3] McAllister M, Dunn G, Todd C. Empowerment: qualitative underpinning of a new patient reported outcome for clinical genetics services. *Eur J Hum Genet*. 2011;19:125–130.

[4] Clarke A. Genetics, ethics and audit. *Lancet*. 1990;1990(335):1145–1147.

[5] McAllister M, Wood A.M, Dunn G, Shiloh S, Todd C. Genetic Counseling Outcome Scale: a new patient-reported outcome measure for clinical genetics

services. *Clin Genet*. 2011;79:413–424.
[6] Clarke A. Is non-directive genetic counseling possible? *Lancet*. 1991;338:998–1001.
[7] Clarke A. The process of genetic counselling: beyond non-directiveness [Chapter 13]. In: Harper P.S, Clarke A, eds. *Genetics, society and clinical practice*. Oxford: Bios Scientific Publishers; 1997:179–200.
[8] Kessler S. Psychological aspects of genetic counseling XI nondirectiveness revisited. *Am J Med Genet*. 1997;72:164–171.
[9] Weil J. Psychosocial genetic counseling in the post-nondirective era: a point of view. *J Genet Counsel*. 2003;12(3):199–211.
[10] Wolff G, Jung C. Nondirectiveness and genetic counseling. *J Genet Counsel*. 1995;4:3–25.
[11] Clarke A.J, Cooper D.N. GWAS: heritability missing in action. *Eur J Hum Genet*. 2010;18:859–861.
[12] Girirajan S, Rosenfeld J.A, Cooper G.M, Antonacci F, et al. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet*. 2010;42:203–209.
[13] McRae J.F, et al. Prevalence and architecture of de novo mutations in developmental disorders. *Nature*. 2017;524:433–438.
[14] Green R.C.M.D, Berg J.S, Grody W.W, Kalia S.S, Korf recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15(7):565–574.

[15] Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, McKelvey KD, Ormond KE, Richards CS, Vlangos CN, Watson M, Martin CL, Miller DT; on behalf of the ACMG Secondary Findings Maintenance Working Group, 2016 Secondary Findings Maintenance Working Group. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update. (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med 2017;19:249–55.

[16] Sarangi S, Bennert K, Howell L, Clarke A, Harper P, C in counselling for Huntington's disease predictive testing: clients' responses to reflective frames. *J Genet Counsel*. 2005;14:29–42.

[17] Parker M, Lucassen A.M. Genetic information: a joint account? *BMJ*. 2004;328:165–167.

[18] Lucassen A, Parker M. Confidentiality and serious harm in genetics – preserving the confidentiality of one patient and preventing harm to relatives. *Eur J Hum Genet*. 2004;12:93–97. .

[19] Hallowell N, Foster C, Eeles R, Ardern-Jones A, Murday V, Watson M. Balancing autonomy and responsibility: the ethics of generating and disclosing genetic information. *J Med Ethics*. 2003;29:74–83.

[20] American Society of Human Genetics. ASHG statement: professional disclosure of familial genetic information. *Am J Hum Genet*. 1998;62:474–483.

[21] Forrest K, Simpson S.A, Wilson B.J, van Teijlingen E.R, McKee L, Haites N, Matthews E. To Tell or Not to Tell: barriers and facilitators in family communication about genetic risk. *Clin Genet*. 2003;64:317–326.

[22] Featherstone K, Bharadwaj A, Clarke A, Atkinson P. *F Relations. Family and Kinship in the Era of New Genetics*. Oxford: Berg Publishers; 2006.

[23] Newson A.J, Humphries S.E. Cascade testing in familial hypercholesterolaemia: how should family members be contacted? *European Journal of Human Genetics*. 2005;13:401–408.

[24] Geelen E, Van Hoyweghen I, Doevendans P.A, Marcelis C.L, Horstm "best interests": genetic testing of children in families with hypertrophic cardiomyopathy. *Am J Med Genet*. 2011;155(8):1930–1938.

[25] BSHG. *Genetic testing of children: report of a working party of the British Society for Human Genetics*. 2010.

[26] Forrest Keenan K, van Teijlingen E, McKee L, Miedzybrodzka Z, Simpson S.A young people find out about their family history of Huntington's disease. *Soc Sci Med*. 2009;68:1892–1900.

[27] Metcalfe A, Plumridge G, Coad J, Shanks A, Gill P. Pa and children's communication about genetic risk: a qualitative study, learning from families' experiences. *Eur J Hum Genet*. 2011 [online].

[28] AAP/ACMG [American Academy of Pediatrics and the American College of Medical Genetics and

Genomics]. Ethical and policy issues in genetic testing and screening of children. *Pediatrics*. 2013;131:620–622.
[29] Jansen L, Friedman Ross L. The ethics of pre-adoption genetic testing. *Am J Med Genet*. 2001;104:214–228.
[30] Binedell J, Soldan J.R, Scourfield J, Harper P.S. Huntir disease predictive testing: the case for an assessment approach to requests from adolescents. *J Med Genet*. 1996;33:912–918.
[31. Gaff C, Lynch E, Spencer L. Predictive testing of eighteen-year olds: counseling challenges. *J Genet Counsel*. 2006;15:245–251.
[32] Swoboda K.J. Seize the day: newborn screening for SMA. *Am J Med Genet*. 2010;152A(7):1605–1607.
[33] Alderson P. Down's syndrome: cost, quality and value of life. *Soc Sci Med*. 2001;53:627–638.
[34] Beulen L, van den Berg M, Faas B.H.W, Feenstra I, Hageman M, van Vugt J.M.G, Bekker N. The effect of a decision aid on informed decisionmaking in the era of non-invasive prenatal testing: a randomised controlled trial. *European Journal of Human Genetics*. 2016;24:1409–1416.
[35] Schoonen H.M.H.J.D, Essink-Bot M.L, van Agt H.M.E, Wildschut H.I, Steegers E.A.P, de Koning H.J. Informed decision-making about the fetal anomaly scan: what knowledge is relevant? *Ultrasound Obstet Gynecol*. 2011;37:649–657.
[36] García E, Timmermans D.R.M, van

Leeuwen E. Reconsidering prenatal screening: an empirical ethical approach to understand moral dilemmas as a question of personal preferences. *J Med Ethics*. 2009;35:410–414.
[37] Scully J.L, Porz R, Rehman-Sutter C. 'You don't make genetic test decisions from one day to the next' – using time to preserve moral space. *Bioethics*. 2007;21:208–217.
[38] Manchanda S, Saikia B, Gupta N, Chowdhary S, Puliy ratio at birth in India, its relation to birth order, sex of previous children and use of indigenous medicine. *PLoS One*. 2011;6(6):e20097.
[39] Puri S, Adams V, Ivey S, Nachtigall R.D. "There is such a thing as too many daughters, but not too many sons": a qualitative study of son preference and fetal sex selection among Indian immigrants in the United States. *Soc Sci Med*. 2011;72(7):1169–1176.
[40] Nuffield Coucil on Bioethics. *Report on non-invasive prenatal testing*. London: Nuffield Council; 2017.
[41] Bekker H, Modell M, Denniss G, Silver A, Mathew C, of cystic fibrosis testing in primary care: supply push or demand pull? *BMJ*. 1993;306:1584–1586.
[42] Elias S, Annas G. Generic consent for genetic screening. *N Engl J Med*. 1994;330:1611–1613.
[43] Modell B, Darr A. Genetic counseling and customary consanguineous marriage. *Nat Rev Genet*. 2002;3:225–229.
[44] Shaw A, Hurst JA. "What is this genetics, anyway?" Understandings of genetics, illness causality and

inheritance among British Pakistani users of genetic services. J Genet Couns August 2008;17(4):373–83.
[45] Armstrong D, Michie S, Marteau T.M. Revealed identity: a study of the process of genetic counseling. *Soc Sci Med*. 1998;47:1653–1658.
[46] Pyeritz R.E. The coming explosion in genetic testing - is there a duty to recontact? *N Engl J Med*. 2011;365:1367–1369.
[47] Dheensa S, Carrieri D, Kelly S, Clarke A, Doheny S, Tı "joint venture" model of recontacting in clinical genomics: challenges for responsible implementation. *Eur J Med Genet*. 2017;60(7):403–409.
[48] Ackerman B.A, Bartos D.C, Kapplinger J.D, Tester D.J promise and peril of precision medicine: phenotyping still matters most. *Mayo Clin Proc*. 2016;91:1606–1616. .
[49] Tudor Hart J. The inverse care law. *Lancet*. 1971;297(7696):405–412.
[50] Marteau T.M, French D.P, Griffin S.J, Prevost A.T, Sut of communicating DNA-based disease risk estimates on risk-reducing behaviours (review). *Cochrane Libr*. 2010;10:1–74.
[51] Clarke A.J, Abdala Sheikh A.P. A perspective on “cure” for Rett syndrome. *Orphanet Journal of Rare Diseases*. 2018;13:44.
[52] Kaye J, Boddington P, de Vries J, Hawkins N, Melham K. Ethical implications of the use of whole genome methods in medical

research. *Eur J Hum Genet*. 2010;18:398–403.
[53] Corrigan O. Empty ethics: the problem of informed consent. *Sociol Health Illness*. 2003;25:768–792.
[54] Homer N, Szelinger S, Redman M, Duggan D, Tembe al. Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays. *PLoS Genet*. 2008;4(8):e1000167. doi: 10.1371/journal.pgen.1(
[55] Lippman A. Prenatal genetic testing. Constructing needs and reinforcing inequities. Am J Law Med 1991;17:15–50 [Reprinted in Clarke, editor. Genetic counselling: practice and principles. London: Routledge; 1994].
[56] Clarke A. The limits to psychiatric and behavioural genetics [Chapter 1]. In: Pilgrim D, Rogers A, Pescosolido B, eds. *The SAGE handbook of mental health and illness*. London: SAGE; 2011:7–25.

14

# Genetics and Genomics in Public Health

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## Abstract

This chapter reviews the emergence and progress in the field of public health genetics/genomics. Firmly rooted in two fields with a rich and disparate history (medical genetics and public health), public health genomics has taken shape only in the past 20 years. Broadly defined, public health genomics is a multidisciplinary field concerned with the responsible and effective translation of genomic science and technologies to improve population health. In contrast to medical genetics, where the unit of intervention is the individual and other family members, public health genomics is concerned with the health and well-being of a whole population or community, no matter how it is defined. We review the goals and activities of public health, discuss public health essential services in the context of genetics/genomics, and give examples of ongoing priorities and activities in the field, such as hereditary cancers, pathogen genomics, precision medicine, and integrating evidence-based genomics applications into the changing landscape of community-based healthcare. Moving forward, the role of public

health genomics is expected to expand due to rapid developments and applications of genomics in healthcare and disease prevention, requiring further assessment, policy development, and assurance efforts.

## Keywords

Counseling; Evidence-based medicine; Genetics; Genomics; Population; Public health; Screening

## 14.1. What Is Public Health Genetics/Genomics?

Public health is defined as "what we, as a society, do collectively to assure the conditions in which people can be healthy". [1] Public health agencies (local, state, and federal) are part of a public health system that links or brings together multiple stakeholders, including healthcare professionals, social services providers, academia, educators, policymakers, community members, and others, to protect and improve the health of families and communities. As an approach, population health focuses on interrelated conditions and factors that influence the health of populations over the life course, identifies systematic variations in their patterns of occurrence, and applies the resulting knowledge to develop and implement policies and actions to improve the health and well being of those populations [2].

Historically, public health focused on public or governmental efforts to protect people from epidemics such as the plague or cholera. Today, the purpose of public health requires a more expanded scope of work (Table 14.1).

Medical genetics services, in contrast, involve genetic testing, diagnoses of genetic conditions, genetic counseling, and treatments for individuals with genetic disorders. These services occur across the life cycle, and influence behavior, disease monitoring, and treatment for individuals being tested as well as their families. But while medical genetics impacts families, attention is still isolated to the

patient and his or her family members, and the focus of traditional medical genetics has been on genetic disorders such as Mendelian single-gene conditions or chromosomal disorders. Increasingly, however, genetic information is being used throughout the practice of medicine, including in pharmacogenetics and prevention and treatment of common chronic diseases such as cancer and heart diseases. This is commonly referred to as genomic medicine.

Public health genetics/genomics places the focus on using genetics or genomics applications to improve the health of the entire community or population, however these terms are defined (i.e., whether it is all children, all pregnant women, a town, city, state, province, or country). It is a multidisciplinary field with established scientific and policy foundations that allows assessment of the potential benefits or harms of using genetic/genomic applications in the population. For public health genetics practitioners, this work may include establishing best practices, policies, or regulations that address quality, enable access to genetic/genomics services, or reduce healthcare disparities. Furthermore, public health practitioners work to implement validated applications with proven health benefits and evaluate their impacts on healthcare or the healthcare delivery system [3].

While the term "genetics" typically refers to single genes and their contribution to a characteristic, trait, or disease and "genomics" is inclusive of the entire genome, the distinction is often inconsequential in the public health arena. In other words, when considering epidemiologic studies, developing regulations or policies, or addressing issues of access to services or healthcare equity, the public health practitioner, with few exceptions, would intentionally be inclusive of both genetic and genomic practices. For this reason, the term genetics/genomics is combined throughout this chapter unless stated otherwise.

## Table 14.1

**Purposes of Public Health**

The fundamental obligation of agencies responsible for population-based health is to:

- prevent epidemics and the spread of disease
- protect against environmental hazards
- prevent injuries
- promote and encourage healthy behaviors and mental health
- respond to disasters and assist communities in recovery
- ensure the quality and accessibility of health services

Reproduced from Wang G, Watts C. The role of genetics in the provision of essential public health services. Am J Public Health 2007;97(4):620–5.

## 14.2. The Purposes of Public Health

As described previously (see Table 14.1), there are six foundational purposes of public health. In this section, current examples of public health genetics/genomics activities are provided for each purpose.

### 14.2.1. Prevent Epidemics and the Spread of Disease

Pathogen genetics/genomics has been used extensively in public health for many years for identifying and tracking pathogens. Rapid, real-time whole genome sequencing and use of pathogen genotypes allows for public health surveillance and tracking of disease outbreaks in both humans and animals. Multiple pathogens associated with infectious diseases have been sequenced, such as Bordetella (whooping cough), Salmonella, Yersinia pestis (plague), Plasmodium (malaria), Ascaris (intestinal worms), and Proteus mirabilis (urinary tract infections) [4]. The Centers for Disease Control and Prevention (CDC) recently launched the Advanced Molecular Detection Initiative to integrate tools of genomics into public health responses across a

wide range of infectious diseases [5]. Knowing a pathogen's sequence or genotype often allows public health personnel to pinpoint the location or source of an outbreak, treat or eliminate the pathogen, and prevent further disease [6].

## 14.2.2. Protect Against Environmental Hazards

As the Human Genome Project (HGP) was foundational to understanding human biology, the sequencing of model organisms has proven to be a catalyst for biotechnology, ecology, and other environmental sciences [7]. Climate change, dwindling natural resources, and increasing pollution are a few environmental hazards that many hope genomic applications will address. By understanding the genome of various species and microbes, researchers utilize the genes and regulatory processes for uptake of, for example, carbon or other environmentally important elements. In essence, these genetically engineered hosts can be the platform for production of biofuels or useful in environmental waste cleanup [8]. Most applications of genetics/genomics for environmental health purposes are currently in the research phase.

## 14.2.3. Prevent Harm and Injuries

When direct-to-consumer (DTC) genetic testing companies initially launched their varied services, there was much concern among both the clinical and public health genetics communities that these tests would lead to anxiety and unwarranted medical treatments/procedures based on limited or erroneous information. These concerns were raised due to the uncertain analytic validity, clinical validity, and clinical utility of many DTC tests and lack of information about how individuals might respond to personal genetic information [9].

Goddard et al. conducted population-based surveys concerning DTC nutrigenomic testing in Michigan, Oregon, and Utah as part of the 2006 Behavioral Risk Factor Surveillance System. This is a CDC-sponsored standardized telephone survey conducted in all states, the

District of Columbia, and three US territories, but states can add their own questions as well. Their results suggested that awareness of DTC tests was fairly low (highest in Oregon at just over 24%) and uptake of DTC for nutrigenomics was quite low—less than 1% of the population surveyed [10]. While these rates may be higher today, there has been no evidence of widespread panic or harm from DTC. Furthermore, work such as the Risk Evaluation and Education for Alzheimer Disease study demonstrated that Alzheimer disease risk assessments with Apolipoprotein E (APOE) genotyping can be given to relatives of people with Alzheimer disease without causing severe adverse psychological or behavioral effects [11]. However, as people become more proactive in managing their health, DTC genetic testing could become more popular in the next decade. With the expanding landscape of such testing, the need for provider education and public genetic literacy has become more important than ever.

More concerning data have come directly from laboratory utilization management programs. Miller and colleagues, from the Genetics Division of ARUP Laboratories, published their findings in 2011 demonstrating that when genetic counselors reviewed test requisitions, they altered the test order for about 26% of complex genetic tests ordered over the course of 21 months. This represented a savings to their referring institutions of $48,000 per month [12]. Similarly, other laboratory utilization management groups studying genetic test ordering patterns concluded that "clinicians without specialty training in genetics make genetic test order errors at a significantly higher rate than geneticists". [13,14] Errors in test requisitions ranged from ordering a more expensive test than necessary (e.g., whole exome sequencing, whole genome sequencing, or a panel microarray when there was a known familial mutation and a targeted test was warranted) to ordering a molecular test for the wrong gene. Since the majority of genetic tests ordered come from clinicians not formally trained in clinical genetics, the potential for misinformation leading to wasteful or potentially harmful treatments as well as the potential for missed prevention opportunities may well

be significant public health concerns.

## 14.2.4. Promote and Encourage Healthy Behaviors and Mental Health

One of the least expensive screening tools for any geneticist or healthcare practitioner, or the general public for that matter, is the family health history. Family history is a risk factor for many human diseases and reflects the combined influences of genetic and environmental factors. While it is part and parcel of medical practice, public health campaigns to educate providers and the public about collection and use of family history have been limited. In 2004 the Surgeon General launched the family health history campaign and declared Thanksgiving Day as Family Health History Day in the United States. A free online tool, the Family Health Portrait, was developed and is available to help the general public collect and share family history information with their healthcare providers. In 2009 the National Institutes of Health State-of-the-Science Conference statement on "Family Health History and Improving Health" concluded that additional research is needed for family health history to become an evidenced-based tool for primary care providers [15]. While it may be true that more robust research is needed for the use of family history in primary care [16], many geneticists can attest to the fact that this history could have prevented a diagnostic odyssey for many families of patients with genetic disease if someone had taken the time to obtain it. A study conducted by Acheson et al. assessing how family practice physicians utilized family health history found that the history was discussed during only 51% of visits with new patients and 22% of visits with established patients. Primary care providers often cite lack of time as a reason for forgoing family health history during the brief time allowed for patient visits. Acheson et al. found that the average time spent discussing the family health history was under 2.5 min [17].

Indeed, one readily available beneficial public health

genetics/genomics intervention is promoting the benefits of knowing one's family health history, updating it frequently, and sharing the information with your healthcare provider. Family health history has been integrated into several evidence-based guidelines in genetics. One prominent example is the US Preventive Services Task Force (USPSTF) recommendation for collecting family history as a way to identify women at high genetic risk for hereditary breast and ovarian cancer and refer them for genetic counseling and testing [18].

## 14.2.5. Respond to Disasters and Assist Communities in Recovery

Tragically, natural and manmade disasters do occur. Public health genetics/genomics programs have assisted, and can assist, primarily in identifying the remains of victims. Providing newborn screening of dried blood spots, laboratory space, and/or partnering with community-based laboratories capable of performing molecular genetic testing for DNA and mitochondrial DNA identification as well as public health staff capable of collecting relevant biologic family histories and potential DNA samples were all instrumental in giving closure for multiple US families following the World Trade Center attacks as well as Hurricane Katrina [19,20].

## 14.2.6. Ensure the Quality and Accessibility of Health Services

Health departments often employ regulatory mechanisms, including licensure, in an attempt to ensure quality. However, licensure alone does not adequately ensure quality. An emerging public health challenge is how to ensure quality in a field with such breadth and in which clinicians not formally trained in genetics are acting within their own scope of practice, yet may be most in need of intervention. Increasingly, public health genetics/genomics professionals convene or participate in national/international advisory groups in an effort to influence best practices or performance guidelines [21].

Distribution of medical genetic services, and therefore access to these services, is dependent on available resources and provider and public awareness. A common public health activity is to conduct a needs assessment: identify existing resources and gaps or barriers to genetic services. Determining how many medical geneticists, nurse geneticists, and genetic counselors are available to serve the population and whether or not that genetics workforce is adequate for the defined population is one example of evaluating access to genetics services. In some areas, public health dollars are used to pay medical geneticists and/or genetics counselors to travel to more remote areas so that clinical genetics services can be provided to residents in those locations. Increasingly, communities are taking advantage of technology to allow patients to be seen in or near their home while the genetics specialist is at a distant location. This is commonly referred to as telemedicine or telegenetics.

## 14.3. The Public Health System Infrastructure

Both science and politics have shaped the evolution of public health over the past 150 years. As scientific advances identified more sources and means of controlling disease, a belief grew that protecting citizens from disease is a governmental, or executive, responsibility. In addition, there was increased public acceptance of interventions to control disease [1].

Fig. 14.1 depicts the US public health infrastructure, although the levels or tiers could easily reflect the interrelatedness of upper-level governmental offices or administrations of any country, which then provides financial support to mid-level organizations (e.g., academic, nongovernmental groups, and states or provinces), which in turn work closely with community-based entities including the medical community. Each of the mid- or local-level entities typically receives funding beyond what the government provides, yet they are often influenced by the executive leadership's direction for public health priorities. In addition, the public health system benefits from multiple partnerships across and between mid- and local-level entities,

including working relationships with hospitals, health, social and mental health practitioners, law enforcement or public safety, and other community-based stakeholders.

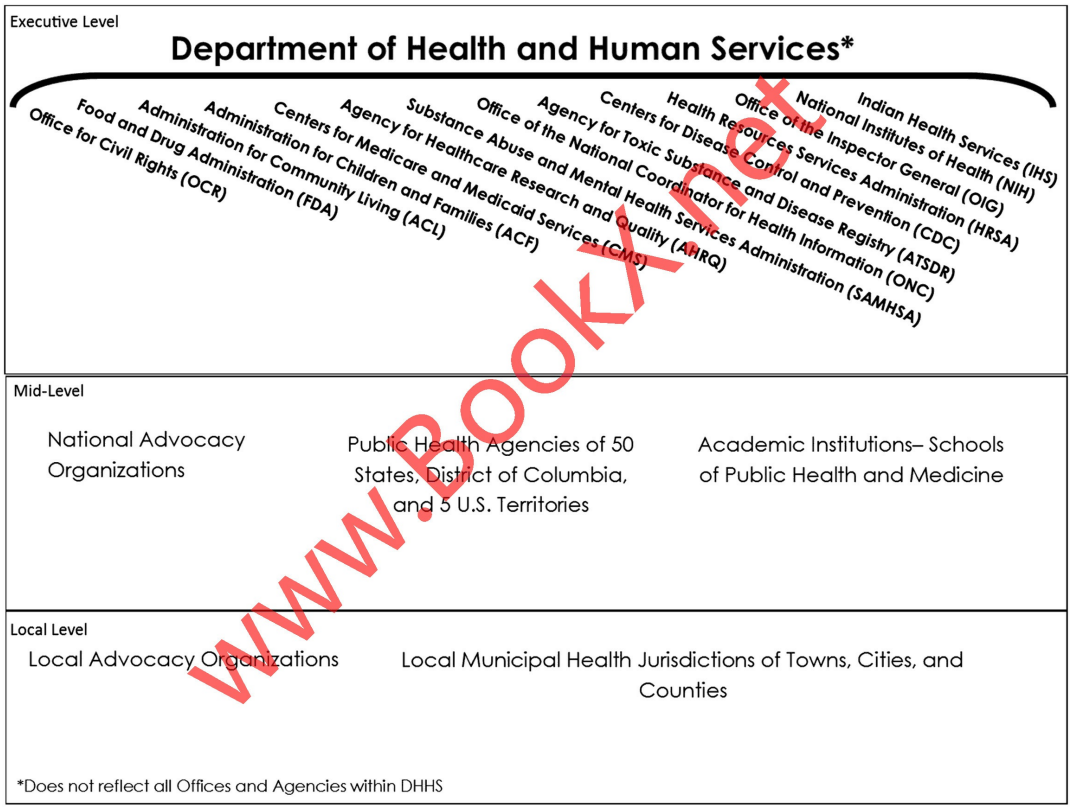



**FIGURE 14.1** Evidenced-Based Public Health Framework.

In 1988 the Institute of Medicine issued a call to action in a report entitled "The Future of Public Health", in which the authors describe a public health system nationally in disarray because of overt politics and lacking "clear delineation of the mission of the public health service system". They called for "improvement in technical, political, managerial and programmatic skills of public health practitioners" [1]. Prior to this report, many local community-based health departments had taken on the role of direct patient care, particularly for those who were uninsured or underinsured. The report urged public health practitioners to return to the core public health functions, including assessment, policy development, and assurance. It also highlighted the dangers of having an ill-prepared public health workforce, and

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counseled readers that assurance did not mean becoming the "provider of last resort", but rather ensured adherence to approved public policy.

The CDC's "10 Essential Public Health Services" further delineated the activities that make up these core functions.

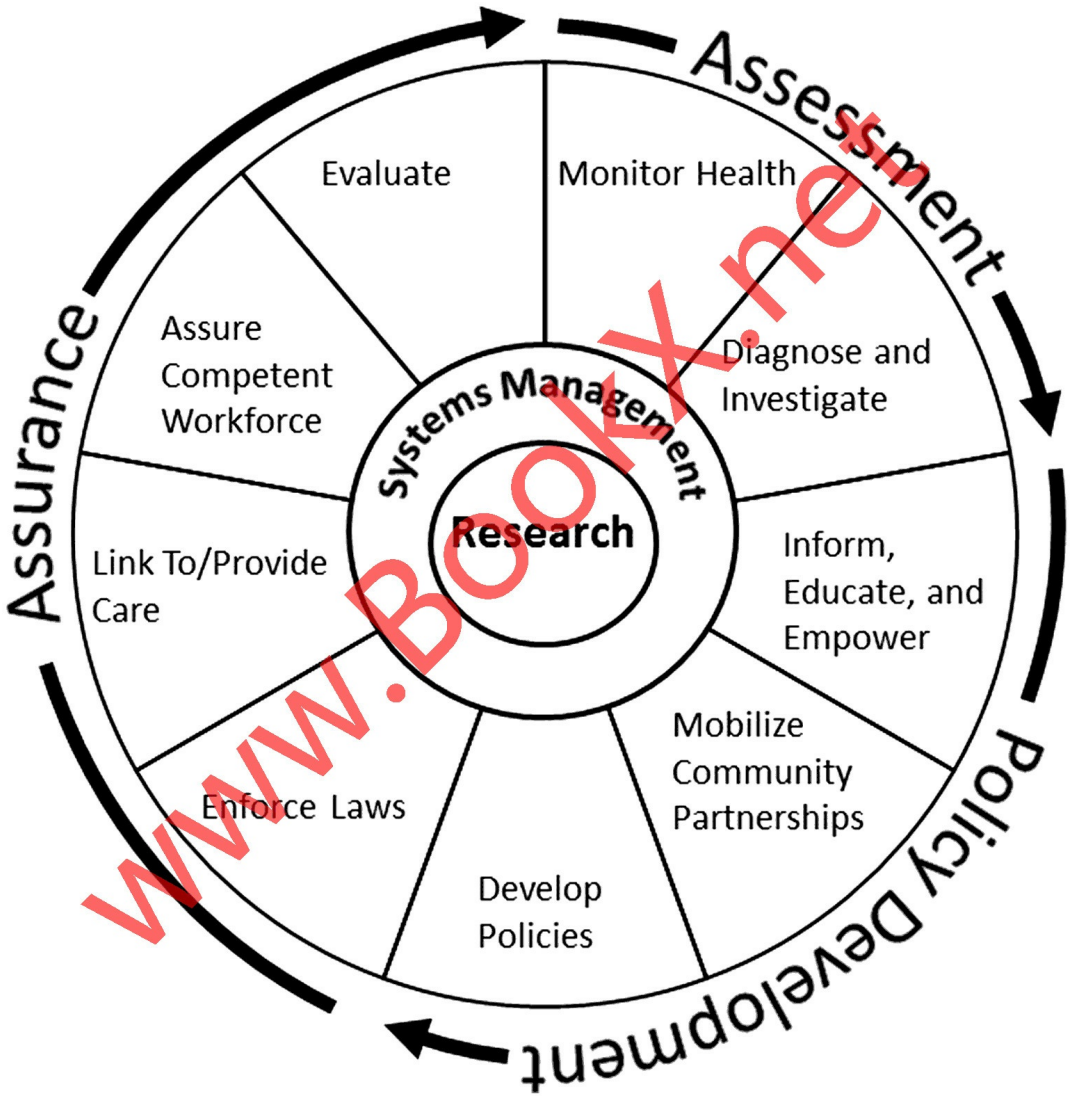
1. Monitoring health status to identify and solve community health problems.
2. Diagnosing and investigating health problems and health hazards in the community.
3. Informing, educating, and empowering people about health issues.
4. Mobilizing community partnerships and action to identify and solve health problems.
5. Developing policies and plans that support individual and community health efforts.
6. Enforcing laws and regulations that protect health and ensure safety.
7. Linking people to needed personal health services.
8. Ensuring that there is a competent public and personal healthcare workforce.
9. Evaluating effectiveness, accessibility, and quality of personal and population-based health services
10. Researching new insights and innovative solutions to health problems.

Collectively, the core public health functions and 10 essential services create the framework for all public health services, including public health genetics/genomics [22] (see Fig. 14.2).

Public health genetics/genomics practices continue to evolve. Although not at all inclusive, the following subsections give some examples of public health genetics/genomics activities using the framework of core public health functions and the 10 essential services [23,24].

## 14.3.1. Assessment

*Monitoring the health status* of a community is achieved by tracking the incidence of health conditions, use of preventive services, and barriers to accessing services. Monitoring can involve compiling demographic and socioeconomic data, combined with vital statistics (i.e., data on births, deaths, marriages, divorces) and immunization data. Monitoring can be further focused for certain groups or conditions by using registry data (e.g., birth defects or cancer registries) and standardized surveillance surveys (e.g., behavioral risk factor surveillance surveys).




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**FIGURE 14.2** Public Health Infrastructure.

The use of genotyping following a disease outbreak demonstrates the essential service of *diagnosing and investigating a health problem*.

## 14.3.2. Policy Development

Multiple interventions support the essential services of *informing, educating, and empowering people* about genetics/genomics health issues. Educational programs that serve to increase people's awareness about the importance of knowing their family health history and sharing the information with healthcare providers are one example. Others are healthcare provider education so people understand the risk factors associated with hereditary cancers and health promotion campaigns that emphasize the benefits of newborn screening—all are intended to inform, educate, and empower.

*Mobilizing community partnerships* is a mainstay of public health practitioners, who routinely call upon both public and private agencies and community members to engage in dialogue and strategic planning to address perceived needs or gaps in genetics/genomics services effectively.

*Policy development* can range from formal and legally binding policies, such as creating regulations concerning what conditions every baby will be screened for, to less formal policies such as creating best practices for communities to support telemedicine efforts for genetic services.

## 14.3.3. Assurance

An example of *enforcing laws to protect the public* could be ensuring that all genetics healthcare practitioners are appropriately licensed.

Having an up-to-date community-based website that includes contact information (e.g., name of facility, address, phone number, and website) for genetics clinics is an easy way to *link people to genetic services*.

Efforts to *ensure a competent workforce* can include public health

genetics/genomics staff hosting periodic educational sessions for health and social service practitioners.

*Evaluating the effectiveness* of all public health genetics/genomics interventions or strategies as exemplified above is crucial and must be ongoing, to improve continuously and generate the largest health impact. Some specific examples include identifying and analyzing the impact of genetic information or applications of genetic/genomics applications on the public, and monitoring the utilization and quality of genetic services.

Research addressing each of these essential services is intended to yield new and innovative solutions to existing and emerging health problems. Since public health genetics/genomics is a young field, there are many areas in need of study.

Finally, building and maintaining the capacity of the public health infrastructure to integrate genetic/genomics into public health practice and research rounds out the overall framework.

## 14.4. Evolution and Convergence of Two Fields of Science—Public Health and Genetics/Genomics

Just as the clinical genetics arena has been shaped by technology and the growth of scientific knowledge concerning genetics/genomic medicine, the field of public health similarly has changed over time, reacting to changing population needs.

To appreciate better how public health and genetics/genomics converged, it is worth looking back at some historical milestones. While not a comprehensive encyclopedia of events over time, consider the following events and how each helped shape these scientific fields over the decades leading up to the 21st century and the HGP [25-28] (see Table 14.2).

During the 1960s the fields of public health and genetics came together largely as a result of the Guthrie test that set the stage for population-based screening of newborns for phenylketonuria (PKU). But this discovery alone may not have resulted in establishing

newborn screening programs if not for the simultaneously occurring groundswell of advocacy for people with disabilities and greater interest in preventing cognitive disabilities [29].

Likewise, in the United States in 1976, Public Law 94-278 established a national genetic disease program entitled "The National Sickle Cell Anemia, Cooley's Anemia, Tay-Sachs, and Genetic Diseases Act". Using funds from PL 94-278, state health departments, medical genetics providers, and others worked together to develop statewide systems for genetic healthcare services. The purpose of these systems was to prevent premature death or disability related to genetic risk factors by establishing statewide genetic healthcare systems that would be accessible to all residents. These systems included early identification, referral, evaluation, screening, diagnostic testing, and genetic counseling, as well as education for health and social services providers and the general public. It is unlikely that such a law would be passed if it were not for rapid advancements in the field of clinical genetics [30].

## Table 14.2

**Evolution of Two Scientific Fields: Public Health and Genetics/Genomics**

| Timeframe | Public Health | Genetics/Genomics |
|---|---|---|
| **1800s** | Industrialization and immigration<br>Advances in bacteriology, and understanding infectious disease and sanitation<br>Proliferation of Medical Schools<br>Schools of Public | Darwin wrote "On the Origin of Species by Means of Natural Selection, or the Preservation of Favored Races in the Struggle for Life"<br>Gregor Mendel's paper published |

| | | |
|---|---|---|
| | Health established | |
| **1900–40s** | Federal government's progressive period<br>Crusade for Children<br>National Foundation for Infantile Paralysis (March of Dimes) | Mendel's paper rediscovered by botanists looking for laws on inheritance<br>Johannsen coins the term "gene" to describe a unit of inheritance<br>Morgan discovers chromosomes carry genes |
| **1950s** | Population explosion<br>Migration from rural to urban communities<br>Several antibiotics discovered | DNA structure characterized and established as basis for heredity<br>46 human chromosomes determined, and Trisomy 21 discovered as cause of Down syndrome<br>Genetic counseling first described<br>Chromosomes visualized |
| **1960s** | President Johnson's "War on Poverty"<br>Heightened awareness for services to people with intellectual disabilities<br>Escalating healthcare costs | Development of Guthrie test for PKU<br>Advances in cytogenetics (staining methods) |

| | | |
|---|---|---|
| **1970s** | Focus on individual rights<br>Government corruption<br>Realization of limited resources<br>Advances in medical technology, newborn intensive care | Ultrasound and Amniocentesis available<br>Public Law 94-278 established a national genetic disease program (Tay Sachs, Beta-thal, and Sickle cell)<br>Restriction Fragment Linked Polymorphisms (RFLPs) described (Kan and Dozy) and DNA sequencing methods reported |
| **1980s** | Continued public distrust in government<br>Increased teen pregnancy and lack of services for the poor<br>AIDSInstitute of Medicine (IOM) report – Future of Public Health | Polymerase Chain Reaction (PCR) invented<br>First disease gene for Huntington's disease mapped to chromosome 4 |
| **1990s** | Courts hear cases for people with disabilities<br>Managed care expansion<br>"Downsizing" and reduced fiscal resources | Human Genome Project initiated<br>Successful cloning of Dolly the sheep<br>State and federal regulations regarding genetics proposed (e.g., bans on human cloning and genetic discrimination in the workplace) |

The HGP researchers had a fairly complete draft by early 2000. They successfully sequenced 90% of the human genome, which they

estimated to be 99.9% accurate. After this, the genetics/genomics floodgates opened as researchers and businesses alike began mining the information, reporting on new discoveries, and launching new genetic tests and new genetic services—not all of which had demonstrated clinical utility or validity. Besides the question of quality surrounding these services, a question regarding their distribution and therefore access to services was also raised.

Schools of public health and medicine have been separated for centuries, and many have argued that this "schism" between the fields results in a less integrated approach to health and healthcare which may affect both individuals and the population [31,32]. The two scientific disciplines of clinical genetics and public health genetics merged in the 1960s with the implementation of newborn screening, often viewed as one of public health's best successes. But aside from newborn screening programs, other public health genetics/genomics activities have been fairly limited despite some calls for greater action [33].

In the United States, Wang and Watts reviewed the state genetics plans from 19 states in 2007. At that time they found "that states' genetics programs do play an essential role in the provision of public health", primarily as they related to "mobilizing community partnerships, educating the public, linking people to needed services and ensuring a competent workforce" [34].

In the United States the Health Resources Services Administration and the CDC have proven to be drivers of the public health genetics/genomics agenda. The Health Resources Services Administration, through its Genetic Services Branch, awarded grants to several states to initiate genetic needs assessments and develop state genetics plans to address gaps identified regarding genetic healthcare services. It later also awarded states with implementation funds to initiate aspects of their state genetics plans—again, largely addressing access to genetic services.

The CDC in 1997 formed the Office of Public Health Genomics, which serves as a resource of genetics/genomics expertise for other CDC programs, other agencies, state health departments, and other

partners by "identifying, evaluating, and implementing evidence-based genomics practices to prevent and control the country's leading chronic, infectious, environmental, and occupational diseases" [35]. The CDC has hosted multiple interdisciplinary meetings and national conferences, and solicited broad community input in an effort not only to identify how best to determine which genetic/genomic applications are ready for widespread implementation, but also address what might be the expected outcomes, who should assist with implementation, what barriers may exist, and what policies may need to be developed for successful implementation [36].

In 2004 the Office of Public Health Genomics supported the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group. This nongovernmental, multidisciplinary independent group utilized a rigorous systematic review of emerging genomic applications and made recommendations based on the findings, either promoting more widespread uptake of services, such as Lynch syndrome screening protocols in 2009 [37], or discouraging routine use, such as factor V Leiden testing in recurrence of venous thrombosis in 2011 [38].

A crucially needed evidenced-based framework for prioritizing the role of human genomics in public health came from Khoury and colleagues in 2011. The framework takes into consideration multiple factors, including the clinical utility and clinical validity of the application along with the potential benefits and harms, and classifies the applications based on tiers. Tier 1 applications can be viewed as ready for implementation and should be promoted, while Tier 2 and Tier 3 applications require further information. Tier 2 applications typically lack evidenced-based guidelines, while Tier 3 applications may not have demonstrated clinical utility or validity and lack evidence-based guidelines. Such a tiered prioritization greatly assists public health organizations in better understanding how best to allocate their limited resources for maximum impact as it relates to genetics/genomics [39].

In the past decade two Tier 1 evidence-based genomic conditions in cancer genetics (hereditary breast ovarian cancer (HBOC) syndrome

and Lynch syndrome) have catalyzed public health action in cancer prevention at the federal and state levels. For HBOC, the US Preventive Services Task Force recommends that primary care providers screen women to identify a family history that may suggest HBOC. High-risk women should be referred for genetic counseling and discussion of genetic testing [18]. Possible clinical interventions include starting breast cancer screening earlier with mammography alone or in combination with breast magnetic resonance imaging, chemo-prevention medications, and risk-reducing surgery [18].

For Lynch syndrome, as mentioned earlier, the EGAPP working group recommends that persons with newly diagnosed colorectal cancer be offered genetic testing for Lynch syndrome to reduce morbidity and mortality in their close relatives. Individuals with Lynch syndrome can talk to their healthcare provider about starting screening for colorectal cancer at a younger age and screening more frequently than the general population [15].

The CDC's cancer program has focused on translating these recommendations into action and implementing them; activities include surveillance, epidemiology research, communication, and partnerships. Knowledge and resources for patients and healthcare providers are shared through the Know:BRCA clinical decision support tool [40] and the Bring Your Brave campaign [41]. Know:BRCA helps women and their providers understand their risk for *BRCA* mutations. Bring Your Brave is a digital media campaign that provides information about HBOC, the importance of receiving genetic counseling, and the usefulness of genetic testing to women, particularly those aged ≤45 years. The CDC Public Health Cancer Genomics Program gives funds to five state public health departments to build capacity for cancer genomics activities. Grantees implement activities that seek to educate the public and providers, monitor the burden associated with hereditary cancers, and improve access to care.

One example, the Michigan Cancer Genomics Program of the Michigan Department of Health and Human Services, seeks to reduce

morbidity and mortality related to hereditary cancers by increasing cancer genetic literacy among the public and healthcare providers, improving use of appropriate cancer risk assessment and clinical genetics services, enhancing communication, and developing partnerships with cancer genetics service providers and other stakeholders. The program collaborated with federal, state, and local partners to develop a free online continuing medical education course where participants can learn to use a variety of cases with different decision options, risks, and outcomes. Following their efforts, the number of persons receiving counseling for HBOC in Michigan increased from roughly 1200 in 2008 to nearly 3500 in 2013 [42].

## 14.5. Future Direction for Public Health Genetics/Genomics

Evidence-based public health (EBPH) arrived about the same time as evidence-based medicine (Fig. 14.3). The EBPH process is not linear, and there could be bidirectional arrows from many of the topics to others. Depending on the maturity of the issue it is also possible to start at different places, or as more information about an issue is learned, previous steps may be revisited and revised. But this model does offer a systematic approach that combines research and surveillance evidence with needed community input. While economic evaluation does not appear in the framework, it actually cuts across all the steps [43].

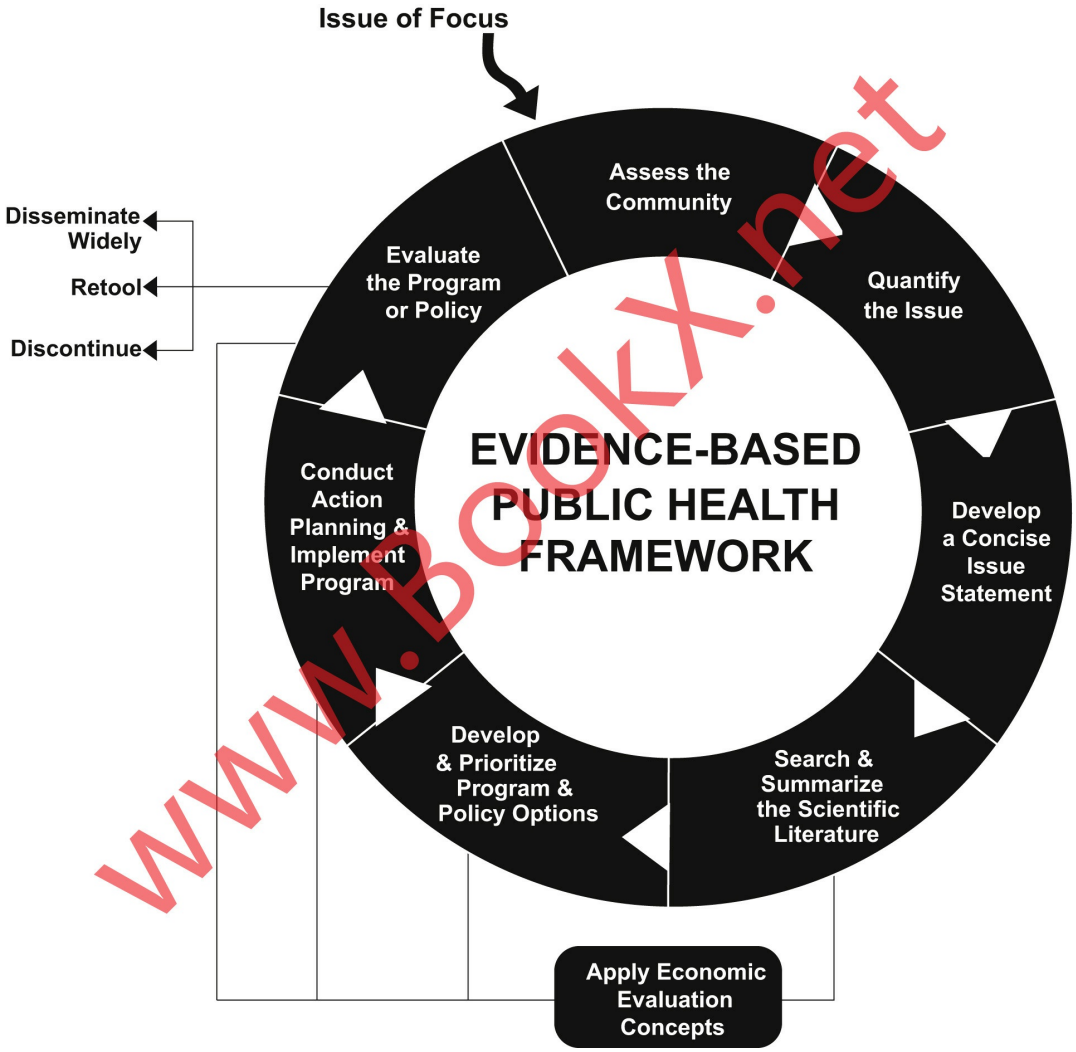



**FIGURE 14.3** Core Public Health Functions and 10 Essential Services.

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While it is relatively easy to apply the EBPH framework to public health genetics/genomics, defining appropriate outcomes for public health genetics/genomics continues to be extremely complex. It requires a program to define a target population and the expected outcomes. In addition, public health genomics interventions are typically part of a myriad events occurring simultaneously in a population. For example, in HBOC the objective may be to increase the number of women with a family history of breast cancer receiving genetic counseling. A measured increase could result from a combination of public health promotion efforts, sponsoring outreach cancer genetics clinics in rural areas, and simultaneously mobilizing

partnerships with patient and support organizations to provide outreach education. In this case, a well-known celebrity figure announcing her own experience [44] could also contribute to increased awareness and testing.

More recently, the National Academies of Sciences, Engineering, and Medicine convened a multidisciplinary group of experts to review the scientific literature for genetic tests, with the specific goal of creating a decision-making framework for use of such tests in clinical care. Their report, entitled "An Evidence Framework for Genetic Testing", was published in 2017 [45]. The group outlined seven evaluation steps.

1. Define genetic test scenarios on the basis of the clinical setting, the purpose of the test, the population, the outcomes of interest, and comparable alternative methods.
2. For each genetic test scenario, conduct an initial structured rapid assessment to determine whether the test should be used in practice or requires additional evaluation.
3. Conduct or support evidence-based systematic reviews for genetic test scenarios that require additional evaluation.
4. Conduct or support a structured decision process to produce clinical guidance for genetic test scenarios.
5. Publicly share resulting decisions and justification about evaluated genetic test scenarios, and retain decisions in a repository.
6. Implement timely review and revision of decisions on the basis of new data.
7. Identify gaps to be addressed by research. [46]

The report also depicts, in essence, a decision tree for moving through the evaluation process.

This evidence framework is an excellent first step in identifying appropriate genetic applications, but it does not cover the broader public health activities like informing, educating, and empowering individuals or policy development, for example, that will be important in the overall implementation to ensure broad access and

utilization of these applications to improve the public's health. For this reason, the broader EBPH framework will still be necessary to ensure maximum health benefit.

In 2007 Khoury et al., argued that a "strong medicine–public health partnership in the genomics era is needed for the translation of all scientific discoveries for the benefit of population health" [31]. Specifically they point to overlapping areas of interest, including "(1) a joint focus on prevention—a traditional public health concern but now a promise of genomics in the realm of individualized primary prevention and early detection, (2) a population perspective, which requires a large amount of population-level data to validate gene discoveries for clinical applications, (3) commitment to evidenced-based knowledge integration with thousands of potential genomic applications in practice, and (4) emphasis on health services research to evaluate outcomes, costs, and benefits in the real world" [31]. Such partnerships will be essential not only due to limited resources but also to maximize the potential cross-cutting benefits.

There has been much discussion over recent years about expanding population-based screening to include *BRCA1* and *BRCA2* testing of women age 30 and older [47], or even perhaps of all adults for Lynch syndrome or other highly penetrant cancer predisposition genes and possibly familial hypercholesterolemia—all currently Tier 1 genes [48]. But how would such population-based screening realistically be assimilated into either the clinical arena or the public health system?

The notion that an executive-level, mid-level, or even local-level public health entity will expand its public health genetics/genomics activities, even newborn screening, to the degree that all the purposes of public health and inherent population-based genetic/genomic problems are addressed within each is unlikely, largely due to limited resources—both human and fiscal. But collaborations and partnerships between "learning healthcare systems" that continuously use data to improve process and outcomes and public health genetics/genomics staff who can help support, influence, or guide healthcare system efforts based on Tier 1 applications, combined with implementation science to study the optimal integration of effective

diagnosis, prevention, and treatment of these Tier 1 conditions, is a more feasible model. Learning healthcare systems are designed to improve patient care over time by using continuous quality improvement techniques while integrating scientific or evidenced-based approaches at the point of patient care [49]. Here, the "population" is everyone served within a health system. Partnering with multiple healthcare systems would lead to an even greater population impact.

It is worth noting that communities exploring innovative ways to reduce healthcare costs and improve care and quality may also be helping to build bridges between medicine and public health and creating meaningful partnerships. For example, in 2011 the Prevention Institute described the Community-Centered Health Home: a three-step process of inquiry, analysis, and action whereby community, clinical, and public health data are used to articulate the social and environmental conditions causing the greatest impact on health outcomes, and how to implement strategies to improve health outcomes at a population level. Another example is the Accountable Care Community—a collaboration between health providers (medicine), community-based organizations, and governmental agencies including public health with the goal of improving the health of the population. It encompasses the healthcare delivery system, the public health system, and the social services delivery system in the hopes that collectively they can affect the entire spectrum of determinants of health [50,51]. How genetics/genomics applications will fit within these community-based models is yet to be defined. But public health genetics/genomics practitioners will be key to promoting the adoption of genetics/genomics applications that evidence has shown clearly have a positive impact on health and health outcomes within such models.

The past two decades have laid the foundation for the emergence of "precision medicine" [52]. Precision medicine is much more than genomics. It takes into account individual differences in people's genes, environments, and lifestyles, allowing the design of targeted disease interventions for both treatment and prevention [52]. The US

Precision Medicine Initiative, launched in 2015, promises a new era of biomedical and health research and its application in healthcare and disease prevention. The initiative is enabled by rapid advances in biomedical sciences, including genomics and bioinformatics, as well as the progress in communication and information technologies and data science. Using a national cohort of at least 1 million people [25], researchers will explore genetic and environmental determinants of health and disease. This information will go a long way to teach us how to scale up from 1 million people to 300 million people in the United States, an ultimate goal of precision medicine at the population level. As recently discussed [53], the initiative will require a public health perspective to help ensure generalizability of findings, assess methods of implementation, focus on prevention, and provide an appropriate balance between generation of long-term knowledge and short-term return on investments in health gains. While skepticism still runs high in the public health community about the value of genomics and precision medicine to population health [54], there is a path forward for using evidence-based approaches to emerging genome-based and other large-scale information (social, environmental) to understand determinants of population health better and target interventions that can improve health outcomes in subpopulations. This could be the beginning of the field of "precision public health" [55].

In summary, public health genetics/genomics is an emerging scientific discipline that supplements medical genetics. Its focus is not on the individual or the family, but on improving the health of populations. Like the field of genetics/genomics, it is evolving rapidly and complements the medical genetics field by way of evaluating the impact of using genetics/genomics applications in populations.

## References

[1] Institute of Medicine. *The future of public health*. Washington, DC: The National Academies Press; 1988 doi: 10.17226/1091.

[2] Kindig D, Stoddart G. *Am J Publ Health*. 2003;93(3):380–383.
[3] Khoury M.J. Public health genomics: the end of the beginning. *Genet Med*. 2011;13(3):206–209.
[4] Welcome Trust Sanger Institute Bacterial Genomes – data download. http://www.sanger.ac.uk/resources/downloads/bacter
[5] Gwinn M, MacCannell D.R, Khabbaz R.F. Integrating advanced molecular technologies into public health. *J Clin Microbiol*. 2017;55(3):703–714. .
[6] Grad Y.H, Lipstitch M. Epidemiologic data and pathogen genome sequences: a powerful synergy for public health. *Genome Biol*. 2014;15:538. http://genomebiology.com/2014/15/1
[7] *Battelle, Economic Impact of the Human Genome Project: how a $3.8 billion investment drove $796 billion in economic impact, created 310,000 jobs and launched the genomic revolution*. Battelle Memorial Institute; 2011. https://www.genome.gov/27544383/ca the-economic-impact-of-the-human-genome-project/.
[8] U.S. Department of Energy. Genomic Science Program. Accessed online at: http://genomicscience.energy.gov/#page=news.
[9] *Federal Trade Commission at-home genetic tests: a healthy dose of skepticism may be the best prescription*. 2006. https://permanent.access.gpo.gov/lp
[10] Goddard K.A.B, Duquette D, Zlot A, Johnson J, Annis Emeott A, Lee P.W, Bland M.P, Edwards K.L, Oehlke awareness and use of direct-to-consumer genetic

tests: results from 3 state population-based surveys, 2006. *Am J Publ Health*. 2009;99(3):442–445.

[11] Green R.C, Roberts J.S, Cupples L.A, Relkin N.R, Whi Deegan R, Farrer L.A. Disclosure of APOW genotype for risk of Alzheimer's disease. *N Engl J Med*. 2009;361:245–254.

[12] Miller C.E, Krautscheid P, Baldwin E.E, Tvrdik T, Ope counselor review of genetic test orders in a reference laboratory reduces unnecessary testing. *Am J Med Genet*. 2014;164A:1094–1101.

[13] Dickerson J.A, Cole B, Conta J.H, Wellner M, Wallace the value of costly genetic reference laboratory testing with active utilization management. *Arch Pathol Lab Med*. 2014;138(1):110–113.

[14] Mathias P.C, Conta J.H, Konnick E.Q, Sternan D.L, Sta genetic testing order errors with a laboratory utilization management program. *Am J Clin Pathol*. 2016;146(2):221–226.

[15] Berg A.O, Baird M.A, Botkin J.R, et al. National Institutes of health state-of-the-science conference statement: family history and improving health. *Ann Intern Med*. 2009;151:872–877.

[16] Pyeritz R.E. The family history: the first genetic test, and still useful after all those years? *Genet Med*. 2012;14(1):3–9.

[17] Acheson L.S, Wiesner G.L, Zyzanski S.J, Goodwin M.

*Med*. 2000;2:180–185.
[18] U.S. Preventive Services Task Force; September 2017. Accessed online at: https://www.uspreventiveservicestaskforce.org/Page/related-cancer-risk-assessment-genetic-counseling-and-genetic-testing#Pod1.
[19] Biesecker L.G, Bailey-Wilson J.E, Ballantyne J, Baum H, Bieber F.R, Brenner identifications after the 9/11 World Trade Center attacks. *Science*. 2005;310:1122–1123.
[20] Dolan S.M, Saaraiya D.S, Donkervoort S, Rogel K, Liel emerging role of genetics professionals in forensic kinship DNA identification after a mass fatality: lessons learned from Hurricane Katrina volunteers. *Genet Med*. 2009;11:414–417.
[21] Doyle D.L, Awwad R.I, Austin J.C, Baty B.J, Bergner A review and update of the genetic counseling practice based competencies by a task force of the accreditation council for genetic counseling. *J Genet Counsel*. 2016;25(5):868–879.
[22] Centers for Disease Control and Prevention; 10 Essential Public Health Services. www.google.com/search?q=10+essential+services&gws_rd=ssl.
[23] Beskow L.M, Khoury M.J, Baker T.G, Thrasher J.F. Th integration of genomics into public health research, policy and practice in the United States. *Community Genet*. 2001;1(4):2–11.

[24] McWalter K, Gaviglio A. Introduction to the special issue: public health genetics and genomics. *J Genet Counsel*. 2015;24:375–380.
[25] *National Institutes of health; all of us*. 2017. https://allofus.nih.gov/.
[26] Advances in genetics timeline from the National Institutes of Health. https://www.genome.gov/pages/education/genetictim
[27] Advances in public health from the World Health Organization Timeline. Last accessed June 23, 201. http://www.who.int/features/history/WHO_60th_ann
[28] Proceedings of symposium on human genetics in public health (University of Minnesota, August 9—11, 1964). https://mchb.hrsa.gov/about/timeline/in-depth-6-genetic-services.asp#Brief-History.
[29] Winter J.A. The development of the disabilities Rights movement as a social problem solver. *Disabil Stud Q*. 2003;23(1):33–61. .
[30] Fineman R.M, Doyle D.L. Public health needs assessment for state-based genetic services delivery. In: Khoury M.J, Burke W, Thomson E.J, eds. *and public health in the 21st century, Oxford monographs on medical genetics No. 40*. Oxford University Press; 2000.
[31] Khoury M.J, Gwinn M, Burke W, Bowen S, Zimmern I genomics widen or help heal the schism between medicine and public health? *Am J Prev Med*. 2007;33(4):310–317.
[32] Ruis A.R, Golden R.N. The schism between medical and public health education: a historical

perspective. *Acad Med*. 2008;83(12):1153–1157.
[33] Bowen M.S, Kolor K, Dotson W.D, Ned R.M, Khoury
health action in genomics is now needed beyond newborn screening. *Public Health Genom*. 2012;15(6):327–334.
[34] Wang G, Watts C. The role of genetics in the provision of essential public health services. *Am J Public Health*. 2007;97(4):620–625.
[35] Centers for Disease Control and Prevention. Office of Public Health Genomics, https://www.cdc.gov/genomics/.
[36] *2012–2017 priorities for public health genomics: stakeholder consultation-priorities conference report*. Center for Public Health and Community Genomics And Genetic Alliance; September 2011.
[37] Berg A.O, Armstrong K, Botkin J, Calogne N, Haddov
from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*. 2009;11(1):35–41.
[38] Berg A.O, Botkin J, Calogne N, Campos-Outcalt D, Haddow J.E, Kaye C, Klein R.D, Offit K, Pa
from the EGAPP Working Group: routine testing for Factor V Leiden (R506Q) and prothrombin (20210G>A) mutations in adults with a history of idiopathic venous thromboembolism and their adult family members. *Genet Med*. 2011;13(1):67–76.
[39]

Khoury M.J, Bowen M.S, Burke W, Coates R.J, Dowlir Pierre J. Current priorities for public health practice in addressing the role of human genetics in improving population health. *Am J Prev Med*. 2011;40:486–493.

[40] Centers for disease control and prevention; KnowBRCA www.knowbrca.org/.

[41] Centers for Disease Control and Prevention; Cancer. www.cdc.gov/cancer/breast/young_women/bringyou

[42] Rodriguez J.L, Thomas C.C, Massetti G.M, Duquette I Grand rounds: family history and genomics as tools for cancer prevention and control. *Morb Mortal Wkly Rep*. November 25, 2016;65(46):1291–1294.

[43] Brownson R.C, Baker E.A, Deshpande A.D, Gillespie I *based public health*. 3rd ed. New York: Oxford University Press; 2018.

[44] Jolie A. *My medical Choice, May 14th opinion page*. The NY Times; 2013.

[45] National Academies of Sciences, Engineering, and Medicine. *An evidence framework for genetic testing*. Washington, DC: The National Academies Press; 2017.

[46] Khoury M.J. No shortcuts on the long Road o evidence-based genomic medicine. *J Am Med Assoc*. 2017:1–1099 [published online (Jama.com) June 1, 2017].

[47] King M.C, Levy-Lahad E, Lahad A. Population-based screening for BRCA1 and BRCA2, 2014 Lasker Award. *J Am Med Assoc*. 2014;312(11):1091–1092.

[48] Evans J.P, Berg J.S, Olshan A.F, Magnuson T, Rimer B screen newborns, don't we?: realizing the promise of public health genomics. *Genet Med*. 2013;15(5):332–334.

[49] Chambers D.A, Feero W.G, Khoury M.J. Convergence of implementation science, precision medicine, and learning health care system a new model for biomedical research. *J Am Med Assoc*. 2016;315(18):1941–1942.

[50] Cantor J, Mikkelsen L, Simons B, Waters R. How can we pay for a healthy population. Oakland, CA 94607: Prevention Institute; 2013. https://www.preventioninstitute.org/si

[51] DeSalvo K.B, O'Carroll P.W, Koo D, Auerbach J.M, M health 3.0: time for an upgrade. *Am J Public Health*. 2016;106(4):621–622.

[52] Collins F.S, Varmus H. A new initiative on precision medicine. *N Engl J Med*. 2015;372(9):793–795.

[53] Khoury M.J, Evans J.P. A public health perspective on a national precision medicine cohort: balancing long-term knowledge generation with early health benefit. *J Am Med Assoc*. 2015;313(21):2117–2118.

[54] Khoury M.J, Galea S. Will precision medicine improve population health? *J Am Med Assoc*. 2016;316(13):1357–1358.

[55] Khoury M.J, Iademarco M.F, Riley W.T. Precision public health for the Era of precision medicine. *Am J Prev Med*. 2016;50(3):398–401.

15

# Implementation of Genomic Medicine

## An International Perspective

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## Abstract

In the "omics" era, incorporation of genomic medicine into clinical practice is greatly anticipated following recent scientific and technological advances. Currently a multidisciplinary agenda that involves government officials, funding agencies, industry leadership, healthcare providers, biomedicine researchers, and the general public is of outmost importance to "translate" human discoveries into human health. But although groundbreaking scientific findings are being revealed, the clinical application of genomic medicine and its acceptance in healthcare cannot be envisaged without sound science and evidence-based facts. A series of challenges exist from both scientific and policy perspectives at a global level. This chapter presents an overview of the current genomic medicine initiatives, both national and worldwide, which once successfully completed will help to

expedite implementation of genomic medicine practices into the clinic.

## Keywords

Developing countries; Genomic medicine; Global large-scale sequencing efforts; Implementation; Worldwide initiatives

## 15.1. Introduction

In the omics era, great scientific and technological advances are anticipated to promote genomic medicine, which constitutes a key component of personalized medicine and tailor-made healthcare. It is now possible to examine a person's genome sequence and define gene expression patterns to make individualized risk predictions and treatment decisions [1]. Health and disease states can now be more precisely characterized via their molecular fingerprints toward patient stratification and disease mechanism elucidation on the basis of genome-wide data. Indeed, hundreds of thousands of genetic variants and their association with disease are being evaluated [2,3]. Omics data is accelerating biomarker discovery [4]. The use of genetic tests is increasing, both in the clinic and for research purposes, at a pace that sometimes raises public concerns [5,6]. Notwithstanding, some human genome discoveries have resulted in evidence-based applications giving health benefits at a population level [7]. The current challenges highlight the role of a multidisciplinary agenda set by enhanced collaboration among government officials, funding agencies, industry leadership, healthcare providers, and biomedical scientists [1,8,9]. Challenges are more profound for developing countries, especially as genomic medicine can be the basis of knowledge-based economies [10–12].

To date, there are several national and international large-scale genomic medicine initiatives aiming to spearhead implementation of genomics into healthcare. These initiatives are often multidisciplinary in nature, not only aiming to elucidate the genetic basis of inherited diseases in certain populations but also approaching genomic

medicine from a more holistic perspective, often dealing with practical issues that are of paramount importance for the implementation of practices such as genome informatics, ethical, legal, societal, and economics issues, reimbursement policies, and so on. This chapter provides an overview of the existing genomic medicine initiatives, both national and worldwide, in an attempt to develop core competences toward a global framework for genomic healthcare.

## 15.2. Large-Scale Genomic Medicine Initiatives

In the context of a global framework of equity for genomics medicine, the existing initiatives aim to strengthen research infrastructure and intellectual property regimes, address healthcare needs, and reduce healthcare costs. How countries choose to capture the benefits of genomics medicine depends on the type of entry points they select [10]. Entry points are dependent on a country's health infrastructure and research status, hence countries that have already embarked on major genomic medicine initiatives will focus on the establishment of state-of-the-art genomics research, data transfer to healthcare applications, and financial benefits. In contrast, for countries that are still devoid of such initiatives, a definition of the entry points is a major concern. In the developing world, in the presence or absence of genomics medicine initiatives, the fundamental question refers to the challenges (and opportunities) that delineate the adoption of genomic medicine and its economic benefit [12]. Considering the nationwide and global demands for implementation of genomic medicine, their interdependent nature becomes evident. Table 15.1 summarizes the major international organizations involved in these efforts.

## 15.3. National Genomic Medicine Initiatives

To date, a number of countries have adopted a nationwide program to analyze the genomes of a large number of individuals, with the goal of implementing genomic medicine interventions in their healthcare systems (Table 15.2).

The Precision Medicine Initiative (PMI) was announced on January

20, 2015 by US President Barack Obama. The PMI is an ambitious research project funded by the National Institutes of Health (NIH), and aims to establish a cohort of 1 million volunteers from diverse population age groups, environments, and societal backgrounds in the United States. Adopting an interactive research approach, participants will be given access to their own results and a summary of all the data the project accrues. The immense size of the cohort, in combination with the genetic, environmental, and lifestyle information available, will accelerate biomedical discoveries and potentially revolutionize how we approach health and treat diseases.

Genome Canada, a nonprofit organization funded by the government of Canada, launched in 2012 the GAPH–GE3LS Initiative (Genomics and Personalized Health—Genomics and Its Ethical, Environmental, Economic, Legal and Social Aspects), comprising a network of 17 different projects in the field of genomics medicine (https://www.genomecanada.ca/en/ge3ls-network-genomics-and-personalized-health). Its aim is to bring these projects together to share best practices, promote collaborative research, and bring GAPH technologies closer to practical application. The network's main topics of discussion are research ethics review; health economics and health technology assessment; knowledge transfer and implementation in health systems for "omics" technologies; and intellectual property and commercialization.

## Table 15.1

**Summary of Major International Organizations, Consortia, and Projects Involved in the Field of Genomic and Precision Medicine**

| Organization | Type | Activities Description | Region |
|---|---|---|---|
| Organizations and Research Networks | | | |
| **European Alliance for Personalized Medicine** | Policy and advocacy group | Aims to position personalized medicine within the broader context of personalized | Europe |

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| | | healthcare, promote understanding of personalized healthcare by patients, professionals, and policymakers, catalyze the shaping of EU policy discussions, introduce incentives for personalized healthcare, and create a favorable reimbursement environment for personalized medicines and companion diagnostics | |
| **European Personalized Medicine Association** | Nonprofit organization | Aims to make personalized medicine a practical reality in Europe by focusing on patients' access to innovations in this area, particularly with respect to codependent drug-companion diagnostic technologies | Europe |
| **European Society for Predictive Medicine** | Scientific society | Aims to promote predictive medicine among healthcare professionals and engage in educational activities to promote the concept of predictive medicine | Europe |
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|---|---|---|---|
| **Personalized Medicine Coalition** | Policy and advocacy group | Represents a broad spectrum of more than 225 innovator, academic, industry, patient, provider, and customer communities; aims to educate both the general public and policymakers by promoting new ways of thinking about healthcare and advancing understanding and adoption of personalized medicine and its products for the benefit of patients | United States |

Table Continued

| Organization | Type | Activities Description | Regi |
|---|---|---|---|
| **Global Genomic Medicine Collaborative** | Nonprofit organization | Action collaborative among global leaders in implementation of genomic medicine in clinical care; a US nonprofit organization, its purpose is to identify opportunities and foster global collaborations to demonstrate value and effective use of genomics in medicine; engaging multiple | Inter |

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| | | stakeholders across the globe, it seeks to improve global health by catalyzing the implementation of genomic tools and knowledge into healthcare delivery globally | |
| **Genomic Medicine Alliance** | Research network | International research network with activities that focus on genomic medicine; under the coordination of the UK Golden Helix Foundation (www.goldenhelix.org), it aims to create collaboration ties between academics, researchers, regulators, and the general public interested in all aspects of genomics and personalized medicine toward advancing the genomic medicine discipline, focusing in particular on translating results from academic research into clinical practice | Inter |
| **South East Asian Pharmacogenomics Research Network** | Research network | Aims to strengthen mutual cooperation and facilitate sustainable | Soutl A |

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| | | and equitable growth of pharmacogenomics community, to promote further cooperation and linkage through seminars, exhibitions, workshops, technical assistance, scholarship schemes, training, and joint R&D projects, and where possible to exchange information, experience, know-how, technology, and best practice in the field of pharmacogenomics | |
| **Global Alliance for Genomics and Health** | Research network | Aims to accelerate progress in human health by helping to establish a common framework of harmonized approaches to enable effective and responsible sharing of genomic and clinical data, and by catalyzing projects that drive data sharing and demonstrate its value | Inter |
| **International Cancer Genome Consortium** | Research network | Goals are to generate comprehensive catalogs of genomic abnormalities in 50 different cancer types and/or subtypes which | Inter |

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|---|---|---|---|
| | | are of clinical and societal importance across the globe, and make the data available to the entire research community as rapidly as possible, with minimal restrictions, to accelerate research into causes and control of cancer | |
| Collaborative Projects and Consortia | | | |
| **PerMed** | European project | Aims to complement existing activities by identifying and promoting promising research topics, developing strategic research and innovation agendas in Europe and beyond, and bringing forward the implementation of personalized medicine for the benefit of society at large | Euro |
| **Lung Genomics Research Consortium** | Research consortium | Consists of top scientists from five top US institutions, who aspire to advance understanding of chronic lung diseases such as chronic obstructive pulmonary | Unite St |

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| | | disease and interstitial lung disease with the help the advanced genomic technologies that have been developed in recent years | |
| **Evidence-Based Network for the Interpretation of Germline Mutant Alleles** | Research consortium | Focuses on suspected breast cancer genes such as *BCRA1* and *BRCA2*, and the clinical significance of sequence variants located in these areas; also works on ways of communicating information to patients and providers | Inter |

Table Co

| Organization | Type | Activities Description | Region |
|---|---|---|---|
| **Exome Aggregation Consortium (ExAC)** | Research consortium | Coalition working on harmonizing and aggregating the incredible amount of exome sequence data being generated around the world; summary data is available at ExAC Browser, which is still in beta | International |

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|---|---|---|---|
| **Psychiatric Genomics Consortium** | Research consortium | Conducts mega-analyses of genome-wide genetic data for psychiatric diseases; with more than 800 investigators from over 38 countries and samples of more than 900,000 individuals analyzed, this is one of the largest consortia in the history of psychiatry | International |
| **Medulloblastoma Advanced Genomics International Consortium** | Research consortium | Through genomic analyses of pediatric medulloblastoma samples, the consortium targets the genes and pathways of the disease, as well as specific biomarkers that can be used for diagnostic purposes | International |
| **International Stroke Genetics Consortium** | Research consortium | With more than 200 members from over 50 countries, | International |

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| | | the consortium is committed to extensive research on the genetic basis of stroke | |
| **International Parkinson's Disease Genomics Consortium** | Research consortium | Strives to increase the understanding of the genetic basis of the disease by identifying the major risk factors involved; newer research of the consortium is targeted at biomarker identification, risk prediction, disease subtyping, and molecular basis of disease | International |
| **International Multiple Sclerosis Genetics Consortium** | Research consortium | Focuses on identification of genes that determine predisposition to multiple sclerosis, mainly through genome-wide association studies | International |

## Table 15.2

### Outline of National Genomic Medicine Initiatives

| Project/Initiative | Country | URL |
|---|---|---|
| Americas | | |
| **Precision Medicine Initiative** | United States | https://www.nih.gov/research-training/allofus-research-program |
| **Genome Canada** | Canada | https://www.genomecanada.ca |
| **EPIGEN Brazil Initiative** | Brazil | https://epigen.grude.ufmg.br |
| Asia and Middle East | | |
| **Chinese Precision Medicine Initiative** | People's Republic of China | http://english.big.cas.cn |
| **Japanese Reference Genome** | Japan | http://www.megabank.tohoku.ac.jp/engli |
| **Qatar Genome Project** | Qatar | http://www.qatarbiobank.org.qa/home |
| **Saudi Human Genome Project** | Saudi Arabia | http://shgp.kacst.edu.sa/site |
| Europe | | |
| **Genomics England** | United Kingdom | http://www.genomicsengland.co.uk/abou genomics-england |
| **Danish** | Denmark | http://www.genomedenmark.dk/english |

| | | |
|---|---|---|
| **Reference Genome Project** | | |
| **Genome of the Netherlands** | The Netherlands | www.nlgenome.nl |
| **Belgian Medical Genomics Initiative** | Belgium | http://www.bemgi.be/about-bemgi |
| **Genomic Medicine France 2025** | France | https://www.aviesan.fr/en |
| **Estonian Genome Project** | Estonia | http://www.geenivaramu.ee/en |
| **Scottish Genomes Partnership** | Scotland | http://www.scottishgenomespartnership |
| **Genome of Greece** | Greece | http://www.gogreece.org.gr |

Genomics England was announced in late 2012 by the UK Prime Minister. Like the PMI, this is an ambitious project aiming to sequence 100,000 whole genomes from NHS patients and their families, focusing on rare diseases as well as common cancers. For this, Genomics England, a wholly owned company of the Department of Health, was established by Jeremy Hunt, Secretary of State for Health, as part of the NHS 65th birthday celebration on July 5, 2013. This initiative is planned to confront and solve current challenges, such as electronic healthcare records and genomic data ownership and handling, and at the same kick-start the development of a sustainable UK genomics industry, enabling new discoveries and medical insights.

Similarly, in January 2016 the Chinese Academy of Sciences

(http://english.cas.cn/) announced a precision medicine research plan which is the first step toward the implementation of China's 15-year multibillion dollar Chinese PMI. The project is coordinated by the Beijing Institute of Genomics, with researchers initially gathering genetic information from 4000 volunteers in the first 4 years. Comprehensive research involving whole genome sequencing analysis, genome sequence health profiles, and early warning and intervention for key chronic diseases, cancer, and diabetes will target 2000 of the volunteers. These genome health profiles will be the basis for this initiative, which will cover the entire Chinese population. This massive sequencing task will be undertaken in large part by three genomics firms that rely on Illumina's HiSeq X-Ten system: WuXi PharmaTech, CloudHealth, and Novogene. WuXi NextCODE, which provided the databases for both Genomics England and the Qatar Genome Project (see below), is also involved in China's national genome project, along with Huawei Technologies, which will develop the cloud infrastructure to store and handle the mass of genomic data associated with the Chinese PMI. The Chinese government is entrusting the establishment of China's National Genebank to BGI, a company with its own sequencing technology and database.

In Japan the Tohoku University Tohoku Medical Megabank Organization was established after the Great East Japan Earthquake to promote industry–academic partnerships, advance genomic research in the area, and eventually construct the Japanese Reference Genome. At the moment it is developing a biobank while providing whole genome sequencing of the participants in its cohort studies. By June 21, 2016 a total of 2049 participants had their whole genome sequenced, with the datasets of all the Single Nucleotide Variations (SNVs) available to researchers through the integrative Japanese Genome Variation Database.

Following the paradigm of United Kingdom, the Qatar Genome Project aims to sequence 350,000 genomes from the Qatari population and integrate that data into Qatar's national health service electronic medical records. In October 2015, the Sidra Medical and Research

Center, the genomics and informatics hub undertaking this task, announced that WuXi NextCODE will be providing the entire infrastructure for this project. In this context, Qatar Biobank, a center within Qatar Foundation, has been established in collaboration with Hamad Medical Corporation and Supreme Council of Health and the expert support of scientists from Imperial College London. Through sample collection and genomics, metabolomics, and pharmacogenomics information on health and lifestyle of the Qatari population, Qatar Biobank is expected to promote research outcomes, both nationally and globally. The joint forces of the Qatar Genome Project and Qatar Biobank promise a 15-year follow-up to enhance genotype to phenotype correlation.

In Saudi Arabia, the Saudi Human Genome Project aims to resolve the genetic basis of inherited diseases in the kingdom, lay the foundations for the development of personalized medicine, and establish capacity for the genomics industry. This 3-year project focuses on the analysis of 20,000 genomes to identify causative genes for the genetic diseases that impact the Saudi population. The Saudi Human Genome Project is funded and organized by the King Abdulaziz City for Science and Technology (Saudi Arabia's national funding agency), and involves the creation of a national network of genome centers [13]. In partnership with Thermo Fisher Scientific, this project also leverages the Ion Proton DNA sequencer and Ion AmpliSeq inherited disease gene panels, with the goal of sequencing 100,000 human genomes through a national network of genome screening centers to investigate both normal and disease-associated genes specific to the Saudi population. In 2015 clinical researchers from the Saudi Human Genome Program presented data that implies the potential to diagnose inherited diseases within a single day, employing 13 gene panels for all known Mendelian rare diseases. The findings were shared at the Personalized Medicine Conference 2015 in Riyadh, and represent a first major national effort at this scale (http://news.thermofisher.com/press-release/life-technologies/research-scientists-release-initial-data-saudi-human-genome-program).

The Danish Reference Genome Project aims to establish a Danish high-quality reference genome derived from the genomes of 150 healthy Danes of strict Danish descent. For this, GenomeDenmark has been established as a consortium of four Danish universities, two hospitals (Herlev and Vendsyssel), and two private firms (Bavarian Nordic and BGI-Europe) to provide the research infrastructure required. This is an excellent paradigm of public–private partnership.

The Genome of the Netherlands Consortium is one of the projects within Biobanking and Biomolecular Research Infrastructure–Netherlands (BBMRI–NL, http://www.bbmri.nl), a research infrastructure funded by the national government of the Netherlands. It is a whole genome sequencing project initiated in 2010 with the aim to sequence 1000 independent genomes from the Dutch population. For this, 250 representative parent-pairs (two parents and one offspring) of Dutch ancestry were chosen from five population-based biobanks (LifeLines Cohort Study, Leiden Longevity Study, Netherlands Twin Registry, Rotterdam Study, and Rucphen Study). The trio design provided information on four genomes (two per parent) capable of establishing an accurately phased haplotype Dutch reference genome as well as creating an imputation pipeline on the European grid network for cohorts taking part in BBMRI–NL. All sequencing was performed by BGI on the Illumina HiSeq 2000 platform, and samples were additionally genotyped on the Illumina Immunochip and at least one other genotyping chip. All data has been deposited at the European Genome–Phenome Archive [14,15]. Additional projects within BBMRI–NL aim to further enrich Dutch biobanks with epigenetic studies, RNA sequencing, and metabolomics studies.

The Belgian Medical Genomics Initiative (BeMGI) aims to establish a vibrant network devoted to medical genomics. It is designed to boost individual research efforts by promoting collaborative exploitation of advanced genomic tools; develop strategies to predict clinical outcomes from genomic information and integrate genomic information in clinical care in Belgium at a pilot level; educate future genomics researchers and medical practitioners; and conduct public

outreach. BeMGI is funded by the phase VII Interuniversity Attraction Poles program of the Belgian Federal Science Policy Office, and comprises top Belgian scientists active in the field of human genetics as well as international partners.

In June 2016 the French government announced plans to invest €670 million in the construction of a medical and industrial system to introduce individualized therapy into the care pathway and develop a national framework for the achievement of this goal. Genomic Medicine France 2025 will be produced by the National Alliance for Life Sciences and Health (Aviesan), while the implementation of the program will be monitored by an interministerial strategic committee. The 10-year French plan, which has the ambitious goal of delivering 235,000 genomes a year by 2020, will initially concentrate its efforts on cancer, diabetes, and rare diseases and then branch out to common diseases. Genomic Medicine France 2025, unlike the UK 100,000 Genomes Project, is proposing the establishment of 12 different sequencing centers throughout the country, with two separate national genomic medicine centers as sites of genomic expertise in clinical data analysis and interpretation. The French project is structured by three targets: to position France among the leading countries in genomic medicine, to prepare for the integration of precision medicine into healthcare, and to establish a national genomics medicine industry that will drive scientific and technological innovation as well as economic growth.

Upon its initiation in 2001, the Estonian Genome Project (EGP) aimed to create a database of health, genealogical, and genetic data representing approximately 10% of Estonia's population [16]. Today the EGP gene bank contains data contributed by over 51,000 donors, with an expected increase to 100,000 within the next 2 or 3 years. Notably, this nationwide genomic medicine initiative set the technological means for Estonia to be considered as an e-country since 2009, supporting several e-health services as well as global collaboration schemes.

In January 2015 the Scottish Genomes Partnership was announced,

consisting of the Universities of Edinburgh and Glasgow in partnership with Illumina. Additional funding in January 2016 by Scotland's Chief Scientist Office and the UK Medical Research Council enables sequencing the genomes of over 3000 Scottish people. The Scottish Genomes Partnership will initially focus on rapid screening of cancer patients; diagnosing childhood illnesses and disorders of the central nervous system; and population studies. Currently, a cancer biobank has been established.

Lastly, the EPIGEN Brazil Initiative is considered the most comprehensive up-to-date genomic study of any Latin American population, consisting of a population-based genome-wide analysis of 6487 individuals. Findings, in the context of worldwide genomic diversity, revealed how ancestry, kinship, and inbreeding interact in three populations from the northeast (African ancestry 50%), southeast, and south (both with European ancestry >70%) of Brazil [17]. In another study supported by the EPIGEN Brazil Initiative it was reported that ethnoracial self-classification is affected by both genome ancestry and nonbiological factors [18].

## 15.4. Large-Scale Regional Genome Initiatives

The 1000 Genomes Project (http://www.internationalgenome.org) remains one of the most comprehensive genomics initiatives to date; its successor is the International Genome Sample Resource. Concluded in 2015, it was truly a global effort that conducted sequencing of individuals from 26 populations in Africa, East Asia, Europe, South Asia, and the Americas. Using a combination of low-coverage whole genome sequencing, deep exome sequencing, and dense microarray genotyping, 2054 genomes were reconstructed by the end of the project [19].

African populations have the highest level of diversity in their genome; along with Asian populations, they remain consistently understudied [20]. For this reason, a welcome effort is the Human Heredity and Health in Africa (H3Africa) initiative (http://h3africa.org), which was launched in 2010. The US National Human Genome Research Institute, the NIH Common Fund, the

Alliance for Accelerating Excellence in Africa, and the UK's Wellcome Trust Sanger Institute in partnership with the African Society of Human Genetics (http://www.afshg.org) introduced H3Africa, which supports African scientists conducting research on the genetic and environmental factors of chronic and infectious diseases in Africa. The program is funded for 5 years or more by these organizations and other local sources (e.g., South African Department of Science and Technology), and consists of 25 projects in 27 African countries. These include biomedical and ethical research projects, training programs, a bioinformatics network (H3ABioNet), and three biorepositories in Nigeria, Uganda, and South Africa that will ultimately house samples from 75,000 individuals (The H3 Consortium [21]).

Even though Asian populations represent 40% of all humanity, they remain vastly understudied compared to others. For this reason, GenomeAsia 100K (http://www.genomeasia100k.com), a nonprofit multinational consortium of companies, academics, and other stakeholders in the field of genetics, aims to generate genomic information for Asian populations, promote genetic understanding to support research and discovery, and pave the way for tailor-made healthcare. As the name suggests, the project intends to sequence 100,000 individuals from 12 South Asian countries and at least seven North and East Asian countries. The first phase of the program will focus on creating reference genomes for all major Asian ethnic groups by sequencing the genomes of 10,000 individuals. The second phase, sequencing an additional 90,000 individuals and genomic data coupled with clinical and phenotype information, will shed light on the genetic and molecular fabric of health and disease in this understudied population. The database will be hosted by Singapore's Nanyang Technological University, and sequencing, data analysis, and research will be carried out with the support of Macrogen, MedGenome, and Illumina.

The Surveillance and Pharmacogenomics of Adverse Drug Reactions project (http://www.saphire.sg) is a 3-year initiative introduced in 2014 and funded by the Biomedical Research Council of

the Agency of Science, Technology and Research (A*STAR) and the Health Sciences Authority in Singapore. The project aims to improve drug safety by genomic biomarker discovery in the Asian population. Close collaboration has been established with local hospitals, the Genome Institute of Singapore, and the Translational Laboratory for Genetic Medicine. Ongoing clinical studies focus on adverse reactions relating to the administration of doxorubicin/Herceptin, statins, and coumadin.

On a European level, the Ubiquitous Pharmacogenomics Consortium (http://upgx.eu), funded by the European Commission's Horizon-2020 program, is an example of international coordination between universities, foundations, and associations; its focus is the implementation of pharmacogenomics knowledge in clinical practice [22]. It has designed a prospective, block-randomized, controlled clinical study (PREPARE) with a total of 8100 patients; this implements preemptive pharmacogenomic testing in routine care, and will be conducted in the healthcare environments of seven European countries (Austria, Italy, Greece, the Netherlands, Slovenia, Spain, and the UK). The goal is to reduce the incidence of adverse drug reactions significantly through pharmacogenomics-guided pharmacotherapy in a cost-effective fashion.

Type 2 Diabetes Genetic Exploration by Next-Generation Sequencing in Multiethnic Samples (T2D-GENES; http://www.type2diabetesgenetics.org/projects/t2dGenes) is an international consortium funded by the United States National Institute of Diabetes and Digestive and Kidney Diseases, with the aim of identifying genetic variants that influence risk of type 2 diabetes. It comprises three projects: whole exome sequencing in 10,000 people from five ethnicities (African-American, East Asian, South Asian, European, and Hispanic); whole genome sequencing of 600 individuals selected from extended Mexican-American pedigrees; and a transethnic fine-mapping "mega-meta-analysis". The research is providing deeper insights into the genetic architecture of type 2 diabetes [23]. Association studies from T2D-GENES and other large-scale type 2 diabetes consortia can be found at the Type 2 Diabetes

Knowledge Portal, an open access repository for data associated with the disease.

## 15.5. Corporate Genomic Medicine Initiatives

In 2014 Regeneron Pharmaceuticals (http://www.regeneron.com) collaborated with Geisinger Health System in Pennsylvania (http://www.geisinger.org) to perform genomic analysis of 100,000 patients, aiming to improve drug development and patient healthcare via genotype–phenotype associations. The latter will be validated by the VelociGene and VelocImmune technologies of Regeneron Pharmaceuticals. This 5-year partnership will be the collaborative basis for the US National Human Genome Research Institute, while an expanded consortium with other academic, government, and integrated medical systems is envisaged.

AstraZeneca, a pharmaceutical company, has embarked on an ambitious plan to gather genomic information and health records from 2 million individuals over the course of the next decade, with the hope of gaining new insight into the biology of diseases (https://www.astrazeneca.com/media-centre/press-releases/2016/AstraZeneca-launches-integrated-genomics-approach-to-transform-drug-discovery-and-development-22042016.html). This initiative aims to revolutionize drug discovery and development; due to the magnitude of the effort, it has collaborated with two research institutes (the UK Wellcome Trust Sanger Institute and the Institute for Molecular Medicine in Finland) and a genomic database (Human Longevity, Inc., HLI). Furthermore, AstraZeneca will establish its own Centre for Genomic Research and develop a database of genome sequences and clinical and drug-response data that have been donated over time by 500,0000 patients in its clinical trials.

23andMe (https://www.23andme.com) is one of the leaders in the field of direct-to-consumer genetic testing and a contributor to over 230 studies. 23andMe has genotyped more than 1 million individuals, with over than 80% of them opting to participate in research, leading to breakthroughs like the identification of two novel loci and a substantial genetic component for Parkinson disease [24]. The sheer

potential of the genetic data available to such companies is tremendous. With the consent of healthy users (controls) and collaboration with communities of people with certain diseases, more studies like this could be realized in a timely manner.

A community-based initiative has been launched by Sequence Bio (https://sequencebio.com), a biotech company in Newfoundland and Labrador, and is planning to conduct its own 100K Genome Project in this specific population. This is a unique, genetically isolated, young founder population with a high incidence of several rare monogenic disorders, and it is being studied for genetic mapping of common diseases [25,26]. However, the genetic structure of this community has not yet been systematically examined. Genomic and phenotypic data collected by this project will be used in combating medical challenges in this community, improving patient outcomes, and implementing data-driven precision medicine. For this reason, Sequence Bio received a government fund and the company's efforts are supported by DNA Genotek, Genospace, GenomeAtlantic, and BIOTECanada.

HLI is also developing initiatives of its own, such as the HLI Health Nucleus, a genomic-powered clinical research program which uses whole genome sequence analysis, advanced clinical imaging, innovative machine learning, and curated personal health information to deliver the most complete picture of individual health. It has also developed Open Search, a browser-based search program that gives researchers access to the data of over 10,000 genomes which have been generated by deep genome sequencing and meet quality standards for clinical use [27].

In Iceland, deCODE (http://www.decode.com) has gathered detailed genetic and medical information on 500,000 individuals from around the globe and on more than 160,000 Icelandic volunteers, amounting to more than 50% of the adult population of the country. It is one of the world leaders in the discovery of genetic factors for common diseases, with strong contributions in genome-wide analyses [28,29].

Google and Autism Speaks are working together in MSSNG (https://www.mss.ng), a collaboration effort aiming to sequence over

10,000 individuals from families affected by autism which will create the world's largest genomic database on autism. This database, built with the help of Google Cloud Platform and Google Genomics, will be available to researchers around the globe and can be used to provide better diagnostics and personalized treatments. MSSNG supports and enables "open science" research.

## 15.6. Studying Founder Populations

The value of studying founder populations is highlighted by the SardiNIA project in Italy (https://sardinia.nia.nih.gov), an effort that started in 2001 with the support of the United States National Institute on Aging, the Italian National Research Council Institute in Cagliari, and scientists from several US universities. The population, due to its isolation for an extended period of time, is characterized by a relatively high frequency of variants which are rare elsewhere, as demonstrated in a study by Sidore and coworkers [30]. SardiNIA is striving to decipher the genetic processes involved in age-related traits and diseases, while simultaneously providing unique insights into diseases that are particularly prevalent in the cohort.

In May 2016 the Discovery Research Investigating Founder Population Traits (DRIFT) project announced an open call for research collaboration to understand the genetic architecture of founder populations throughout the world. The program is coordinated by the Regeneron Genetics Center (https://www.regeneron.com/genetics-center), and aims to identify rare loss-of-function mutations in founder populations to delineate further the genetic factors that underpin health and disease. This initiative is also addressed at developing countries and those in resource-limiting environments, under the coordination of the Genomic Medicine Alliance (http://www.genomicmedicinealliance.org), a founding partner of the DRIFT project, to provide the opportunity to take part in advanced genomics research and make a positive impact on alleviating disease in their populations.

There is a perception that the benefits of genomics are attainable both inside and outside the industrialized world, as large-scale

sequencing efforts exploring human genomic variation have been initiated in several emerging markets across the globe, following the paradigm of the countries that have already embarked on major genomics initiatives.

## 15.7. Conclusions and Future Perspectives

Population-based genomic medicine studies must be carried out on both global and local scales; failing to do so inevitably leads to sampling bias. Sampling bias not only prevents scientists from discovering rare population-specific variants in Genome-Wide Association Studies (GWAS) that might be able to explain the heritability of some diseases, but also perpetuates the health disparities of medical systems around the world [31,32].

The Broad Institute of MIT and Harvard (https://www.broadinstitute.org/genomics) has been a pioneer in the field of genomics for years, and recently combined all the genomics and data science platforms to form Broad Genomics. Over the past 5 years Broad Genomics has been the largest producer of human genomic information in the world, having processed more than 1.5 million samples from over 1400 groups in over 50 countries. An integrated and industry-scale working methodology has already produced numerous publications as a result of GWAS analyses [33]. The Genome Analysis Toolkit (https://software.broadinstitute.org/gatk), a software package for processing and analyzing sequence data, and the Ricopili (https://data.broadinstitute.org/mpg/ricopili), a tool for visualizing regions of interest in select GWAS datasets, have also been developed by the institute.

Genomic medicine and research, as with all health and social services, should not restrictively serve the benefit of a privileged few ethnicities, countries, or individuals. We need to close the genetic knowledge gap among all the world's populations and at the same time reduce the bias of modern medicine application toward Western populations. To achieve this goal, national genomic medicine

initiatives must be designed in a way that allows them to include minority groups and people from diverse ancestries (e.g., East London Genes and Health, which aims to analyze the genome and improve health among 100,000 people of Pakistani and Bangladeshi heritage in East London, http://www.genesandhealth.org), while researchers in developing countries must be given incentives to unearth the genetic portrait of their own populations.

We feel that the value of genomic medicine research in healthcare improvement and knowledge-based economies can be only appreciated when institutional leadership meets political will to developing a vision and, ultimately, a plan—nationally or globally. The major international organizations involved in the genomic medicine efforts summarized in Table 15.1 and the large-scale human genotyping projects described here would be unlikely to occur otherwise. Evidently, both are setting the cost-beneficial and evidence-based platform needed for the implementation of genomic medicine findings.

## 15.8. Competing Interests

The authors declare no conflict of interests.

## References


[1] Ginsburg G.S, Willard H.F. Genomic and personalized medicine: foundations and applications. *Transl Res*. 2009;154:277–287.

[2] Christensen K, Murray J.C. What genome-wide association studies can do for medicine. *N Engl J Med*. 2007;356:1094–1097.

[3] Feero W.G, Guttmacher A.E, Collins F.S. Genomic medicine—an updated primer. *N Engl J Med*. 2010;362:2001–2011.

[4] Altar C.A. The biomarkers consortium: on the

critical path of drug discovery. *Clin Pharmacol Therapeut*. 2008;83:361–364.

[5] Pagon R.A. GeneTests: an online genetic information resource for health care providers. *J Med Libr Assoc*. 2006;94:343–348.

[6] Patrinos G.P, Baker D.J, Al-Mulla F, Vasiliou V, Cooper D.N. Genetic tests obtainable through pharmacies: the good, the bad, and the ugly. *Hum Genom*. 2013;7:17.

[7] Burke W, Khoury M.J, Stewart A, Zimmern R.L. The path from genome-based research to population health: development of an international public health genomics network. *Genet Med*. 2006;8:451–458.

[8] Dove E.S, Barlas I.O, Birch K, Boehme C, Borda-Rodriguez A, Byne W.M, Chaverneff F, Coşkun Y, Da L, Dereli T. An appeal to the global health community for a tripartite innovation: an "essential diagnostics List," "Health in all policies," and "See-through 21st century science and ethics". *OMICS*. 2015;19:435–442.

[9] Manolio T.A, Chisholm R.L, Ozenberger B, Roden D.M genomic medicine in the clinic: the future is here. *Genet Med*. 2013;15:258–267.

[10] Hardy B.-J, Seguin B, Goodsaid F, Jimenez-Sanchez G, Singer P.A, Daar A.S. The next steps for genomic medicine: challenges and opportunities for the developing world. *Nat Rev Genet*. 2008;9 Suppl 1:S23-S27.

[11] Seguin B, Hardy B.-J, Singer P.A, Daar A.S. Genomic medicine and developing countries: creating a room

of their own. *Nat Rev Genet*. 2008;9:487–493.
[12] Mitropoulos K, Al
Jaibeji H, Forero D.A, Laissue P, Wonkam A, Lopez-Correa C, Mohamed Z, Chantratita W, Lee M.T, Llere stories in genomic medicine from resource-limited countries. *Hum Genom*. June 18, 2015;9:11.
[13] Abu-Elmagd M, Assidi M, Schulten H.-J, Dallol A, Pushparaj P.N, Ahmed F, Scherer S, Al-Qahtani M. Individualized medicine enabled by genomics in Saudi Arabia. *BMC Med Genom*. 2015 8 Suppl 1:S3.
[14]
Boomsma D.I, Wijmenga C, Slagboom E.P, Swertz M. Dijk F, Francioli L.C, Hottenga J.J, Laros J.F, Li Q, Li Y Setten J, Menelaou A, Pulit S.L, Hehir-Kwa J.Y, Beekman M, Elbers C.C, Byelas H, de Craen A.J, Deelen P, Dijkstra M, den Dunnen J.T, de Knijff P, Houwing-Duistermaat J, Koval V, Estrada K, Hofman A, Kanter Leeuwen E.M, Neerincx P.B, Oostra B, Rivadeneira F, Bakker P.I, van Ommen G.J, van Duijn C.M. The genome of The Netherlands: design, and project goals. *Eur J Hum Genet*. 2014;22:221–227. .
[15] Genome of the Netherlands C. Whole-genome sequence variation, population structure and demographic history of the Dutch population. *Nat Genet*. 2014;46:818–825.
[16] Metspalu A. Estonian Genome Project—before the take-off and take-off. *Bioinformatics*. 2002;18:S152.
[17]
Kehdy F.S.G, Gouveia M.H, Machado M, Magalhães V

Souza G.B, Rodrigues-
Soares F, Araújo G.S, Zamudio R, Sant
Anna H.P, Santos H.C, Duarte N.E, Fiaccone R.L, Figu
Costa M.F, Pereira A.C, Rodrigues M.R, Tarazona-
Santos E, Consortium TBEP. Origin and dynamics of admixture in Brazilians and its effect on the pattern of deleterious mutations. *Proc Natl Acad Sci U S A*. 2015;112:8696–8701.

[18] Lima-
Costa M.F, Rodrigues L.C, Barreto M.L, Gouveia M, H
Soares F, Victora C.G, Tarazona-Santos E. Genomic ancestry and ethnoracial self-classification based on 5,871 community-dwelling Brazilians (The Epigen Initiative). *Sci Rep*. 2015;5:9812.

[19] 1000 Genomes Project
Consortium, Auton A, Brooks L.D, Durbin R.M, Garri
global reference for human genetic
variation. *Nature*. 2015 Oct 1;526(7571):68–74.

[20] Genome
Project C, Abecasis G.R, Altshuler D, Auton A, Brooks
map of human genome variation from population-scale sequencing. *Nature*. 2010;467:1061–1073.

[21] T.h.e H3A. Consortium. Enabling the genomic revolution in Africa. *Nature*. 2014;344 (6190):1346-8.

[22] van der Wouden C.H, Cambon-
Thomsen A, Cecchin E, Cheung K.-C, Dávila-
Fajardo C.L, Deneer V.H, Dolžan V, Ingelman-
Sundberg M, Jönsson S, Karlsson M.O, Kriek M, Mitro
Plassmann G, Toffoli G, Turner R.M, van
Rhenen M.H, Swen J.J, Guchelaar H.-J, on behalf of the Ubiquitous Pharmacogenomics

Consortium. Implementing pharmacogenomics in Europe: design and implementation strategy of the ubiquitous pharmacogenomics consortium. *Clin Pharmacol Ther*. 2017 Mar;101(3):341–358.

[23]
Fuchsberger C, Flannick J, Teslovich T.M, Mahajan A, de Bunt M, Pearson R.D, Kumar A, Müller-Nurasyid M, Grarup N, Stringham H.M, Gamazon E.I Williams A.G, Ferreira T, Fingerlin T, Horikoshi M, H der
Schouw Y.T, Loh M, Musani S.K, Puppala S, Scott W.I G, Wong T.Y, Njølstad P.R, Levy J.C, Mangino M, Bor Jensen J, Brandslund I, Christensen C, Koistinen H.A, Melander M, Qi L, Qi Q, Roden M, Rolandsson O, Sw Angelis M.H, Deloukas P, Gjesing A.P, Jun G, Nilsson genetic architecture of type 2 diabetes. *Nature*. 2016 Aug 4;536(7614):41–47.

[24]
Do C.B, Tung J.Y, Dorfman E, Kiefer A.K, Drabant E.M al. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet*. 2011;7(6):e1002141.

[25]
Rahman P, Jones A, Curtis J, Bartlett S, Peddle L, Fern Newfoundland population: a unique resource for genetic investigation of complex diseases. *Hum Mol Genet*. 2003;12 Spec No 2:R167–R172.

[26]
Zhai G, Zhou J, Woods M.O, Green J.S, Parfrey P, Rah structure of the Newfoundland and Labrador

population: founder effects modulate variability. European journal of human genetics. *Eur J Hum Genet*. 2016;24:1063–1070.

[27] Telenti A, Pierce L.C, Biggs W.H, di Iulio J, Wong E.H, Fabani M.M, Kirkness E.F, Moustaf sequencing of 10,000 human genomes. *Proc Natl Acad Sci U S A*. October 18, 2016;113(42):11901–11906. .

[28] Gudbjartsson D.F, Helgason H, Gudjonsson S.A, Zink scale whole-genome sequencing of the Icelandic population. *Nat Genet*. May 2015;47(5):435–444.

[29] Lo M.T, Hinds D.A, Tung J.Y, Franz C, Fan C.C, Wang Price V, Smith D.J, O'Donovan M, Stefansson H, Bjorr wide analyses for personality traits identify six genomic loci and show correlations with psychiatric disorders. *Nat Genet*. January 2016;49(1):152–156.

[30] Sidore C, Busonero F, Maschio A, Porcu E, Naitza S, Z Vecchyo V.D.O, Chiang C.W.K, Bragg-Gresham J, Pitzalis M, Nagaraja R, Tarrier B, Brennan sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers. *Nat Genet*. November 2015;47(11):1272–1281.

[31] Bustamante C.D, Burchard E.G, De la Vega F.M. Genomics for the world. *Nature*. 2011;475:163–165.

[32] Need A.C, Goldstein D.B. Next generation disparities in human genomics: concerns and remedies. *Trends Genet*. 2009;25:489–494.

[33]
Lane J.M, Liang J, Vlasac I, Anderson S.G, Bechtold D wide association analyses of sleep disturbance traits identify new loci and highlight shared genetics with neuropsychiatric and metabolic traits. *Nat Genet*. December 19, 2016.

## Further Reading

Lévy Y. Genomic medicine 2025: France in the race for precision medicine. *Lancet*. December 10, 2016;388(10062):2872.

Mensah G.A, Peprah E.K, Sampson U.K.A, Cooper R.S. H3Africa comes of age. *Cardiovasc J Afr*. March-April 2015;26(2 H3Africa Suppl.):s3–s5.

Nagasaki M, Yasuda J, Katsuoka F, Nariai N, Kojima K, Kawai Y, Yama Kabata Y, Yokozawa J, Danjoh I, Saito S, Sato Y, Mimori T, Tsuda K, Engel J, Kinoshita K, Kure S, Yaegashi N, Yamamoto M. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. *Nat Commun*. August 21, 2015;6:8018.

Kan Z, Zheng H, Liu X, Li S, Barber T.D, Gong Z, Gao H, Hao K, Willa genome sequencing identifies recurrent mutations in hepatocellular carcinoma. *Genome Res*. September 2013;23(9):1422–1433.

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# Index

'*Note:* Page numbers followed by "f" indicate figures, "t" indicate tables'.


**A**

Aarskog syndrome, 102, 106f

AAV vectors, *See* Adeno-associated viral vectors (AAV vectors)

ABI, *See* Ankle/brachial blood pressure index (ABI)

Abnormal body size and proportion

  approach to patient with abnormal stature, 82–84

    prenatal-onset growth deficiency disorders, 82t

  mechanisms of growth, 84–100

  pathologic overgrowth, 118–128

  pathologic short stature, 100–118

Absolute risk, 20

ACCE, *See* Analytic validity, Clinical validity, Clinical utility and Ethical, legal and social issues (ACCE)

ACE inhibitors, *See* Angiotensin-converting enzyme inhibitors (ACE inhibitors)

ACMG, *See* American College of Medical Genetics and Genomics (ACMG)

ACOG, *See* American College of Obstetrics and Gynecology (ACOG)

Acromesomelic dysplasia, 85
Activated phosphatidylinositol 3-kinase δ syndrome (APDS), 124–125
Activated protein C (APC), 175–176
Active CDS, 320
Acute myeloid leukemia, 249–250
AD, *See* Alzheimer disease (AD)
Adeno-associated viral vectors (AAV vectors), 217–218
ADHD, *See* Attention-deficit/hyperactivity disorder (ADHD)
ADI-R, *See* Autism Diagnostic Inventory-Revised (ADI-R)
ADOS, *See* Autism Diagnostic Observation Schedule (ADOS)
"Adult" stem cells, 255
Advanced sleep phase syndrome (ASPS), 300–301
Adverse effects of nongenetic factors, 15
Ag-NOR, *See* Silver stain-NOR (Ag-NOR)
Age for carrier screening, 294
AHG, *See* Anti-human globulin (AHG)
AIDS, 28–29
AKT, *See* Oncogene protein kinase B (AKT)
Allele-specific oligonucleotide hybridization (ASO hybridization), 169
Alleles, 229–230
Allelic heterogeneity, 81–82
Allogeneic grafts, 250
Allografts, 235
ALS/FTD, *See* Amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD)

Alzheimer disease (AD), 268, 304–305
American Academy of Sleep Medicine, 306–307
American College of Medical Genetics and Genomics (ACMG), 173–174, 194, 285
American College of Obstetrics and Gynecology (ACOG), 285
American Society for Histocompatibility and Immunogenetics, 237
American Society of Human Genetics (ASHG), 294
Aminoglycosides, 38
Aminopterin, 37
Amniocentesis, 33
Amnion rupture sequence, 8, 8f
AMP, *See* Association for Molecular Pathology (AMP)
AMP-activated protein kinase (AMPK), 302–303
Amplification refractory mutation system (ARMS), 169–170, 240
Amplified restriction fragment polymorphisms (AmpFLPs), 169–170
AMR, *See* Antibody-mediated rejection (AMR)
Amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD), 184
Analytic validity, Clinical validity, Clinical utility and Ethical, legal and social issues (ACCE), 274
Anasarca, 120
Angelman syndrome (AS), 185, 317–318
Angiotensin II receptor inhibitors, 43–44
Angiotensin-converting enzyme inhibitors (ACE inhibitors), 17, 43
Animal studies, 22
Ankle/brachial blood pressure index (ABI), 269

Antenatal screening, 340–344
*See also* Carrier screening
decisions in social context, 341–342
disability issues, health economics, and sex selection, 343–344
entry to, 342–343
future, 344
goals, 340–341
reprogenetics, 344
Anti-human globulin (AHG), 245–246
Anti-human IgG antibody, 247
Anti-infective agents, 38–39
Anti-inflammatory drugs, 36
Antibiotics, 38–39
Antibody reactivity of patient awaiting transplant, 244–245
Antibody-mediated rejection (AMR), 247
Anticancer agents, 37
Anticonvulsant agents, 39–40
Antigen presenting cells (APCs), 229
Antigens, 247
Antimalarial agents, 38–39
Antithyroid agents, 41
APC, *See* Activated protein C (APC)
APCs, *See* Antigen presenting cells (APCs)
APDS, *See* Activated phosphatidylinositol 3-kinase δ syndrome (APDS)

*ApoE*4, *See* Apolipoprotein E4 (*ApoE*4)
Apolipoprotein E4 (*ApoE*4), 266–268
ARMS, *See* Amplification refractory mutation system (ARMS)
Array, 159–162
  array-based CGH, 10
  array-based genomic analysis, 159
    array-CGH analysis in patient with epilepsy, 160f
    genome-wide SNP array results, 161f
  array-based techniques, 149
Arthrogryposis, 29
AS, *See* Angelman syndrome (AS)
ASDs, *See* Autism spectrum disorders (ASDs)
ASHG, *See* American Society of Human Genetics (ASHG)
Ashkenazi Jewish carrier screening panels, 190
ASO hybridization, *See* Allele-specific oligonucleotide hybridization (ASO hybridization)
ASPS, *See* Advanced sleep phase syndrome (ASPS)
Assist communities, public health genetics/genomics responding to, 357
Association for Molecular Pathology (AMP), 191–192, 194
AstraZeneca, 376
Asynchronous CDS, 319–320
Attention, arousal, affect, and action of infancy (four As of infancy), 35
Attention-deficit/hyperactivity disorder (ADHD), 34
Autism Diagnostic Inventory-Revised (ADI-R), 69
Autism Diagnostic Observation Schedule (ADOS), 69

Autism spectrum disorders (ASDs), 39, 65–66, 69–70
clinical findings in, 70t
X-chromosome genes, 71t
Autografts, 235
Autoimmune disease, 45
Autosomal recessive disorders, 102, 284–286, 286t, 346
Autosomal recessive primary microcephaly, 86

**B**

B lymphocytes, 237
BACs, *See* Bacterial artificial chromosomes (BACs)
Bacteria, 29–30
Bacterial artificial chromosomes (BACs), 148
Baller-Gerold syndrome, 107–108
Bannayan-Riley-Ruvalcaba syndrome (BRRS), 127–128
Bayes theorem, 284
BBMRI–NL, *See* Biobanking and Biomolecular Research Infrastructure–Netherlands (BBMRI–NL)
Bead-based assays, 248
Becker type muscular dystrophies (BMD), 184–185
Beckwith-Wiedemann syndrome (BWS), 99, 100f, 120–121
Belgian Medical Genomics Initiative (BeMGI), 374
BeMGI, *See* Belgian Medical Genomics Initiative (BeMGI)
Bendectin, 44
Benign allele, 283
Binding protein (BiP), 213–214

Biobanking and Biomolecular Research Infrastructure–Netherlands (BBMRI–NL), 374

Birth defects, 19–20, 22–23, 31, 44

Birth weight of infants, 82

"Blood relationship", 235

Bloom syndrome, 82t, 85, 88t–97t, 102

Bloom-Torre-Machacek syndrome, 102

BMAL1 transcription factor, 303

BMD, *See* Becker type muscular dystrophies (BMD), Bone mineral density (BMD)

BMPs, *See* Bone morphogenetic proteins (BMPs)

BMT, *See* Bone marrow transplantation (BMT)

Bone marrow transplant, 250

Bone marrow transplantation (BMT), 205, 209–210, 237–238

Bone mineral density (BMD), 87

Bone morphogenetic proteins (BMPs), 86

  antagonists, 87

  BMP4, 255

*Borrelia burgdorferi*, 30

Brachmann-de Lange syndrome, 82t, 102

Brain imaging, 72–75

*BRCA*1 gene, 187, 277, 332

*BRCA*1/2 genes, 186

*BRCA*2 gene, 187, 277, 332

Breast cancer, 187, 271, 272f

Breech deformation sequence, 7–8, 8f

BRRS, *See* Bannayan-Riley-Ruvalcaba syndrome (BRRS)

*N*-Butyldeoxynojirimycin (NB-DNJ), 210

BWS, *See* Beckwith-Wiedemann syndrome (BWS)

**C**

C-type natriuretic peptide (CNP), 85

C9orf72, *See* Chromosome 9 open reading frame-72 (C9orf72)

Caffeine, 35–36

CAID syndrome, *See* Chronic atrial intestinal dysrhythmia syndrome (CAID syndrome)

Cancer, 165, 265, 304

  biobank, 375

  chemotherapy, 37

  chromosomal aberrations, 145–146

  familial breast/ovarian, 168–169, 186–187

  hereditary nonpolyposis colon cancer, 187–188

  suppressor genes, 156–157

CAP, *See* College of American Pathologists (CAP)

CAPSeq, *See* Clustering and alignment of polymorphic sequences (CAPSeq)

Carbamazepine, 40

Cardiofaciocutaneous syndrome (CFC syndrome), 117–118

Cardiovascular disorders, 302–304

  circadian clock and cardiovascular disorders, 304

β-Carotene, 41

Carotenoids, 41

Carrier screening, 167–168, 283, 344–345
*See also* Antenatal screening
in clinical practice, 284–285
cascade testing, 285
reducing disease incidence, 285
reproductive screening and testing options, 284–285
cost and feasibility, 293
in individuals of defined subpopulation groups, 285–292
β-thalassemia, 288
CF, 289
ECS, 290–292
ethnic-specific carrier screening panels, 287t
frequency and incidence for selected autosomal recessive disorders, 286t
hemoglobinopathies, 288
SCD, 288–289
SMA, 289–290
TSD, 287–288
sensitivity and specificity, 292–293
Carrier testing, 167–168
of children, 337–338
Cas9 nuclease, 218–219
Cascade testing, 285
Case reports, 22
Case series, 22

Case–control studies, 23–24
Cathepsin K, 87
Caudal regression, 44–45
CDA, *See* Clinical document architecture (CDA)
CDC, *See* Centers for Disease Control and Prevention (CDC), Complement-dependent lymphocytotoxicity (CDC)
CdLS, *See* Cornelia de Lange syndrome (CdLS)
cDNAs, *See* Complementary DNAs (cDNAs)
CDs, *See* Cyclodextrins (CDs)
CDS systems, *See* Clinical decision support systems (CDS systems)
Cell
  culture, 148–149
  replication defects, 85–86
  transplanting, 234
"Cell-based assay", 244–245
Cellularly characterized *HLA* alleles and loci, 237–238
Centers for Disease Control and Prevention (CDC), 192, 356
Central clock, 299–300, 303
Central nervous system (CNS), 17, 64
  anomalies, 29
  degenerative states, 12
  dysgenesis, 72–73
  malformations, 72–75
Cerebral gigantism, 121–123, 122f
Cerebrocostomandibular syndrome, 9

Ceroid lipofuscinosis type 2 (CLN2), 207–208
CF, *See* Cystic fibrosis (CF)
CFC syndrome, *See* Cardiofaciocutaneous syndrome (CFC syndrome)
CGH, *See* Comparative genomic hybridization (CGH)
CHARGE syndrome, 66
CHD8 gene, *See* Chromo-domain-helicase-DNA-binding 8 gene (CHD8 gene)
Chemiluminescence, 239
Childhood, genetic testing in, 336–338
Chimeras, 234
Chinese Academy of Sciences, 373
Chorionic villus sampling (CVS), 33, 166
Chromatin remodeling, 65
  by nucleosome complexes, 98
Chromo-domain-helicase-DNA-binding 8 gene (CHD8 gene), 121
Chromosome 9 open reading frame-72 (C9orf72), 184
Chromosome microarray analysis (CMA), 66, 145, 159
Chromosomal/chromosome, 9, 145–146
  abnormalities, 9–10, 146, 152
  banding, 149
  chromosome SNP microarrays, 65–66
  chromosome-6 homozygous, 238
  fiber, 157–158
  heteromorphisms, 146–147
  number, 146

Chronic atrial intestinal dysrhythmia syndrome (CAID syndrome), 85–86
Chronic diseases of adulthood, 268–277
Chronotherapy, 306–307
  pharmaceutical treatment scheduling therapy, 307
  sleep-phase therapy, 306–307
"Chronotype", 300
Circadian clock, 299–300, 304
Circadian rhythm disruption, 303–304
Circadian rhythms and disease
  cancer, 304
  central and peripheral clocks, 299–300
  chronotherapy, 306–307
  extrinsic circadian disorders, 302
  intrinsic circadian disorders, 300–301
  metabolic and cardiovascular disorders, 302–304
  molecular mechanisms, 299
  psychologic and neurologic diseases and, 304–306
Cleft palate, 7–8
CLIA guidelines, *See* Clinical Laboratory Improvement Amendments guidelines (CLIA guidelines)
Clinical decision support systems (CDS systems), 318–319
Clinical document architecture (CDA), 321–322
Clinical genetics, 327
  confidentiality, 335–336
  counseling, testing, and screening, 328–331

goals and outcomes of genetics services, 329–330
nondirectiveness in genetic counseling, 330–331
diagnostic genetic testing, 331–334
historical context, 328
population genetic screening, 338–345
predictive genetic testing, 334
testing in childhood, 336–338
Clinical Laboratory Improvement Amendments guidelines (CLIA guidelines), 191–192
Clinical teratology, 15
characterization of teratogenic exposures, 17–19, 18t
evaluating patient and exposure, 21–25
assessing scientific literature, 21–25
pregnant patient evaluation for teratogenic exposures, 21
future perspective, 46
paternal and maternal exposures before or shortly after conception, 46
teratogenesis mechanisms, 16, 16t
teratogenic effects
genetic susceptibility to, 16
risk assessment and counseling for, 19–21
teratogenic exposures, 25–45
Clinical utility, 274
ClinVar database, 194
CLN2, *See* Ceroid lipofuscinosis type 2 (CLN2)
Clomiphene, 41

Clustered regularly interspaced short palindromic repeats-CRISPR associated endonuclease system (CRISPR/Cas9), 218–219

Clustering and alignment of polymorphic sequences (CAPSeq), 244, 244f

CMA, *See* Chromosome microarray analysis (CMA)

CMV, *See* Cytomegalovirus (CMV)

CNP, *See* C-type natriuretic peptide (CNP)

CNS, *See* Central nervous system (CNS)

CNVs, *See* Copy number variants (CNVs)

Cocaine, 6–7, 35

Cockayne syndrome, 102, 107f

Codeine, 43

Coffin-Siris syndrome (CSS), 98, 98f

Cohesinopathies, 85–86, 108–111

Cohesion, 85–86

Cohort studies, 23

College of American Pathologists (CAP), 171–174, 194

Commercialization, 347–348

Common complex disorders, 347–348

Communicable disease, 331

Community-Centered Health Home, 365

Comparative genomic hybridization (CGH), 4, 147–148, 158

Complement-dependent lymphocytotoxicity (CDC), 244–245

Complementary DNAs (cDNAs), 206

Confidentiality, 335–336

  privacy within family, 335–336

third parties, 335
Confidentiality, 349
Congenital cytomegalovirus infection, 26–27
Congenital hearing loss, 189–190
Congenital hip dislocation, 5–6
Congenital lipomatous asymmetric overgrowth of the trunk, lymphatic, capillary, venous, and combined-type vascular malformations, epidermal nevi, skeletal and spinal anomalies (CLOVES syndrome), 124–126
Congenital rubella syndrome, 26
Congenital syphilis, 30
Congenital toxoplasmosis, 31
Congenital Zika syndrome, 27, 28f–29f
Connective tissue disorders, 12
Consent, 348–349
Constraint, 32–33
Copy number abnormalities, 9–10
Copy number variants (CNVs), 69, 159, 333
Core molecular feedback loop, 299
Cornelia de Lange syndrome (CdLS), 85–86, 103f, 108–111
Corporate genomic medicine initiatives, 376–377
Corticosteroids, 41
Cowden syndrome, 127, 271
Cranial MRI, 34
Craniosynostosis, 4
CREB-binding protein (*CREBBP*), 11–12

*CREBBP*, *See* CREB-binding protein (*CREBBP*)
"Cri du chat" syndrome, 346
CRISPR, 218–219
CRISPR/Cas9, *See* Clustered regularly interspaced short palindromic repeats-CRISPR associated endonuclease system (CRISPR/Cas9)
Cross-cultural genetic counseling, 346
*Cryptochrome* (*Cry*) genes, 299
CSS, *See* Coffin-Siris syndrome (CSS)
Culture
  cells, 148
  challenges in genetic counseling, 345–346
Cushing disease, 117
Customary consanguineous marriage, 346
CVS, *See* Chorionic villus sampling (CVS)
Cyclic AMP/MAP kinase, 87
Cyclodextrins (CDs), 212
  SRT with, 212
Cystic fibrosis (CF), 167, 174–175, 284, 289, 329, 332
Cytogenetic analysis, 147
  chromosome abnormalities, 152
  chromosome banding, 149
  indications for, 148
  ISH, 152–162
  milestones in human cytogenetics, 145–148
  normal human karyotype, 149–152

tissue samples and cell culture, 149
Cytogenomic copy number abnormalities, 65–66
Cytogenomic microarray, 66
Cytomegalovirus (CMV), 26–27, 102

**D**

D-box binding PAR bZIP transcription factor (*Dbp*), 302
Danish Reference Genome Project, 374
Data synthesis, 24–25
*Dbp*, *See* D-box binding PAR bZIP transcription factor (*Dbp*)
Decision support rules implementation, 321–322
Decision-making process, 265, 346
deCODE, 377
Deformations, 5–6
extrinsically derived prenatal-onset, 6
intrinsically derived prenatal-onset, 6
Degenerate oligonucleotide-primed (DOP), 153–154
Delayed sleep phase syndrome (DSPS), 300–301
Dentatorubral-pallidoluysian atrophy (DRPLA), 181
Deoxynucleotide triphosphates (dNTPs), 239
DES, *See* Diethylstilbestrol (DES)
Desert hedgehog (DHH), 86
Detection of structural variation by genome and exome sequencing, 162
"166DG" epitope, 252
DHH, *See* Desert hedgehog (DHH)

Diabetes mellitus, 44–45, 265
Diagnostic genetic testing, 331–334
  implications for others, 331–332
  information of uncertain significance, 332–334
Diagnostic molecular genetics
  of commonly tested diseases, 174–189
  future directions, 195–196
  indications for molecular genetic testing, 166–169
  internet resources for molecular genetic testing, 193–194
  mitochondrial DNA disorders, 189
  pharmacogenetic testing, 190–191
  QA, reimbursement, and regulatory issues, 191–193
  societal impact of genetic technology, 194–195
  targets of molecular genetic screening, 189–190
  technical approaches to molecular genetic testing, 169–174
Diclectin, 44
Diethylstilbestrol (DES), 40
Diffusion distance, 244
DiGeorge syndrome, 65–66
Direct fetal infection, 25
Direct-to-consumer genetic testing (DTC genetic testing), 356
Disasters, public health genetics/genomics responding to, 357
Discovery Research Investigating Founder Population Traits project (DRIFT project), 377
Disease genes detection, 171–173

Disruption, 5
Disruptive defects, 6–7
DM, *See* Myotonic dystrophy (DM)
DMD, *See* Duchenne muscular dystrophy (DMD)
DMEG, *See* Dysplatic megalencephaly (DMEG)
*DMPK* gene, *See* Dystrophica myotonica protein kinase gene (*DMPK* gene)
DNA
  DNA-based methods, 256
  fiber-FISH, 157–158, 158f
  methylation, 98–99
  probes and applications, 153–157
    chromosome painting, 153f
    chronic myeloid leukemia, 157f
    diagnosis of microdeletion syndromes, 156f
    FISH probes, 154f
    human metaphase showing telomeric probes, 156f
    recurrent segmental deletions/duplications associated syndromes, 155t
  repair defects, 83t–84t, 85
  replication, 146–147
  segment, 230
  sequencing, 169, 171f
    equipment, 244
    technology for HLA typing, 241–242, 243f
*DNMT3A* Tatton-Brown-Rahman syndrome, 123

dNTPs, *See* Deoxynucleotide triphosphates (dNTPs)
Dominantly inherited cancer syndromes, 188–189
Donohue syndrome, 104–106
Donor-specific antibodies (DSAs), 235–236, 244–245
DOP, *See* Degenerate oligonucleotide-primed (DOP)
L-Dopa, *See* Levodopa (L-Dopa)
Double-strand break repair (DSBR), 107–108
Double-stranded DNA, 152
Down syndrome, 5, 9–10, 65–66, 145–146, 329, 341
DRIFT project, *See* Discovery Research Investigating Founder Population Traits project (DRIFT project)
DRPLA, *See* Dentatorubral-pallidoluysian atrophy (DRPLA)
Drug
  and chemical agents, 33–45
    autoimmune and isoimmune disease, 45
    environmental agents, 33–34
    maternal metabolic factors, 44–45
    nonprescription drugs, 36
    prescription drugs, 36–44
    "recreational" drugs, 34–36
  delivery systems, 307
  metabolism, 191
DSAs, *See* Donor-specific antibodies (DSAs)
DSBR, *See* Double-strand break repair (DSBR)
DSPS, *See* Delayed sleep phase syndrome (DSPS)

DSPs, *See* Dual specificity phosphatases (DSPs)
DTC genetic testing, *See* Direct-to-consumer genetic testing (DTC genetic testing)
Dual specificity phosphatases (DSPs), 124
Dubowitz syndrome, 82t, 88t–97t
Duchenne muscular dystrophy (DMD), 170, 184–185, 255, 332
  large deletions detection, 184–185
  microlesions detection, 185
Dysmorphic child, 1
  developmental problems
    postnatal onset of, 1–4, 12
    prenatal onset of, 1–12
  single primary defects in development, 4t
  with structural defects, 2f
Dysmorphology, 64
Dysplasia, 5, 7
Dysplatic megalencephaly (DMEG), 124–125
Dystrophica myotonica protein kinase gene (*DMPK* gene), 183

**E**

Early congenital syphilis, 30
Early invasive prenatal diagnosis, 33
EBPH, *See* Evidence-based public health (EBPH)
Ecological fallacy, 24
Ecological studies, 24
ECS, *See* Expanded carrier screening (ECS)

EED, *See* Embryonic ectoderm development (EED)

EGP, *See* Estonian Genome Project (EGP)

EHRs, *See* Electronic health records (EHRs)

Electronic health records (EHRs), 315, 318

electronic Medical Records and Genomics network (eMERGE network), 319

Elejalde syndrome, 83t–84t, 112t–116t

Eliglustat, 211

ELISA, *See* Enzyme-linked immunosorbent assay (ELISA)

EMA, *See* European Medicines Agency (EMA)

Embryonic ectoderm development (EED), 99–100

Embryonic stem cells (ES cells), 253–255

eMERGE network, *See* electronic Medical Records and Genomics network (eMERGE network)

Empowerment, 330

Endocrine agents, 40–41

Endocrine disrupters, 33–34

Endocrine-active agents, 41

Endoplasmic reticulum (ER), 213–214

Endoplasmic reticulum–associated protein degradation (ERAD), 213

Energy homeostasis, interactions between circadian system and, 303

Enhancer of zeste (EZH2), 99–100

Environmental agents, 33–34

  environmental chemicals, 33–34

  organic mercury compounds, 33

Environmental hazards, protection against, 356

Enzyme replacement therapy (ERT), 205–209, 206t, 217
factors affecting selection of candidate lysosomal disorders, 207–208
FDA and EMEA approval, 208t
principles for effective treatment, 208–209, 208t
Enzyme-linked immunosorbent assay (ELISA), 247–248, 248f
*EP*300 gene, 11–12
EPIGEN Brazil Initiative, 375
Epigenetic contributions to abnormal growth, 87–100
chromatin remodeling by nucleosome complexes, 98
DNA methylation and imprinted genes, 98–99
gene silencing, 99–100
Epitope-based matching, 250–253
artistic representation of chimera, 254f
Eplet, 250–252, 252f
ER, *See* Endoplasmic reticulum (ER)
ERAD, *See* Endoplasmic reticulum–associated protein degradation (ERAD)
ERT, *See* Enzyme replacement therapy (ERT)
ES cells, *See* Embryonic stem cells (ES cells)
Estonian Genome Project (EGP), 374–375
Estrogens, 40
Ethnicity-based screening, 277, 283
Ethyl alcohol, 34
Etiologic diagnosis, 62
Etiology, 1, 3–4, 10

Eugenics, 328
Eukaryotic phosphatases, 124
European Medicines Agency (EMA), 205
Evidence-based genomics practices, 362
Evidence-based public health (EBPH), 363
Exome Aggregation Consortium (ExAC), 194, 370t–372t
Exome sequencing, 162
Expanded carrier screening (ECS), 283, 290–292, 291t
  carrier screening and heterozygote testing modalities, 292t
  methods and tissues, 291–292
Exposure cohort studies, 23
Extrinsic circadian disorders, 302
Extrinsic mechanisms of proportionate postnatal short stature, 117
Extrinsically derived prenatal-onset deformations, 6
ExVivo gene therapy, HSCT for, 218
EZH2, *See* Enhancer of zeste (EZH2)

**F**

Fabry disease, 211, 213
  PCT for, 216
    clinical trials of, 216
Factor V Leiden mutation, 175–177, 176f
Familial adenomatous polyposis, 188
Familial ASPS, 300–301
Familial breast/ovarian cancer, 168–169, 186–187

Familial cancer syndromes, 186–189
Familial melanoma, 188
Family history assessment, 269–271, 270f
Fanconi pancytopenia syndrome, 85, 102, 108
FCXM, *See* Flow cytometry crossmatch (FCXM)
FDA, *See* US Food and Drug Administration (FDA)
Feedback in human genetics project, 348–349
Female sex hormones, 40–41
Fetal
  abnormalities, 43–44
  alcohol syndrome, 34, 35f
  anticonvulsant syndrome, 39–40
  crowding, 6
  valproate syndrome, 39
  warfarin syndrome, 37–38, 38f
Fetal growth restriction (FGR), *See* Prenatal growth deficiency
FGFs, *See* Fibroblast growth factors (FGFs)
Fibroblast growth factors (FGFs), 86
"File drawer problem", 24–25
FISH, *See* Fluorescent in situ hybridization (FISH)
FISH nomenclature, 158
Flow cytometry crossmatch (FCXM), 247
Fluconazole, 39
Fluorescent in situ hybridization (FISH), 65–66, 145, 147
*FMR*1 gene, *See* Fragile X mental retardation gene (*FMR*1 gene)

FMRP, *See* Fragile X mental retardation protein (FMRP)
Focal femoral hypoplasia, 44–45
Folic acid antagonists, 37
Fragile X mental retardation gene (*FMR*1 gene), 181, 182f
Fragile X mental retardation protein (FMRP), 69
Fragile X syndrome (FXS), 66, 69, 170, 181–183
Frataxin gene (*FXN* gene), 183–184
Friedreich ataxia, 183–184
*FXN* gene, *See* Frataxin gene (*FXN* gene)
FXS, *See* Fragile X syndrome (FXS)

**G**

G-banding, 149
  human idiogram based on G-banding pattern, 150f
  metaphase with marker chromosomes, 149f
α-Gal A activity, *See* α-Galactosidase A activity (α-Gal A activity)
α-Galactosidase A activity (α-Gal A activity), 207
GATK, *See* Genome Analysis Toolkit (GATK)
GCPS, *See* Greig cephalopolysyndactyly syndrome (GCPS)
GDB, *See* Genome Data Base (GDB)
GDD, *See* Global developmental delay (GDD)
Geisinger Health System in Pennsylvania, 376
Gene
  silencing, 99–100
  therapy, 217–219, 255

gene editing and genomic therapy, 218–219
HSCT for ExVivo gene therapy, 218
in vivo gene therapy, 218
GeneCards, 193
Generalized dysplasias, 5
Generalized overgrowth disorders, 120–128
overgrowth syndromes diagnosed by sequencing, 128
prenatal-onset overgrowth
BWS, 120–121
overgrowth syndromes linked to epigenetic mechanisms, 121
PI3K–related overgrowth disorders, 124–127
PP2A related overgrowth, 124
PTEN hamartoma tumor syndrome, 127–128
Simpson-Golabi-Behmel syndrome, 121
GeneReviews, 193
Genetic Testing Registry (GTR), 193
Genetic(s), 322, 350–351
consultation process, 268–277, 268f
clinical assessment, 269
family history assessment, 269–271
management and prevention strategies, 275–276
counseling process, 269, 276–277, 293–294, 355, 362–363
challenges in, 345–352
nondirectiveness in, 330–331
determinism, 350

disorders with known genetic etiology, 10–11
education, 276–277
and environmental factors contribute to development, 266f
health care
models, 277
services, 265–266, 266t–267t
heterogeneity, 265, 283
mechanisms of ID, 65
mutations, 152
outcomes of genetic services, 265–268
services, 265–268, 327, 329–330
societal impact of genetic technology, 194–195
susceptibility to teratogenic effects, 16
testing, 271–275, 273f–274f, 273t
Geneticization, 350–351
Genetics/genomics
evolution and convergence, 360–363, 361t
public health, 355–356
future direction, 363–365
Genome
editing, 217–219
instability, 85–86
cell replication defects, 85–86
DNA repair defects, 85
sequencing, 162

Genome Analysis Toolkit (GATK), 242–244
Genome Canada, 370–373, 372t
Genome Data Base (GDB), 158
Genome of Netherlands Consortium, 374
Genome-wide association studies (GWAS), 81–82
Genomic ancillary system, 317
Genomic diagnosis, 62
Genomic DNA, 238–239
Genomic health record
  anticipate fundamental changes, 322
  clinical care and discovery science, 322–323
  compact representation of clinically actionable subsets, 318–320
  data compression from primary molecular observations, 317
  elements necessary for genomic data integration, 316t
  human-viewable formats and machine-readable formats, 321–322
  maintaining linkage of molecular observations, 317–318
  maintaining separation of primary molecular observations, 316–317
Genomic imprinting, 98–99
Genomic medicine, 355
  age, 315–316
  corporate genomic medicine initiatives, 376–377
  large-scale genomic medicine initiatives, 370
  large-scale regional genome initiatives, 375–376
  national genomic medicine initiatives, 370–375
  studying founder populations, 377

Genomic Medicine Alliance, 370t–372t, 377
Genomic Medicine France (2025), 374
Genomic testing technologies, 265
Genomic therapy, 218–219
Genomics England, 373
Genotype, 230–231, 244
Germline genetic testing for common diseases, 271
GH, *See* Growth hormone (GH)
Global developmental delay (GDD), 61–62
Globin synthesis disorders, 178–179
Glossoptosis, 7–8
Glucocorticoid receptor (GR), 117
Glucocorticoid response elements (GREs), 117–118
Glycosphingolipidoses, 211
gnomAD database, 194
GR, *See* Glucocorticoid receptor (GR)
Graft-versus-host disease (GVHD), 238
Greig cephalopolysyndactyly syndrome (GCPS), 86–87
GREs, *See* Glucocorticoid response elements (GREs)
Growth hormone (GH), 84–85
Growth mechanisms, 84–100
  epigenetic contributions to abnormal growth, 87–100
  genome instability, 85–86
  signaling pathways in growth regulation, 86–87
GTR, *See* Genetic Testing Registry (GTR)

GVHD, *See* Graft-versus-host disease (GVHD)

GWAS, *See* Genome-wide association studies (GWAS)

**H**

H3Africa initiative, *See* Human Heredity and Health in Africa initiative (H3Africa initiative)

H3K36me, *See* Histone 3 lysine 36 (H3K36me)

Hallermann-Streiff syndrome, 102

Haplotype, 231–233

Hb, *See* Hemoglobin (Hb)

HBOC syndrome, *See* Hereditary breast ovarian cancer syndrome (HBOC syndrome)

HD, *See* Huntington disease (HD)

HDAC3, *See* Histone deacetylase 3 (HDAC3)

Health Level-7 (HL7), 322

Healthy behaviors, 357

Heat in fetal hyperthermia, 32

Hedgehog signaling (Hh signaling), 86–87

Hematopoietic stem cell transplantation (HSCT), 209

  for ExVivo gene therapy, 218

Hematopoietic stem cells (HSCs), 255

Hemihyperplasia, 126

Hemihyperplasia lipomatosis syndrome (HHML), 124–126

Hemochromatosis, 111

Hemoglobin (Hb), 178, 288

  Hb S, 288–289

Hemoglobinopathies, 288
Hereditary breast ovarian cancer syndrome (HBOC syndrome), 271, 362
Hereditary hemochromatosis, 177–178
Hereditary hemoglobinopathies, 178–179
  globin synthesis disorders, 178–179
  structural globin disorders, 178
Hereditary nonpolyposis colon cancer (HNPCC), 187–188
Hereditary thrombophilias, 175–177
Heterogeneous detection of mutations, 170–171
Heterozygote
  testing, 283–284
  therapeutic implications for, 292
β-Hexosaminidase A (HexA), 287–288
HFD, *See* High-fat diet (HFD)
HGMD, *See* Human gene mutation database (HGMD)
HGP, *See* Human Genome Project (HGP)
Hh signaling, *See* Hedgehog signaling (Hh signaling)
HHML, *See* Hemihyperplasia lipomatosis syndrome (HHML)
High-fat diet (HFD), 303
Histiocytosis X, 111
Histocompatibility definition, *ITER* to
  cellularly characterized *HLA* alleles and loci, 237–238
  DNA sequencing technology for *HLA* typing, 241–242
  HLA antigens, 234–236

microsphere-based technology for *HLA* typing, 241
molecularly characterized *HLA* alleles and loci, 238–241
next-generation DNA sequencing for HLA typing, 242–244
serologically characterized *HLA* alleles and loci, 236–237
Histone 3 lysine 36 (H3K36me), 121–123
Histone deacetylase 3 (HDAC3), 98
HIV infection, 28–29
HL7, *See* Health Level-7 (HL7)
HLAs, *See* Human leukocyte antigens (HLAs)
HLI, *See* Human Longevity, Inc (HLI)
HNPCC, *See* Hereditary nonpolyposis colon cancer (HNPCC)
Holoprosencephy, 44–45
Homologous recombination (HR), 107–108
HPβCD, *See* Hydroxypropyl-β-cyclodextrins (HPβCD)
HR, *See* Homologous recombination (HR)
HSCs, *See* Hematopoietic stem cells (HSCs)
HSCT, *See* Hematopoietic stem cell transplantation (HSCT)
Huawei Technologies, 373
Human cytogenetics, 145–148
Human gene mutation database (HGMD), 193
Human Genome Project (HGP), 46, 159–162, 242, 356
Human Heredity and Health in Africa initiative (H3Africa initiative), 375
Human karyotype, normal, 149–152
Human leukocyte antigens (HLAs), 229, 246f

antigens, 234–236
healing of Justinian by Saint Cosmas and Saint Damian, 235f
cellularly characterized *HLA* alleles and loci, 237–238
methods for *HLA* typing
antibody reactivity of patient awaiting transplant, 244–245
CDC crossmatch, 245–247
epitope-based matching, 250–253
flow cytometry crossmatch, 247
SPIs, 247–249
virtual crossmatch, 247
microsphere-based technology for *HLA* typing, 241
molecularly characterized *HLA* alleles and loci, 238–241
next-generation DNA sequencing for *HLA* typing, 242–244
serologically characterized *HLA* alleles and loci, 236–237
Human Longevity, Inc (HLI), 376–377
Human molecular variation, 322
Human-viewable format documents, 321–322
Huntington disease (HD), 168–169, 179–181, 332
Hybridization approaches, 242
Hydrocephalus, 3
Hydroxypropyl-β-cyclodextrins (HPβCD), 212
Hyperplasia, 120
Hypertrophy, 120
Hypervariable segments, 229
Hypogammaglobulinemia, 26

**I**

ICRs, *See* Imprinting control regions (ICRs)

ID, *See* Intellectual disability (ID)

IEMs, *See* Inborn errors of metabolism (IEMs)

IGF, *See* Insulin-like growth factor (IGF)

IGF insensitivity (IGFIR), 111

IGFIR, *See* IGF insensitivity (IGFIR)

IgG, *See* Immunoglobulin G (IgG)

IHH, *See* Indian hedgehog (IHH)

ILO, *See* Isolated lateralized overgrowth (ILO)

IMAGEN, *See* International MHC and Autoimmunity Genetics Network (IMAGEN)

Immune responses, 235–236

Immunoglobulin G (IgG), 207

Immunologic chimerism, 253

Immunosuppressive drugs, 256–258

Imprinted genes, 98–99

Imprinting control regions (ICRs), 99

In situ hybridization (ISH), 147, 152–162

- array nomenclature, 159–162
- array-based genomic analysis, 159
- comparative genomic hybridization, 158
- detection of structural variation by genome and exome sequencing, 162
- DNA fiber-FISH, 157–158
- DNA probes and applications, 153–157

FISH nomenclature, 158
molecular substitutes for multiprobe FISH tests, 157
In vitro fertilization (IVF), 167, 351
In vivo gene therapy, 218
Inadequate folic acid intake, 44
Inborn errors of metabolism (IEMs), 70–72, 72t
Indian hedgehog (IHH), 86
Induced pluripotent stem (iPS), 255
Infectious agents, 25–31
*See also* Physical agents
bacteria, 29–30
parasites, 30–31
viruses, 26–29
InfoButtons approach, 319–320
Informed consent process, 293–294
age for carrier screening, 294
timing of carrier screening, 294
Institute of Medicine (IOM), 322
Insulin, 104–106
Insulin-like growth factor (IGF), 99
Intellectual disability (ID), 61, 72–75, 123
diagnostic testing of patients, 65
genetic mechanisms of ID, 65
IEMs and, 70–72
medical genetics evaluation for, 64t

recurrent interstitial CMA deletions and duplications, 67t–68t
International Human Genome Project, 154–156
International MHC and Autoimmunity Genetics Network (IMAGEN), 240–241
International System for Human Cytogenetic Nomenclature (ISCN), 149–151, 151t
Intracellular signaling pathways, 87
Intrauterine diagnosis of fetal rubella infection, 26
Intrinsic circadian disorders, 300–301
comparison of sleep profiles of circadian rhythm disorders, 300f
Intrinsic systemic mechanisms of proportionate postnatal short stature, 111–117
Intrinsically derived prenatal-onset deformations, 6
Iodides, 41
IOM, *See* Institute of Medicine (IOM)
Ionizing radiation, 18, 31–32
iPS, *See* Induced pluripotent stem (iPS)
ISCN, *See* International System for Human Cytogenetic Nomenclature (ISCN)
ISH, *See* In situ hybridization (ISH)
Isofagamine, 217
Isoimmune disease, 45
Isolated hemihyperplasia, 126
Isolated hemihypertrophy, 126
Isolated lateralized overgrowth (ILO), 126
Isotretinoin embryopathy, 41, 42f

IUGR, *See* Prenatal growth deficiency
IVF, *See* In vitro fertilization (IVF)

**J**

Janus kinase (JAK), 98
Johanson-Blizzard syndrome, 102

**K**

Karyotype, 149–151
Kennedy disease, 179
Killer Ig-like receptors (KIRs), 249–250
Kleinfelter syndrome, 85, 146
Klippel-Trenaunay syndrome (KTS), 124–126
Kruppel-like factor 15 (Klf15), 304

**L**

Laboratory-developed tests (LDTs), 170
Large deletions detection, 170
  in patients and carriers, 184–185
Large-scale genomic medicine initiatives, 370, 370t–372t
Large-scale regional genome initiatives, 375–376
LD, *See* Linkage disequilibrium (LD)
LDTs, *See* Laboratory-developed tests (LDTs)
LEOPARD syndrome, 117–118
Leprechaunism, *See* Donohue syndrome

Leukemia, 256
Levodopa (L-Dopa), 305–306
Li–Fraumeni syndrome, 188, 271
Limb malformations, 102
Linkage disequilibrium (LD), 231–233
Lipomatosis, 126
"Liquid biopsy", 169
Lithium carbonate, 42
Localized dysplasias, 5
Loci
  cellularly Characterized *HLA* alleles and, 237–238
  serologically characterized *HLA* alleles and, 236–237
Long-term anticoagulant therapy, 175–176
Loss of methylation (LOM), 99
Lyme disease, 30
Lynch syndrome, 187–188, 268, 362
Lysosomal diseases
  mutations causing, 214–215
  PCT for, 216–217
Lysosomal diseases, 209–210
Lysosomal disorders
  experimental studies of PCT in, 215–216
    PCT for Fabry disease, 216
  factors affecting selection of candidate, 207–208
Lysosomal enzyme biosynthesis and degradation, 213–214

Lysosomal storage diseases (LSDs)
BMT, 209–210
emerging therapies, 217–219
ERT, 205–209
pharmacologic chaperone therapy, 213–217
Lysosomal storage disorders, SRT for, 211–212

**M**

Machine-readable formats, 321–322
Magnetic resonance imaging (MRI), 27, 72
Major histocompatibility complex (MHC), 229–234, 230f–231f
HLA typing determine HLA phenotype, 233f
map of mouse and human MHC loci, 232f
MHC-encoded polypeptide chains, 234
Malan syndrome, 121–123
Male sex hormone agents, 41
Malformations, 5
Mandibular hypoplasia, 7–8
MAP, *See* Mitogen activated protein (MAP)
MAPH, *See* Multiplex amplifiable probe hybridization (MAPH)
Marshall-Smith syndrome, 123
Maternal birth weight (MBW), 120
Maternal metabolic factors, 44–45
diabetes mellitus, 44–45
inadequate folic acid intake, 44

obesity, 45

PKU, 45

Maternal smoking during pregnancy, 35

MBW, *See* Maternal birth weight (MBW)

MCAP syndrome, *See* Megalencephaly-capillary malformation syndrome (MCAP syndrome)

MCPH, 86

MDC, *See* Muscle-derived cell (MDC)

Mean fluorescence intensity, 248

Mechanistic target of rapamycin complex (mTOR complex), 87

Medical genetics services, 355

Megaencephaly, 126–127

Megalencephaly-capillary malformation syndrome (MCAP syndrome), 124–127, 128f

Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH syndrome), 126–127

MELAS episodes, *See* Myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS episodes)

MEN, *See* Multiple endocrine neoplasia (MEN)

Mendelian

diseases, 283–284

disorder, 269, 271

forms, 265, 269

inheritance, 5

Mental health, 357

Mental retardation, 65

Messenger RNA processing (mRNA processing), 179
Metabolic conditions, 12
Metabolic disorders, 285, 302–304
  circadian rhythm disruption and metabolic disorders, 303–304
  interactions between circadian system and energy homeostasis, 303
  molecular interactions between clock network and metabolic processes, 302–303
Metabolic dysfunction, 283–284
Metabolic syndrome, 301
Methotrexate, 37
Methylene blue, 44
Methylenetetrahydrofolate reductase gene (*MTHFR* gene), 16, 177
Metropolitan Atlanta Congenital Defects Program, 22–23
MHC, *See* Major histocompatibility complex (MHC)
Microcephalic osteodysplastic primordial dwarfism, type II (MOPDII), 102
Microcephaly, 86
Microchimerism, 253
Microdeletion syndrome, 10
Microduplication syndrome, 10
Microlesions detection in patients and carriers, 185
Microlymphocytotoxicity assay, 236–237
MicroRNAs (miRNAs), 87–98
Microsatellite instability (MSI), 187–188
Microsphere-based technology for HLA typing, 241
Migalastat, 213

Miglustat, SRT with, 212
Minor malformations, 3–4
miRNAs, *See* MicroRNAs (miRNAs)
Mismatch repair deficiency, *See* Replication error
Misoprostol, 43
Missing heritability, 332–333
Mitochondria, 189
Mitochondrial DNA disorders, 189
Mitogen activated protein (MAP), 87
Mixed lymphocyte reaction (MLR), 237–238
MLPA, *See* Multiplex ligation-dependent probe amplification (MLPA)
MLR, *See* Mixed lymphocyte reaction (MLR)
Mobilizing community partnerships, 360
Molecular
  basis, 229–230
  biology technologies, 238
  clock, 299
  etiology, 283–284
  interactions between clock network and metabolic processes, 302–303
  mechanisms, 299
  observations to laboratory methods, 317–318
  scissors, 218
  substitutes for multiprobe FISH tests, 157
  techniques, 145

Molecular genetic diagnosis of commonly tested diseases
CF, 174–175
factor V Leiden and hereditary thrombophilias, 175–177
familial cancer syndromes, 186–189
hereditary hemochromatosis, 177–178
hereditary hemoglobinopathies, 178–179
PWS and AS, 185
repeat expansion disorders, 179–185
DMD and BMD, 184–185
selecting specific repeat expansion disorders, 179–184
Molecular genetic screening targets, 189–190
Molecular genetic technology, 66, 284
Molecular genetic testing
indications for, 166–169
carrier testing and screening, 167–168
diagnostic testing, 166
newborn screening, 166
prenatal diagnosis, 167
presymptomatic/predisposition testing, 168–169
technical approaches to, 169–174
internet resources for, 193–194
ACMG, 194
AMP, 194
CAP's, 194
ClinVar, 194

ExAC and gnomAD, 194
GeneCards, 193
GeneReviews, 193
GTR, 193
HGMD, 193
OMIM, 193
Molecularly characterized HLA alleles and loci, 238–241
Monoamine neurotransmitters, 306
Monozygotic twins, 126
Mood disorders, 306
MOPDII, *See* Microcephalic osteodysplastic primordial dwarfism, type II (MOPDII)
Mouse anti-human monoclonal antibodies, 237
MPPH syndrome, *See* Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH syndrome)
MRI, *See* Magnetic resonance imaging (MRI)
mRNA processing, *See* Messenger RNA processing (mRNA processing)
MSI, *See* Microsatellite instability (MSI)
MSSNG, 377
*MTHFR* gene, *See* Methylenetetrahydrofolate reductase gene (*MTHFR* gene)
mTOR complex, *See* Mechanistic target of rapamycin complex (mTOR complex)
*MTRR* gene, 16
Multifactorial forms, 265
Multifactorial inheritance, 4

Multifactorial model, 4
Multiple endocrine neoplasia (MEN), 188
Multiple malformation syndrome, 5, 9
Multiplex amplifiable probe hybridization (MAPH), 147, 157
Multiplex ligation-dependent probe amplification (MLPA), 147, 170
Multiprobe FISH tests, 157
Muscle-derived cell (MDC), 255
Muscular dystrophies, 167
Mutation-specific DNA-based testing, 292
Mutator phenotype, *See* Replication error
Mycophenolate mofetil, 43
Myocarditis, 29
Myopathies, 12
Myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS episodes), 189
Myositis, 29
Myotonic dystrophy (DM), 183

**N**

NAD(H), *See* Nicotinamide adenine dinucleotide (NAD(H))
NAMPT, *See* Nicotinamide phosphoribosyltransferase (NAMPT)
Nanotechnologies, 46
Narcotic withdrawal symptoms, 43
NAS, *See* Neonatal abstinence syndrome (NAS)
National Academy of Medicine, 322
National genomic medicine initiatives, 370–375, 372t

National Human Genome Research Institute (NHGRI), 319
National Institutes of Health (NIH), 370
National Marrow Donor Program (NMDP), 238
National Society of Genetic Counselors (NSGC), 290–291
Natriuretic peptide precursor-C (NPPC), 85
Natural ability, 328
Natural killer cells (NK cells), 234
NB-DNJ, *See* *N*-Butyldeoxynojirimycin (NB-DNJ)
nBAF complex, *See* Neuron-specific Brg1/hBrm-associated factor complex (nBAF complex)
NCBRS, *See* Nicolaides-Baraitser syndrome (NCBRS)
Neimann-Pick disease (NPD), 207–208
Neonatal abstinence syndrome (NAS), 43
Neural tube defects, 341
Neurocutaneous melanosis sequence, 9, 9f
Neurodegeneration, 305
Neurodevelopmental disabilities, 62
  ASDs, 69–70, 70t
  clinical studies of whole exome sequencing in, 63t
  CNS malformations, ID and brain imaging, 72–75
  cytogenomic copy number abnormalities, 65–66
  diagnosis, 62
  diagnostic testing of patients with ID, 65
  FXS, 69
  GDD, 61–62

genetic mechanisms of ID, 65
ID, 61
inborn errors of metabolism and ID, 70–72, 72t
phenotyping, 64–65
WES, 63–64
WGS, 64
X-linked ID, 66
Neurologic diseases
AD, 304–305
mood disorders, 306
PD, 305–306
Neuromuscular disorders, 265
Neuron-specific Brg1/hBrm-associated factor complex (nBAF complex), 98
Nevo syndrome, 112t–116t
Newborn screening, 166, 273, 277, 339–340, 360–362
*See also* Antenatal screening
Next-generation DNA sequencing for HLA typing, 242–244
Next-generation sequencing (NGS), 162, 167, 172f–173f, 284, 333
NF-κB, *See* Nuclear factor-κB (NF-κB)
*NFIX* gene, 121–123
NGS, *See* Next-generation sequencing (NGS)
NHGRI, *See* National Human Genome Research Institute (NHGRI)
Nicolaides-Baraitser syndrome (NCBRS), 98
Nicotinamide adenine dinucleotide (NAD(H)), 302–303

Nicotinamide phosphoribosyltransferase (NAMPT), 302–303
NIH, *See* National Institutes of Health (NIH)
NIPS, *See* Noninvasive prenatal screening (NIPS)
NIPT, *See* Noninvasive prenatal testing (NIPT)
Nitric oxide synthase gene (*NOS*3 gene), 16
NK cells, *See* Natural killer cells (NK cells)
NMDP, *See* National Marrow Donor Program (NMDP)
*Noggin*, 87
Non-24-h sleep-wake disorder (non-24SWD), 301
non-24SWD, *See* Non-24-h sleep-wake disorder (non-24SWD)
Non-HLA antibodies, 244–245
Nondirectiveness in genetic counseling, 330–331
Nongenetic healthcare providers, 277
Noninvasive prenatal screening (NIPS), 167
Noninvasive prenatal testing (NIPT), 344
Nonirradiated "responders", 237–238
Nonprescription drugs, 36
*See also* Prescription drugs
Nonsyndromic XLID (NS-XLID), 66
Noonan syndrome, 102, 106f, 118
Normal human karyotype, 149–152
NORs, *See* Nucleolar organizing regions (NORs)
*NOS*3 gene, *See* Nitric oxide synthase gene (*NOS*3 gene)
NPD, *See* Neimann-Pick disease (NPD)
NPPC, *See* Natriuretic peptide precursor-C (NPPC)

NS-XLID, *See* Nonsyndromic XLID (NS-XLID)
NSGC, *See* National Society of Genetic Counselors (NSGC)
Nuclear factor-κB (NF-κB), 87
Nuclear receptors, 299
Nucleases, 218
Nucleolar organizing regions (NORs), 149

**O**

Obesity, 45, 301
OBW, *See* Offspring birth weight (OBW)
Ocular anomalies, 29
Odds ratios, *See* Relative risk
Offspring birth weight (OBW), 120
"Oligotyping" technique, 239
OMIM, *See* Online Mendelian Inheritance in Man (OMIM)
Oncogene protein kinase B (AKT), 87
Online Mendelian Inheritance in Man (OMIM), 85, 193
Openinfobutton, 319
Opioid analgesics, 43
Opitz syndrome, 82t, 88t–97t
Organic mercury compounds, 33
Organism level, circadian rhythm disruption and metabolic disorders at, 303–304
"Orphan" genetic test, 193
OS, *See* Osteosarcoma (OS)
Osteoblast hyperactivity, 87

Osteoporosis, 265
Osteosarcoma (OS), 107–108
Osterix, 124
Overgrowth syndromes
  diagnosed by sequencing, 128
  linked to epigenetic mechanisms, 121
Ownership, 349–350

**P**

p110δ-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI), 124–125
PACS, *See* Picture archiving and communication system (PACS)
Pan-ethnic screening, 283
Panel reactive antibody (PRA), 244–245
PAR1, *See* Pseudoautosomal region1 (PAR1)
Parasites, 30–31
Parathyroid hormone–related protein (PTHrP), 86
Parental karyotypes, 10
Parkes-Weber syndrome (PWS), 126
Parkinson disease (PD), 305–306
Parvovirus B19, 29
PASLI, *See* p110δ-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI)
Passive CDS, 319
Patch homologues 1 (Ptch1), 86
Patch homologues 2 (Ptch2), 86

Patchy phenotype, 9
Pathogen genetics/genomics, 356
Pathogenesis, 6–7
Pathologic overgrowth, 118–128
  generalized overgrowth disorders, 120–128
Pathologic short stature, 100–118
  disproportionate short stature, 118
  proportionate prenatal-onset short stature, 100–111
    chromosomal disorders, 102–106
    cohesionopathies, 108–111
    defects of DNA repair, 107–108
    syndromic short stature, 102
  proportionate short stature of postnatal onset, 111–118
    extrinsic mechanisms, 117
    intrinsic systemic mechanisms, 111–117
    RASopathies, 117–118
PCR, *See* Polymerase chain reaction (PCR)
PCT, *See* Pharmacologic chaperone therapy (PCT)
PD, *See* Parkinson disease (PD)
Penicillin, 30
Peptides, 234
*Per*2 gene, 300–301
Perceived personal control, 330
*Period* genes (*Per* genes), 299
Peripheral clocks, 299–300, 303

Perlman syndrome, 83t–84t, 112t–116t
Persistent congenital anemia, 29
Peutz–Jeghers syndrome, 271
*Pgc*-1$\alpha$, *See* PPAR$\gamma$ coactivator 1$\alpha$; (*Pgc*-1$\alpha$)
PGD, *See* Preimplantation genetic diagnosis (PGD)
Pharmaceutical treatment scheduling therapy, 307
Pharmacogenetics, 347–348
  testing, 190–191, 271–272
Pharmacologic chaperone therapy (PCT), 205, 213–217
  cotherapy, 217
  criteria for rescuable mutations by, 215
  criteria for selection of pharmacologic chaperones, 215
  experimental studies of PCT in lysosomal disorders, 215–216
  for lysosomal diseases, 216–217
  lysosomal enzyme biosynthesis and degradation, 213–214
  mutations causing lysosomal diseases, 214–215
Phase-delay technique, 306–307
Phenobarbital, 40
Phenotype, 230–231
Phenotyping, 64–65
Phenylketonuria (PKU), 45, 285, 328–329, 339
Philadelphia chromosome, 145–146
Phosphatases (PSPs), 124
Phosphatidylinositol-3-kinase (PI3K), 87, 124–125
  PI3K–related overgrowth disorders, 124–127

Phosphoinositide-3 kinase, *See* Phosphatidylinositol-3-kinase (PI3K)
Phosphorylated S6 ribosomal protein (Phospho-S6 protein), 126–127
Physical agents, 31–33
  *See also* Infectious agents
  heat, 32
  ionizing radiation, 31–32
  mechanical factors, 32–33
Phytohemagglutinin, 146–147
PI3K, *See* Phosphatidylinositol-3-kinase (PI3K)
PI3K-related overgrowth spectrum (PROS), 124–125
Picture archiving and communication system (PACS), 316
Pierre Robin syndrome, 7–8
*PIK3CA* gene, 124–125
Pilot carrier screening programs, 345
PKU, *See* Phenylketonuria (PKU)
Plant cytogenetics, 146
PMI, *See* Precision Medicine Initiative (PMI)
Point mutations detection, 169–170
Policy development, 360
Polycomb repressive complex (PCR), 99–100
Polycomb-group proteins, 99–100
Polymerase chain reaction (PCR), 165, 183f, 239
  amplification, 170
  multiplex PCR for detection of dystrophin deletions, 184f
  PCR-based methylation analysis, 185f

Polymorphisms, 173, 229–230, 300
Polystyrene, 248
Population
  genetic screening, 338–345
    antenatal screening, 340–344
    carrier screening, 344–345
    newborn screening, 339–340
  health, 355
  population-attributable risk, 20
  population-based carrier screening, 284
  population-based cohort studies, 23
Postnatal growth deficiency syndromes, 109t–110t
Postnatal onset of developmental problems, 1–4, 12
  environmental factors, 12
  genetic, 12
    central nervous system degenerative states, 12
    metabolic conditions, 12
    myopathies and connective tissue disorders, 12
Potter sequence, 32–33
PP1, *See* Protein phosphatase 1 (PP1)
PP2A, *See* Protein phosphatase 2A (PP2A)
PPARγ coactivator 1α (*Pgc*-1α), 302
PRA, *See* Panel reactive antibody (PRA)
Prader-Willi and Angelman syndromes, 65–66
Prader–Willi syndrome (PWS), 185, 317–318

Precision medicine, 365
Precision Medicine Initiative (PMI), 370
Predictive genetic testing, 334
  *See also* Diagnostic genetic testing
Predictive testing of children, 337
Pregnancy, 21
  maternal smoking during, 35
  registries, 22–23
  termination, 285
Pregnant patient evaluation for teratogenic exposures, 21
Preimplantation genetic diagnosis (PGD), 351
Premutation alleles, 69
Prenatal diagnosis, 167
Prenatal growth
  deficiency, 101
  retardation, 35
Prenatal onset of developmental problems, 1–12
  chromosomal abnormalities, 9–10
  deformations, 5–6
  disorders
    causing by teratogens, 11
    with known genetic etiology, 10–11
  disruptive defects, 6–7
  dysplasia, 7
  malformations, 5

multiple malformation syndromes, 9
recognized patterns, 11–12
sequence, 7–9
single primary defect in development, 5
Prenatal-onset growth deficiency disorders, 82t, 88t–97t
Prenatal-onset overgrowth syndromes, 112t–116t
Prescription drugs, 36–44
antibiotics and anti-infective agents, 38–39
anticancer agents, 37
anticonvulsants, 39–40
bendectin, 44
codeine and opioid analgesics, 43
endocrine agents, 40–41
folic acid antagonists, 37
inhibitors of renin-angiotensin system, 43–44
lithium carbonate, 42
methylene blue, 44
misoprostol, 43
mycophenolate mofetil, 43
retinoids and vitamin A, 41–42
SSRIs, 42–43
thalidomide, 36
warfarin anticoagulants, 37–38
Presymptomatic/predisposition testing, 168–169
Primary molecular observations, 316–317

Primidone, 40
Privacy within family, 335–336
Proband, 283–284
Progestins, 40
Prognosis, 5–7
PROS, *See* PI3K-related overgrowth spectrum (PROS)
Protein phosphatase 1 (PP1), 124
Protein phosphatase 2A (PP2A), 124
  related overgrowth, 124
Proteus syndrome, 126
PSBT, *See* Pyrosequence-based typing (PSBT)
Pseudoautosomal region1 (PAR1), 85
Pseudodeficiency allele, 283
PSPs, *See* Phosphatases (PSPs)
Psychologic diseases, 304–306
Psychosocial dwarfism, 117
Ptch1, *See* Patch homologues 1 (Ptch1)
PTEN, 87
  hamartoma tumor syndrome, 127–128
PTHrP, *See* Parathyroid hormone–related protein (PTHrP)
PTPs, *See* Tyrosine phosphatases (PTPs)
Public health, 356–358, 356t
  ensuring quality and accessibility of health services, 358
  evolution and convergence of science fields, 360–363, 361t
  genetics/genomics, 355–356

future direction, 363–365
preventing epidemics and spread of disease, 356
preventing harm and injuries, 356–357
promoting and encouraging healthy behaviors and mental health, 357
protection against environmental hazards, 356
responding to disasters and assist communities in recovery, 357
system infrastructure, 358–360
assessment, 359–360
assurance, 360
policy development, 360
Publication bias, 24–25
PWS, *See* Parkes-Weber syndrome (PWS), Prader–Willi syndrome (PWS)
Pyloric stenosis, 5
Pyrosequence-based typing (PSBT), 241–242

## Q

Q-bands, *See* Quinacrine bands (Q-bands)
Q-PCR, *See* Quantitative polymerase chain reaction (Q-PCR)
QA, *See* Quality assurance (QA)
Qatar Biobank, 373
Qatar Genome Project, 373
Quality assurance (QA), 191–193
Quantitative polymerase chain reaction (Q-PCR), 147
Quinacrine bands (Q-bands), 147

Quinine, 38–39

**R**

Rabbit anti-mouse erythrocyte sera, 235–236
Randomized controlled trials, 23
RANKL, *See* Receptor activator of NF-κB ligand (RANKL)
RAPADILINO syndrome, 107–108
Ras, *See* Rat sarcoma (Ras)
Ras/MAPK signaling cascade, 87, 119f
RASopathies, 87, 117–118
  Wolf-Hirschhorn syndrome, 118f
Rat sarcoma (Ras), 87
Recall of risk information, 329
Receptor activator of NF-κB ligand (RANKL), 87
Receptor tyrosine kinase (RTK), 87
Recognized patterns, 11–12
Recontact, duty to, 347
Record linkage studies, 24
RecQ proteins, 85
"Recreational" drugs, 34–36
  caffeine, 35–36
  cocaine, 35
  ethyl alcohol, 34
  tobacco smoking, 34–35
Recurrence risk, 4–5

Reduced disease incidence, 285
Regeneron Pharmaceuticals, 376
Reimbursement, 191–193
Relative risk, 20
Religion, 345–346
Renin-angiotensin system, inhibitors of, 43–44
Replication error, 187–188
Reproductive plans, 330
Reproductive screening and testing options, 284–285
Reproductive technologies, 351–352
Reprogenetics, 344, 351–352
*REPROTOX* resources, 15
Research in human genetics, 348–350
  confidentiality, 349
  consent and feedback, 348–349
  control and ownership, 349–350
Restriction fragment length polymorphism (RFLP), 178, 238–239
Retinoblastoma, 189
Retinoids, 41–42
REV-ERB$\alpha$, 302
"Reverse dot blot", 239
"Reverse" techniques, 240
RFLP, *See* Restriction fragment length polymorphism (RFLP)
RhoGTPase pathway, 65
Risk, 20

RNA–based testing methods, 291

Roberts syndrome, 85–86

Robin malformation sequence, 7–8, 7f

Robinow syndrome, 82t, 88t–97t

Rothmund-Thomson syndrome (RTS), 107–108

RSS, *See* Russell-Silver syndrome (RSS)

RT-PCR, 28

RTK, *See* Receptor tyrosine kinase (RTK)

RTS, *See* Rothmund-Thomson syndrome (RTS), Rubenstein–Taybi syndrome (RTS)

Rubella, 26

  embryopathy, 26, 26f

Rubenstein–Taybi syndrome (RTS), 11–12, 82t, 102, 105f

*Runt-related transcription factor* 2 (*RUNX*2), 86–87

Russell-Silver syndrome (RSS), 82t, 99, 99f, 102

**S**

S-XLID, *See* Syndromic XLID (S-XLID)

SABs, *See* Single-antigen beads (SABs)

Salicylates, 36

SardiNIA project in Italy, 377

Saudi Human Genome Project, 373–374

SBMA, *See* Spinobulbar muscular atrophy (SBMA)

SCA, *See* Spinocerebellar ataxia (SCA)

Scc1, *See* Sister chromatid cohesion 1 (Scc1)

Scc2–Scc4 complex, *See* Sister chromatid cohesion 2 and 4 complex

(Scc2–Scc4 complex)
SCD, *See* Sickle cell disease (SCD)
SCN, *See* Suprachiasmatic nucleus (SCN)
Scottish Genomes Partnership, 375
Screening tools, 357
SCT, *See* Sickle cell trait (SCT)
Seckel syndrome spectrum disorder, 86
Segmental overgrowth syndromes, 126
Selective serotonin reuptake inhibitors (SSRIs), 42–43
Sensitivity, 292–293
Sensorineural hearing loss, 27
Sequence, 7–9
Bio, 376–377
interpretation of novel sequence variants, 173–174
Sequence-specific oligonucleotide probe (SSOP), 239
Sequence-specific primer (SSP), 240
Serologically characterized HLA alleles and loci, 236–237
SGA, *See* Small for gestational age (SGA)
SHH, *See* Sonic hedgehog (SHH)
Short stature, 81–82
pathologic, 100–118
Sickle cell disease (SCD), 288–289, 339
Sickle cell trait (SCT), 284
Signal transducer and activator of transcription (STAT), 85
Signaling pathways in regulation of growth, 86–87

BMP antagonists, 87
hedgehog signaling, 86–87
signaling cascades, 87
prenatal-onset growth deficiency disorders, 88t–97t
Silver stain-NOR (Ag-NOR), 149
Simons Simplex Collection (SSC), 70
Simpson-Golabi-Behmel syndrome, 121
Single nucleotide polymorphisms (SNPs), 10, 81–82, 148, 229–230, 332–333
Single primary defect in development, 5
Single-antigen beads (SABs), 248–249
Single-stranded DNA, 152
Sister chromatid cohesion 1 (Scc1), 108
Sister chromatid cohesion 2 and 4 complex (Scc2–Scc4 complex), 108
Sleep-phase therapy, 306–307
SMA, *See* Spinal muscular atrophy (SMA)
Small for gestational age (SGA), 3, 82
SMC proteins, *See* Structural maintenance of chromosome proteins (SMC proteins)
Smith-Lemli-Opitz syndrome, 63t, 67t–68t, 70t
Smith-Magenis syndrome, 65–66
*SMN*1 gene, *See* Survival motor neuron 1 gene (*SMN*1 gene)
Smoothened protein (Smo protein), 86
SNPs, *See* Single nucleotide polymorphisms (SNPs)
Society, 350–351
Solid-phase immunoassays (SPIs), 244–245, 247–249

Somatic cell nuclei, 253–255
Sonic hedgehog (SHH), 86
Sotos syndrome, *See* Cerebral gigantism
Sotos syndrome 2, 121–123
Specificity, 292–293
Spinal muscular atrophy (SMA), 189–190, 289–290
Spinobulbar muscular atrophy (SBMA), 179
Spinocerebellar ataxia (SCA), 166, 181
SPIs, *See* Solid-phase immunoassays (SPIs)
Spondyloepiphyseal dysplasia congenita, 9
SRT, *See* Substrate reduction therapy (SRT)
SSC, *See* Simons Simplex Collection (SSC)
SSOP, *See* Sequence-specific oligonucleotide probe (SSOP)
SSP, *See* Sequence-specific primer (SSP)
SSRIs, *See* Selective serotonin reuptake inhibitors (SSRIs)
STAT, *See* Signal transducer and activator of transcription (STAT)
Steinert myotonic dystrophy, 6
Stem cells and transplantation, 253–255, 256f
Steroids, 117
Stickler syndrome, 9
Stimulators, 237–238
Structural directiveness, 330–331
Structural Globin disorders, 178
Structural maintenance of chromosome proteins (SMC proteins), 108
Structural variation detection by genome and exome sequencing, 162

Structured data, 315
Substrate reduction therapy (SRT), 205, 210–213
with cyclodextrins, 212
for lysosomal storage disorders, 211–212
with miglustat, 212
for type 1 Gaucher disease, 211
Suppressor of zeste (SUZ), 99–100
Suprachiasmatic nucleus (SCN), 299–300
Surveillance and Pharmacogenomics of Adverse Drug Reactions project, 375–376
Survival motor neuron 1 gene (*SMN*1 gene), 289–290
SUZ, *See* Suppressor of zeste (SUZ)
SWItch/sucrose nonfermentable-like chromatin remodeling complex (SWI/SNF-like chromatin remodeling complex), 98
Syndromes naming, 346
Syndromic short stature, 102
Syndromic XLID (S-XLID), 66
Syphilis, 30

**T**

T-cell receptor (TCR), 229
TALENs, *See* Transcription activator-like effector nucleases (TALENs)
Talipes equinovarus, 5–6
Tall stature, 81–82
Tandem mass spectrometry (TMS), 339
Tandem repeat disorders, mutation ranges for selecting, 180t

Tay–Sachs disease (TSD), 284, 287–288
TCR, *See* T-cell receptor (TCR)
Telegenetics, 358
Telemedicine, 358
Teratogen, 15
  risk counseling, 20–21
Teratogenesis mechanisms, 16, 16t
Teratogenic disorder, 82t, 88t–97t, 100
Teratogenic effects, 19–21
  characterization, 19t
  clinical settings, 19–20
  dealing with uncertainty, 21
  risk, 20
  teratogen risk counseling, 20–21
Teratogenic exposures, 25–45
  characterization, 17–19, 18t
  drug and chemical agents, 33–45
  infectious agents, 25–31
  physical agents, 31–33
Teratogenicity, 15, 18
Teratogens, disorders causing by, 11
Teratology, 15
Teratology information services (TIS), 22
*TERIS* resources, 15
Tetracyclines, 38

*TGFA* gene, 16
$\alpha$-Thalassemia, 178
$\beta$-Thalassemia, 178–179, 288, 345
Thalassemias, 178–179
Thalidomide, 17, 36
  embryopathy, 36
Third parties of confidentiality, 335
Thymic hypoplasia, 26
Thyroid hormone deficiency, 117
Time-restricted feeding (tRF), 303
Timing of carrier screening, 294
TIPRL, *See* TOR signaling pathway regulator-like (TIPRL)
TIS, *See* Teratology information services (TIS)
Tissue samples, 149
TMS, *See* Tandem mass spectrometry (TMS)
TNF$\alpha$, *See* Tumor necrosis factors $\alpha$ (TNF$\alpha$)
TNF$\beta$, *See* Tumor necrosis factors $\beta$ (TNF$\beta$)
Tobacco smoking, 34–35
Tohoku University Tohoku Medical Megabank Organization, 373
TOR signaling pathway regulator-like (TIPRL), 124
"TORCH" screening, 25–26
Total donor lymphocytes, 246
*Toxoplasma* infection, 30–31
Toxoplasmosis, 30–31
Traditional model, 277

Transcription activator-like effector nucleases (TALENs), 218–219
Transplacental parvovirus B19 infection, 29
Transplantation, 253
  genetics
    clinical significance of HLA molecular typing, 249–253
    historical ITER toward histocompatibility definition, 234–244
    induction of donor-specific immune tolerance with thymus organoids, 257f
    major histocompatibility complex, 229–234
    methods for HLA typing, 244–249
    stem cells and transplantation, 253–255
  stem cells and, 253–255
  surgery, 235
tRF, *See* Time-restricted feeding (tRF)
Trimethoprim, 37
Trinucleotide repeat expansions detection, 170
"Triplet", 250–252, 252f
TSD, *See* Tay–Sachs disease (TSD)
Tumor necrosis factors $\alpha$ (TNF$\alpha$), 230
Tumor necrosis factors $\beta$ (TNF$\beta$), 230
"Two-hit" model of developmental disorders, 333
Type 1 Gaucher disease, 207, 211
Type 2 diabetes, 45, 301
Type 2 Diabetes Genetic Explorationby Next-Generation Sequencing in Multiethnic Samples (T2D-GENES), 376
Tyrosine phosphatases (PTPs), 124

**U**

Ubiquitin ligases, 86

Ubiquitous Pharmacogenomics Consortium, 376

Ultrasound, 126

Unified approach, 82–84

Uniparental disomy (UPD), 11, 185

Uniparental disomy for chromosome 7 (UPD7), 99

United Network for Organ Sharing (UNOS), 250

UPD, *See* Uniparental disomy (UPD)

UPD7, *See* Uniparental disomy for chromosome 7 (UPD7)

US Food and Drug Administration (FDA), 170, 205, 208t, 320, 321f

US Food and Drug Administration Pregnancy and Lactation Labeling Rule, 21

US National Human Genome Research Institute, 375

US Precision Medicine Initiative, 365

US Preventive Services Task Force (USPSTF), 357

**V**

Valproic acid, 39

Variants of uncertain significance (VUS), 166, 173

Vascular disruption, 35, 43

Very low birth weight infants (VLBW infants), 82

Virtual crossmatch (VXM), 247

Viruses, 26–29

Vitamin A, 41–42

Von Hippel–Lindau syndrome (VHL syndrome), 189

**W**

Warfarin anticoagulants, 37–38
Weak folic acid antagonists, 37
Weaver syndrome, 123, 124f
Werdnig–Hoffmann disease, 6
Whole exome sequencing (WES), 4, 63–64, 317–318
Whole genome sequencing (WGS), 10–11, 64, 162, 333
Williams syndrome, 102, 105f
Williams-Beuren syndrome, 65–66
Wolf-Hirschhorn syndrome, 118f
WuXi NextCODE, 373

**X**

X-linked disorder, 216, 284–285
X-linked ID (XLID), 66
X-linked MR (XLMR), 66
Xenografts, 235

**Y**

Yeast artificial chromosome (YAC), 154–156

**Z**

Zika virus (ZIKV), 20, 27–28
Zinc finger nucleases (ZFNs), 218–219
Zinc finger protein 9 gene (*ZNF9* gene), 183