Human Genetics

PART VIII

Chapter 95

Genetics in Pediatric Medicine

Daryl A. Scott and Brendan Lee

Since the completion of the Human Genome Project, there has been an unprecedented expansion in the understanding of how human health is impacted by variations in genomic sequence and epigenetic, non-sequence-based changes that affect gene expression. There has also been the development and implementation of accurate clinical tests that makes it easier for physicians to detect pathogenic variant changes. In addition, there has been a dramatic increase in the availability of information about the genetic aspects of pediatric diseases, particularly on the internet (Table 95.1).

THE BURDEN OF GENETIC DISORDERS IN CHILDHOOD

Medical problems associated with genetic disorders can appear at any age, with the most obvious and serious problems typically manifesting in childhood. It has been estimated that 53/1,000 children and young adults can be expected to have diseases with an important genetic component. If congenital anomalies are included, the rate increases to 79/1,000. In 1978 just over half of admissions to pediatric hospitals were for a genetic condition. By 1996, because of changes in healthcare delivery and a greater understanding of the genetic basis of many disorders, that percentage increased to 71% in one large pediatric hospital in the United States, with 96% of chronic disorders leading to admission having an obvious genetic component or influenced by genetic susceptibility.

Major categories of genetic disorders include single-gene, genomic, chromosomal, and multifactorial conditions.

Individually, each **single-gene disorder** is rare, but collectively they represent an important contribution to childhood disease. The hallmark of a single-gene disorder is that the phenotype is overwhelmingly determined by changes that affect an individual gene. The character and severity of a phenotype associated with some single-gene disorders can vary from one patient to another. In some cases, this may be due to differences in the severity of the change affecting the gene. In other cases, variability is seen in individuals who carry the same pathogenic variant or variants. This is termed **variable expressivity**; the same pathogenic variants in *GJB2* can cause mild, moderate, severe, or profound sensorineural hearing loss in different individuals. This variability may be due to differences in other genetic, epigenetic, environmental, and/or stochastic factors. If this variability results in some individuals being completely unaffected, we say that the disorder shows **incomplete penetrance**. Some single-gene disorders are associated with phenotypes that affect different organ systems or biologic functions that may appear unrelated. Pendred syndrome is caused by pathogenic variants in *SLC26A4* and is characterized by both hearing loss and thyroid enlargement (goiter). This feature of some genetic diseases is referred to as **pleiotropy**.

Common single-gene disorders include sickle cell anemia and cystic fibrosis. Some identifiable syndromes and diseases can be caused by more than one gene. Noonan syndrome can be caused by pathogenic variants in at least 12 different genes. In addition, pathogenic variants

Table 95.1 Useful Internet Genetic Reference Sites

RESOURCE	WEB ADDRESS
National Center for Biotechnology Information A general reference maintained by the National Library of Medicine	www.ncbi.nlm.nih.gov
Online Mendelian Inheritance in Man A useful resource for clinicians containing information on all known mendelian disorders and >12,000 genes. Information focuses on the relationship between phenotype and genotype.	www.ncbi.nlm.nih.gov/omim
Genetic Testing Registry A resource that provides information on individual genes, genetic tests, clinical laboratories, and medical conditions. This resource also provides access to GeneReviews, a collection of expert-authored reviews on a variety of genetic disorders.	www.ncbi.nlm.nih.gov/gtr/
Genetics — MedlinePlus A resource that provides consumer-friendly information about the effects of genetic variations on human health	http://medlineplus.gov/genetics/
National Human Genome Research Institute A resource for information about human genetics and ethical issues	www.genome.gov
Human Gene Mutation Database A searchable index of all described pathogenic variants in human genes with phenotypes and references	www.hgmd.cf.ac.uk
DECIPHER A database designed to aid physicians in determining the potential consequences of chromosomal deletions and duplications	http://decipher.sanger.ac.uk
Database of Genomic Variants A database of chromosomal alterations seen in normal controls	http://dgv.tcag.ca/dgv/app/home
American Society of Human Genetics	www.ashg.org
American College of Medical Genetics and Genomics	www.acmg.net

affecting a single gene may cause more than one disorder. Variants in *SCN5A* can cause Brugada syndrome type 1, long QT syndrome type 3, dilated cardiomyopathy type 1E, familial atrial fibrillation type 10, familial ventricular fibrillation type 1, progressive heart block type 1A, nonprogressive heart block, and sick sinus syndrome type 1.

Single-gene disorders tend to occur when changes in a gene have a profound effect on the *quantity* of the gene product produced, either too much or too little, or the *function* of the gene product, either a loss of function or a harmful gain of function. Single-gene disorders can be caused by de novo sequence changes that are not found in the unaffected parents of the affected individual, or they may be caused by inheriting the affected gene. When a single-gene disorder is known to be caused by changes in only one gene, or a small number of individual genes, searching for deleterious changes is classically performed by directly sequencing that gene and, in some cases, looking for small deletions and/or duplications in that gene. When multiple genes can cause a particular disorder, it is sometimes more efficient and cost-effective to screen large numbers of disease-causing genes using a disease-specific panel, which takes advantage of massively parallel or next-generation sequencing technology, rather than screening genes individually. When such panels are not available, or when the diagnosis is in question, physicians may consider screening the protein-coding regions of all genes by **exome sequencing (ES),** also termed whole exome sequencing (WES), on a clinical basis. In many circumstances, ES is less expensive than sequencing multiple individual genes. **Whole genome sequencing (WGS)**, in which an individual's entire genome is sequenced, is another clinical option (see Chapter 98; Table 95.2).

The risk of having a child with a particular single-gene disorder can vary from one population to another. This may be the result of a **founder effect**, in which a specific change affecting a disease-causing gene becomes relatively common in a population derived from a small number of founder individuals. This high frequency is maintained when there is relatively little interbreeding with persons outside that population because of social, religious, or physical barriers. This is the case for Tay-Sachs disease in Ashkenazi Jews and French Canadians. Other changes may be subject to **positive selection** when found in the heterozygous carrier state. In this case, individuals who carry a single copy of a genetic change **(heterozygotes)** have a survival advantage over noncarriers. This can occur even when individuals who inherit two copies of the change **(homozygotes)** have severe medical problems. This type of positive selection is evident among individuals in sub-Saharan Africa who carry a single copy of a hemoglobin pathogenic variant that confers relative resistance to malaria but causes sickle cell anemia in homozygotes.

Genomic disorders are a group of diseases caused by alterations in the genome, including **deletions** (copy number loss), **duplications** (copy number gain), **inversions** (altered orientation of a genomic region), and **chromosomal rearrangements** (altered location of a genomic region). **Contiguous gene disorders** are caused by changes that affect two or more genes that contribute to the clinical phenotype and are located near one an other on a chromosome. DiGeorge syndrome, which is caused by deletions of genes located on chromosome 22q11, is a common example. Some genomic disorders are associated with distinctive phenotypes whose patterns can be recognized clinically. Other genomic disorders do not have a distinctive pattern of anomalies but can cause developmental delay, cognitive impairment, structural birth defects, abnormal growth patterns, and changes in physical appearance.

Fluorescent in situ hybridization (FISH) can provide information about the copy number and location of a specific genomic region. **Array-based copy number detection assays**, sometimes referred to as chromosomal microarray analyses, can be used to screen for chromosomal deletions and duplications (large and small) across the genome but do not provide information about the orientation or location of genomic regions. A **chromosome analysis (karyotype)** can detect relatively large chromosomal deletions and duplications and can also be useful in identifying inversions and chromosomal rearrangements even when they are **copy number neutral** changes that do not result in a deletion or duplication of genomic material (e.g., balanced translocations).

Table 95.2 Indications for Single Gene, Gene Panel, Exome, and Genome Sequencing

INDICATIONS	EXAMPLES
SINGLE GENE	
Minimal locus heterogeneity (only one or a small number of genes known to cause the condition)	*CFTR* for cystic fibrosis
Distinctive clinical findings that clearly point to specific gene	*PAH* for phenylketonuria
GENE PANEL	
Locus heterogeneity (multiple genes are known to cause the same or similar conditions)	Muscular dystrophy panel
Disorders with overlapping phenotypes	Cardiomyopathy panel
Disorders that share one manifestation but can have very different presentations	Epilepsy panel
Disorders associated with genes from a common pathway or structure	*RAS*opathy panel
EXOME	
Extreme heterogeneity and de novo mutations common	Autism, intellectual disability
Two or more unrelated phenotypes in one patient	Oculocutaneous albinism and neutropenia
No distinctive phenotypic feature present	Kabuki syndrome
Phenotype indistinct and underlying cause not clear	Congenital diarrhea, Zellweger syndrome
GENOME	
As above for Exome, plus:	
Noncoding variation is suspected as a cause	Hypertrophic cardiomyopathy
Structural variation is suspected as a cause	DiGeorge syndrome
Exome sequencing already performed and was nondiagnostic	Undiagnosed Diseases Network
Rapid generation of sequencing data needed for patients who are critically ill	Neonates and children in intensive care
Suspected mitochondrial disorder: multisystem (heart, brain, muscle, liver) disorder	Whole genome and/or mitochondrial DNA (tissue) sequencing

From Manolio TA, Rowley R, Williams MS, et al. Opportunities, resources, and techniques for implementing genomics in clinical care. *Lancet*. 2019;394:511–520. Table 2.

Deletions, duplications, and chromosomal rearrangements that affect whole chromosomes, or large portions of a chromosome, are typically referred to as **chromosomal disorders**. One of the most common chromosomal disorders is Down syndrome, which is most often associated with the presence of an extra copy, or **trisomy**, of an entire chromosome 21. When all or part of a chromosome is missing, the disorder is referred to as **monosomy**. **Translocations** are a type of chromosomal rearrangement in which a genomic region from one chromosome is transferred to a different location on the same chromosome or on a different (nonhomologous) chromosome. Translocations can be *balanced*, meaning that no genetic material has been lost or gained, or they can be *unbalanced*, in which some genetic material has been deleted or duplicated.

In some cases, only a portion of cells that make up a person's body are affected by a single-gene defect, a genomic disorder, or a chromosomal defect. This is referred to as **mosaicism** and indicates that the individual's body is made up of two or more distinct cell populations. Through use of technologies with high sensitivity, it has become

evident that there can be significant low-level mosaicism in *somatic* tissues.

Polygenic disorders are caused by the cumulative effects of variations in more than one gene. **Multifactorial** disorders are caused by the cumulative effects of variations in multiple genes or the combined effects of genetic, epigenetic, and/or environmental factors. Spina bifida and isolated cleft lip or palate are common birth defects that display multifactorial inheritance patterns. Multifactorial inheritance is seen in many common pediatric disorders, such as asthma and diabetes mellitus. These traits can cluster in families but do not have a mendelian pattern of inheritance (see Chapter 97). In these cases, the genetic changes or variations that are contributing to a particular disorder are often unknown, and genetic counseling is based on empirical data and estimates.

THE CHANGING PARADIGM OF GENETICS IN MEDICINE

Genetic testing is increasingly available for a wide variety of both rare and relatively common genetic disorders. Genetic testing is typically used in pediatric medicine to resolve uncertainty regarding the underlying etiology of a child's medical problems. Identifying a molecular cause often provides the basis for improved genetic counseling, may alter medical management, and may suggest that a specific therapy should be employed. Even in cases where a specific treatment is not available, identifying a genetic cause can aid physicians in providing individuals and families with accurate prognostic and recurrence risk information, can often help to relieve unfounded feelings of guilt, and may stem the tide of misdirected blame.

Genetic tests are influencing an increasing number of medical decisions and are becoming a seamless part of routine medical care. Although most genetic testing is presently aimed at identifying or confirming a diagnosis, in the future genetic testing may find wider application as a means of determining whether an individual is predisposed to develop a particular disease. Another area in which genetic testing could make a significant impact is on individualized drug treatment (pharmacogenetics). It has long been known that genetic variation in the enzymes involved in drug metabolism underlies differences in the therapeutic effect and toxicity of some drugs. When the genetic changes that underlie these variations are identified, new genetic tests can be developed that allow physicians to tailor treatments based on individual variations in drug metabolism, responsiveness, and susceptibility to toxicity. It is likely that the expansion of such testing will depend, at least in part, on the extent to which such tests can be linked to strategies to prevent disease or improve outcome (see Chapter 98).

Long-standing and highly successful carrier screening programs have existed for disorders such as Tay-Sachs disease and many other rare, single-gene disorders that are prevalent in specific populations. Couples are usually offered screening for a variety of conditions based, in part, on ancestry (Tay-Sachs disease, hemoglobinopathies, cystic fibrosis). Couples found to be at increased risk for such disorders can be offered preconception or prenatal testing aimed at detecting specific disease-causing variants.

Prenatal screening is routinely offered for chromosomal disorders such as trisomy 13, trisomy 18, and Down syndrome. Pregnancies affected by these, and possibly other genetic disorders, are being recognized by noninvasive screening tests targeting **fetal cell-free DNA** in maternal blood and by fetal ultrasound. When genetic disorders are suspected, chorionic villus sampling at 10-12 weeks of gestation or amniocentesis at 16-18 weeks of gestation can provide material for genetic testing. When a couple are at risk for a specific genetic defect, **preimplantation genetic diagnosis** can sometimes be used to select unaffected early embryos, which are then implanted as part of an in vitro fertilization procedure.

Although prenatally obtained genetic material can be used to identify single-gene disorders, genomic disorders, and chromosomal anomalies, the information obtained on any pregnancy depends on the tests that are ordered. It is important that physicians select the most appropriate prenatal tests and that couples understand the limitations of these tests. No amount of genetic testing can guarantee the birth of a healthy child.

Specific treatments are not available for most genetic disorders, although some important exceptions exist (see Chapter 98). **Inborn errors of metabolism** were the first genetic disorders to be recognized, and many are amenable to treatment by dietary manipulation (see Chapter 104). These conditions result from genetically determined deficiency of specific enzymes, leading to the buildup of toxic substrates and/or deficiency of critical end products.

Individual metabolic disorders tend to be very rare, but their combined impact on the pediatric population is significant. Tandem mass spectrometry has made it relatively inexpensive to screen for a large number of these disorders in the newborn period. Use of this technology not only dramatically increases the number of metabolic disorders identified within a population but also allows treatment to be initiated at a much earlier stage in development. Broader screening in the postnatal period, in the form of metabolomic screening, has increased the potential for identifying an increasing list of rare inborn errors of metabolism, especially when combined with exome or genome sequencing.

Progress in genetic therapies has improved the treatment of some **lysosomal storage disorders** (see Chapter 106.4). These metabolic diseases are caused by defects in lysosomal function. Lysosomes are cellular organelles that contain specific digestive enzymes. Some of these disorders that were characterized by early lethal or intractable chronic illness can now be treated using specially modified enzymes administered by intravenous infusion. These enzymes are taken up by cells and incorporated into lysosomes. Conditions such as Gaucher disease and Fabry disease are routinely treated using **enzyme replacement**; similar therapies are being developed for other lysosomal disorders.

Therapeutic advances are also being made in the treatment of nonmetabolic genetic disorders. Improvements in surgical techniques and intensive care medicine are extending the survival of children with life-threatening birth defects such as congenital diaphragmatic hernia and severe cardiac defects. In many cases, the life expectancy of children with debilitating genetic disorders is also increasing. Improvements in nutrition and the management of chronic pulmonary disease in patients with cystic fibrosis allow an increasing percentage of affected patients to survive into adulthood, creating a need to transition care from pediatric to adult providers.

Gene replacement therapies are being used in the treatment of a variety of genetic disorders (see Chapter 98). Stem cell–based therapies have also been employed as a potential treatment for a number of intractable disorders.

DIRECT-TO-CONSUMER GENETIC TESTING

In most cases, healthcare providers order genetic tests, interpret the results, communicate these results to their patients, and then document these finding in the medical record. The costs of these tests are often covered completely or in part by a health insurance company. In contrast, some companies are offering direct-to-consumer genetic tests in which individuals order their own tests and receive test results directly from the company. The individual usually pays for these tests, and the results are not recorded in their medical record unless the individual chooses to share this information with their healthcare providers. Some direct-to-consumer genetic tests only provide information about common traits or information about an individual's ancestry or relationship to others **(ancestry testing)**. Other direct-to-consumer tests are designed to make predictions about health and/or to identify pathogenic variants associated with specific genetic disorders. In some cases, individuals ordering these tests may have the option of reviewing their results with a healthcare provider employed by the company performing the test.

One of the main benefits of direct-to-consumer testing is that it allows individuals to privately access their genetic information without having to involve a healthcare provider or their insurance company. Although this could be seen as a means of empowering individuals with regards to their healthcare, the benefits derived from such testing depend heavily on the appropriateness of the test ordered, the quality of

the testing/reporting services, the capacity of the individual to understand the test results, and their ability to make informed choices based on those results.

ETHICS ISSUES

Genetic testing, diagnosis, and treatment should be performed *confidentially*. Nothing is as personal as one's genetic information, and all efforts should be made to avoid any stigma for the patient. Many people worry that results of genetic testing will put them, or their child, at risk for genetic discrimination. **Genetic discrimination** occurs when people are treated unfairly because of a difference in their DNA that suggests they have a genetic disorder or they are at an increased risk of developing a certain disease. In the United States the Genetic Information Nondiscrimination Act of 2008 protects individuals from genetic discrimination at the hands of health insurers and employers but does not extend protection against discrimination from providers of life, disability, or long-term care insurance.

The decisions about genetic testing should be based on a careful evaluation of the potential benefits and risks. In the pediatric setting, these decisions may be more difficult because physicians and parents are often called on to make decisions for a child who cannot directly participate in discussions about testing. Molecular diagnostic tests are often used to diagnose malformation syndromes, cognitive delay, or other disabilities in which there is a clear benefit to the child. In other cases, such as genetic testing for susceptibility to adult-onset diseases, it is appropriate to wait until the child or adolescent is mature enough to weigh the potential risks and benefits and make their own decisions about genetic testing.

Policies regarding genetic testing of children have been developed collaboratively by the American Academy of Pediatrics (AAP) and the American College of Medical Genetics and Genomics (ACMG; *Pediatrics* 131[3]:620–622, 2013). These recommendations are outlined in the following list.

General Recommendations

1. Decisions about whether to offer genetic testing and screening should be driven by the best interest of the child.
2. Genetic testing is best offered in the context of genetic counseling. Genetic counseling can be performed by clinical geneticists, genetic counselors, or any other healthcare provider with appropriate training and expertise. AAP and ACMG support the expansion of educational opportunities in human genomics and genetics for medical students, residents, and practicing pediatric primary care providers.

Diagnostic Testing

3. In a child with symptoms of a genetic condition, the rationale for genetic testing is similar to other medical diagnostic evaluations. Parents or guardians should be informed about the risks and benefits of testing, and their permission should be obtained. Ideally, and when appropriate, the assent of the child should be obtained.
4. When performed for therapeutic purposes, pharmacogenetic testing of children is acceptable, with permission of parents or guardians and, when appropriate, the child's assent. If a pharmacogenetic test result carries implications beyond drug targeting or dose responsiveness, the broader implications should be discussed before testing.

Newborn Screening

5. AAP and ACMG support the mandatory offering of newborn screening for all children. After education and counseling about the substantial benefits of newborn screening, its remote risks, and the next steps in the event of a positive screening result, parents should have the option of refusing the procedure, and an informed refusal should be respected.

Carrier Testing

6. AAP and ACMG do not support routine carrier testing in minors when such testing does not provide health benefits in childhood. The AAP and ACMG advise against school-based testing or screening programs, because the school environment is unlikely to be conducive to voluntary participation, thoughtful consent, privacy, confidentiality, or appropriate counseling about test results.
7. For pregnant adolescents or for adolescents considering reproduction, genetic testing and screening should be offered as clinically indicated, and the risks and benefits should be explained clearly.

Predictive Genetic Testing

8. Parents or guardians may authorize predictive genetic testing for asymptomatic children at risk of childhood-onset conditions. Ideally, the assent of the child should be obtained.
9. Predictive genetic testing for adult-onset conditions generally should be deferred unless an intervention initiated in childhood may reduce morbidity or mortality. An exception might be made for families for whom diagnostic uncertainty poses a significant psychosocial burden, particularly when an adolescent and the parents concur in their interest in predictive testing.
10. For ethical and legal reasons, healthcare providers should be cautious about providing predictive genetic testing to minors without the involvement of their parents or guardians, even if a minor is mature. Results of such tests may have significant medical, psychologic, and social implications, not only for the minor but also for other family members.

Histocompatibility Testing

11. Tissue compatibility testing of minors of all ages is permissible to benefit immediate family members but should be conducted only after thorough exploration of the psychosocial, emotional, and physical implications of the minor serving as a potential stem cell donor. A donor advocate or similar mechanism should be in place from the outset to avert coercion and safeguard the interests of the child.

Adoption

12. The rationale for genetic testing of children in biological families should apply for adopted children and children awaiting placement for adoption. If a child has a known genetic risk, prospective adoptive parents must be made aware of this possibility. In rare cases, it may be in a child's best interest to undergo predictive genetic testing for a known risk before adoption to ensure the child's placement with a family capable of and willing to accept the child's potential medical and developmental challenges. In the absence of such indications, genetic testing should not be performed as a condition of adoption.

Disclosure

13. At the time of genetic testing, parents or guardians should be encouraged to inform their child of the test results at an appropriate age. Parents or guardians should be advised that, under most circumstances, a request by a mature adolescent for test results should be honored.
14. Results from genetic testing of a child may have implications for the parents and other family members. Healthcare providers have an obligation to inform parents and the child, when appropriate, about these potential implications. Healthcare providers should encourage patients and families to share this information and offer to help explain the results to the extended family or refer them for genetic counseling.
15. Misattributed paternity, use of donor gametes, adoption, or other questions about family relationships may be uncovered "incidentally" whenever genetic testing is performed, particularly when testing multiple family members. This risk should be discussed, and a plan about disclosure or nondisclosure should be in place before testing.

Direct-to-Consumer Testing

16. AAP and ACMG strongly discourage the use of direct-to-consumer and home-kit genetic testing of children because of the lack of oversight on test content, accuracy, and interpretation.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

Chapter 96

Principles of Human Genetics

Daryl A. Scott and Brendan Lee

THE HUMAN GENOME

The human genome has approximately 20,000 genes that encode the wide variety of proteins found in the human body. Reproductive or germline cells contain one copy (N) of this genetic complement and are **haploid**, whereas somatic (nongermline) cells contain two complete copies (2N) and are **diploid**. Genes are organized into long segments of deoxyribonucleic acid (**DNA**), which, during cell division, are compacted into intricate structures together with proteins to form chromosomes. Each somatic cell has 46 chromosomes: 22 pairs of **autosomes**, or nonsex chromosomes, and one pair of **sex chromosomes** (XY in a male, XX in a female). Germ cells (ova or sperm) contain 22 autosomes and one sex chromosome, for a total of 23. At fertilization, the full diploid chromosome complement of 46 is again realized in the embryo.

Most of the genetic material is contained in the cell's nucleus. The mitochondria (the cell's energy-producing organelles) contain their own unique genome. The **mitochondrial chromosome** consists of a double-stranded circular piece of DNA, which contains 16,568 base pairs (bp) of DNA and is present in multiple copies per cell. The proteins that occupy the mitochondria are produced either in the mitochondria, using information contained in the mitochondrial genome, or are produced outside of the mitochondria, using information contained in the nuclear genome, and then transported into the organelle. Sperm do not usually contribute mitochondria to the developing embryo, so all mitochondria are maternally derived, and a child's mitochondrial genetic makeup derives exclusively from the child's biologic mother (see Chapter 108).

FUNDAMENTALS OF MOLECULAR GENETICS

DNA consists of a pair of chains of a sugar-phosphate backbone linked by pyrimidine and purine bases to form a **double helix** (Fig. 96.1). The sugar in DNA is deoxyribose. The pyrimidines are cytosine (C) and thymine (T), and the purines are guanine (G) and adenine (A). The bases are linked by hydrogen bonds such that A always pairs with T and G with C. Each strand of the double helix has polarity, with a free phosphate at one end (5′) and an unbonded hydroxyl on the sugar at the other end (3′). The two strands are oriented in opposite polarity in the double helix.

The replication of DNA follows the pairing of bases in the parent DNA strand. The original two strands unwind by breaking the hydrogen bonds between base pairs. Free nucleotides, consisting of a base attached to a sugar-phosphate chain, form new hydrogen bonds with their complementary bases on the parent strand; new phosphodiester bonds are created by enzymes called **DNA polymerases.** Replication of chromosomes begins simultaneously at multiple sites, forming replication bubbles that expand bidirectionally until the entire DNA molecule (chromosome) is replicated. Errors in DNA replication, or pathogenic variants induced by environmental mutagens such as irradiation or chemicals, are detected and potentially corrected by DNA repair systems.

Information encoded in DNA, predominantly located in the cell's nucleus, is transcribed into **messenger** ribonucleic acid **(mRNA)**, which is then transported to the cytoplasm, where it is translated into protein. A prototypical gene consists of a regulatory region, segments called **exons** that encode the amino acid sequence of a protein, and intervening segments called **introns** (Fig. 96.2).

Transcription is initiated by attachment of ribonucleic acid (RNA) polymerase to the promoter site upstream of the beginning of the coding sequence. Specific proteins bind to the region to repress or activate transcription by compacting or opening chromatin, which is a complex of DNA and histone proteins. It is the action of these regulatory proteins **(transcription factors)** that determines, in large part, when a gene is turned on or off. Some genes are also turned on and off by methylation of cytosine bases that are adjacent to guanine bases **(cytosine-phosphate-guanine bases** [**CpGs**]). Methylation is an example of an **epigenetic** change, meaning a change that can affect gene expression, and possibly the characteristics of a cell or organism, but that *does not* involve a change in the underlying genetic sequence. Gene regulation is flexible and responsive, with genes being turned on or off during development and in response to internal and external environmental conditions and stimuli. Another epigenetic mechanism that controls gene expression is the biochemical modification of histone proteins around which DNA is wound. These modifications can affect the relative accessibility of chromatin by enzymes for transcription.

Transcription proceeds through the entire length of the gene in a 5′ to 3′ direction to form an mRNA transcript whose sequence is complementary to that of one of the DNA strands. RNA is a sugar-phosphate chain with pyrimidines and purines. In RNA the sugar is ribose, and uracil replaces the thymine found in DNA. A "cap" consisting of 7-methylguanosine is added to the 5′ end of the RNA in a 5′-5′ bond and, for most transcripts, several hundred adenine bases are enzymatically added to the 3′ end after transcription.

mRNA processing occurs in the nucleus and consists of excision of the introns and splicing together of the exons. Specific sequences at the start and end of introns mark the sites where the splicing machinery will act on the transcript. In some cases, there may be tissue-specific patterns to splicing, so that the same primary transcript can produce multiple distinct proteins.

The processed transcript is next exported to the cytoplasm, where it binds to ribosomes, which are complexes of protein and **ribosomal** RNA **(rRNA)**. The genetic code is then read in triplets of bases, each triplet corresponding with a specific amino acid or providing a signal that terminates **translation**. The triplet codons are recognized by **transfer** RNAs **(tRNAs)** that include complementary anticodons and bind the corresponding amino acid, delivering it to the growing peptide. New amino acids are enzymatically, sequentially attached to the growing peptide. Each time an amino acid is added, the ribosome moves one triplet codon step along the mRNA. Eventually a **stop codon** is reached, at which point translation ends and the peptide is released. In some proteins, there are **posttranslational modifications**, such as attachment of sugars **(glycosylation)**. The protein is then delivered to its destination within or outside the cell by trafficking mechanisms that recognize portions of the peptide.

Another mechanism of gene regulation is **noncoding** RNAs, which are RNAs transcribed from DNA but not translated into proteins. Noncoding RNAs function in many contexts, including mediating splicing, the processing of coding RNAs in the nucleus, and the translation of coding mRNAs in ribosomes. The roles of *long* noncoding RNAs (>200 bp) and *short* noncoding RNAs (<200 bp) extend beyond these processes to impact a diverse set of biologic functions, including the regulation of gene expression. **MicroRNAs (miRNAs)** are a class of small RNAs that control gene expression in the cell by directly targeting specific sets of coding RNAs by direct RNA–RNA binding. This RNA–RNA interaction can lead to degradation of the target-coding RNA or inhibition of translation of the protein specified by that coding RNA. miRNAs often target and regulate several hundred mRNAs.

GENETIC VARIATION

The process of producing protein from a gene is subject to disruption at multiple levels because of alterations in the DNA sequence (Fig. 96.3). Changes in the regulatory region can lead to altered gene expression, including increased or decreased rates of transcription, failure of gene activation, or activation of the gene at inappropriate times or in inappropriate cells. Changes in the coding sequence can lead to **substitution** of one amino acid for another (**missense variant** or **nonsynonymous variant**) or creation of a stop codon in the place of an amino acid codon (**nonsense** or **stop-gain variant**).

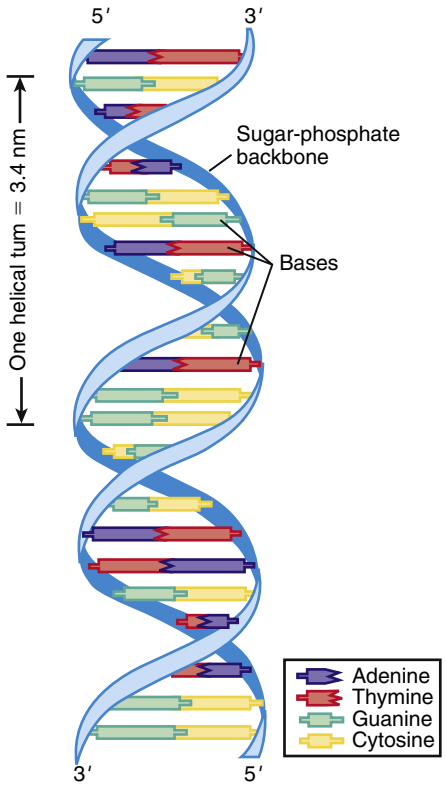


Fig. 96.1 DNA double helix, with sugar-phosphate backbone and nitrogenous bases. (*From Jorde LB, Carey JC, Bamshad MJ, et al., eds. Medical Genetics, 2nd ed. St Louis: Mosby; 1999: p. 8.*)

Overall, missense and nonsense variants are the most common (56% of variants); small deletions or insertions represent approximately 24% of variants (Table 96.1). Some single-base changes do not affect the amino acid (**silent**, **wobble**, or **synonymous variants**) because there may be several triplet codons that correspond to the same single amino acid. Amino acid substitutions can have a profound effect on protein function if the chemical properties of the substituted amino acid are markedly different. Other substitutions can have a subtle or no effect on protein function, particularly if the substituted amino acid is chemically similar to the original. Single nucleotide changes, whether silent or missense, are termed **single nucleotide variants (SNV)**.

Genetic changes can also include **insertions** or **deletions** ("**indels**"). Insertions or deletions of a nonintegral multiple of three bases into the coding sequence leads to a **frameshift,** altering the grouping of bases into triplets. This leads to translation of an incorrect amino acid sequence and often a premature stop to translation. Insertion or deletion of an integral multiple of three bases into the coding sequence will insert or delete a corresponding number of amino acids from the protein, leading to **in-frame** alterations that maintain the amino acid sequence outside the deleted or duplicated amino acids. Larger scale insertions or deletions can disrupt a coding sequence or result in complete deletion of an entire gene or group of genes.

Pathogenic variants usually can be classified as causing a loss of function or a gain of function. **Loss-of-function variants** lead to a reduction in the level of protein function as a result of decreased expression or production of a protein that does not work as efficiently. In some cases, loss of protein function from one of two alleles is sufficient to cause disease. **Haploinsufficiency** describes the situation in which maintenance of a normal phenotype requires the proteins produced by both alleles of a gene, and a 50% decrease in gene function results in an abnormal phenotype. *Haploinsufficient phenotypes are dominantly inherited.* Loss-of-function variants can also have a **dominant**

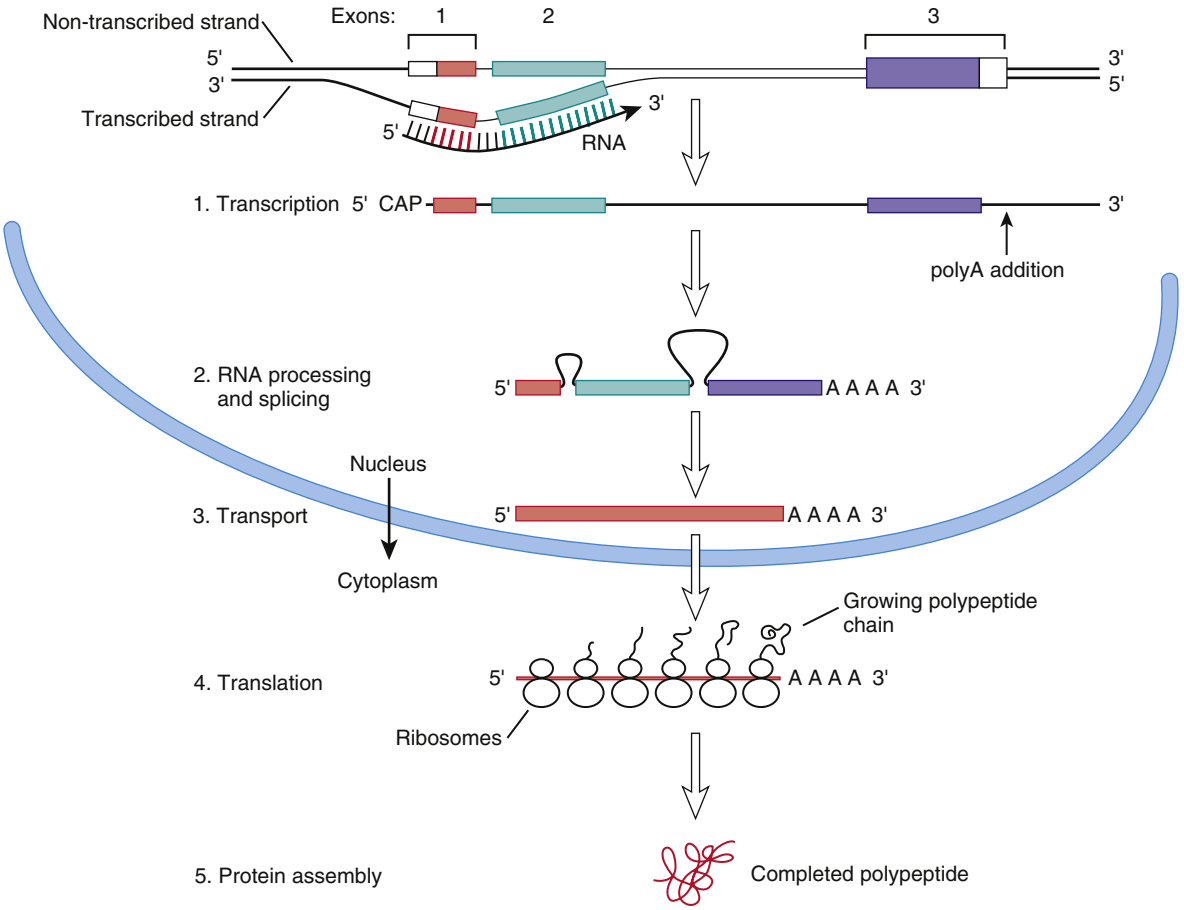


Fig. 96.2 Flow of information from DNA to RNA to protein for a hypothetical gene with three exons and two introns. Within the exons, *colored regions* indicate coding sequences. Steps include transcription, RNA processing and splicing, RNA transport from the nucleus to the cytoplasm, translation, and protein assembly. (*From Nussbaum RL, McInnis RR, Willard HF, Hamosh A, eds. Thompson & Thompson Genetics in Medicine, 7th ed. Philadelphia: Saunders; 2007: p. 31.*)

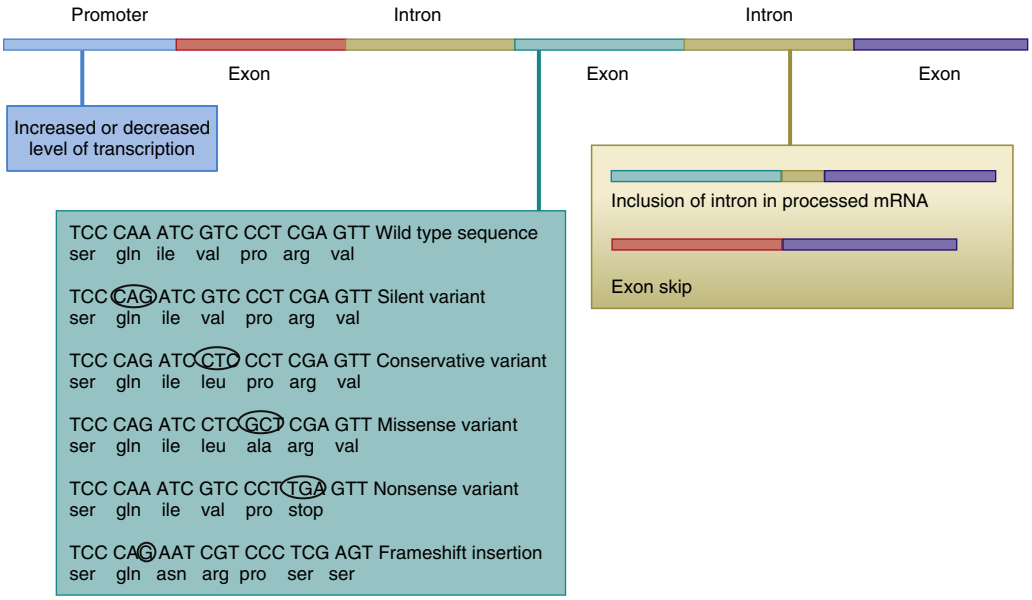


Fig. 96.3 Various types of intragenic sequence variants. Promoter variants alter rate of transcription or disrupt gene regulation. Base changes within exons can have various effects, as shown. Variants within introns can lead to inclusion of some intronic sequence in the final processed mRNA, or it can lead to exon skipping.

Table 96.1 Main Classes, Groups, and Types of Sequence Variants and Their Effects on Protein Products

CLASS	GROUP	TYPE	EFFECT ON PROTEIN PRODUCT
Substitution	Synonymous	Silent*	Same amino acid
	Nonsynonymous	Missense*	Altered amino acid—may affect protein function or stability
		Nonsense or stop-gain*	Stop codon—loss of function or expression from degradation of mRNA
		Splice site	Aberrant splicing—exon skipping or intron retention
		Promoter	Altered gene expression
Deletion	Multiple of three (codon)		In-frame deletion of one or more amino acid(s)—may affect protein function or stability
	Not multiple of three	Frameshift	Likely to result in premature termination with loss of function or expression
	Large deletion	Partial gene deletion	May result in premature termination with loss of function or expression
		Whole gene deletion	Loss of expression
Insertion	Multiple of three (codon)		In-frame insertion of one or more amino acid(s)—may affect protein function or stability
	Not multiple of three	Frameshift	Likely to result in premature termination with loss of function or expression
	Large insertion	Partial gene duplication	May result in premature termination with loss of function or expression
		Whole gene duplication	May have an effect because of increased gene dosage
	Expansion of trinucleotide repeat	Dynamic pathogenic variant	Altered gene expression or altered protein stability or function

*Some have been shown to cause aberrant splicing.
From Turnpenny P, Ellard S, eds. *Emery's Elements of Medical Genetics*, 14th ed. Philadelphia: Churchill Livingstone; 2012: p. 23.

negative effect when the abnormal protein product actively interferes with the function of the normal protein product. Both situations lead to diseases inherited in a dominant fashion. Haploinsufficiency of type I collagen can cause relatively mild osteogenesis imperfecta (OI) type I. In contrast, heterozygous, missense glycine substitutions in type I collagen can act in a dominant negative fashion to cause severe OI type II, III, and IV. In other cases, loss-of-function variants must be present in both copies of a gene before an abnormal phenotype results. This situation typically results in diseases inherited in a **recessive** fashion (see Chapter 97). Some conditions are **semidominant** where one affected copy (heterozygous pathogenic variant) causes a milder phenotype than when both copies are affected (compound heterozygous or homozygous pathogenic variants). This is seen in classical achondroplasia vs homozygous achondroplasia

Gain-of-function variants typically cause dominantly inherited diseases. These variants can result in production of a protein molecule with an increased ability to perform a normal function or can confer a novel property on the protein. The gain-of-function variant

in **achondroplasia**, the most common of the disproportionate, short-limbed short stature disorders, exemplifies the enhanced function of a normal protein. Achondroplasia results from a pathogenic variant in the fibroblast growth factor (FGF) receptor three gene *(FGFR3)*, which leads to activation of the receptor, even in the absence of FGF. In **sickle cell disease**, an amino acid is substituted into the hemoglobin molecule and has little effect on the ability of the protein to transport oxygen. However, sickle hemoglobin chains have a novel property. Unlike normal hemoglobin, sickle hemoglobin chains aggregate under conditions of deoxygenation, forming fibers that deform the red cells.

Other gain-of-function variants result in overexpression or inappropriate expression of a gene product. Many cancer-causing genes **(oncogenes)** are normal regulators of cellular proliferation during development. However, expression of these genes in adult life and/or in cells in which they usually are not expressed can result in neoplasia.

In some cases, changes in gene expression are caused by changes in the number of copies of a gene that are present in the genome (Fig. 96.4). Although some **copy number variations** are common and do not appear to cause or predispose to disease, others are clearly disease causing. **Charcot-Marie-Tooth disease type 1A**, the most common inherited form of chronic peripheral neuropathy of childhood, is caused by duplications of the gene for peripheral myelin protein 22, resulting in overexpression due to the existence of three active copies of this gene (see Chapter 653.1). Deletions of this same gene, leaving only one active copy, are responsible for a different disorder, **hereditary neuropathy with liability to pressure palsies**.

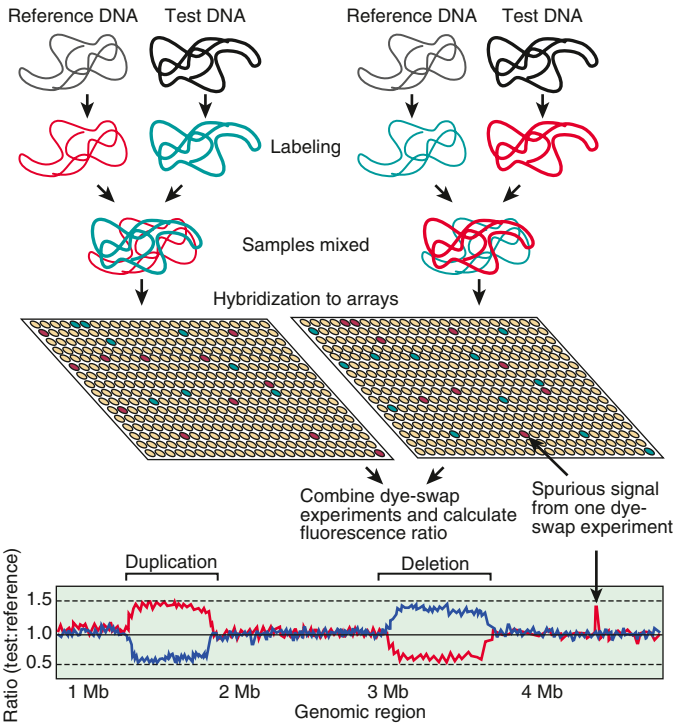


Fig. 96.4 Array comparative genomic hybridization. Test and reference DNA samples are differentially labeled, mixed, and passed over a target array of probes (e.g., bacterial artificial chromosome clones or oligonucleotides) containing DNA fragments from across the whole human genome. The experiment is often repeated with reversal of the test and reference dyes to detect dye effects or identify spurious signals. DNA samples hybridize with their corresponding probe, and the ratio of fluorescence from each probe *(test:reference)* is used to detect regions that vary in copy number between the test and the reference sample (*red line*, original hybridization; *blue line*, dye-swapped hybridization). Equal copy number for both the test and the reference DNA is identified by equal binding, resulting in a ratio of 1:1. Duplication in a genomic region of the test sample is identified by an increased ratio, and a deletion is identified by a decreased ratio, but a deletion in the test sample is indistinguishable from a duplication in the reference sample. These ratios are usually converted to log2 scale for further analysis. *Mb*, megabase. (*Adapted from Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet 2006;7:85–97.*)

Deletions and duplications can vary in their extent and can involve several genes, even when they are not detectable using a traditional chromosome analysis. Such changes are commonly called **microdeletions** and **microduplications**. When deletion or duplication of two or more genes in the same chromosomal region each play a role in the resulting clinical features, the condition can also be referred to as a **contiguous gene disorder**.

In some cases, the recognition of a specific constellation of features leads clinicians to suspect a specific microdeletion or microduplication syndrome. Examples of such disorders include Smith-Magenis, DiGeorge, and Williams syndromes. In other cases, the clinician may be alerted to this possibility by an unusually diverse array of clinical features in one patient or the presence of unusual features in a person with a known condition. Because of the close physical proximity of a series of genes, different deletions involving the short arm of the X chromosome can produce individuals with various combinations of ichthyosis, Kallmann syndrome, ocular albinism, intellectual disability, chondrodysplasia punctata, and short stature.

DNA rearrangements can also take place in **somatic cells** (cells that do not go on to produce ova or sperm). Rearrangements that occur in **lymphoid** cells are required for the normal formation of functional immunoglobulin in B cells and antigen-recognizing receptors on T cells. Large segments of DNA, which code for the variable and the constant regions of either immunoglobulin or the T-cell receptor (TCR), are physically joined at a specific stage in the development of an immunocompetent lymphocyte. These rearrangements take place during development of the lymphoid cell lineage in humans and result in the extensive diversity of immunoglobulin and TCR molecules. Because of this postgermline DNA rearrangement, no two individuals, not even identical twins, are fully identical, because mature lymphocytes from each will have undergone random DNA rearrangements at these loci.

The human genome demonstrates that any two individuals will differ in about one base in 1,000. Some of these differences are silent; some result in changes that explain phenotypic differences (hair or eye color, physical appearance); and some have medical significance, causing single-gene disorders such as sickle cell anemia or explaining susceptibility to common pediatric disorders such as asthma. Genetic variants in a single gene that occur at a frequency of >1% in a population are often referred to as **polymorphisms**. These variations may be silent or subtle or may have significant phenotypic effects.

GENOTYPE-PHENOTYPE CORRELATIONS IN GENETIC DISEASE

The term **genotype** is used to signify the internally coded, heritable information of an individual and can also be used to refer to which particular alternative version **(allele)** of a gene is present at a specific location **(locus)** on a chromosome. A **phenotype** is the observed structural, biochemical, and physiologic characteristics of an individual, determined by the genotype, and can also refer to the observed structural and functional effects of a variant allele at a specific locus. Many sequence variants result in predictable phenotypes; physicians can predict clinical outcomes and plan appropriate treatment strategies based on a patient's genotype. There is also phenotypic expansion where multiple alleles (variants) within a gene can be associated with often diverse and distinct clinical presentations.

Long QT syndrome exemplifies a disorder with predictable associations between a patient's genotype and his or her phenotype (see Chapter 484.5). Long QT syndrome is genetically heterogeneous, meaning that pathogenic variants in *several different genes* can cause the same disorder. The risk for cardiac events (syncope, aborted cardiac arrest, or sudden death) is higher in patients with long QT syndrome involving *KCNQ1* (63%) or *KCNH2* (46%) than in those with pathogenic variants in *SCN5A* (18%). In addition, individuals with *KCNQ1* variants experience most of their episodes during exercise and rarely during rest or sleep. In contrast, individuals with pathogenic variants in *KCNH2* and *SCN5A* are more likely to have episodes during sleep or rest and rarely during exercise. Therefore variants in specific genes

(genotype) are correlated with specific manifestations (phenotype) of long QT syndrome. These types of relationships are commonly referred to as *genotype-phenotype correlations.*

Pathogenic variants in the fibrillin-1 gene associated with **Marfan syndrome** represent another example of predictable genotype-phenotype correlations (see Chapter 743). Marfan syndrome is characterized by the combination of skeletal, ocular, and aortic manifestations, with the most devastating outcome being aortic root dissection and sudden death. The fibrillin-1 gene consists of 65 exons, and pathogenic variants have been found in almost all these. The location of the variant within the gene (genotype) might play a significant role in determining the severity of the condition (phenotype). Neonatal Marfan syndrome is often caused by variants in exons 24-27 and in exons 31 and 32, whereas milder forms are caused by variants in exons 59-65 and in exons 37 and 41.

Genotype-phenotype correlations have also been observed in some complications of **cystic fibrosis** (CF; see Chapter 454). Although pulmonary disease is the major cause of morbidity and mortality, CF is a multisystem disorder that affects not only the epithelia of the respiratory tract but also the exocrine pancreas, intestine, male genital tract, hepatobiliary system, and exocrine sweat glands. CF is caused by pathogenic variants in the CF transmembrane conductance regulator *(CFTR)* gene. More than 1,600 different pathogenic *CFTR* variants have been identified. The most common is a deletion of three nucleotides that removes the amino acid phenylalanine (F) at the 508th position on the protein (ΔF508 variant), which accounts for approximately 70% of all pathogenic CF variants and is associated with severe disease. The best genotype-phenotype correlations in CF are seen in the context of pancreatic function, with common pathogenic variants being classified as either *pancreatic sufficient* or *pancreatic insufficient.* Persons with pancreatic sufficiency usually have either one or two pancreatic-sufficient alleles, indicating that pancreatic-sufficient alleles are dominant. In contrast, the genotype-phenotype correlation in pulmonary disease is much weaker, and persons with identical genotypes have wide variations in the severity of their pulmonary disease. This finding may be accounted for, in part, by genetic modifiers or environmental factors.

In many disorders, the effects of variants on phenotype can be modified by changes in the other allele of the same gene, by changes in specific **modifier genes**, and/or by variations in a number of unspecified genes **(genetic background)**. When sickle cell anemia is co-inherited with the gene for hereditary persistence of fetal hemoglobin, the sickle cell phenotypic expression is less severe. Modifier genes in CF can influence the development of congenital meconium ileus, or colonization with *Pseudomonas aeruginosa.* Modifier genes can also affect the manifestations of Hirschsprung disease, neurofibromatosis type 2, craniosynostosis, and congenital adrenal hyperplasia. The combination of genetic variants producing glucose-6-phosphate dehydrogenase deficiency and longer versions of the TATAA element in the uridine diphosphate–glucuronosyltransferase gene promoter exacerbates neonatal physiologic hyperbilirubinemia.

HUMAN GENOME PROJECT

A rudimentary genetic map can be made using genetic linkage, which is based on the principle that alleles at two genetic loci that are located near each other segregate together in a family unless they are separated by genetic **recombination**. In contrast to linkage maps, physical maps rely on overlapping DNA fragments to determine the location of loci with respect to one another. Overlapping segments of the genomic regions can be identified and used to piece together a map composed of overlapping DNA fragments to obtain the DNA sequence of the entire region. An alternative strategy involves breaking the entire genome into random fragments, sequencing the fragments, and then computationally assembling them into overlapping segments. This whole genome approach in combination with next-generation sequencing technologies has resulted in a dramatic reduction in the cost of sequencing an individual's entire genome.

Analysis of the human genome has produced some surprising results. The number of genes appears to be ~20,000. *However, the number of protein products encoded by the genome is far greater than the number of genes.* This is a result of the presence of alternative promoter regions, alternative splicing, and posttranslational modifications, which can allow a single gene to encode a number of protein products.

Fig. 96.5 Microarray containing 36,000 oligonucleotides. The microarray was exposed to RNA from normal fibroblasts (labeled in *red*; see *arrows*) and fibroblasts from a patient with Niemann-Pick disease, type C (labeled *green*). *Arrows* point to regions in which there was a strong hybridization signal with either normal or disease RNA. This microarray was used to search for genes that are highly expressed in the fibroblasts of patients. (*From Jorde LB, Carey JC, Bamshad MJ, White RL. Medical Genetics, 3rd ed. St Louis: Mosby; 2006: p. 116.*)

It is also apparent that most of the human genome *does not* encode protein, with <5% being transcribed and translated, although a much larger percentage may be transcribed without translation. Many transcribed sequences are not translated but represent genes that encode RNAs that serve *regulatory* roles. A large fraction of the genome consists of repeated sequences that are interspersed among the genes. Some of these are transposable genetic elements that can move from place to place in the genome. Others are static elements that were expanded and dispersed in the past during human evolution. Other repeated sequences might play a structural role. There are also regions of *genomic duplications.* Such duplications are substrate for evolution, allowing genetic motifs to be copied and modified to serve new roles in the cell. Duplications can also play a role in chromosomal rearrangement, permitting nonhomologous chromosome segments to pair during meiosis and exchange material. This is another source of evolutionary change and a potential source of chromosomal instability leading to congenital anomalies or cancer. Low copy repeats also play an important role in causing genomic disorders. When low copy repeats flank unique genomic segments, these regions can be duplicated or deleted through a process known as *nonallelic homologous recombination.*

Availability of the entire human genomic sequence permits the study of large groups of genes, looking for patterns of gene expression or genome alteration. Studies of gene expression are performed using next-generation sequencing techniques to obtain information about all RNA transcripts in a tissue or single cell. The patterns of gene expression may provide signatures for particular disease states, such as cancer, or change in response to therapy (Fig. 96.5).

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

Chapter 97

Patterns of Genetic Transmission

Daryl A. Scott and Brendan Lee

FAMILY HISTORY AND PEDIGREE NOTATION

The family history remains the most important screening tool for pediatricians in identifying a patient's risk for developing a wide range of diseases, from multifactorial conditions such as diabetes and attention-deficit/hyperactivity disorder, to single-gene disorders such as sickle cell anemia and cystic fibrosis. Through a detailed family history, the physician can often ascertain the mode of genetic transmission and the risks to family members. Because not all familial clustering of disease is caused by genetic factors, a family history can also identify common environmental and behavioral factors that influence the occurrence of disease. The main goal of the family history is to identify genetic susceptibility, and the cornerstone of the family history is a systematic and standardized pedigree.

A **pedigree** provides a graphic depiction of a family's structure and medical history. It is important when taking a pedigree to be systematic and use standard symbols and configurations so that anyone can read and understand the information (Figs. 97.1-97.4). In the pediatric setting, the **proband** is typically the child or adolescent who is being evaluated. The proband is designated in the pedigree by an arrow.

A three- to four-generation pedigree should be obtained for every new patient as an initial screen for genetic disorders segregating within the family. The pedigree can provide clues to the inheritance pattern of these disorders and can aid the clinician in determining the risk to the proband and other family members. The closer the relationship of the proband to the person in the family with the genetic disorder, the greater is the shared genetic complement. **First-degree** relatives, such as a parent, full sibling, or child, share one half their genetic information on average; first cousins share one eighth. Sometimes the person providing the family history may mention a distant relative who is affected with a genetic disorder. In such cases a more extensive pedigree may be needed to identify the risk to other family members. For example, a history of a distant maternally related cousin with intellectual disability caused by fragile X syndrome can still place a male proband at an elevated risk for this disorder.

MENDELIAN INHERITANCE

There are three classic forms of genetic inheritance: **autosomal dominant, autosomal recessive,** and **X-linked**; these are referred to as **mendelian inheritance** forms.

Autosomal Dominant Inheritance

Autosomal dominant inheritance is determined by the presence of one abnormal gene on one of the **autosomes** (chromosomes 1-22). Autosomal genes exist in pairs, with each parent contributing one copy. In an autosomal dominant trait, a change in one of the paired genes affects the phenotype of an individual, even though the other copy of the gene is functioning correctly. A **phenotype** can refer to a physical manifestation, a behavioral characteristic, or a difference detectable only through laboratory tests.

The pedigree for autosomal dominant disorders demonstrates certain characteristics. These disorders show a **vertical transmission** (parent-to-child) pattern and can appear in multiple generations (Fig. 97.5). An affected individual has a 50% chance of passing on the deleterious gene in *each* pregnancy and, therefore, of having a child affected by the disorder. This is referred to as the **recurrence risk** for the disorder. Unaffected individuals (family members who do not manifest the trait and do not harbor a copy of the deleterious gene) do not pass the disorder to their children. Males and females are equally affected.

Although not a characteristic per se, the finding of male-to-male transmission essentially confirms autosomal dominant inheritance. Vertical transmission can also be seen with X-linked traits. However, because a father passes on his Y chromosome to a son, male-to-male transmission cannot be seen with an X-linked trait. Therefore male-to-male transmission eliminates X-linked inheritance as a possible explanation. Although male-to-male transmission can occur with Y-linked genes as well, there are very few Y-linked disorders, compared with thousands having the autosomal dominant inheritance pattern.

Although parent-to-child transmission is a characteristic of autosomal dominant inheritance, many patients with an autosomal dominant disorder have no history of an affected family member, for several possible reasons. First, the patient may have the disorder due to a **de novo** (new) pathogenic variant that occurred in the DNA of the egg or sperm that formed that individual. Second, many autosomal dominant conditions demonstrate **incomplete penetrance**, meaning that not all individuals who carry the pathogenic variant have phenotypic manifestations. In a pedigree, this can appear as a **skipped generation**, in which an unaffected individual links two affected persons (Fig. 97.6). There are many potential reasons that a disorder might exhibit incomplete penetrance including the effect of modifier genes, environmental factors, gender, and age. Third, individuals with the same autosomal dominant variant can manifest the disorder to different degrees. This is termed **variable expression** and is a characteristic of many autosomal dominant disorders.

Fourth, some spontaneous genetic pathogenic variants occur not in the egg or sperm that forms a child, but rather in a *cell* in the developing embryo or in a tissue after birth. Such events are referred to as **somatic pathogenic variants**. Because only a subset of cells in the body are affected, the change is said to be **mosaic**. The phenotypes caused by a somatic pathogenic variant can vary but are usually milder than if all cells were affected by the pathogenic variant. Some cancers typically arise from cells that have been affected by multiple somatic pathogenic variants. In **germline mosaicism,** the pathogenic variant occurs in a subset of cells that ultimately produce ova or sperm. An individual who is germline mosaic for a pathogenic variant might not have any manifestations of the associated disorder but may pass that change on to his or her offspring by producing ova or sperm that carry the pathogenic variant. Children whose bodies derive from those ova or sperm will carry the pathogenic variant in all of their cells unless the change undergoes a rare reversion of the somatic pathogenic variant. Recurrence of a dominantly inherited condition to apparently unaffected parents can sometimes be explained by germline mosaicism for the dominantly transmitted variant in one of the parents.

Autosomal Recessive Inheritance

Autosomal recessive inheritance requires deleterious variants in both copies of a gene to cause disease. Examples include cystic fibrosis and sickle cell disease. Autosomal recessive disorders are characterized by **horizontal transmission**, the observation of multiple affected members of a kindred in the same generation, but no affected family members in other generations (Fig. 97.7). They are associated with a recurrence risk of 25% for carrier parents who have had a previous affected child. Male and female offspring are equally likely to be affected, although some traits exhibit differential expression between sexes. The offspring of consanguineous parents are at increased risk for rare, autosomal recessive traits due to the increased chance that both parents may carry a gene affected by a deleterious variant that they inherited from a common ancestor. **Consanguinity** between parents of a child with a suspected genetic disorder implies, but certainly does not prove, autosomal recessive inheritance. Although consanguineous unions are uncommon in Europe and the Americas, in other parts of the world (southern India, Japan, and the Middle East) as high as 50% of all children may be conceived in consanguineous unions. The risk of a genetic disorder in the offspring of a first-cousin union (6–8%) is about double the risk in the general population (3–4%).

Instructions:
—Key should contain all information relevant to interpretation of pedigree (e.g., define fill/shading)
—For clinical (non-published) pedigrees include:
a) name of proband/consultand
b) family names/initials of relatives for identification, as appropriate
c) name and title of person recording pedigree
d) historian (person relaying family history information)
e) date of intake/update
f) reason for taking pedigree (e.g., abnormal ultrasound, familial cancer, developmental delay, etc.)
g) ancestry of both sides of family
—Recommended order of information placed below symbol (or to lower right)
a) age; can note year of birth (e.g., b.1978) and/or death (e.g., d. 2007)
b) evaluation (see Fig. 97.4)
c) pedigree number (e.g., I-1, I-2, I-3)
—Limit identifying information to maintain confidentiality and privacy

	Male	Female	Gender not specified	Comments
1. Individual	b.1925	30 y	4 mo	Assign gender by phenotype (see text for disorders of sex development, etc.). Do not write age in symbol.
2. Affected individual				Key/legend used to define shading or other fill (e.g., hatches, dots, etc.). Use only when individual is clinically affected.
			With ≥2 conditions, the individual's symbol can be partitioned accordingly, each segment shaded with a different fill and defined in legend.	
3. Multiple individuals, number known	5	5	5	Number of siblings written inside symbol. (Affected individuals should not be grouped.)
4. Multiple individuals, number unknown or unstated	n	n	n	"n" used in place of "?".
5. Deceased individual	d. 35	d. 4 mo	d. 60's	Indicate cause of death if known. Do not use a cross (†) to indicate death to avoid confusion with evaluation positive (+).
6. Consultand				Individual(s) seeking genetic counseling/testing.
7. Proband	P	P		An affected family member coming to medical attention independent of other family members.
8. Stillbirth (SB)	SB 28 wk	SB 30 wk	SB 34 wk	Include gestational age and karyotype, if known.
9. Pregnancy (P)	P LMP: 7/1/2007 47,XY,+21	P 20 wk 46, XX	P	Gestational age and karyotype below symbol. Light shading can be used for affected; define in key/legend.

Pregnancies not carried to term	Affected	Unaffected	
10. Spontaneous abortion (SAB)	17 wks female cystic hygroma	<10 wks	If gestational age/gender known, write below symbol. Key/legend used to define shading.
11. Termination of pregnancy (TOP)	18 wks 47, XY,+18		Other abbreviations (e.g., TAB, VTOP) not used for sake of consistency.
12. Ectopic pregnancy (ECT)	ECT		Write ECT below symbol.

Fig. 97.1 Common pedigree symbols, definitions, and abbreviations. (*From Bennett RL, French KS, Resta RG, et al. Standardized human pedigree nomenclature: update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Couns. 2008;17:424–433.*)

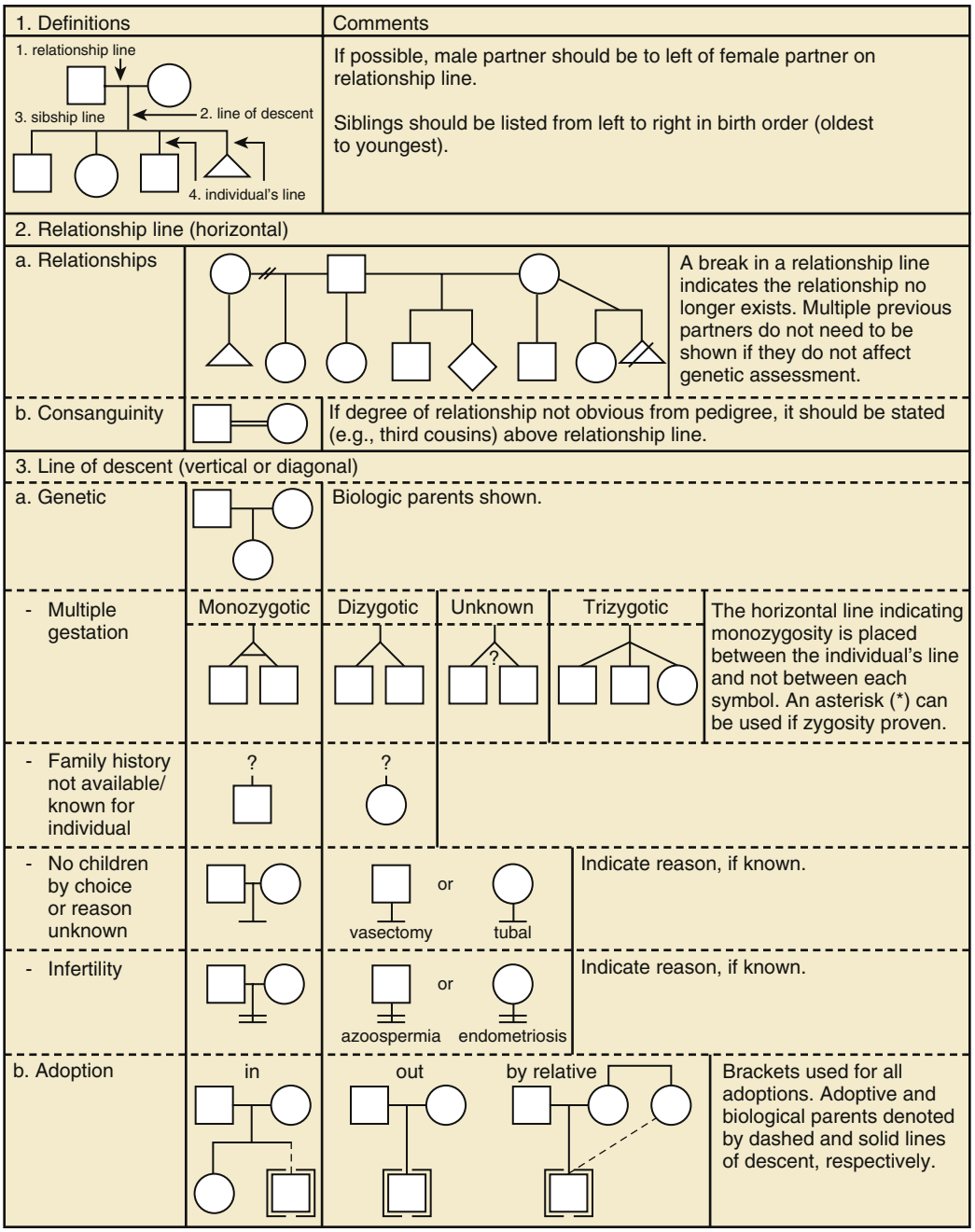


Fig. 97.2 Pedigree line definitions. (*From Bennett RL, French KS, Resta RG, et al. Standardized human pedigree nomenclature: update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Couns. 2008;17:424–433.*)

Every individual probably carries several rare, deleterious recessive pathogenic sequence variants. Because most pathogenic variants carried in the general population occur at a very low frequency, it does not make economic sense to screen the entire population to identify the small number of persons who carry these variants. These variants typically remain undetected unless an affected child is born to a couple who both carry pathogenic variants affecting the same gene.

However, in some **genetic isolates** (small populations isolated by geography, religion, culture, or language), certain rare recessive pathogenic variants are much more common than in the general population. Even though there may be no known consanguinity, couples from these genetic isolates have a greater chance of sharing pathogenic alleles inherited from a common ancestor. Screening programs have been developed among some such groups to detect persons who carry common disease-causing variants and, therefore, are at increased risk for having affected children.

A variety of autosomal recessive conditions are more common among Ashkenazi Jews than in the general population. Couples of Ashkenazi Jewish ancestry should be offered prenatal or preconception screening for Gaucher disease type one (carrier rate 1:14), cystic fibrosis (1:25), Tay-Sachs disease (1:25), familial dysautonomia (1:30), Canavan disease (1:40), glycogen storage disease type 1A (1:71), maple syrup urine disease (1:81), Fanconi anemia type C (1:89), Niemann-Pick disease type A (1:90), Bloom syndrome (1:100), mucolipidosis IV (1:120), and possibly neonatal familial hyperinsulinemic hypoglycemia.

The prevalence of carriers of certain autosomal recessive variants in some larger populations is unusually high. In such cases, **heterozygote advantage** is postulated. The carrier frequencies of sickle cell disease in the African population and of cystic fibrosis in the northern European population are much higher than would be expected from the rate of new pathogenic variants. In these populations,

Instructions:
— D represents egg or sperm donor
— S represents surrogate (gestational carrier)
— If the woman is both the ovum donor and a surrogate, in the interest of genetic assessment, she will only be referred to as a donor (e.g., 4 and 5); the pregnancy symbol and its line of descent are positioned below the woman who is carrying the pregnancy
— Available family history should be noted on the gamete donor and/or gestational carrier

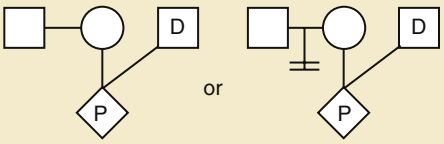
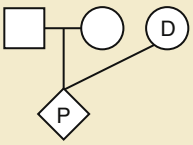
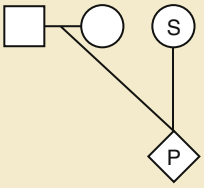
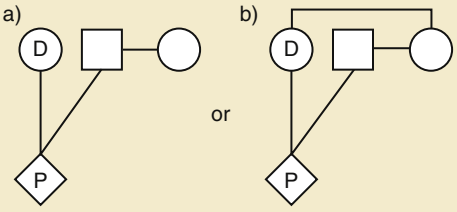
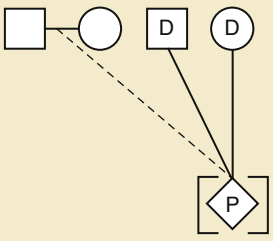
Possible Reproductive Scenarios		Comments
1. Sperm donor		Couple in which woman is carrying pregnancy using donor sperm. No relationship line is shown between the woman carrying the pregnancy and the sperm donor.
2. Ovum donor		Couple in which woman is carrying pregnancy using a donor egg and partner's sperm. The line of descent from the birth mother is solid because there is a biologic relationship that may affect the fetus (e.g., teratogens).
3. Surrogate only		Couple whose gametes are used to impregnate a woman (surrogate) who carries the pregnancy. The line of descent from the surrogate is solid because there is a biological relationship that may affect the fetus (e.g., teratogens).
4. Surrogate ovum donor		Couple in which male partner's sperm is used to inseminate (a) an unrelated woman or (b) a sister who is carrying the pregnancy for the couple.
5. Planned adoption		Couple contracts with a woman to carry a pregnancy using ovum of the woman carrying the pregnancy and donor sperm.

Fig. 97.3 Assisted reproductive technology symbols and definitions. (*From Bennett RL, French KS, Resta RG, et al. Standardized human pedigree nomenclature: update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Couns. 2008;17:424–433.*)

heterozygous carriers may have had an advantage in terms of survival and reproduction over noncarriers. In sickle cell disease, the carrier state is thought to confer some resistance to malaria. In cystic fibrosis, the carrier state has been postulated to confer resistance to cholera or enteropathogenic *Escherichia coli* infections. Population-based **carrier screening** for cystic fibrosis is recommended for persons of northern European and Ashkenazi Jewish ancestry, and population-based screening for sickle cell disease is recommended for persons of African ancestry.

If the frequency of an autosomal recessive disease is known, the frequency of the heterozygote or carrier state can be calculated from the **Hardy-Weinberg formula:**

$$p^2 + 2pq + q^2 = 1$$

where p is the frequency of one of a pair of alleles and q is the frequency of the other. For example, if the frequency of cystic fibrosis among White Americans is one in 2,500 (p^2), then the frequency of the heterozygote ($2pq$) can be calculated: If $p^2 = 1/2{,}500$, then $p = 1/50$ and $q = 49/50$; $2pq = 2 \times (1/50) \times (49/50) = 98/2{,}500$, or 3.92%.

Pseudodominant Inheritance

Pseudodominant inheritance refers to the observation of apparent dominant (parent to child) transmission of a known autosomal recessive disorder (Fig. 97.8). This occurs when a homozygous affected individual has a partner who is a heterozygous carrier. This is most likely to occur for relatively common recessive traits within a population, such as sickle cell anemia or nonsyndromic autosomal recessive hearing loss caused by deleterious variants in *GJB2*, the gene that encodes connexin 26.

X-Linked Inheritance

X-linked inheritance describes the inheritance pattern of most disorders caused by deleterious changes in genes located on the X chromosome (Fig. 97.9). In X-liked disorders, males are more commonly

Instructions:
- E is used for evaluation to represent clinical and/or test information on the pedigree
  a. E is to be defined in key/legend
  b. If more than one evaluation, use subscript (E_1, E_2, E_3) and define in key
  c. Test results should be put in parentheses or defined in key/legend
- A symbol is shaded only when an individual is clinically symptomatic
- For linkage studies, haplotype information is written below the individual. The haplotype of interest should be on left and appropriately highlighted
- Repetitive sequences, trinucleotides, and expansion numbers are written with affected allele first and placed in parentheses
- If variant known, identify in parentheses

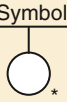
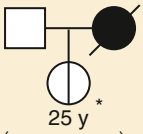
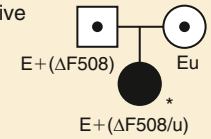
Definition	Symbol	Scenario
1. Documented evaluation (*) Use only if examined/evaluated by you or your research/clinical team or if the outside evaluation has been reviewed and verified.		Woman with negative echocardiogram. 
2. Carrier—not likely to manifest disease regardless of inheritance pattern		Male carrier of Tay-Sachs disease by patient report (* not used because results not verified). 
3. Asymptomatic/presymptomatic carrier—clinically unaffected at this time but could later exhibit symptoms		Woman age 25 with negative mammogram and positive BRCA1 DNA test. 
4. Uninformative study (u)		Man age 25 with normal physical exam and uninformative DNA test for Huntington disease (E_2). 
5. Affected individual with positive evaluation (E+)		Individual with cystic fibrosis and positive variant study; only one variant has currently been identified. 
		10 week male fetus with a trisomy 18 karyotype. 

Fig. 97.4 Pedigree symbols of genetic evaluation and testing information. (*From Bennett RL, French KS, Resta RG, et al. Standardized human pedigree nomenclature: update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Couns. 2008;17:424–433.*)

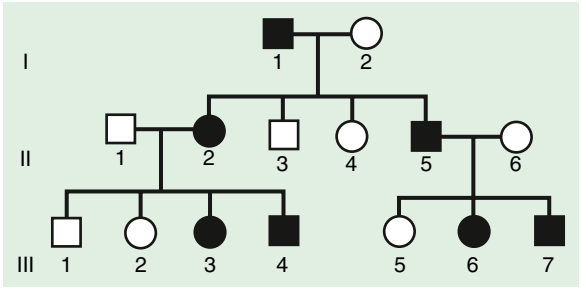


Fig. 97.5 Autosomal dominant pedigree. Pedigree showing typical inheritance of a form of achondroplasia *(FGFR3)* inherited as an autosomal dominant trait. *Black,* Affected patients.

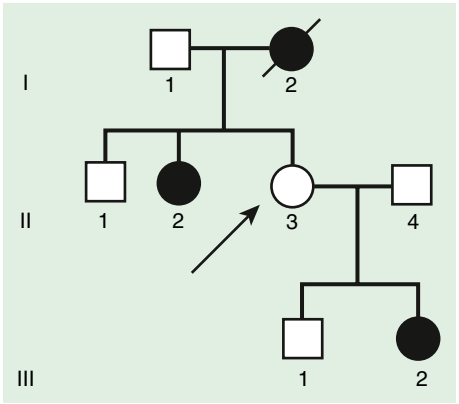


Fig. 97.6 Incomplete penetrance. This family segregates a familial cancer syndrome, familial adenomatous polyposis. Individual II.3 is an obligate carrier *(arrow),* but there are no findings to suggest the disorder. This disorder is nonpenetrant in this individual. *Black,* Affected patients.

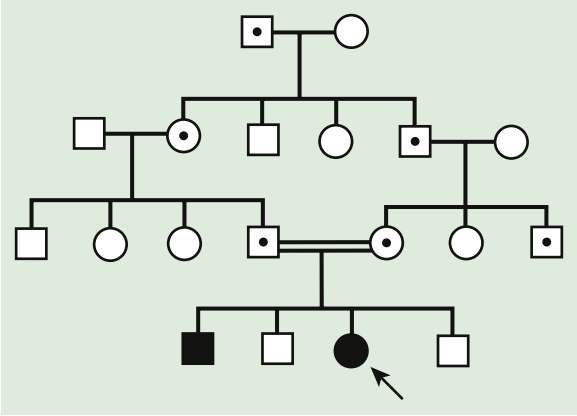


Fig. 97.7 Autosomal recessive pedigree with parental consanguinity. *Central dot,* Carriers; *Black,* affected patients.

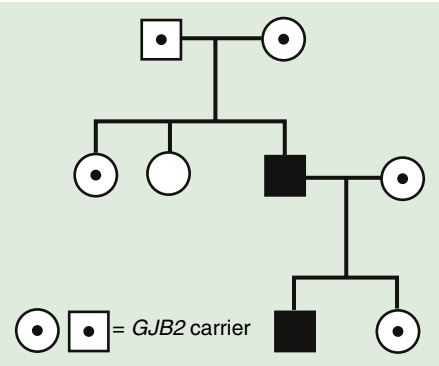


Fig. 97.8 Pseudodominant inheritance. *Black,* Affected (deaf); *central dot* shows carrier who is asymptomatic (unaffected).

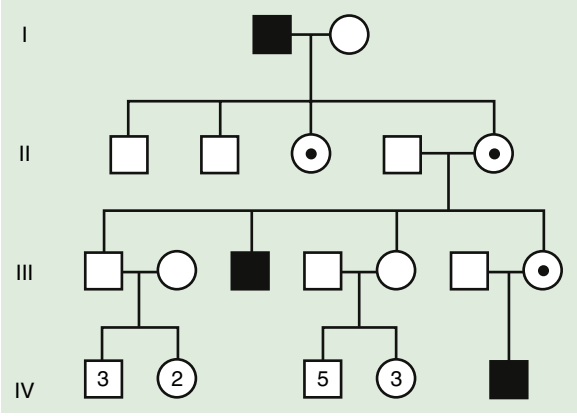


Fig. 97.9 Pedigree demonstrating X-linked recessive inheritance. *Central dot,* Carriers; *black,* affected patients.

affected than females. Female carriers of these disorders are generally unaffected, or if affected, they are affected more mildly than males. In each pregnancy, female carriers have a 25% chance of having an affected son, a 25% chance of having a carrier daughter, and a 50% chance of having a child that does not inherit the mutated X-linked gene. Because affected males pass their X chromosome to all their daughters and their Y chromosome to all their sons, they have a 50% chance of having a daughter who is a carrier. Male-to-male transmission excludes X-linked inheritance but is seen with autosomal dominant and Y-linked inheritance.

A female occasionally exhibits signs of an X-linked trait similar to a male. This occurs rarely from homozygosity for an X-linked trait or the presence of a sex chromosome abnormality (45,X or 46,XY female) but more commonly from skewed or nonrandom X-inactivation. **X chromosome inactivation** occurs early in development and involves the random and irreversible inactivation of most genes on one X chromosome in female cells (Fig. 97.10). In some cases, a preponderance of cells inactivates the same X chromosome, resulting in phenotypic expression of an X-linked pathogenic variant if it resides on the active chromosome. This can occur because of chance, selection against cells that have inactivated the X chromosome carrying the normal gene, or an X chromosome abnormality that results in inactivation of the X chromosome carrying the normal gene.

In some X-linked disorders, both **hemizygous** males and **heterozygous** females who carry an affected X-linked gene have similar phenotypic manifestations. In these cases, an affected male will have a 50% chance of having an affected daughter and a 50% chance of having an unaffected son in each pregnancy, whereas half the male and female offspring of an affected mother will be affected (Fig. 97.11). Some X-linked conditions are lethal in a high percentage of males, such as **incontinentia pigmenti**. In such cases the pedigree typically shows only affected females and an overall female/male ratio of 2:1, with an increased number of miscarriages (Fig. 97.12).

Y-LINKED INHERITANCE

There are few Y-linked traits. These demonstrate *only* male-to-male transmission, and only males are affected (Fig. 97.13). Most Y-linked genes are related to male sex determination and reproduction and are associated with infertility. Therefore it is rare to see familial transmission of a Y-linked disorder. However, advances in assisted reproductive technologies might make it possible to have familial transmission of male infertility.

INHERITANCE ASSOCIATED WITH PSEUDOAUTOSOMAL REGIONS

There are pseudoautosomal regions on the X and Y chromosomes. Because these regions are made up of homologous sequences of nucleotides, genes that are located in these regions are present in equal numbers among both males and females. *SHOX* is one of the best-characterized disease genes located in these regions. Heterozygous *SHOX* pathogenic variants cause **Leri-Weil dyschondrosteosis**, a rare skeletal dysplasia that involves bilateral bowing of the forearms with dislocations of the ulna at the wrist and generalized short stature. Homozygous *SHOX* pathogenic variants cause the much more severe **Langer mesomelic dwarfism**.

DIGENIC INHERITANCE

Digenic inheritance explains the occasional occurrence of **retinitis pigmentosa (RP)** in children of parents who each carry a pathogenic variant in a different RP-associated gene (Fig. 97.14). Both parents have normal vision, as would be expected, but their offspring who are **double heterozygotes**—having inherited both variants—develop RP. Digenic pedigrees can exhibit characteristics of both autosomal dominant (vertical transmission) and autosomal recessive inheritance (one in four recurrence risk). A couple in which the two unaffected partners are carriers for variants in two different RP-associated genes that show digenic inheritance have a one in four risk of having an affected child, similar to what is seen in autosomal recessive inheritance. However, their affected children, and affected children in subsequent generations, have a one in four risk of transmitting both variants to their offspring, who would be affected (vertical transmission).

PSEUDOGENETIC INHERITANCE AND FAMILIAL CLUSTERING

Sometimes nongenetic causes of a particular disease in multiple family members can produce a pattern that mimics genetic transmission. These nongenetic factors can include identifiable environmental factors, teratogenic exposures, or undetermined or undefined factors.

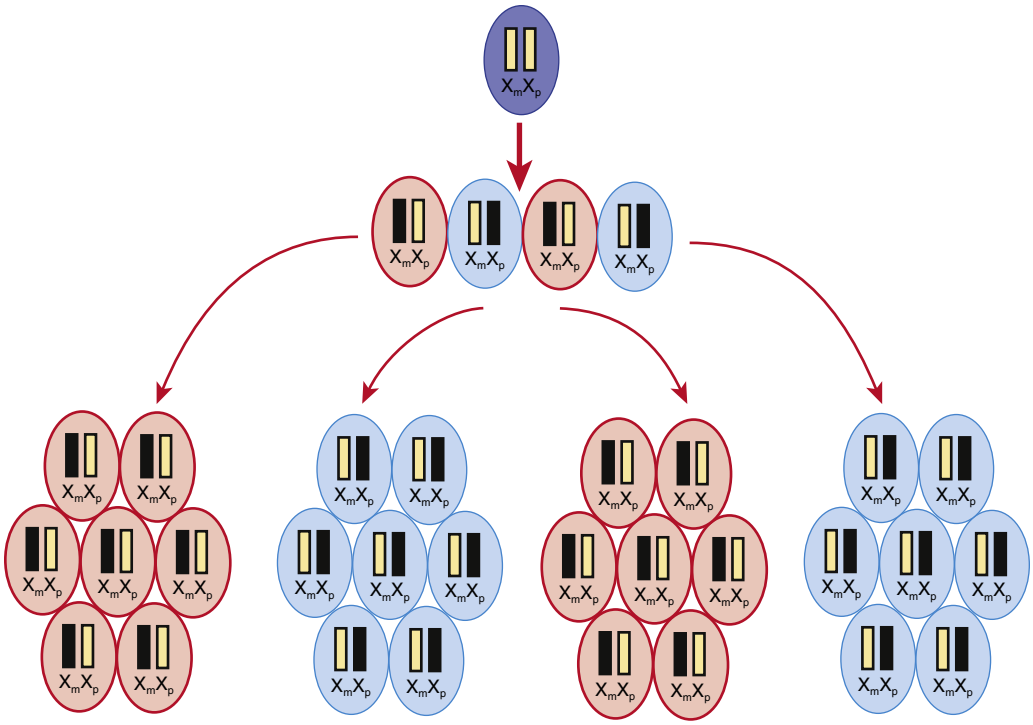


Fig. 97.10 X-inactivation. *Black* marks the active X chromosome. Color of the cell represents its active X chromosome is paternally (X_p, *blue*) or maternally (X_m, *pink*) derived.

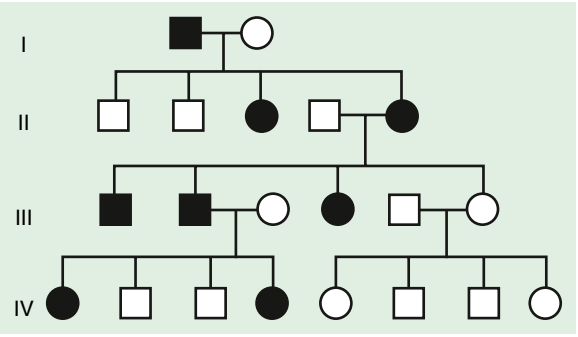


Fig. 97.11 Pedigree pattern demonstrating X-linked dominant inheritance. *Black,* Affected patients. Note there is no father-to-son transmission in this situation, and hemizygosity (i.e., X-linked gene in a male) is not lethal. In some X-linked dominant conditions, X-linked males have a more severe phenotype and might not survive. In that case, only females manifest the disease (see Fig. 97.12).

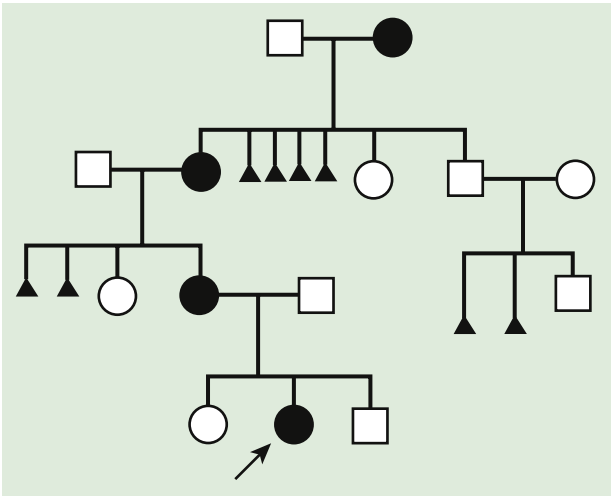


Fig. 97.12 Pedigree of an X-linked dominant disorder with male lethality, such as incontinentia pigmenti. *Black,* Affected patients.

Examples of identifiable factors might include multiple siblings in a family having asthma because of exposure to cigarette smoke from their parents or having failure to thrive, developmental delay, and unusual facial appearance caused by exposure to alcohol during pregnancy.

In some cases, the disease is sufficiently common in the general population that some familial clustering occurs simply by chance. Breast cancer affects 11% of all women, and it is possible that several women in a family will develop breast cancer even in the absence of a specific genetic predisposition. However, hereditary breast cancer associated with pathogenic variants in *BRCA1* and *BRCA2* should be suspected in any individual who has a personal history of breast cancer with onset before age 50, early-onset breast and ovarian cancer at any age, bilateral or multifocal breast cancer, a family history of breast cancer or breast and ovarian cancer consistent with autosomal dominant inheritance, or a personal or family history of male breast

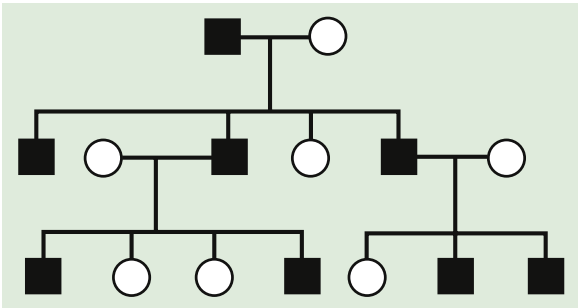


Fig. 97.13 Y-linked inheritance. *Black,* Affected patients.

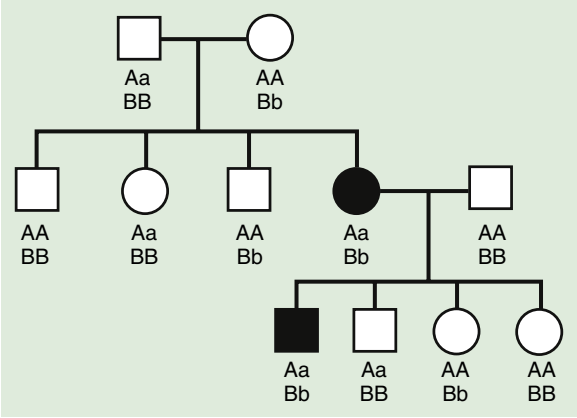


Fig. 97.14 Digenic pedigree. Here, the disease alleles are *a* and *b* and they reside on distinct genetic loci or genes. For a person to have the disease, heterozygosity for variant alleles in both genes (A/a; B/b) is required. *Black,* Affected patients.

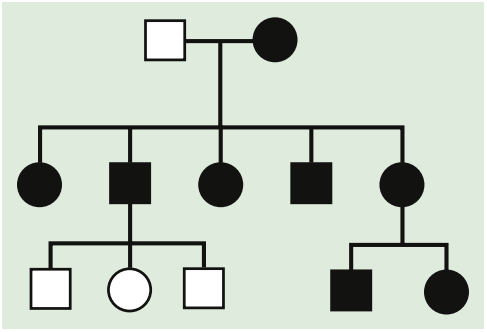


Fig. 97.15 Pedigree of a mitochondrial disorder, exhibiting maternal inheritance. *Black,* Affected patients.

cancer. In other cases, clustering within a family may be caused by undefined genetic factors or unidentified pathogenic sequence variants (nuclear or mitochondrial).

NONTRADITIONAL INHERITANCE

Some genetic disorders are inherited in a manner that does not follow classical mendelian patterns. Nontraditional inheritance is seen in mitochondrial disorders, triplet repeat expansion diseases, and imprinting defects.

Mitochondrial Inheritance

An individual's mitochondrial genome is entirely derived from the mother because sperm contain relatively few mitochondria, which are degraded after fertilization. It follows that **mitochondrial inheritance** is, essentially, **maternal inheritance**. A female with a mitochondrially inherited genetic disorder can have affected offspring of either sex, but an affected father cannot pass on the disease to his offspring (Fig. 97.15). Mitochondrial DNA pathogenic variants are often deletions or point pathogenic variants. Overall, one person in 400 has a maternally inherited pathogenic mitochondrial DNA pathogenic variant (see Chapter 108). In individual families, mitochondrial inheritance may be difficult to distinguish from autosomal dominant or X-linked inheritance, but in many cases, the sex of the transmitting and nontransmitting parents can suggest a mitochondrial basis (Table 97.1).

Mitochondria are the cell's suppliers of energy; the organs that are most affected by the presence of abnormal mitochondria are those that have the greatest energy requirements, such as the brain, muscle, heart, and liver (see Chapters 107.4, 409, 638.2, and 651.4; Fig. 97.16). Common manifestations include developmental delay, seizures, cardiac dysfunction, decreased muscle strength and tone, and hearing and vision problems.

Mitochondrial diseases can be highly variable in their clinical manifestations. This is partly because cells can contain multiple mitochondria, each bearing several copies of the mitochondrial genome. Thus a cell can have a mixture of normal and abnormal mitochondrial genomes, which is referred to as **heteroplasmy**. In contrast, **homoplasmy** refers to a state in which all copies of the mitochondrial genome carry the same sequence variant. Unequal segregation of mitochondria carrying normal and abnormal genomes and replicative advantage can result in varying degrees of heteroplasmy in the cells of an affected individual, including the individual ova of an affected female. Because of this, a mother may be asymptomatic and yet have children who are severely affected. The level of heteroplasmy at which disease symptoms typically appear can also vary based on the type of mitochondrial variant.

Detection of variants in the mitochondrial genome may require sampling of the affected tissue for DNA analysis. In some tissues, such as blood, testing for mitochondrial DNA variants may be inadequate because the variant may be found primarily in affected tissues such as muscle (Fig. 97.17). Growth and differentiation factor 15 (GDF-15) and blood lactate levels are screening tests for mitochondrial disorders. GDF-15 is only accurate in children ≥1 year of age and in the absence of heart, liver, or renal disease.

Triplet Repeat Expansion Disorders

Triplet repeat expansion disorders are distinguished by the special dynamic nature of the disease-causing variant. Triplet repeat expansion disorders include fragile X syndrome, myotonic dystrophy, Huntington disease, and spinocerebellar ataxias (Table 97.2 and Fig. 97.18). These disorders are caused by expansion in the number of 3-bp repeats. The fragile X gene, *FMR1,* normally has 5-40 CGG triplets. An error in replication can result in expansion of that number to a level in the gray zone between 41 and 58 repeats or to a level referred to as a **prepathogenic variant**, which comprises 59-200 repeats. Some prepathogenic variant carriers, more often males, develop fragile X–associated tremor/ataxia syndrome (FXTAS) as adults. Female prepathogenic variant carriers are at risk for fragile X–associated primary ovarian insufficiency (FXPOI). Persons, especially females, with a prepathogenic variant are also at risk for having the repeat expand further in subsequent meiosis, thus crossing into the range of a **full pathogenic variant** (>200 repeats) in offspring. With this number of repeats, the *FMR1* gene becomes hypermethylated, and protein production is lost.

Some triplet expansions associated with other genes can cause disease through a mechanism other than decreased protein production. In Huntington disease, the expansion causes the gene product to have a new, toxic effect on the neurons of the basal ganglia. For most triplet repeat disorders, there is a clinical correlation to the size of the expansion, with a greater expansion causing more severe symptoms and having an earlier age of disease onset. The observation of increasing severity of disease and early age at onset in subsequent generations is termed **genetic anticipation** and is a defining characteristic of many triplet repeat expansion disorders (Fig. 97.19).

Genetic Imprinting

The two copies of most autosomal genes are functionally equivalent. However, in some cases, only one copy of a gene is transcribed, and the second copy is silenced. This gene silencing is typically associated with methylation of DNA, which is an **epigenetic** modification; it does not change the nucleotide sequence of the DNA (Fig. 97.20). In **imprinting**, gene expression depends on the *parent of origin* of the chromosome (see Chapter 99.7). Imprinting disorders result from an imbalance of active copies of a given gene, which can occur for several reasons. Prader-Willi and Angelman syndromes,

Table 97.1 Representative Examples of Disorders Caused by Pathogenic Variants in Mitochondrial DNA and Their Inheritance

DISEASE	PHENOTYPE	MOST FREQUENT PATHOGENIC VARIANT IN mtDNA MOLECULE	HOMOPLASMY vs HETEROPLASMY	INHERITANCE
Leber hereditary optic neuropathy	Rapid optic nerve atrophy, leading to blindness in young adult life; sex bias approximately 50% males with visual loss, only 10% females	Substitution p.Arg340His in *ND1* gene of complex I of electron transport chain; other complex I missense pathogenic variants	Homoplasmic (usually)	Maternal
NARP, Leigh disease	*N*europathy, *a*taxia, *r*etinitis *p*igmentosa, developmental delay, intellectual disability lactic academia	Point pathogenic variants in ATPase subunit six gene	Heteroplasmic	Maternal
MELAS	*M*itochondrial encephalomyopathy, *l*actic *a*cidosis, and *s*trokelike episodes; may manifest only as diabetes mellitus or deafness	Point pathogenic variant in $tRNA^{Leu}$	Heteroplasmic	Maternal
MERRF	*M*yoclonic *e*pilepsy, *r*agged *r*ed *f*ibers in muscle, ataxia, sensorineural deafness	Point pathogenic variant in $tRNA^{Lys}$	Heteroplasmic	Maternal
Deafness	Progressive sensorineural deafness, often induced by aminoglycoside antibiotics	m.1555A>G pathogenic variant in 12S rRNA	Homoplasmic	Maternal
	Nonsyndromic sensorineural deafness	m.7445A>G pathogenic variant in 12S rRNA	Homoplasmic	Maternal
Chronic progressive external ophthalmoplegia (CPEO)	Progressive weakness of extraocular muscles, cardiomyopathy, ptosis, heart block, ataxia, retinal pigmentation, diabetes	The common MELAS point pathogenic variant in $tRNA^{Lys}$; large deletions similar to KSS	Heteroplasmic	Maternal if point pathogenic variants
Pearson syndrome	Pancreatic insufficiency, pancytopenia, lactic acidosis	Large deletions	Heteroplasmic	Sporadic, somatic pathogenic variants
Kearns-Sayre syndrome (KSS)	PEO of early onset with heart block, retinal pigmentation	5-kb large deletion	Heteroplasmic	Sporadic, somatic pathogenic variants

mtDNA, Mitochondrial DNA; rRNA, ribosomal RNA; tRNA, transfer RNA.
From Nussbaum RL, McInnes RR, Willard HF, eds. *Thompson & Thompson Genetics in Medicine*, 6th ed. Philadelphia: Saunders; 2001: p. 246.

two distinct disorders associated with developmental impairment, are caused by microdeletions of chromosome 15q11-12. The microdeletion in **Prader-Willi syndrome** is always on the *paternally* derived chromosome 15, whereas in **Angelman syndrome** it is on the *maternal* copy. *UBE3A* is the gene responsible for Angelman syndrome. The paternal copy of *UBE3A* is transcriptionally silenced in the brain, but the maternal copy continues to be transcribed. If an individual has a maternally derived deletion, an insufficient amount of UBE3A protein is produced in the brain, resulting in the neurologic deficits seen in Angelman syndrome.

Uniparental disomy (UPD), the rare occurrence of a child inheriting both copies of a chromosome from the *same parent*, is another genetic mechanism that can cause Prader-Willi and Angelman syndromes. Inheriting both chromosomes 15 from the mother is functionally the same as deletion of the paternal 15q12 region and results in Prader-Willi syndrome. Approximately 30% of cases of Prader-Willi syndrome are caused by maternal UPD15, whereas paternal UPD15 accounts for only 3% of Angelman syndrome (see Chapter 99.7). Pathogenic variants in *UBE3A* account for ~11% of patients with Angelman syndrome and result in familial transmission. The least common cause is a pathogenic variant affecting the imprinting center, which results in an inability to correctly imprint *UBE3A*. In a mother, inability to reset the imprinting on her paternally inherited chromosome 15 imprint results in a 50% risk of passing on an incorrectly methylated copy of *UBE3A* to a child, who would then develop Angelman syndrome.

Other imprinted regions of clinical interest include the short arm of chromosome 11, where the genes for Beckwith-Wiedemann syndrome and nesidioblastosis map, and the long arm of chromosome seven with maternal UPD of 7q being associated with some cases of idiopathic short stature and Russell-Silver syndrome.

Imprinting of a gene can occur during gametogenesis or early embryonic development **(reprogramming)**. Genes can become inactive or active by various mechanisms including DNA methylation or demethylation or histone acetylation or deacetylation, with different patterns of (de)methylation noted on paternal or maternal imprintable chromosome regions. Some genes demonstrate tissue-specific imprinting (see Fig. 97.20). There is a small but increased incidence of imprinting disorders, specifically Beckwith-Wiedemann and Angelman syndrome, following assisted reproductive technologies such as in vitro fertilization and intracytoplasmic sperm injection. The overall incidence of these disorders in children conceived using assisted reproductive technologies is likely to be <1%.

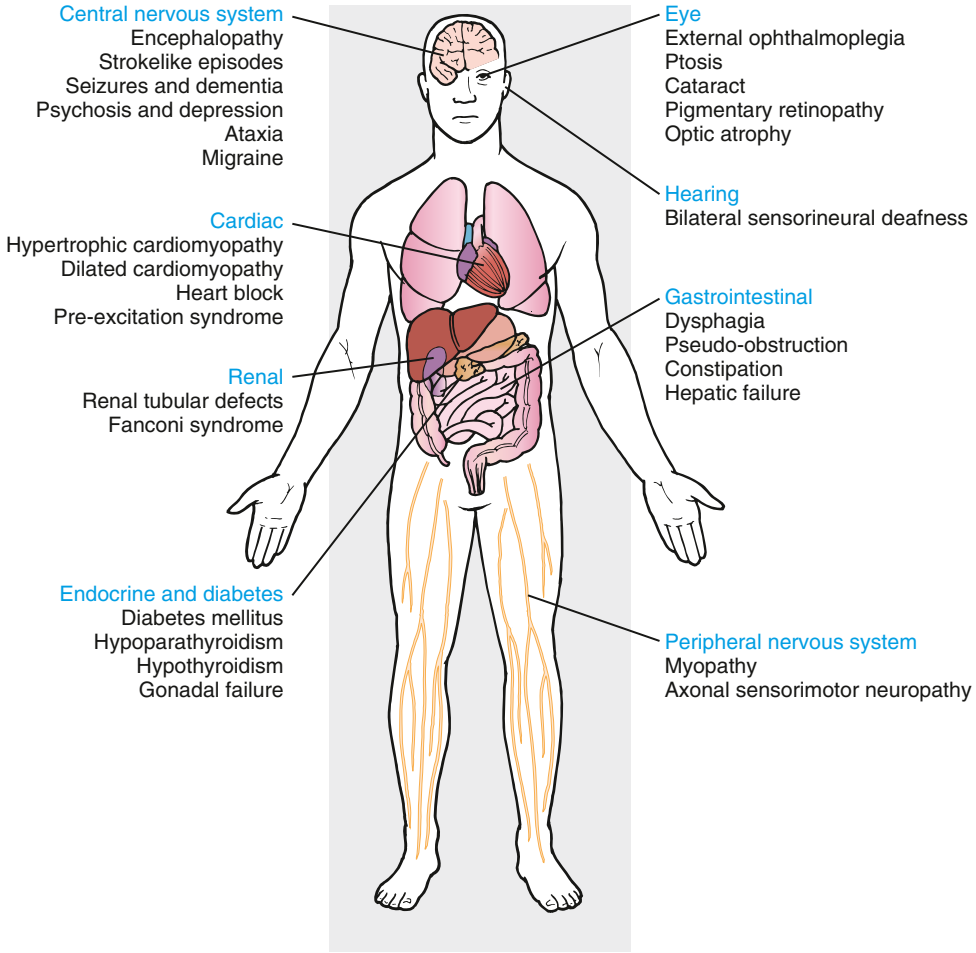


Fig. 97.16 The range of affected tissues and clinical phenotypes associated with pathogenic variants in mitochondrial DNA (mtDNA). (*Modified from Chinnery PF, Turnbull DM. Mitochondrial DNA and disease. Lancet. 1999;345:SI17–SI21.*)

MULTIFACTORIAL AND POLYGENIC INHERITANCE

Multifactorial inheritance refers to traits that are caused by a combination of inherited, environmental, and stochastic factors (Fig. 97.21). Multifactorial traits differ from polygenic inheritance, which refers to traits that result from the additive effects of multiple genes. Multifactorial traits segregate within families but do not exhibit a consistent or recognizable inheritance pattern. Characteristics include the following:

- There is a similar rate of recurrence among all first-degree relatives (parents, siblings, offspring of affected child). It is unusual to find a substantial increase in risk for relatives related more distantly than second degree to the index case.
- The risk of recurrence is related to the incidence of the disease.
- Some disorders have a sex predilection, as indicated by an unequal male:female incidence. Pyloric stenosis, for example, is more common in males, whereas congenital dislocation of the hips is more common in females. With an altered sex ratio, the risk is higher for the relatives of an index case whose gender is less often affected than relatives of an index case of the more frequently affected gender. The risk to the son of an affected female with infantile pyloric stenosis is 18%, compared with the 5% risk for the son of an affected male. An affected female presumably has a greater genetic susceptibility, which can be passed on to offspring.
- The likelihood that both identical twins will be affected with the same malformation is <100% but much greater than the chance that both members of a nonidentical twin pair will be affected. This contrasts with the pattern seen in mendelian inheritance, in which identical twins almost always share fully penetrant genetic disorders.
- The risk of recurrence is increased when multiple family members are affected. A simple example is that the risk of recurrence for unilateral cleft lip and palate is 4% for a couple with one affected child and increases to 9% with two affected children. It is sometimes difficult to distinguish between a multifactorial and mendelian etiology in families with multiple affected individuals.
- The risk of recurrence may be greater when the disorder is more severe. For example, an infant who has long-segment Hirschsprung disease has a greater chance of having an affected sibling than the infant who has short-segment Hirschsprung disease.

There are two types of multifactorial traits. One exhibits **continuous variation**, with "normal" individuals falling within a statistical range—often defined as having a value two standard deviations (SDs) above and/or below the mean—and "abnormals" falling outside that range. Examples include such traits as intelligence, blood pressure, height, and head circumference. For many of these traits, offspring values can be estimated based on a modified average of their parental values, with nutritional and environmental factors playing an important role.

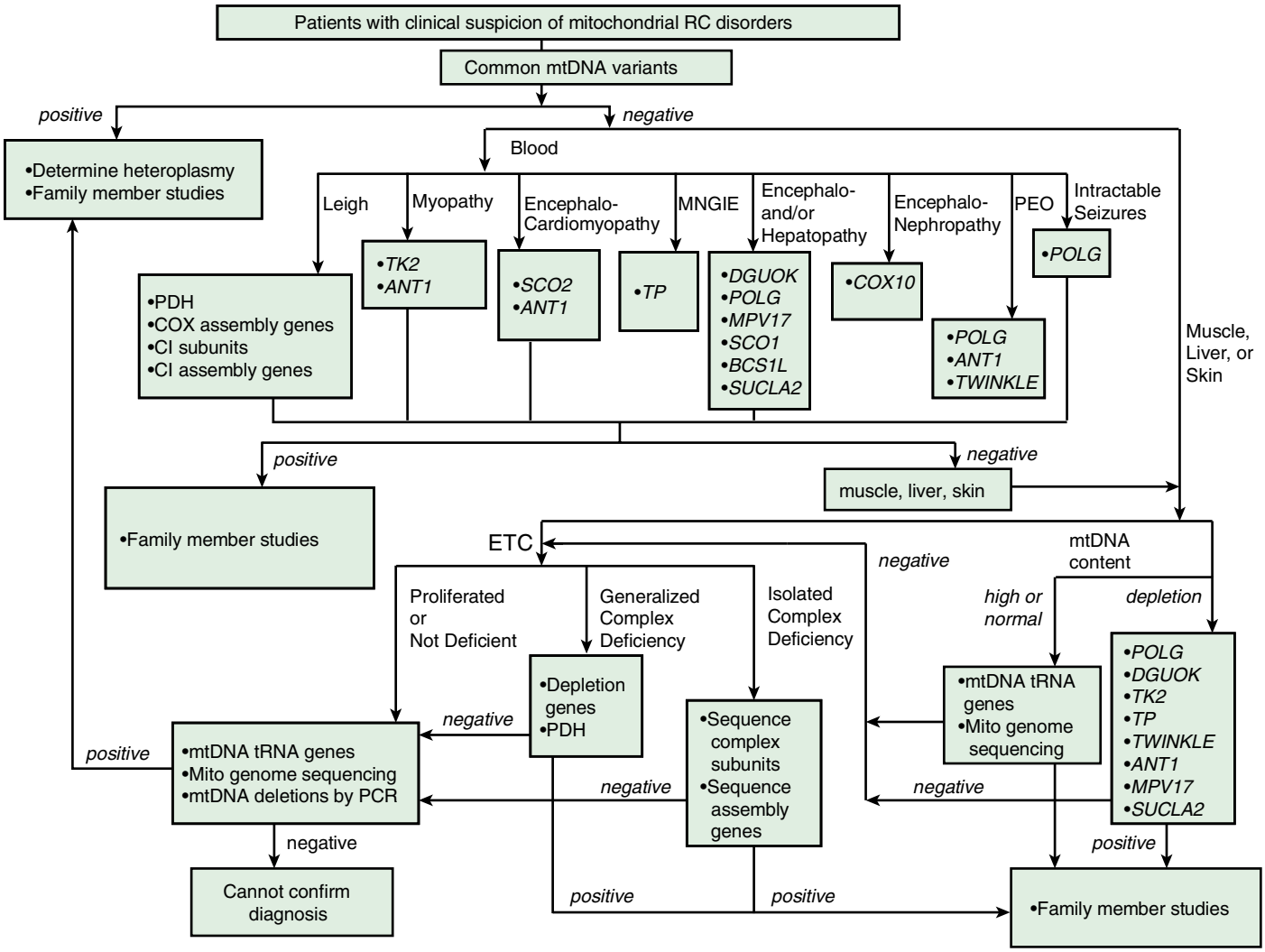


Fig. 97.17 Clinical algorithm for genetic diagnostic testing of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) genes in patients suspected of mitochondrial disorders (Baylor College of Medicine, Mitochondrial Diagnostics Laboratory). RC, Respiratory chain; MNGIE, mitochondrial neurogastrointestinal encephalopathy; PEO, progressive external ophthalmoplegia; PDH, pyruvate dehydrogenase; CI, respiratory complex I; ETC, electron transport chain; PCR, polymerase chain reaction. (*From Haas RH, Parikh S, Falk MJ, et al. The in-depth evaluation of suspected mitochondrial disease. Mol Genet Metab. 2008;94:16–37.*)

Table 97.2 Diseases Associated with Polynucleotide Repeat Expansions

DISEASE	DESCRIPTION	REPEAT SEQUENCE	NORMAL RANGE	ABNORMAL RANGE	PARENT IN WHOM EXPANSION USUALLY OCCURS	LOCATION OF EXPANSION
CATEGORY 1						
Huntington disease	Loss of motor control, dementia, affective disorder	CAG	6-34	36-100 or more	More often through father	Exon
Spinal and bulbar muscular atrophy	Adult-onset motor-neuron disease associated with androgen insensitivity	CAG	11-34	40-62	More often through father	Exon
Spinocerebellar ataxia type 1	Progressive ataxia, dysarthria, dysmetria	CAG	6-39	41-81	More often through father	Exon
Spinocerebellar ataxia type 2	Progressive ataxia, dysarthria	CAG	15-29	35-59	—	Exon
Spinocerebellar ataxia type three (Machado-Joseph disease)	Dystonia, distal muscular atrophy, ataxia, external ophthalmoplegia	CAG	13-36	68-79	More often through father	Exon

Continued

Table 97.2 Diseases Associated with Polynucleotide Repeat Expansions—cont'd

DISEASE	DESCRIPTION	REPEAT SEQUENCE	NORMAL RANGE	ABNORMAL RANGE	PARENT IN WHOM EXPANSION USUALLY OCCURS	LOCATION OF EXPANSION
CATEGORY 1						
Spinocerebellar ataxia type 6	Progressive ataxia, dysarthria, nystagmus	CAG	4-16	21-27	—	Exon
Spinocerebellar ataxia type 7	Progressive ataxia, dysarthria, retinal degeneration	CAG	7-35	38-200	More often through father	—
Spinocerebellar ataxia type 17	Progressive ataxia, dementia, bradykinesia, dysmetria	CAG	29-42	47-55	—	Exon
Dentatorubral-pallidoluysian atrophy/Haw River syndrome	Cerebellar atrophy, ataxia, myoclonic epilepsy, choreoathetosis, dementia	CAG	7-25	49-88	More often through father	Exon
CATEGORY 2						
Pseudoachondroplasia, multiple epiphyseal dysplasia	Short stature, joint laxity, degenerative joint disease	GAC	5	6-7	—	Exon
Oculopharyngeal muscular dystrophy	Proximal limb weakness, dysphagia, ptosis	GCG	6	7-13	—	Exon
Cleidocranial dysplasia	Short stature, open skull sutures with bulging calvaria, clavicular hypoplasia, shortened fingers, dental anomalies	GCG, GCT, GCA	17	27 (expansion observed in one family)	—	Exon
Synpolydactyly	Polydactyly and syndactyly	GCG, GCT, GCA	15	22-25	—	Exon
CATEGORY 3						
Myotonic dystrophy (DM1; chromosome 19)	Muscle loss, cardiac arrhythmia, cataracts, frontal balding	CTG	5-37	100 to several thousand	Either parent, but expansion to congenital form through mother	3′ untranslated region
Myotonic dystrophy (DM2; chromosome 3)	Muscle loss, cardiac arrhythmia, cataracts, frontal balding	CCTG	<75	75-11,000	—	3′ untranslated region
Friedreich ataxia	Progressive limb ataxia, dysarthria, hypertrophic cardiomyopathy, pyramidal weakness in legs	GAA	7-2	200-900 or more	Autosomal recessive inheritance, so disease alleles are inherited from both parents	Intron
Fragile X syndrome (FRAXA)	Intellectual impairment, large ears and jaws, macroorchidism in males	CGG	6-52	200-2,000 or more	Exclusively through mother	5′ untranslated region
Fragile site (FRAXE)	Mild intellectual impairment	GCC	6-35	>200	More often through mother	5′ untranslated region
Spinocerebellar ataxia type 8	Adult-onset ataxia, dysarthria, nystagmus	CTG	16-37	107-127	More often through mother	3′ untranslated region
Spinocerebellar ataxia type 10	Ataxia and seizures	ATTCT	12-16	800-4,500	More often through father	Intron
Spinocerebellar ataxia type 12	Ataxia, eye movement disorders; variable age at onset	CAG	7-28	66-78	—	5′ untranslated region
Progressive myoclonic epilepsy type 1	Juvenile-onset seizures, myoclonus, dementia	12-bp repeat motif	2-3	30-75	Autosomal recessive inheritance, so transmitted by both parents	5′ untranslated region

From Jorde LB, Carey JC, Bamshad MJ, White, RL. *Medical Genetics*, 3rd ed. St Louis: Mosby; 2006: p. 82.

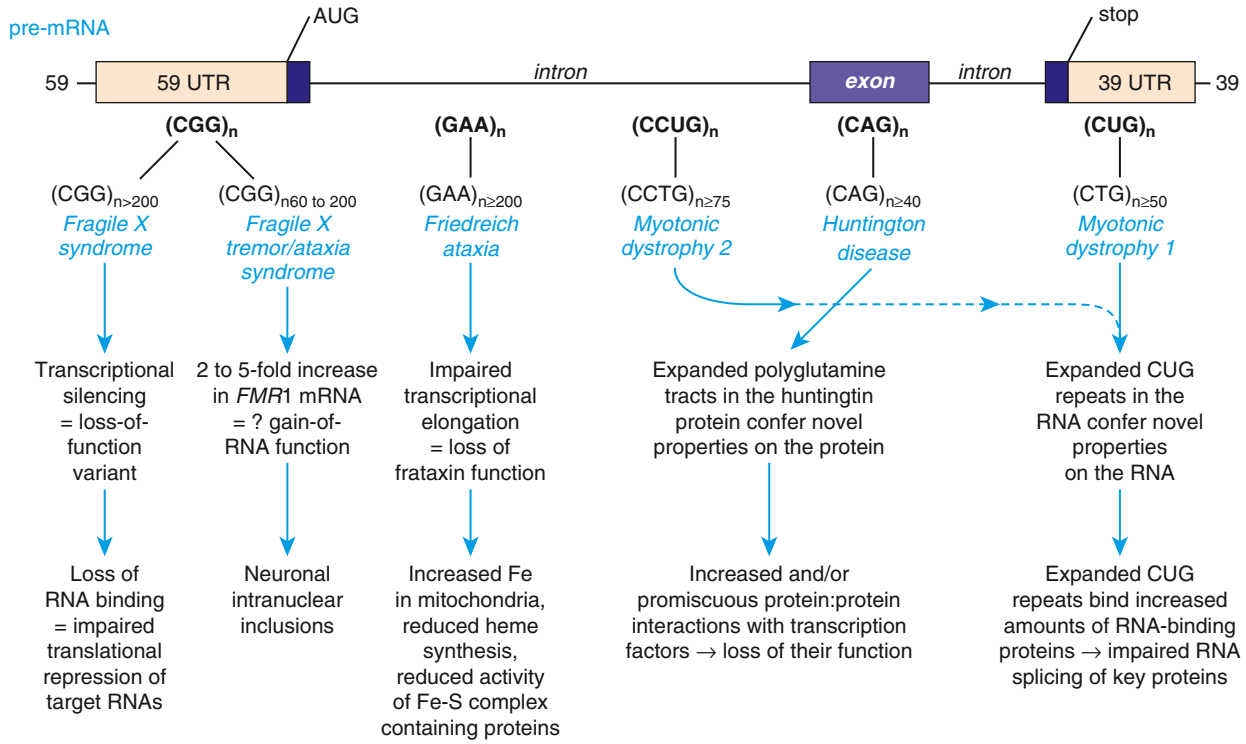


Fig. 97.18 The locations of the trinucleotide repeat expansions and the sequence of each trinucleotide in five representative trinucleotide repeat diseases, shown on a schematic of a generic pre–messenger RNA (mRNA). The minimal number of repeats in the DNA sequence of the affected gene associated with the disease is also indicated, as well as the effect of the expansion on the mutant RNA or protein. (*From Nussbaum RL, McInnes RR, Willard HF, eds. Thompson & Thompson Genetics in Medicine, 8th ed. Philadelphia: Elsevier; 2016; based partly on an unpublished figure from John A. Phillips III, Vanderbilt University.*)

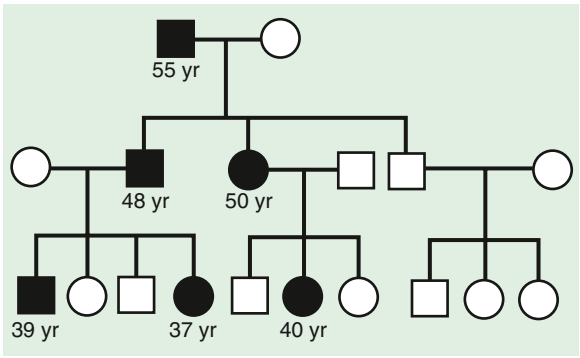


Fig. 97.19 Myotonic dystrophy pedigree illustrating genetic anticipation. In this case the age at onset for family members affected with an autosomal dominant disease is lower in more recent generations. *Black,* Affected patients.

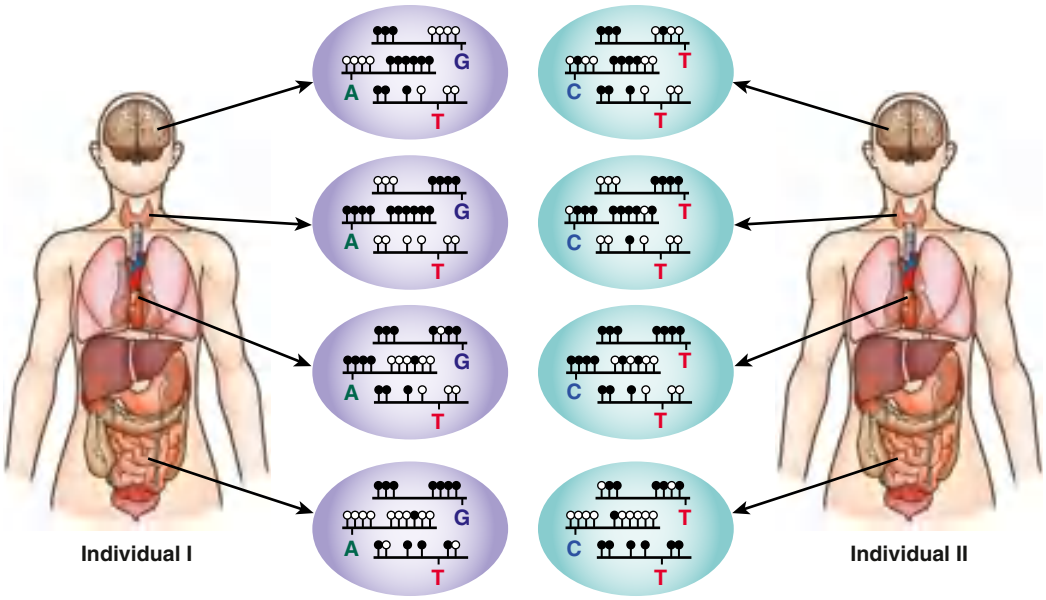


Fig. 97.20 Tissue-specific DNA methylation and epigenetic heterogeneity among individuals. A subset of the DNA methylation patterns within a cell is characteristic of that cell type. Cell type–specific and tissue-specific DNA methylation patterns are illustrated by organ-to-organ variations in the clusters of methylated cytosine-phosphate-guanine bases (CpGs) within the same individual. Despite overall consistency in tissue-specific DNA methylation patterns, variations in these patterns exist among different individuals. Methylated CpGs are indicated by a *filled circle* and unmethylated CpGs by an *open circle*. Single nucleotide polymorphisms (SNPs) are indicated by the corresponding base. (*Redrawn from Brena RM, Huang THM, Plass C. Toward a human epigenome. Nat Genet. 2006;38:1359–1360.*)

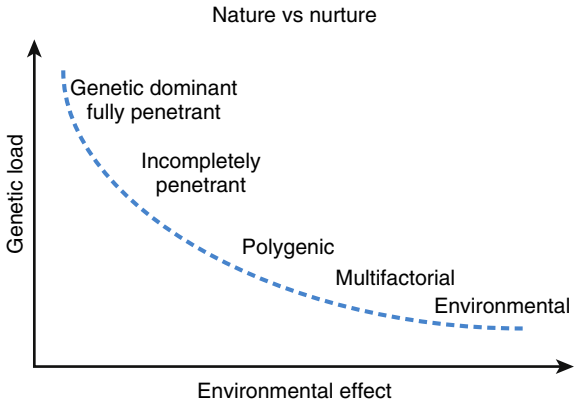


Fig. 97.21 The progressive decrease in the genetic load contributing to the development of a disease creates a smooth transition in the distribution of illnesses on an etiologic diagram. In theory, no diseases are completely free from the influence of both genetic and environmental factors. (*From Bomprezzi R, Kovanen PE, Martin R. New approaches to investigating heterogeneity in complex traits. J Med Genet. 2003;40:553–559.*)

With other multifactorial traits, the distinction between normal and abnormal is based on the **qualitative** presence or absence of a particular trait. Examples include pyloric stenosis, neural tube defects, congenital heart defects, and cleft lip and cleft palate. Such traits follow a **threshold model** (see Fig. 97.15). A distribution of liability because of genetic and nongenetic factors is postulated in the population. Individuals who exceed a threshold liability develop the trait, and those below the threshold do not.

The balance between genetic and environmental factors is demonstrated by neural tube defects. Genetic factors are implicated by the increased recurrence risk for parents of an affected child compared with the general population, yet the recurrence risk is about 3%, less than what would be expected if the trait was caused by a single, fully penetrant pathogenic variant. The role of nongenetic environmental factors is shown by the recurrence risk decreasing up to 87% if the mother-to-be takes 4 mg of folic acid daily starting 3 months before conception.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

Chapter 98

Integration of Genetics into Pediatric Practice

Brendan Lee and Nicola Brunetti-Pierri

Genetic testing involves analyzing genetic material to obtain information related to a person's health status using chromosomal (cytogenetic) analysis (see Chapter 99) and nucleic acid (primarily DNA but also RNA)-based testing.

DIAGNOSTIC TESTING

Diagnostic genetic testing helps to aggregate a set of signs and symptoms (phenotype) for disease diagnosis. The list of disorders for which specific genetic tests are available is extensive and growing. The website http://www.ncbi.nlm.nih.gov/gtr/ provides one database of reported available tests.

Single-gene disorders can be tested by at least three different approaches: linkage analysis (a classical approach now rarely used), chromosome microarray (CMA), and direct pathogenic variant analysis, usually by DNA sequencing (Table 98.1). Linkage analysis is used if the responsible gene is mapped but not yet identified, or if it is impractical to find specific pathogenic variants, usually because of the large size and large number of different pathogenic variants in some genes. CMA can be used to detect large, multigene deletions or duplications **(copy number variations, or CNVs)**. In addition, with increasing resolution, single-gene or smaller intragenic deletions or duplications can be detected by CMA, although it is important to note that coverage of each gene varies by array type used. *Direct DNA pathogenic variant identification is readily available with the advent of the complete human genome sequence next-generation sequencing (NGS) tools.* We also recognize the relatively frequent co-occurrence of multiple disorders, each caused by distinct pathogenic variants, in patients with complex or "blended" features. This may include partial to minimal clinical manifestations in a substantial percentage of individuals harboring pathogenic variants in known disease genes that remain undiagnosed. The ability to simultaneously sequence hundreds to thousands of genes (via NGS approaches) has provided insight into this complexity of disease pathogenesis.

Linkage testing involves tracking a genetic trait through a family using closely linked polymorphic markers as a surrogate for the trait (Fig. 98.1). It requires testing an extended family and is vulnerable to several pitfalls, such as genetic recombination, genetic heterogeneity, and incorrect diagnosis in the proband or family member. **Genetic recombination** occurs between any pair of loci, with the frequency being proportional to the distance between them. This problem is minimized using very closely linked markers and, if possible, using markers that flank the specific gene. **Genetic heterogeneity** can be problematic for a linkage-based test if there are multiple distinct genomic loci that can cause the same phenotype, resulting in the risk that the locus tested for is not the one responsible for disease in the family. **Incorrect diagnosis** in the proband also leads to tracking the wrong gene. Linkage testing remains useful for several genetic conditions, but it has been superseded by the availability of DNA sequencing of either single gene, panel, or exome sequencing.

CMAs can detect CNVs in a patient's DNA (see Chapter 99). CMA provides a level of genetic resolution between that available with DNA sequencing (single nucleotide) and that available with chromosome analysis (~5 million base pairs). CMA can resolve deletions or duplications of several kilobases within one gene. In theory, this approach can detect deletion and duplication pathogenic variants that would be missed by either chromosome analysis or direct pathogenic variant testing by DNA sequencing. However, because the specific resolution and coverage of different CMA platforms can vary tremendously for different gene regions, the sensitivity for detecting deletions and duplications can vary for different diseases and laboratories. The highest resolution used is detection of a deletion or duplication at the single exon level.

Direct DNA-based pathogenic variant testing avoids the pitfalls of linkage testing by detecting the specific gene pathogenic variant (i.e., sequence change). The specific approach used is customized to the biology of the gene being tested. In some disorders, one or a few distinct pathogenic variants occur in all affected individuals and a specific assay for that variant could be used. This is the case in sickle cell anemia, in which the same single-base substitution occurs in everyone with the disorder. In other conditions, many possible pathogenic variants may account for the disorder in different individuals. In cystic fibrosis, for example, >1,000 distinct pathogenic variants have been found in the *CFTR* gene. Pathogenic variant analysis is challenging because no single technique can detect all possible pathogenic variants (e.g., single nucleotide variation [SNV]), small (several nucleotide) insertion-deletions ("indels"), CNV (larger deletion or duplications), complex structural variations (e.g., translocations, inversions, etc.), DNA repeat expansions (e.g., variable repeat expansions), and epigenetic alterations (e.g., changes in DNA methylation without changing the underlying DNA sequence). However, with high-throughput DNA sequencing technology, the most frequently used approach is to

Table 98.1 Approaches for Genetic Testing

TYPE OF TESTING	RESOLUTION	ADVANTAGES	DISADVANTAGES	SAMPLE REQUIREMENTS
Linkage analysis	Depends on location of polymorphic markers near putative disease gene	Possible when specific disease-causing genetic pathogenic variant is not identifiable or found	Can give only diagnostic probability based on likelihood of genetic recombination between presumed DNA pathogenic variant and polymorphic markers	Requires multiple family members with documented Mendelian pattern of inheritance within family
Chromosome microarray (CMA)	Several hundred base pairs to several hundreds of kilobases	Able to detect small deletion or duplications within one or more genes	Can miss small deletions or insertions depending on the resolution of the array used	Single patient sample sufficient, but having sample from biological parents can help with interpretation
Direct DNA-based testing (e.g., DNA sequencing)	Single–base-pair changes	High specificity if previously described deleterious pathogenic variant is found	Can miss deletion or duplication of a segment of gene	Single patient sample sufficient, but having sample from biological parents and siblings (affected or unaffected) can help with interpretation

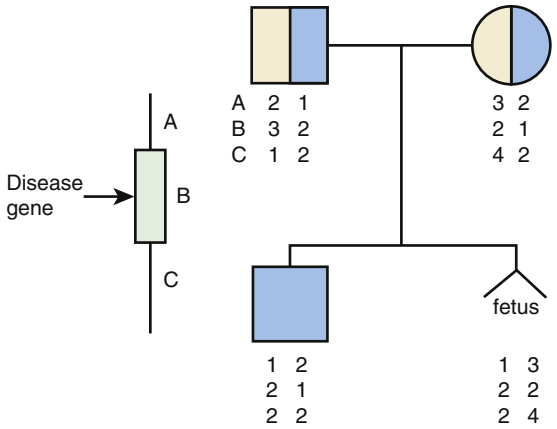


Fig. 98.1 Use of linkage analysis in prenatal diagnosis of an autosomal recessive disorder. Both parents are carriers, and they have one affected son. The numbers below the symbols indicate alleles at three polymorphic loci: A, B, and C. Locus B resides within the disease gene. The affected son inherited the 1-2-2 chromosome from his father and the 2-1-2 chromosome from his mother. The fetus has inherited the same chromosome from the father but the 3-2-4 chromosome from the mother and therefore is most likely to be a carrier.

directly sequence DNA segments "captured" and amplified from DNA isolated from peripheral blood white blood cells. The limitation of this approach is that only amplified DNA segments are sequenced and are usually restricted to the *coding exonic* regions of a gene. Because pathogenic variants sometimes occur in the *noncoding* and *intronic* regions, failure to detect a pathogenic variant does not fully exclude the diagnosis. In addition, deleted genes or regions may not be detected.

NGS tests panels of genes that target disease symptoms (e.g., epilepsy, brain malformations, sensorineural deafness, or skeletal dysplasias) or the majority of the exome **(exome sequencing)**. **Genome sequencing**, where both *coding* and *noncoding* sequences are sequenced, can provide even more information. However, clinical interpretation is limited predominantly to the coding sequences of the approximately 20,000 human genes, the "digital exome," as it is extracted electronically from genome data. Genome sequencing, compared with exome sequencing, also has the advantage of providing improved detection of CNV, structural variation, and repeat expansions, although this is highly dependent on the bioinformatics algorithms used in interpretation.

With NGS, the challenge is the interpretation of enormous genetic variation within a single individual. Direct sequencing of tens to hundreds of genes in NGS panels offers a potentially higher sensitivity because the "depth" of read is higher without complicating high discovery rate of **variants of unknown sequences (VUS)** (Fig. 98.2). Exome and genome sequencing also offer the potential for identifying new disease-gene associations as well as the ability to detect clinical presentations caused by more than one altered gene (i.e., oligogenic phenotypes).

An important ethical consideration is the reporting of *incidental findings,* whether medically actionable or not medically actionable in a patient. For example, exome and genome sequencing may identify pathogenic variants that cause aminoglycoside-sensitive hearing loss, which would be medically actionable but unrelated to the primary indication for which the test was ordered. At the same time, the discovery of apolipoprotein E variants in a child that increase Alzheimer disease risk susceptibility would not typically be medically actionable. Therefore counseling for patients undergoing these tests is important so that only requested results are reported back to the patient. Guidelines continue to evolve for reporting of incidental findings from exome and genome sequencing by the American College of Medical Genetics and Genomics (www.acmg.net). Practice and recommendations continue to vary among international genetic organizations revealing incidental findings to patients, and many strongly advocate the engagement of the patient and family in the decision. Some groups require revealing to the patient and/or family significant diseases (actionable) with a specific and successful treatment or prevention strategy (Table 98.2).

Genetic testing is interpreted by three factors: analytic validity, clinical validity, and clinical utility. **Analytical validity** is test accuracy: Does the test correctly detect the presence or absence of pathogenic variant? Most genetic tests have a very high analytical validity assuming human error has not occurred. Human errors are possible, and unlike most medical tests, a genetic test is unlikely to be repeated because it is assumed that the result will not change over time. Therefore human errors can go undetected for long periods of time. *In addition, variants may be reinterpreted over time as our knowledge base of disease-causing pathogenic variants and genes increases.*

Clinical validity is the degree to which the test correctly predicts presence or absence of disease. False-positive and false-negative test results can occur. **False-positive results** are more likely for predictive tests than for diagnostic tests. An important contributing factor is **nonpenetrance**, where an individual with an at-risk genotype might not clinically express the condition. Another factor is the finding of a **VUS**. Detection of a base sequence variation in an affected patient does not prove that it is the cause of the patient's disorder. Exome sequencing of an individual may identify more than 30,000 VUS and more than 3,000,000 VUS for genome sequencing. Various lines of evidence are used to establish pathogenicity. These include the absence of the variant in large populations of control individuals, finding the variant in affected individuals, demonstration or inference that the variant alters the function of the gene product, noting that the amino acid altered by the pathogenic variant is conserved in evolution, and segregation of the variant with disease in a family. For many variants, it is possible to be certain whether the variant is pathogenic or benign, but for others it might be impossible to definitively assign causality or noncontribution with 100% confidence. For these reasons, they are noted as **VUS.** *Notably, interpretation of pathogenicity for a specific VUS may change over time as our knowledge base increases, underscoring the importance of counseling and reinterpretation.*

False-negative results reflect an inability to detect a pathogenic variant in an affected patient. This occurs principally in disorders with genetic heterogeneity—**allelic** (different pathogenic variants occur in one causative gene) or **locus** (>1 gene can cause a disease) heterogeneity. It is often difficult to detect all possible pathogenic variants within a gene because pathogenic variants vary, both in location within the gene and in the type of variant. Direct sequencing can miss gene deletions or rearrangements (i.e., structural variants), and pathogenic variants may be found within noncoding sequences such as introns or promoter. Therefore a negative DNA test does not necessarily exclude a diagnosis.

Clinical utility is the degree to which the results of a test guide clinical management. For genetic testing, clinical utility includes establishing a diagnosis that obviates the need for additional workup or guiding surveillance or treatment. Test results may also be used as a basis for genetic counseling. For some disorders, genetic testing is possible, but the test results do not add to the clinical assessment. If the diagnosis and genetic implications are already clear, it might not be necessary to pursue genetic testing.

Predictive genetic testing involves performing a test in a person at risk for developing a genetic disorder **(presymptomatic)**, usually on the basis of family history, yet who does not manifest signs or symptoms. This is usually done for disorders that display age-dependent penetrance (e.g., the likelihood of manifesting signs and symptoms increases with age, as in cancer genetic syndromes or Huntington disease).

A major caution with predictive testing is that the presence of a gene pathogenic variant does not necessarily mean that the disease will develop. Many of the disorders with age-dependent penetrance display *incomplete penetrance*. A person who inherits a pathogenic variant may never develop signs of the disorder. There is concern that a positive DNA test could result in stigmatization of the person and might not provide information that will guide medical management. Stigmatization might include psychologic stress, but it could also include

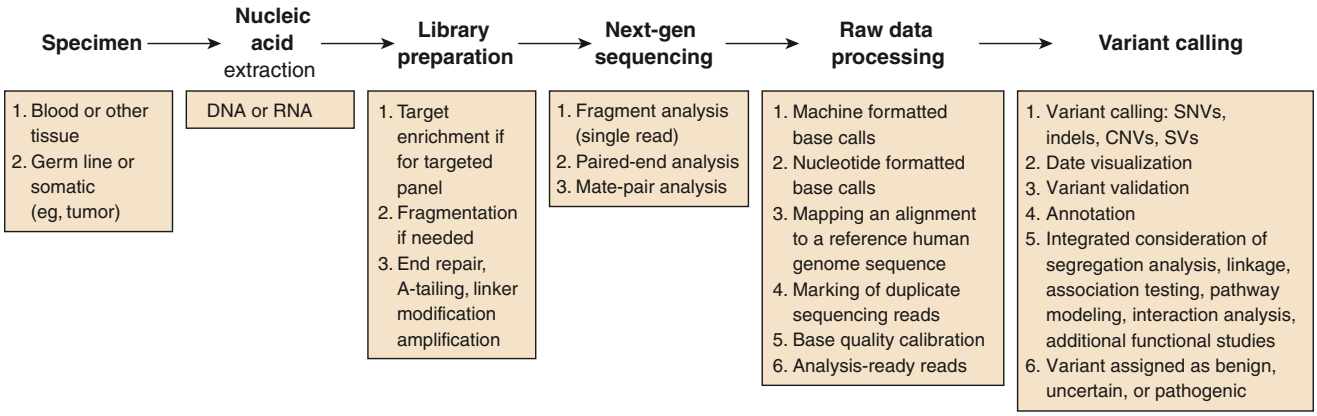


Fig. 98.2 Overview of the next-generation DNA sequencing workflow. CNV, Copy number variant; Indels, insertions and deletions; SNV, single nucleotide variant; SV, structural variant. (*Adapted from Casey G, Conti D, Haile R, et al. Next generation sequencing and a new era of medicine. Gut. 2013;62[6]:920–932.*)

Table 98.2 Variants That Are Incidental Findings Are Assigned to One of Four Categories

Childhood onset	Medically actionable*
Childhood onset	Not medically actionable†
Adult onset	Medically actionable*
Adult onset	Not medically actionable†

*"Medically actionable" refers to a variant in a gene in which knowledge of the particular variant will affect medical decision-making, such as initiation of a treatment or family planning.

†"Not medically actionable" refers to variants that increase the individual's risk for a disease in which no treatment is proven to significantly change the disease natural history.

From Bick D, Dimmock D. Whole exome and whole genome sequencing. *Curr Opin Pediatr.* 2011;23:594–600.

discrimination, including denial of health, life, or disability insurance, or employment (see Chapter 95).

It is generally agreed that predictive genetic tests should be performed for children only if the results of the test will benefit the medical management during childhood; otherwise, the test should be deferred until the individual understands the risks and benefits of testing and can provide their own informed consent. Individual states offer varying degrees of protection from discrimination on the basis of genetic testing. A major milestone in the prevention of genetic discrimination was the passage of the **Genetic Information Nondiscrimination Act** (GINA) in 2008, which is a U.S. federal law that prohibits discrimination in health coverage or employment based on genetic information. *Of note, it does not* protect against refusal of life insurance.

Predispositional genetic testing is available with the goal of predicting risk of disease. The rationale for predispositional testing is that the results would lead to strategies aimed at risk reduction as part of a *personalized approach* to healthcare maintenance. This might include avoidance of environmental exposures that would increase risk of disease (cigarette smoking and α_1-antitrypsin deficiency), medical surveillance (familial breast cancer and mammography), or in some cases, pharmacologic treatment (statins and hypercholesterolemia).

Common disorders are multifactorial in etiology, and many different genes may contribute to risk of any specific condition (see Chapter 103). Most genetic variants found to correlate with risk of a common disease add small increments of relative risk and, in most cases, too little to guide management based on a single variant.

Statistical models for predicting risk based on a collection of DNA variants has been integrated into approaches such as *polygenic risk scores*. By combining the relative risk conferred by a group of DNA variants, the goal is to quantify a larger proportion of risk for a specific disease. These predictions are confounded by the source of data from which they are generated, and current population genetic data have limitations including lack of ethnic diversity and population stratification.

PHARMACOGENETICS

Polymorphisms in drug metabolism genes can result in distinctive patterns of drug absorption, metabolism, excretion, or effectiveness. Knowledge of individual genotypes can guide pharmacologic therapy, allowing customization drug choice and dosage to avoid toxicity and provide a therapeutic response. Well-known examples include testing for polymorphisms within the methylenetetrahydrofolate reductase *(MTHFR)* gene for susceptibility of potentially increased toxicity to methotrexate antimetabolite therapy and thiopurine *S*-methyltransferase (*TPMT)* to avoid adverse effects with 6-mercaptopurine therapy (Fig. 98.3).

Pediatric Pharmacogenomics

Pharmacogenomics is the study of how variants contribute to interindividual variability in drug response. The finding that drug responses can be influenced by the patient's genetic profile offers great hope for realizing *individualized* pharmacotherapy, in which the relationship between genotype and phenotype (either disease and/or drug response) is predictive of drug response. In addition to genetic differences, environmental factors (e.g., diet, concomitant drug or toxic exposure), physiologic variables (age, sex, pregnancy), and patient adherence all contribute to variations in drug metabolism and response. Interindividual differences in children are further complicated by the changing patterns of gene expression occurring during the developmental processes from birth through adolescence. Combining these genetic and nongenetic individual-specific variables, it is expected that children will benefit from the promise of precision medicine, e.g., identifying the right drug for the right patient at the right time and dosing (Fig. 98.4).

Examples of pharmacogenomic traits include specific adverse drug reactions, such as prolonged respiratory muscle paralysis due to succinylcholine, hemolysis associated with antimalarial therapy, carbamazepine-induced Stevens-Johnson syndrome, isoniazid-induced neurotoxicity, and others (Table 98.3). The pharmacokinetic properties of a drug are determined by genes that control drug absorption, distribution, metabolism, and excretion. Drug-metabolizing enzymes and drug transporters play a particularly important role in this process. One of the better recognized clinical manifestations of pharmacogenomic variability in drug biotransformation is an increased risk of concentration-dependent toxicity caused by reduced clearance and consequent tissue drug accumulation. Equally important is the lack of efficacy caused by variations in metabolism of prodrugs that require biotransformation to be converted into a pharmacologically active form. The pharmacogenomics of drug receptors and other target proteins involved in signal transduction or disease pathogenesis are also expected to contribute to interindividual variability in drug disposition and response.

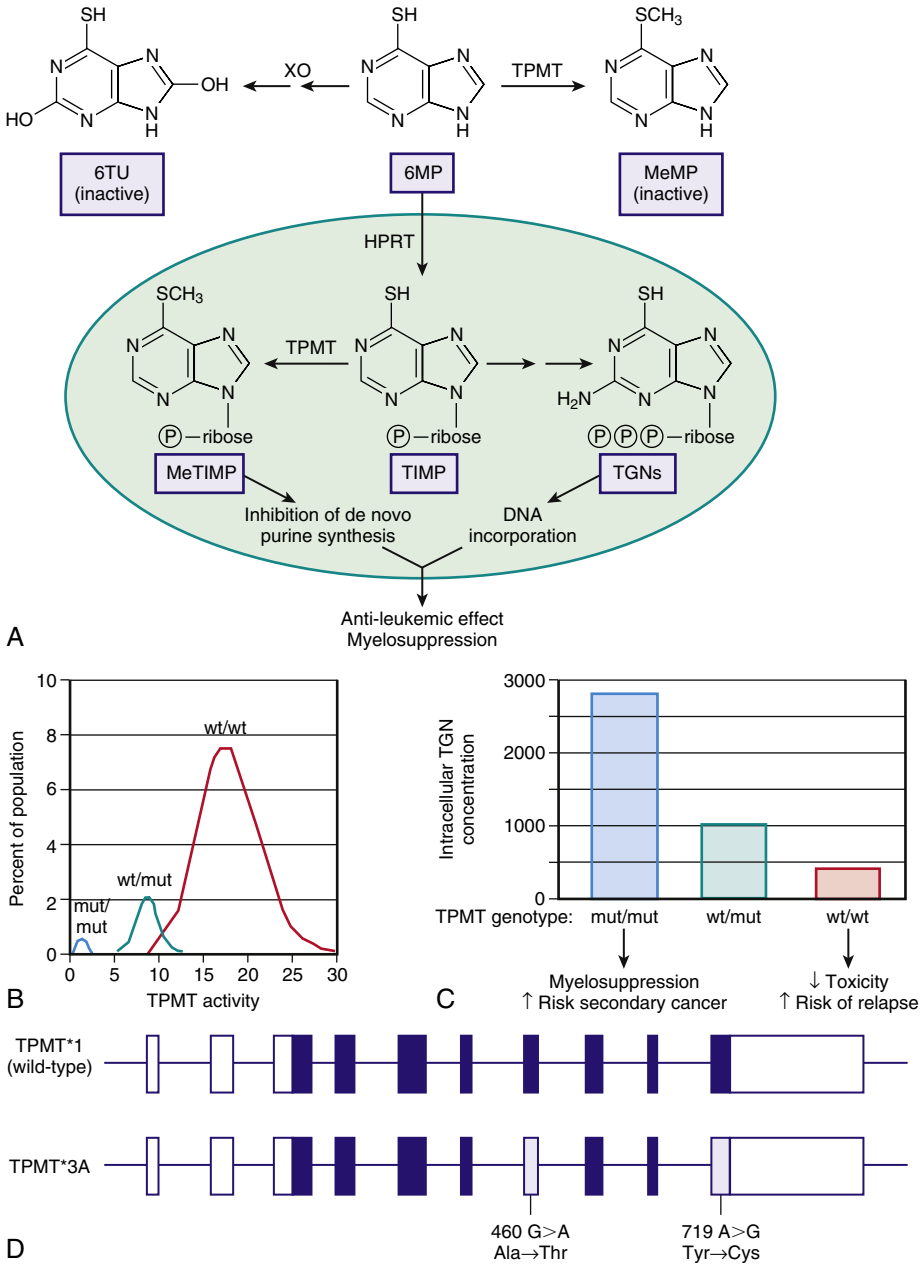


Fig. 98.3 Thiopurine *S*-methyltransferase (TPMT) polymorphism. **A,** 6-Mercaptopurine (6MP) undergoes metabolism to thioguanine nucleotides (TGNs) to exert its cytotoxic effects. TPMT and xanthine oxidase reduce the amount of 6MP available for the bioactivation pathway to TGNs. TPMT can also methylate 6-thioinosine 5′-monophosphate (TIMP) to generate a methylated compound capable of inhibiting de novo purine synthesis. **B,** Distribution of TPMT activity in humans. Of the population, 89% has high activity, whereas 11% has intermediate activity. Approximately 1 in 300 individuals homozygous for two loss-of-function alleles has very low activity. **C,** Correlation between the TPMT genotype and intracellular TGN concentrations. In TPMT-poor metabolizers, more 6MP is available to go down the bioactivation pathway to form TGNs; this situation is associated with an increased risk of myelosuppression. **D,** The most common variant TPMT allele is the result of two pathogenic variants that give rise to an unstable protein product that undergoes proteolytic degradation. Each box represents an exon. Non-coding sequences are shown as white boxes and colored boxes indicate coding sequences. 6TU, 6-Thiouric acid; MeMP, 6-methylmercaptopurine; HPRT, 6-thiomethylinosine 5-monophosphate; MeTIMP, hypoxanthine-guanine phosphoribosyl transferase; wt, wild type; mut, mutant; Ala, alanine; Thr, threonine; Tyr, tyrosine; Cys, cysteine. (*Modified from Relling MV, Dervieux T. Pharmacogenetics and cancer therapy. Nat Rev Cancer. 2001;11:99–108.*)

Individuals are classified as "fast," "rapid," or "extensive" metabolizers at one end and "slow" or "poor" metabolizers at the other end of the continuum. For biotransformation, fetuses and newborns may be phenotypically "slow" or "poor" metabolizers for certain drug-metabolizing pathways because of their stage of development, and they may acquire a phenotype consistent with their genotype at some point later in the developmental process. Moreover, not all infants acquire drug metabolism activity at the same rate, and there is interindividual variability in the trajectory (i.e., rate and extent) of drug biotransformation capacity (Fig. 98.5). The primary organ responsible for drug metabolism is the liver, although the kidney, intestine, lung, adrenals, blood (phosphatases, esterases), and skin can also biotransform certain compounds. Drug biotransformation is characterized by three important features: (1) broad substrate specificity, in which a single isozyme may metabolize a large variety of chemically diverse compounds; (2) many different enzymes may be involved in the biotransformation of a single drug (enzyme multiplicity); and (3) a given drug may undergo several different types of reactions. Drug biotransformation reactions are classified into phase I reactions that introduce or reveal (through oxidation, reduction, or hydrolysis) a functional group within the substrate drug molecule that serves as a site for phase II reactions. These reactions involve conjugation with endogenous substrates (such as acetate, glucuronic acid, glutathione, glycine, and sulfate), making the compound more water soluble to be excreted in urine. A supergene family with at least 16 primary cytochrome P450 (CYP) enzymes are quantitatively the most important phase I enzymes and catalyze the metabolism of many lipophilic endogenous (steroids, fatty acids, fat-soluble vitamins, prostaglandins, leukotrienes, thromboxanes) and exogenous compounds, including a multitude of drugs and environment toxins. The specific CYP isoforms responsible for the majority of human drug metabolism are CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. These enzymes are the products of genes that in some cases are polymorphically expressed, with allelic variants producing enzymes generally resulting in either no or reduced catalytic activity. At birth the activities of drug-oxidizing enzymes in the liver are reduced, which results in slow clearance (and prolonged elimination) of several substrate drugs (e.g., phenytoin, caffeine, diazepam). Phase

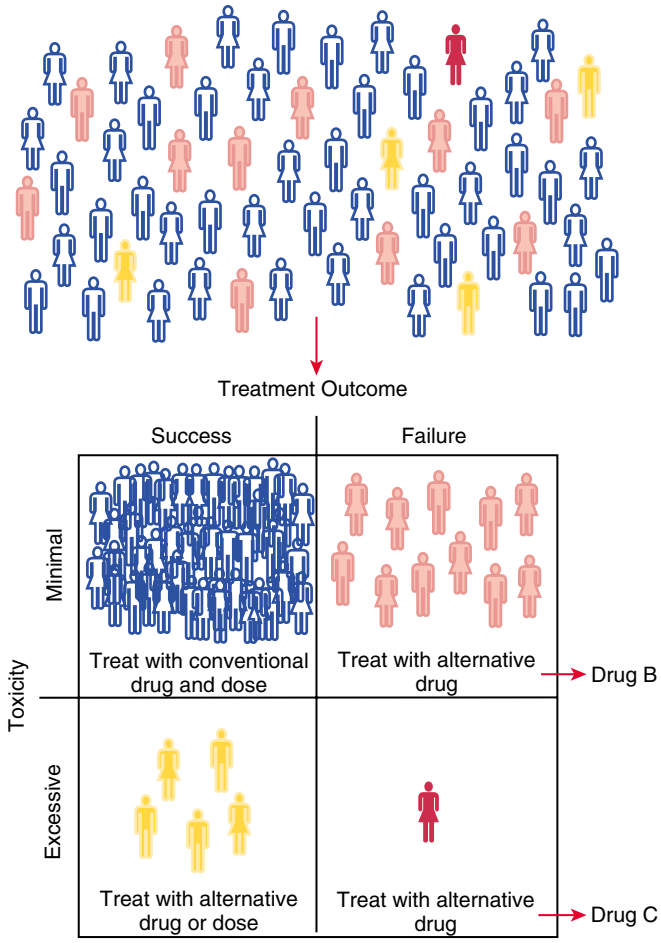


Fig. 98.4 The promise of genomic medicine to human health and disease. The goal of personalized medicine is to identify subgroups of patients who will respond favorably to a given drug with minimum side effects, as well as those who will not respond or who will show toxicity with standard doses. A further benefit of pharmacogenomics is to select the most appropriate alternative drug for patients who cannot be treated successfully with conventional drugs and doses. (*Adapted from Yaffe SJ, Aranda JV. Neonatal and Pediatric Pharmacology, 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2004.*)

II enzymes include arylamine *N*-acetyltransferases (NAT1, NAT2), uridine diphospho-glucuronosyltransferases (UGTs), epoxide hydrolase, glutathione *S*-transferases (GSTs), sulfotransferases (SULTs), and methyltransferases (catechol *O*-methyltransferase, thiopurine *S*-methyltransferase, several *N*-methyltransferases). Phase II enzyme activity is decreased in the newborn and increases into childhood. Conjugation of compounds metabolized by isoforms of UGT (e.g., morphine, bilirubin, chloramphenicol) is reduced at birth but can exceed adult values by 3-4 years of age.

Membrane transporters are involved in drug disposition and actively transport substrate drugs between organs and tissues. They include organic anion transporters (OATs), organic anion–transporting polypeptides (OATPs), organic cation transporters (OCTs), and the adenosine triphosphate–binding cassette (ABC) transporters, such as P-glycoprotein and the multidrug-resistant proteins. Drug transporters are expressed at numerous barriers, such as intestinal epithelial cells, hepatocytes, renal tubular cells, and the blood-brain barrier (Fig. 98.6). Transporters are often determinants of drug resistance, and many drugs work by affecting the function of transporters. Polymorphisms in the genes encoding these transporters might affect drug absorption, distribution, metabolism, and excretion.

98.1 Genetic Counseling

Brendan Lee and Pilar L. Magoulas

Genetic counseling is a communication process in which the genetic contribution to health, specific risks of transmission of a trait, and options to manage the condition are explained to individuals and their family members (Table 98.4). Genetic counselors are specialized healthcare providers trained in the psychosocial aspects of counseling and the science of medical genetics who may serve as members of medical teams in many different specialties. The genetic counselor is expected to present information in a neutral, nondirective manner while providing resources and psychosocial support to the individual and family to cope with decisions that are made (see Table 98.4).

In the prenatal setting, a common indication for genetic counseling is to assess risk of occurrence or recurrence of having a child with a genetic condition and to discuss management or treatment options that might be available before, during, or after the pregnancy, such as preimplantation genetic testing, noninvasive prenatal screening, prenatal diagnosis or fetal intervention, and perinatal management. In pediatric and adult genetics practices, the goals of genetic counseling are to help establish a diagnosis in an individual, provide longitudinal care and psychosocial support to the family, and discuss the genetic basis and inheritance of the condition as it relates to immediate and distant family members.

The genetic counseling role has expanded, particularly with advances in understanding the genetics of adult-onset or common and rare disease therapeutics. In the former context, genetic counseling has a major role in risk assessment for cancer, especially breast, ovarian, or colon cancer, for which well-defined risk models and genetic tests are available to assess risk to an individual. In the latter, the genetic counselor may discuss developments in rare disease therapeutics and make appropriate referral for medical therapies.

There are several situations in which genetic counseling plays a particularly important role. The first situation is the **prenatal diagnosis** of a congenital anomaly or genetic disease. The need for information is urgent because a family must often make time-sensitive decisions about treatment and management options, such as fetal intervention or continuation of a pregnancy in the context of fetal anomalies. Risks to the mother must also be considered. The second type of situation occurs when a child is born with a life-threatening **congenital anomaly** or suspected **genetic disease**. Decisions must be made immediately on how much support should be provided to the child and whether certain types of therapy should be attempted. The third situation arises when there are concerns about a **genetic condition** affecting one later in life. This may occur in an adolescent or young adult with a family history of an adult-onset genetic disorder (e.g., Huntington disease, hereditary breast/ovarian cancer), in an individual with a suspected yet undiagnosed genetic condition, or if a couple with a personal or family history of a genetic condition (or a carrier) is planning a family. In these situations, it is often necessary to have several meetings with a family to discuss possible testing, screening, and management options. Urgency is not as much of an issue as being sure that they have as much information and as many options as are available. Last, with the advent of genomic testing in all areas of clinical care, **pretest genetic counseling** before testing is essential to provide individuals with accurate information regarding the types of results they may receive. During this process, individuals are also given the option of what type of results they want reported back to them, such as medically actionable results unrelated to the primary indication for testing and incidental findings.

GENETIC COUNSELING ELEMENTS

Components of a genetic counseling session and providing accurate information to families requires the following:

- Taking a targeted family history and constructing a pedigree that diagrams the patient's relatives (including miscarriages, abortions, stillbirths, deceased persons) with their sex, age, ethnicity, and state of health, up to and including third-degree relatives.

Table 98.3 Examples of Effects of Gene Polymorphisms on Drug Response

GENE	ENZYME/TARGET	DRUG	CLINICAL RESPONSE
BCHE	Butyrylcholinesterase	Succinylcholine	Prolonged paralysis
CYP2C9	Cytochrome P450 2C9	Warfarin	Individuals having ≥1 reduced function alleles require lower doses of warfarin for optimal anticoagulation, especially initial anticoagulant control.
CYP2C19	Cytochrome P450 2C19	Clopidogrel	Individuals having ≥1 loss-of-function alleles have reduced capacity to form pharmacologically active metabolite of clopidogrel and reduced antiplatelet effect.
CYP2D6	Cytochrome P450 2D6	Codeine	Poor metabolizers (individuals with two loss-of-function alleles) do not metabolize codeine to morphine and thus experience no analgesic effect. Ultrarapid metabolizers (individuals with ≥3 functional alleles) may experience morphine toxicity.
G6PD	Glucose-6-phosphate dehydrogenase	Primaquine (others)	Hemolysis
*HLA-A*3101*	Human leukocyte antigen A31	Carbamazepine	Carriers of *HLA-A*3101* allele have increased risk of SJS and TEN from carbamazepine.
*HLA-B*1502*	Human leukocyte antigen B15	Allopurinol	Han Chinese carriers of *HLA-B*1502* allele have increased risk of SJS and TEN from carbamazepine.
*HLA-B*5701*	Human leukocyte antigen B57	Abacavir Flucloxacillin	Carriers of *HLA-B*5701* allele have increased risk of hypersensitivity reactions to abacavir- and flucloxacillin-induced liver injury.
*HLA-B*5801*	Human leukocyte antigen B58	Allopurinol	Carriers of *HLA-B*5801* allele have increased risk of severe cutaneous adverse reactions to allopurinol, including hypersensitivity reactions, SJS, and TEN.
NAT2	*N*-Acetyltransferase 2	Isoniazid, hydralazine	Individuals homozygous for "slow acetylation" polymorphisms are more susceptible to isoniazid toxicity, or hydralazine-induced systemic lupus erythematosus.
SLCO1B1	Organic anion–transporting protein (OATP) 1B1	Simvastatin	Carriers of the *SLCO1B1*5* allele are at increased risk for musculoskeletal side effects from simvastatin.
TPMT	Thiopurine *S*-methyltransferase	Azathioprine 6-Mercaptopurine	Individuals homozygous for an inactivating pathogenic variant have severe toxicity if treated with standard doses of azathioprine or 6-mercaptopurine; rapid metabolism causes undertreatment.
UGT1A1	Uridine diphospho-glucuronosyltransferase 1A1	Irinotecan	*UGT1A1*28* allele is associated with decreased glucuronidation of SN-38, the active metabolite of irinotecan, and increased risk of neutropenia.
VKORC1	Vitamin K oxidoreductase complex 1	Warfarin	Individuals with a haplotype associated with reduced expression of VKORC1 protein (therapeutic target of warfarin) require lower doses of the drug for stable anticoagulation.

SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

- Gathering information from hospital records about the affected individual and, in some cases, about other family members.
- Documenting prenatal, pregnancy, and delivery histories.
- Reviewing the latest available medical, laboratory, and genetic information concerning the disorder.
- Reviewing a careful physical examination of the affected individual (photographs, measurements) and of apparently unaffected individuals in the family (typically performed by a physician rather than a genetic counselor).
- Reviewing genetic testing and screening options.
- Establishing or confirming the diagnosis by the diagnostic tests available.
- Providing psychosocial support to the individual and family throughout the diagnostic process.
- Giving the family information about support groups and local and national resources.
- Providing new information to the family as it becomes available (a mechanism for updating needs to be established).

Genetic counseling sessions often include anticipatory guidance regarding the occurrence or risk of occurrence of a specific condition, knowledge of the diagnosis of the particular condition, natural history of the condition, genetic aspects of the condition, risk of recurrence, prenatal diagnosis and reproductive options, therapies and referrals, and provision of support resources.

The Diagnostic Process

If a specific diagnosis is made and confirmed, this should be discussed with the family and information provided in writing. Often, however, the disorder fits into a spectrum (e.g., one of many types of arthrogryposis) or the diagnosis is clinical rather than confirmed with molecular testing. In these situations, the family needs to understand the limits of the diagnostic process and present knowledge, and that additional research will probably lead to better information in the future.

Although it is not always possible to make an exact diagnosis, having a diagnosis *as accurate as possible* is important. Estimates of recurrence risk for various family members depend on an accurate diagnosis that

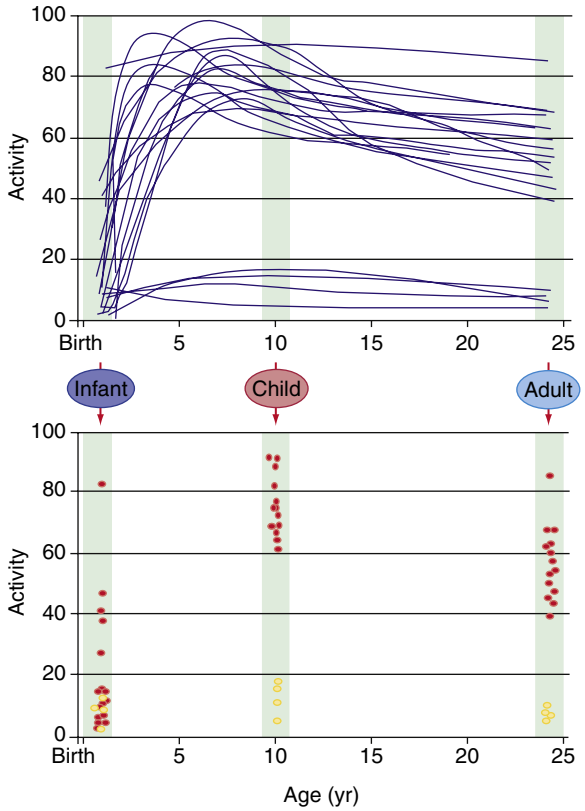


Fig. 98.5 "Developmental" phenotypes. Variability in developmental changes in gene expression and functional enzyme activity are superimposed on pharmacogenetic determinants. **Top,** Developmental profile of a theoretical drug-metabolizing enzyme over a 25-year span in 20 individuals. **Bottom,** At maturity (adults), allelic variation within the coding region of the gene gives rise to two distinct phenotypes: high activity in 92% of the population ("extensive metabolizers"; *red circles*) and low activity in 8% of the population ("poor metabolizers"; *yellow circles*). However, there is also interindividual variability in the rate at which functional activity is acquired after birth. For example, the two phenotypes may not be readily distinguishable in newborn infants. Furthermore, there may be discrete periods during childhood in which the genotype-phenotype relationship may differ from that observed in adults (e.g., developmental stages at which enzyme activity appears to be greater in children than in adults). (*Adapted from Leeder JS. Translating pharmacogenetics and pharmacogenomics into drug development for clinical pediatric and beyond. Drug Discov Today. 2004;9:567–573.*)

considers the likelihood that a particular finding is isolated, associated with a syndrome, or nonsyndromic (e.g., isolated cleft lip and palate). When a specific diagnosis cannot be made (as in many cases of multiple congenital anomalies), the various possibilities in the differential diagnosis should be discussed with the family and empirical information provided. If available, specific diagnostic tests should be discussed. Often, empirical recurrence risks can be given even without a specific laboratory-based diagnosis. At the same time, even negative laboratory testing can further modify this risk.

Natural History of the Condition

It is important to discuss the natural history of the specific genetic disorder in the family. Affected persons and their families have questions regarding the prognosis and potential management or therapy that can be answered only with knowledge of the natural history. If there are other possible diagnoses, their natural history may also be discussed. If the disorder is associated with a spectrum of clinical outcomes or complications, the range of possible outcomes and variability of the condition, as well as treatment and referral to the appropriate specialist, should be addressed.

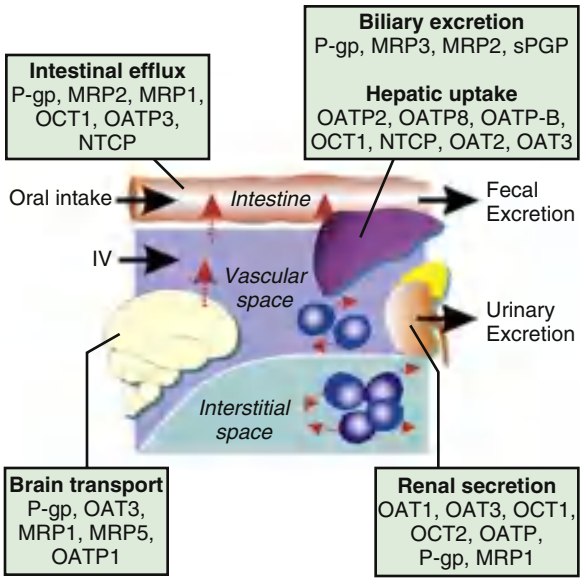


Fig. 98.6 Schematic diagram of important transport proteins and their known locations in humans. *Spheres* correspond to drug molecules. (*From American Pharmacists Association. Ritschel WA, Kearns GL, eds. Handbook of Basic Pharmacokinetics Including Clinical Applications, 7th ed. Washington, DC: American Pharmacists Association, 2009. p 45.*)

Genetic Aspects of the Condition and Recurrence Risk

The genetic aspects and risk of recurrence are important because all family members should be informed of their reproductive choices. The genetic basis of the disorder can be explained with visual aids (e.g., diagrams of chromosomes and inheritance patterns). It is important to provide accurate occurrence and recurrence risks for various members of the family, including unaffected individuals. If a definite diagnosis cannot be made, it is necessary to use empirical recurrence risks. Genetic counseling gives patients the necessary information to understand the various options and to make their own informed decisions regarding pregnancy, adoption, assistive reproductive technologies, prenatal diagnosis, screening, carrier detection, or termination of pregnancy. It may be necessary to have more than one counseling session. Even if a specific molecular diagnosis has been made and a well-defined inheritance pattern has been described for the condition, it is important to recognize that the principles of incomplete penetrance, variable expressivity, and germline/somatic mosaicism can contribute uncertainty to the recurrence risk and severity of a potentially affected pregnancy conception or child.

Prenatal Diagnosis and Prevention

Many different methods of prenatal screening and diagnosis are available, depending on the specific genetic disorder (see Chapter 117). The use of ultrasonography allows prenatal screening of anatomic abnormalities such as congenital heart defects. Amniocentesis and chorionic villus sampling are used to obtain fetal tissue for analysis of chromosomal abnormalities, biochemical disorders, and DNA studies. Maternal blood or serum sampling is used for some types of screening, including noninvasive prenatal screening by direct analysis of cell-free fetal DNA found in maternal blood, which is used for screening of conditions such as trisomy 21 and other aneuploidies. In addition, this source of cell-free fetal DNA has also been used clinically for DNA sequencing for selected dominant de novo conditions in the fetus that may occur with increased frequency with increasing paternal age. Current tests of fetal DNA from maternal blood should be considered screening tests, and invasive testing like amniocentesis or chorionic villus sampling should be considered for confirmatory diagnostic testing.

Table 98.4 Indications for Genetic Counseling

Advanced parental age
• Maternal age ≥35 years
• Paternal age ≥40 years
Previous child with or family history of:
• Congenital abnormality
• Dysmorphology
• Intellectual disability
• Isolated birth defect
• Metabolic disorder
• Chromosome abnormality
• Single-gene disorder
Adult-onset genetic disease (presymptomatic testing)
• Cancer
• Huntington disease
Pharmacogenomics
Consanguinity
Teratogen exposure (occupational, abuse)
Repeated pregnancy loss or infertility
Pregnancy screening abnormality
• Maternal serum α-fetoprotein
Maternal first-trimester screen
• Maternal triple or quad screen or variant of this test
• Fetal ultrasonography
Noninvasive prenatal testing (NIPT)
• Fetal karyotype
Heterozygote screening based on ethnic risk
• Sickle cell anemia
• Tay-Sachs, Canavan, and Gaucher diseases
• Thalassemias
Universal carrier screening panels
Follow-up to abnormal neonatal genetic testing
Prior to whole genome or exome sequencing
Prior to preimplantation genetic testing

Therapies and Referral

Some genetic disorders require the care of multiple specialists. Many genetic conditions have diagnosis and management guidelines to aid in the treatment and management of these complex patients. Prevention of known complications is a priority, so close follow-up with the necessary specialists involved in the child's care is essential to identify any potentially concerning issues early. The psychologic adjustment of the family might also require specific intervention. Some challenges may involve when to discuss the diagnosis of a chronic disease with the patient, siblings, and other family members or friends. The decision to do so should always involve the parents and an assessment of the maturity and capacity of the child or adolescent.

Alternative medicines or nontraditional therapies are often brought to attention by parents after exhaustive internet searches. Such treatments should not necessarily be dismissed since the physician and genetic counselor should serve as an important resource for helping parents navigate the maze of nonstandard treatments. Instead, the relative merits of treatments should be framed in the context of cost and benefit, scientific rationale, evidence from controlled and observational studies, the placebo effect, safety of the treatment, and the gaps in our own scientific knowledge base.

Support Groups

A large number of community and online lay disease–specific support groups have been formed to provide information and to fund research on specific genetic and nongenetic conditions. An important part of genetic counseling is to give information about these groups to patients and to suggest a contact person for the families. Many groups have established websites and social media platforms with very helpful information. With the rise of social media and its ability to connect families with rare syndromes from around the world, it is important to stress to families that their individual disease course will be unique and significant biases of reporting occur on such platforms. This should be balanced by the benefit of sharing potential important natural history elements of the underlying rare disease.

Follow-Up

Families should be encouraged to continue to ask questions and keep up with new information about the specific disorder. New developments often influence the diagnosis and therapy of specific genetic disorders.

Nondirective Counseling

Genetic counseling is usually nondirective; choices about reproduction are left to the family to decide what is right for them. The role of the counselor (physician, genetic counselor, nurse, medical geneticist) is to provide information in understandable terms and outline the range of options available.

98.2 Principles of Management and Treatment of Genetic Disorders

Brendan Lee and Nicola Brunetti-Pierri

TREATMENT OF GENETIC DISEASES

Genetic conditions are often chronic disorders. Some are amenable to curative therapies, although there has been a rapid increase in the number of treatable disorders. Based on ongoing preclinical and clinical investigations, new therapies for a growing number of diseases are expected to become available in the near future. Surgical management is available for many conditions that are associated with congenital anomalies or predisposition to tumors. All patients and families should be provided information about the disorder, genetic counseling, anticipatory guidance, and appropriate medical surveillance.

Resources for patients include the National Organization of Rare Disorders (www.rarediseases.org), the Genetic Alliance (www.geneticalliance.org), the National Library of Medicine (www.nlm.nih.gov/medlineplus/geneticdisorders.html), and a large number of disease-specific websites. A current listing of federally and privately funded clinical trials, including many for genetic diseases, is available at www.ClinicalTrials.gov.

Specific medical therapies for genetic disorders can be classified into **physiologic** and **replacement** therapies. Another approach to *correct protein misfolding* induced by missense pathogenic variants is through use of small molecules that specifically bind to mutant proteins, stabilizing their conformation, thereby preventing early degradation, and allowing proper cellular trafficking and localization. This strategy has found successful applications for therapy of cystic fibrosis caused by specific *CFTR* pathogenic variants, including the F508del (see Chapter 454).

PHYSIOLOGIC THERAPIES

Physiologic therapies attempt to ameliorate the phenotype of a genetic disorder by modifying the physiology of the affected individual. The underlying defect itself is not altered by treatment. Physiologic therapies are used in the treatment of **inborn errors of metabolism** (see Chapter 104). These include dietary manipulations, such as reducing phenylalanine intake by persons with phenylketonuria, coenzyme supplementation for some patients with methylmalonic acidemia and mitochondrial diseases, stimulation of alternative pathways to excrete ammonia for those with urea cycle disorders, phototherapy to increase excretion of neurotoxic unconjugated bilirubin in Crigler-Najjar syndrome, bisphosphonate treatment for those with osteogenesis imperfecta to reduce bone fractures, and avoiding cigarette smoking by persons with α_1-antitrypsin deficiency or specific foods and drugs by persons with glucose-6-phosphate dehydrogenase deficiency or acute intermittent porphyria. Physiologic treatments can be highly effective, but they usually need to be maintained for a lifetime because they do not affect the underlying genetic disorder. Many of these treatments are most effective early in life

before irreversible damage has occurred. This is the rationale for comprehensive newborn screening for inborn errors of metabolism.

Many physiologic therapies use small-molecule pharmaceuticals (e.g., to remove ammonia in those with urea cycle disorders). **Pharmacologic treatments** directly target a defective cellular pathway that is altered by an abnormal or a missing gene product. One approach is the inhibition of an enzyme reaction that is upstream of the deficient enzyme to prevent accumulation of the toxic metabolites, such as the nitisinone (NTBC) for therapy of tyrosinemia type I. A similar approach focuses on partially reducing the synthesis of the substrate of the abnormal enzyme or its precursors in lysosomal storage disorders (see Chapter 106.4). Other examples include targeting the FGFR3 signaling pathway by the C-type natriuretic peptide in achondroplasia, FGF23 by the monoclonal antibody burosumab in X-linked hypophosphatemia, or the angiopoietin-like protein 3 (ANGPTL3) by the monoclonal antibody evinacumab in homozygous familial hypercholesterolemia.

REPLACEMENT THERAPIES

Replacement therapies include replacement of a missing metabolite, an enzyme, an organ, or even a specific gene.

Enzyme Replacement

Enzyme replacement therapy is a component of the treatment of cystic fibrosis to manage intestinal malabsorption. Pancreatic enzymes are easily administered orally, because they must be delivered to the gastrointestinal tract. Recombinant alkaline phosphatase coupled to a bone-targeting motif is available for intravenous therapy of hypophosphatasia, a skeletal disorder caused by alkaline-phosphatase deficiency.

Enzyme replacement strategies are effective for several lysosomal storage disorders. Enzymes are targeted for the lysosome by modification with mannose-6-phosphate, which binds to a specific receptor. This receptor is also present on the cell surface, so lysosomal enzymes with exposed mannose-6-phosphate residues can be infused into the blood and are taken into cells and delivered to lysosomes. Enzyme replacement therapies are available for Gaucher disease and Fabry disease, most of the mucopolysaccharidoses (MPS I, II, IVA, VI, VII), acid lipase deficiency, α-mannosidosis, neuronal ceroid lipofuscinosis late infantile (CLN2), and Pompe disease, and are being tested for MPS IIIA and IIIB, metachromatic leukodystrophy, and Niemann-Pick disease type B. Other examples include enzyme replacement therapy with pegylated recombinant phenylalanine ammonia lyase for phenylketonuria that is effective at reducing blood phenylalanine concentrations in most patients, such that most of them can come off a phenylalanine-restricted diet.

One complication of enzyme replacement therapy is antibody response to the infused recombinant enzyme. The magnitude of this response is not always predictable and varies depending on the enzyme preparation and the disease. In most cases, the patient's antibody response does not affect the treatment's efficacy (e.g., Gaucher disease), but in other situations it may be a significant hurdle (e.g., Pompe disease and phenylketonuria).

Transplantation

Cell transplantation and organ transplantation are potentially effective approaches to replacement of a defective gene. Aside from transplantation to replace damaged tissues, transplantation of stem cells, liver, or bone marrow is also used for several diseases, mainly inborn errors of metabolism, and hematologic or immunologic disorders. A successful transplant can be essentially curative, although there may be significant risks and side effects (see Chapters 177-181). Cell and tissue transplantation is effective in many clinical scenarios, but there is always short-term morbidity, often associated with either surgical (liver) or preparative (bone marrow) regimens, and long-term morbidity related to chronic immunosuppression and graft failure. Bone marrow transplantation is the best example of stem cell therapy, but much effort also is focused on identifying, characterizing, expanding, and using other tissue stem cells for regenerative therapies. In contrast to transplantation from a healthy donor, the infused cells are the patient's own cells in ex vivo gene therapy of hematopoietic stem cells. Therefore, although it requires preparative myeloablation akin to regular bone marrow transplantation from healthy donors, engraftment of genetically modified hematopoietic stem cells is devoid of risks of rejection or graft-versus-host disease. Increasingly, this approach is combined with gene therapy after genetic correction of autologous stem cells.

Gene Therapy

Another approach focuses on replacing or correcting a defective gene **(gene therapy)**. In theory, if one can target the specific tissue that has a deficiency in the gene or gene product, this can offer a less invasive means of achieving a cure for a genetic disorder compared to transplantations. Ultimately, gene therapy depends on the unique interaction of the disease pathophysiology, which is specific to the patient, and the gene delivery vehicle.

Gene-transfer vehicles include viral and nonviral vectors administered through ex vivo or in vivo approaches. In ex vivo approaches the patient's cells are removed and after gene correction or replacement are infused into the patient. An example of this is the US Food and Drug Administration (FDA)-approved chimeric antigen receptor (CAR) T-cell therapy for lymphomas and leukemias. In the in vivo approaches the gene therapy vector is directly injected into the body by either systemic (e.g., intravenous) or localized (e.g., intracerebral, intraocular) injections. Most human clinical trials have used viral vectors because of their efficiency of gene delivery to tissues. In some diseases, such as X-linked and adenosine deaminase–deficient severe combined immunodeficiency, chronic granulomatous disease, and Wiskott-Aldrich syndrome, clinical gene therapy is a viable and effective option. Ex vivo gene transfer of hematopoietic stem cells can be considered at least as effective to allogenic hematopoietic stem cell transplantation in presymptomatic patients with X-linked adrenoleukodystrophy, metachromatic leukodystrophy and Hurler syndrome.

In vivo gene therapy is also promising for Leber congenital amaurosis by intraocular delivery, and hemophilias and several inborn errors of liver metabolism by systemic intravenous injection. In vivo gene therapy is FDA approved for treatment of a specific RPE65-deficient form of retinitis pigmentosa using adeno-associated virus (AAV)-mediated expression of the normal RPE65 gene via subretinal injections. In vivo AAV-mediated gene therapy is also an approved treatment that has significantly changed the early course of the disease and improved motor function in children with spinal muscular atrophy (SMA) (see Chapter 652.2). Although patients with SMA typically present with muscle atrophy, respiratory failure, and die before 2 years of age, treated patients have remained healthy and attained motor milestones that are typically not achieved in any of the untreated patients. Similar efficacy has also been achieved by intrathecal delivery of antisense oligonucleotides (ASOs) correcting *SMN2* splicing. Brain-directed gene therapy is available for aromatic L-amino acid decarboxylase (AADC) deficiency by intracerebral injections of an AAV-based vector. Systemic intravenous AAV-based gene therapy vectors have been approved for both hemophilia A and B.

Gene editing with *direct correction* of a disease-causing pathogenic variant is another possible approach to genetic therapy. Various nucleases with specific DNA-recognition sequences are available for genome editing; they include zinc finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated nine (Cas9). They all permit permanent gene modification of genes in cells. This is achieved by DNA site-specific double-strand breaks (DSBs) induced by the endonuclease and a template encompassing the wild-type sequence to be used as a substrate for repair by **homology-directed repair (HDR)**. Following site-specific DSB, DNA repair is mediated by either nonhomologous end joining (NHEJ) or HDR that repairs DNA in the presence of a donor sequence (Fig. 98.7A and B). NHEJ repairs the DSB by joining the two ends of the DSB, often introducing small insertions or deletions (indels) at the DSB site that generally inactivate gene function (see Fig. 98.7A). Compared with NHEJ, HDR is less efficient and requires a donor DNA template (see Fig. 98.7C). The wild-type copy of the mutated gene can be integrated into the endogenous locus or into "safe harbors" that allow high expression levels of the therapeutic gene (see Fig. 98.7A). Based on

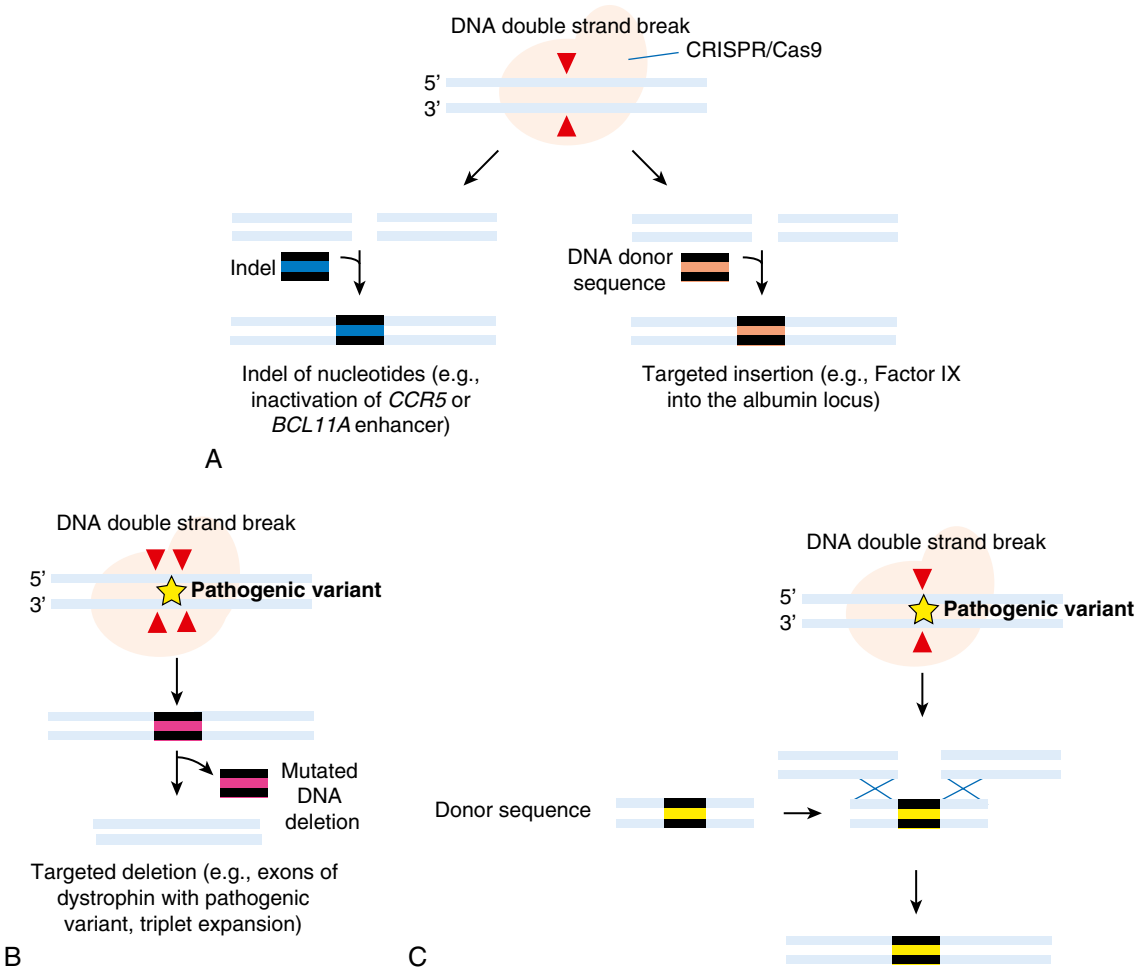


Fig. 98.7 A, Double-strand breaks can be repaired by nonhomologous end-joining (NHEJ) repair mechanisms, giving rise to small insertions and deletions (Indel) that can disrupt gene expression *(blue)*. B, Two double-strand breaks can also be corrected by NHEJ and can eliminate the DNA carrying the pathogenic variant *(pink)* located between the two breaks. C, Double-strand breaks can be repaired by homology-directed repair mechanisms in the presence of a donor template allowing the incorporation of a donor sequence *(yellow)* that correct the mutated gene. CRISPR/Cas9, Clustered regularly interspaced short palindromic repeat/CRISPR-associated nine (Cas9).

these features, genome editing has the potential to overcome several limitations of gene replacement therapy. First, genotoxicity due to ectopic activation of nearby protooncogenes or knockout of tumor suppressor genes does not occur with on-target editing. Second, genome editing allows physiologic regulation of the expression of the corrected gene in contrast to gene replacement therapy. Third, gene editing is maintained in proliferating cells, and it overcomes the dilution effect due to cell division that is observed with gene replacement therapy by nonintegrating vectors such as AAV vectors.

Genome editing tools have corrected the gene defect in several preclinical murine models and are in clinical trials. ZFNs have been used ex vivo to disrupt CCR5 expression in human T cells to induce resistance to HIV infection. ZFNs and CRISPR/Cas9 are being used to boost fetal hemoglobin in β-thalassemia and sickle cell disease by disrupting the enhancer of the *BCL11A* gene, which suppresses fetal hemoglobin production. Moreover, ZFN-mediated targeted introduction of therapeutic genes downstream of the highly active albumin promoter in hepatocytes is currently under clinical investigation for several diseases.

RNA-Targeted Therapy

Antisense oligonucleotides (ASOs) are short synthetic nucleic acids that hybridize with cellular RNA using classic base pairing to modulate gene expression. To ensure specificity, their sequences are complementary to their target sequences (Fig. 98.8). Through binding to pre-mRNA or mRNA, ASOs can posttranscriptionally regulate protein synthesis by mechanisms including modification of pre-mRNA processing and splicing, competitive inhibition, steric blockade of translational machinery, and degradation of the target RNA. Clinical ASOs have been developed for treatment of Duchenne muscular dystrophy and SMA, respectively (Table 98.5). In SMA, *SMN1* and *SMN2* genes are identical (99% homology), except for an 11-nucleotide sequence in exon seven that alters splicing (see Chapter 652.2). Splicing of the *SMN2* pre-mRNA leads to the exclusion of exon seven, generating a truncated, unstable protein that is rapidly degraded. Therefore *SMN2* cannot compensate for *SMN1* loss in SMA. The ASO designed to correct *SMN2* splicing by promoting the inclusion of exon seven allows SMN2 to compensate for the loss of expression caused by the *SMN1* pathogenic variant. ASOs are also effective for treatment of homozygous familial hypercholesterolemia and have been also designed to inhibit HTT mRNA to reduce concentrations of mutant huntingtin in Huntington disease.

Conjugation of siRNA with GalNAc to target the asialoglycoprotein receptor is an efficient strategy to facilitate cell uptake and to increase siRNA liver accumulation (see Fig. 98.8). A GalNAc-siRNA targeting the mRNA for aminolevulinate synthase 1 (ALAS1), the first enzyme in the heme synthesis pathway, has been developed for treatment of acute hepatic porphyria, and a similar strategy targeting glycolate oxidase is effective at reducing oxalate levels for treatment of primary hyperoxaluria type 1.

Lipid nanoparticles (LNPs) are spherical structures with a composition very similar to cell membranes and are suitable carriers for

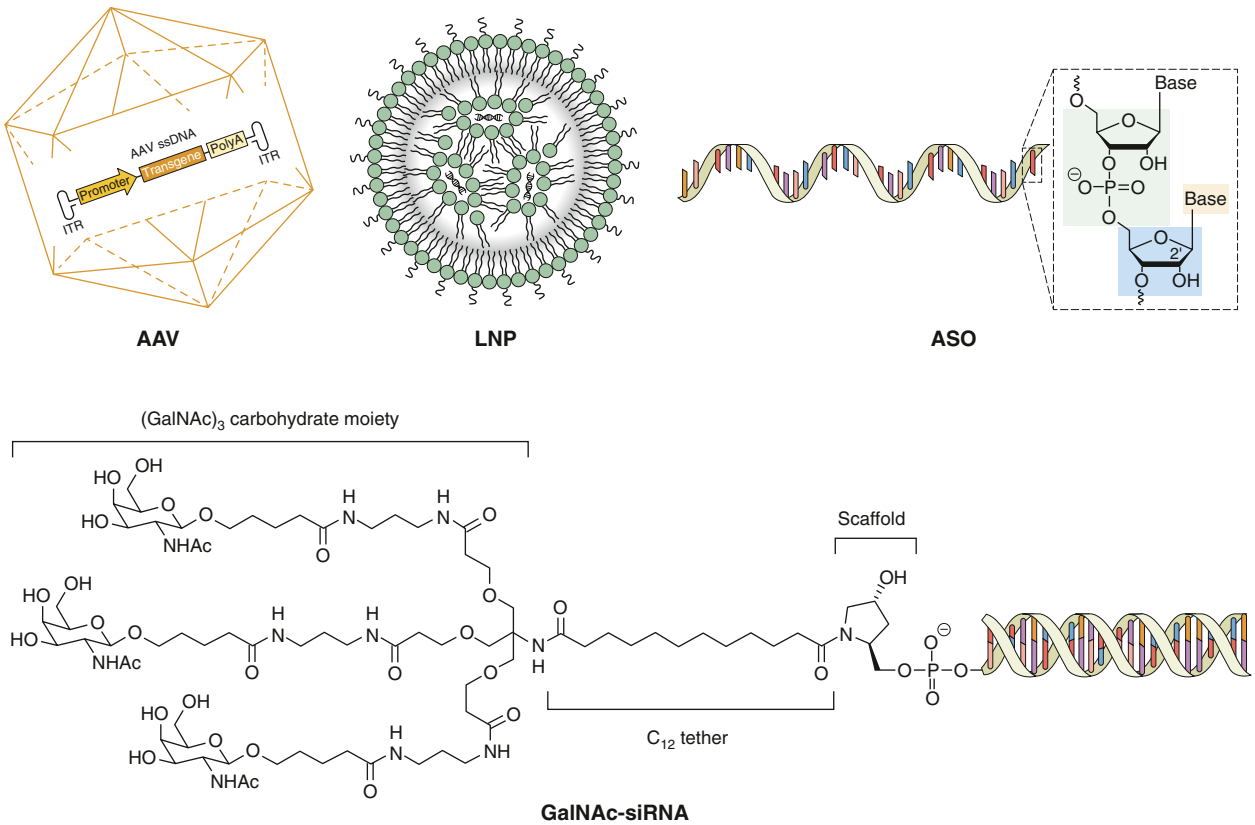


Fig. 98.8 Approved delivery technologies for in vivo gene therapy. Four platform technologies have been clinically approved for gene-based therapies of genetic diseases: adeno-associated virus (AAV) vector containing a 4.7-kb single-stranded DNA with inverted terminal repeats (ITRs), antisense oligonucleotides (ASO) therapeutics, lipid nanoparticle (LNP) containing siRNA or mRNA including key lipid components, and N-acetyl-galactosamine-short-interfering RNA (GALNAc-siRNA) therapeutics made of a trivalent ligand with terminal GalNAc moieties covalently linked to siRNA at the 3′-end of the sense strand.

Table 98.5 Approved In Vivo Gene-Based (DNA and RNA) Therapies for Genetic Disorders

PRODUCT	GENE TARGET	DISEASE	ROUTE OF ADMINISTRATION
ASO			
Eteplirsen	Dystrophin (exon 51)	Duchenne muscular dystrophy	Intrathecal
Golodirsen	Dystrophin (exon 53)	Duchenne muscular dystrophy	Subcutaneous
Casimersen	Dystrophin (exon 45)	Duchenne muscular dystrophy	Subcutaneous
Inotersen	Transthyretin (TTR)	TTR-mediated amyloidosis	Subcutaneous
Nusinersen	Survival of motor neuron two (SMN2)	Spinal muscular atrophy	Intrathecal
Mipomersen	Apolipoprotein B-100	Hypercholesterolemia	Subcutaneous
Volanesorsen	Apolipoprotein CIII	Familial chylomicronemia	Subcutaneous
GALNAc-sIRNA CONJUGATES			
Givosiran	ALAS1	Acute hepatic porphyrias	Subcutaneous
Inclisiran	PCSK9	Hypercholesterolemia	Subcutaneous
Lumasiran	Glycolate oxidase	Primary hyperoxaluria type 1	Subcutaneous
LNP-RNA			
Patisiran	TTR siRNA	TTR-mediated amyloidosis	Intravenous
AAV VECTORS			
Voretigene neparvovec-rzyl	RPE65 (AAV2)	Leber congenital amaurosis	Subretinal
Onasemnogene abeparvovec	SMN1 (AAV9)	Spinal muscular atrophy	Intravenous
Eladocagene exuparvovec	DDC (AAV2)	AADC deficiency	Bilateral intraputaminal infusions
Valoctocogene roxaparvovec	Factor VIII (AAV5)	Hemophilia A	Intravenous
Etranacogene dezaparvovec	Factor IX (AAV5)	Hemophilia B	Intravenous

AAV, Adeno-associated virus; AADC, aromatic L-amino acid decarboxylase; ASO, antisense oligonucleotides; LNP, lipid nanoparticle.

nucleic acid delivery, such as siRNA and mRNAs (see Fig. 98.8). A liver targeting LNP carrying an siRNA targeting transthyretin has been effective for treatment of hereditary transthyretin amyloidosis that has been recently approved. In various preclinical models, LNPs have been shown to deliver mRNA molecules to hepatocytes with high efficiency. LNPs are being used for the delivery of gene editing molecules such as ZFNs and Cas9 mRNA together with a single guide (sg)RNA. In addition, LNPs have been used for delivery of genome editing tools such as ZFNs and CRISPR/Cas9 together with a viral vector carrying a promoterless DNA sequence capable of homologous recombination that can result in high levels of the integrated sequence and short-term expression of the endonucleases.

Correction of Genetic Diseases in the Human Germline

Prevention of genetic diseases has been accomplished by **preimplantation genetic diagnosis (PGD)**. This procedure requires in vitro fertilization and single–embryo cell genetic testing of the known families' pathogenic variant and is performed with polymerase chain reaction (PCR) amplification of the affected gene. To avoid disease recurrence, only the unaffected embryos are implanted. With the advent of efficient genome editing by CRISPR/Cas9, editing human embryos is technically possible. The announcement of the birth of "CRISPR babies" has led to calls for a moratorium on human germline genome editing, and currently, germline and/or embryonic gene editing studies in humans have not been approved. Given the limited understanding of the consequences of CRISPR/Cas9-mediated DSBs on the germline human genome, it is unlikely that these approaches will become available any time soon.

In contrast, **mitochondrial replacement therapies** to avoid mitochondrial DNA pathogenic variants are available. In one technique, the pathogenic variant carrier mother's nuclear DNA is removed from the unfertilized oocyte and transferred to an unaffected mitochondrial donor oocyte (minus that cell's nuclear DNA). In another approach, the pronucleus from the pathogenic variant-carrier mother's fertilized oocyte is transferred to the unaffected mitochondrial donor's fertilized oocyte (minus the pronucleus).

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

Table 99.1 Incidence of Chromosomal Abnormalities in Newborn Surveys

TYPE OF ABNORMALITY	NUMBER	APPROXIMATE INCIDENCE
SEX CHROMOSOME ANEUPLOIDY		
Males *(43,612 newborns)*		
47,XXY	45	1/1,000*
47,XYY	45	1/1,000
Other X or Y aneuploidy	32	1/1,350
Total	**122**	**1/360 male births**
Females *(24,547 newborns)*		
45,X	6	1/4,000
47,XXX	27	1/900
Other X aneuploidy	9	1/2,700
Total	**42**	**1/580 female births**
AUTOSOMAL ANEUPLOIDY (68,159 NEWBORNS)		
Trisomy 21	82	1/830
Trisomy 18	9	1/7,500
Trisomy 13	3	1/22,700
Other aneuploidy	2	1/34,000
Total	**96**	**1/700 live births**
STRUCTURAL ABNORMALITIES (68,159 NEWBORNS)		
Balanced Rearrangements		
Robertsonian	62	1/1,100
Other	77	1/885
Unbalanced Rearrangements		
Robertsonian	5	1/13,600
Other	38	1/1,800
Total	**182**	**1/375 live births**
All chromosome abnormalities	**442**	**1/154 live births**

*Recent studies show the prevalence is currently 1:580 (Morris JK, Alberman E, Scott C, Jacobs P. Is the prevalence of Klinefelter syndrome increasing? *Eur J Hum Genet.* 2008;16(2):163–170.)

Data from Hsu LYF. Prenatal diagnosis of chromosomal abnormalities through amniocentesis. In: Milunsky A, ed, *Genetic Disorders and the Fetus*, 4th ed. Baltimore: Johns Hopkins University Press; 1998: pp. 179–248.

Chapter 99

Chromosome Disorders

Carlos A. Bacino and Brendan Lee

99.1 Methods of Chromosome Analysis

Carlos A. Bacino and Brendan Lee

Clinical cytogenetics is the study of chromosomes, including their structure, function, inheritance, and abnormalities. Chromosome abnormalities are very common and occur in approximately 1–2% of live births, 5% of stillbirths, and 50% of early fetal losses in the first trimester of pregnancy (Table 99.1). Chromosome abnormalities are more common among individuals with intellectual disability and play a significant role in the development of some neoplasias.

Chromosome analyses are indicated in persons presenting with multiple congenital anomalies, dysmorphic features, and/or intellectual disability. The specific indications for studies include prenatal testing in conceptuses of women with advanced maternal age (>35 years), multiple abnormalities on fetal ultrasound, multiple congenital anomalies, unexplained growth restriction in the fetus, postnatal problems in growth and development, ambiguous genitalia, unexplained intellectual disability with or without associated anatomic abnormalities, primary amenorrhea or infertility, recurrent miscarriages (≥3) or prior history of stillbirths and neonatal deaths, a first-degree relative with a known or suspected structural chromosome abnormality, clinical findings consistent with a known anomaly, some malignancies, and chromosome breakage syndromes (e.g., Bloom syndrome, Fanconi anemia).

Cytogenetic studies are usually performed on peripheral blood lymphocytes, although cultured fibroblasts obtained from a skin biopsy may also be used. Prenatal (fetal) chromosome studies are performed with cells obtained from the amniotic fluid (amniocytes), chorionic villus tissue, and fetal blood or, in the case of preimplantation diagnosis, by analysis of a *blastomere* (cleavage stage) biopsy, polar body biopsy, or blastocyst biopsy. Cytogenetic studies of bone marrow have an important role in tumor surveillance, particularly among patients with leukemia. These are useful to determine induction of remission and success of therapy, or in some cases the occurrence of relapses.

Chromosome anomalies include abnormalities of number and structure and are the result of errors during cell division. There are two types of cell division: mitosis, which occurs in most somatic cells, and meiosis, which is limited to the germ cells. In **mitosis,** two genetically identical daughter cells are produced from a single parent cell. DNA replication (duplication of DNA material) has already occurred during **interphase** in the S phase of the cell cycle (DNA synthesis). Therefore, at the beginning of mitosis, the chromosomes consist of two double DNA strands joined together at the centromere, known as *sister chromatids*. Mitosis can be divided into four stages: prophase, metaphase, anaphase, and telophase. **Prophase** is characterized by condensation of the DNA. Also during prophase, the nuclear membrane and the nucleolus disappear and the mitotic spindle forms. In **metaphase** the chromosomes are maximally compacted and are clearly visible as distinct structures. The chromosomes align at the center of the cell, and spindle fibers connect to the centromere of each chromosome and extend to centrioles at the two poles of the mitotic figure. In **anaphase** the chromosomes divide along their longitudinal axes to form two separate daughter chromatids, which then migrate to opposite poles of the cell. **Telophase** is characterized by formation of two new nuclear membranes and nucleoli, duplication of the centrioles, and cytoplasmic cleavage to form the two daughter cells.

Meiosis begins in the female oocyte during fetal life and is completed years to decades later. In males it begins in a spermatogonial cell sometime between adolescence and adult life and is completed in a few days. Meiosis is preceded by DNA replication so that at the outset, each of the 46 chromosomes consists of two chromatids. In meiosis, a **diploid cell** (2n = 46 chromosomes) divides to form four **haploid cells** (n = 23 chromosomes). Meiosis consists of two major rounds of cell division. In **meiosis I**, each of the homologous chromosomes pair precisely so that **genetic recombination**, involving exchange between two DNA strands **(crossing over)**, can occur. This results in reshuffling of the genetic information for the recombined chromosomes and allows further genetic diversity. Each daughter cell then receives one of each of the 23 homologous chromosomes. In oogenesis, one of the daughter cells receives most of the cytoplasm and becomes the egg, whereas the other smaller cell becomes the first polar body. **Meiosis II** is similar to a mitotic division but without a preceding round of DNA replication. Each of the 23 chromosomes divides longitudinally, and the homologous chromatids migrate to opposite poles of the cell. This produces four spermatogonia in males, or an egg cell and a second polar body in females, each with a haploid (n = 23) set of chromosomes. Consequently, meiosis fulfills two crucial roles: It enables genetic recombination, and it reduces the chromosome number from diploid (46) to haploid (23) so that on fertilization a diploid number is restored.

Two common errors of cell division may occur during meiosis or mitosis, and either can result in an abnormal number of chromosomes. The first error is **nondisjunction**, in which two chromosomes fail to separate during meiosis and thus migrate together into one of the new cells, producing one cell with two copies of the chromosome and another with no copy. The second error is **anaphase lag**, in which a chromatid or chromosome is lost during mitosis because it fails to move quickly enough during anaphase to become incorporated into one of the new daughter cells (Fig. 99.1).

For chromosome analysis, cells are cultured (for varying periods depending on cell type), with or without stimulation, and then artificially arrested in mitosis during metaphase (or prometaphase), later subjected to a hypotonic solution to allow disruption of the nuclear cell membrane and proper dispersion of the chromosomes for analysis, fixed, banded, and finally stained. The most commonly used banding and staining method is the **GTG banding** (G bands by trypsin using Giemsa), also known as **G banding**, which produces a unique combination of dark (G-positive) and light (G-negative) bands that permits recognition of all individual 23 chromosome pairs for analysis.

Metaphase chromosome spreads are first evaluated microscopically, and then their images are photographed or captured by a video camera and stored on a computer for later analysis. Humans have 46 chromosomes or 23 pairs, which are classified as *autosomes* for chromosomes

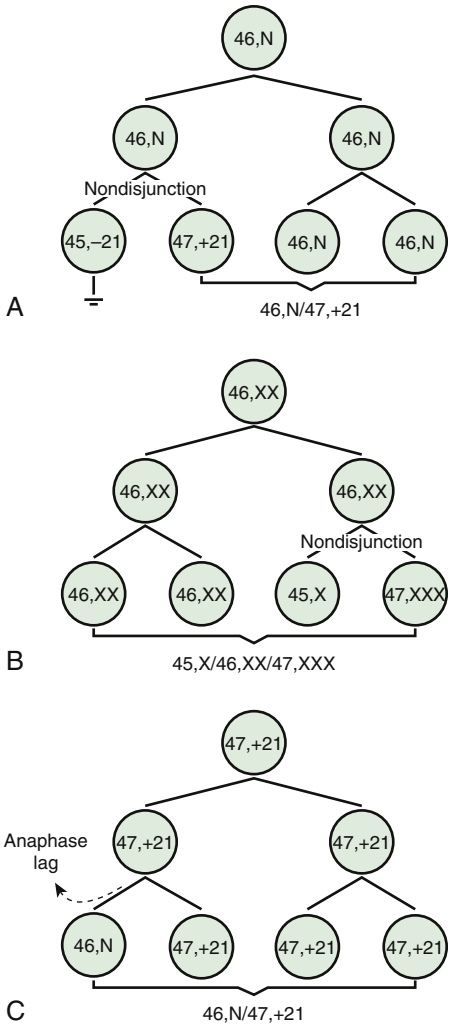


Fig. 99.1 Generation of mosaicism. **A,** Postzygotic nondisjunction in an initially normal conceptus. In this example, one cell line (monosomic 21) is subsequently lost, with the final karyotype 46,N/47,+21. **B,** Postzygotic nondisjunction in an initially 46,XX conceptus, resulting in 45,X/46,XX/47,XXX mosaicism. **C,** Postzygotic anaphase lag in an initially 47,+21 conceptus. *(From Gardner RJM, Sutherland GR. Chromosome Abnormalities and Genetic Counseling, 3rd ed. New York: Oxford University Press; 2003: Fig. 43.1, p. 33.)*

1-22, and the *sex chromosomes*, often referred as *sex complement*: XX for females and XY for males. The homologous chromosomes from a metaphase spread can then be paired and arranged systematically to assemble a karyotype according to well-defined standard conventions such as those established by International System for Human Cytogenetic Nomenclature (ISCN), with chromosome 1 being the largest and 22 the smallest. According to nomenclature, the description of the karyotype includes the total number of chromosomes followed by the sex chromosome constitution. A normal karyotype is 46,XX for females and 46,XY for males (Fig. 99.2). Abnormalities are noted after the sex chromosome complement.

Although the internationally accepted system for human chromosome classification relies largely on the length and banding pattern of each chromosome, the position of the centromere relative to the ends of the chromosome also is a useful distinguishing feature (Fig. 99.3). The centromere divides the chromosome in two, with the short arm designated the **p arm** and the long arm designated the **q arm**. A plus or minus sign before the number of a chromosome indicates that there is an extra or missing chromosome, respectively. Table 99.2 lists some of the abbreviations used for the descriptions of chromosomes and their abnormalities. A metaphase chromosome spread usually

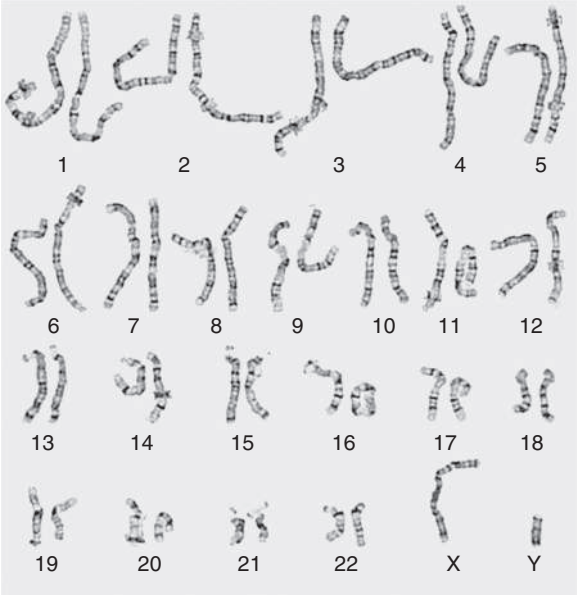


Fig. 99.2 Karyotype of a normal male at the 550-600 band level. The longer the chromosomes are captured at metaphase or sometimes prometaphase, the more bands can be visualized.

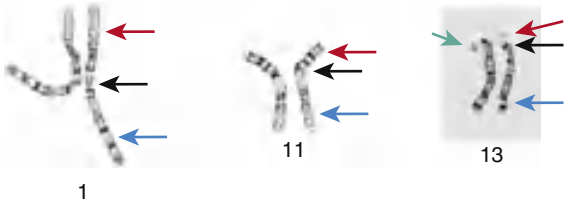


Fig. 99.3 Example of different chromosome types according to the position of the centromere. On the *left* is a chromosome one pair with the centromere equidistant from the short and long arm (also known as *metacentric*). In the *center* is a chromosome 11 pair that is submetacentric. On the *right* is a chromosome 13 pair that is an example of an acrocentric chromosome. Acrocentric chromosomes contain a very small short arm, stalks, and satellite DNA. The *black arrow* indicates the position of the centromere. The *blue arrow* shows the long arm of a chromosome. The *red arrow* shows the short arm of a chromosome. The *green arrow* highlights the satellite region, which is made of DNA repeats. The *light area* between the short arm and the satellite is known as the *stalk*.

shows 450-550 bands. Prophase and prometaphase chromosomes are longer, are less condensed, and often show 550-850 bands. High-resolution analysis may detect small chromosome abnormalities, although this has been mostly replaced by chromosome **microarray analysis (CMA)**, also called array comparative genomic hybridization (aCGH).

Molecular techniques (e.g., fluorescence in situ hybridization [FISH], CMA) identify subtle abnormalities that are often below the resolution of standard cytogenetic studies. **FISH** is used to identify the presence, absence, or rearrangement of *specific* DNA segments and is performed with gene- or region-specific DNA probes. Several FISH probes are used in the clinical setting: unique sequence or single-copy probes, repetitive-sequence probes (alpha satellites in the pericentromeric regions), and multiple-copy probes (chromosome specific or painting) (Fig. 99.4A,B). FISH involves using a unique, known DNA sequence or probe labeled with a fluorescent dye that is complementary to the studied region of disease interest. The labeled probe is exposed to the DNA on a microscope slide, typically metaphase or interphase chromosomal DNA. When the probe pairs with its complementary DNA sequence, it can then be visualized by fluorescence microscopy (Fig. 99.5). In metaphase chromosome spreads, the exact chromosomal location of each probe copy can be documented, and often the *number of copies* (deletions, duplications) of the DNA sequence as well. When the interrogated segments (as in genomic duplications) are close together, interphase cells can accurately determine the presence of two or more copies or signals, because in chromosome-condensed metaphase cells some duplications might falsely appear as a single signal.

Chromosome rearrangements <5 million bp (5 Mbp) cannot be detected by conventional cytogenetic techniques. FISH has facilitated the clinical characterization of several **microdeletion syndromes** (50-200 kb of DNA). Some FISH probes hybridize to repetitive sequences located in the pericentromeric regions. Pericentromeric probes are still used for rapid identification of certain trisomies in interphase cells of blood smears, or even in the rapid analysis of prenatal samples from cells obtained through amniocentesis. Such probes are available for chromosomes 13, 18, and 21 and for the sex pair X and Y (see Fig. 99.4C and D). FISH is *no longer* the first line of testing, and its role has also mostly changed to the confirmation of chromosome microarray findings. FISH is reserved for (1) confirmation studies of abnormalities detected by CMA, (2) rapid prenatal screening on interphase amniotic fluid cells, and (3) interphase blood smear for sex assignment of newborns who present with ambiguous genitalia.

Array comparative genomic hybridization (aCGH) is a type of **chromosomal microarray (CMA)** that uses a molecular-based technique that differentially labels the patient's DNA with a fluorescent dye (green fluorophore) and a normal reference DNA with a red fluorophore (Figs. 99.6 and 99.7). Oligonucleotides (short DNA segments) encompassing the entire genome are spotted onto a slide or **microarray** grid. Equal amounts of the two-labeled DNA samples are mixed, and the green:red fluorescence ratio is measured along each tested area. Regions of amplification of the patient's DNA display an excess of green fluorescence, and regions of loss show excess red fluorescence. If the patient's and the control DNA are equally represented, the green:red ratio is 1:1, and the tested regions are represented as yellow (see Chapter 96 and Fig. 96.5).

Another frequently used chromosome microarray type in the clinical setting is the **single nucleotide polymorphism (SNP) array**. SNPs are polymorphic variations between two nucleotides and, when analyzed in massive parallel fashion, they can provide valuable clinical information. Several million SNPs normally occur in the human genome. SNP arrays can help with the detection of **uniparental disomies** (i.e., genetic information derived from only one parent), as well as consanguinity in the family. Many arrays used in clinical practice combine the use of oligonucleotides for the detection of copy number variations (CNVs) in conjunction with SNPs.

The detection of abnormalities by CMA is possible at the single-exon resolution level, depending on the configuration of the array used. The many advantages of CMA include its ability to test for duplications or deletions in critical disease-causing regions of the genome at once, including single-gene and contiguous gene deletion syndromes. In addition, CMA does not require cell cultures to generate sufficient DNA, which may be important in the context of prenatal testing because of timing. However, CMA cannot detect balanced translocations or inversions and may not detect low levels of chromosomal mosaicism. **Targeted CMAs** can detect clinically known cryptic chromosomal aberrations associated with known disease phenotypes. CNV detection can also be diagnosed by next-generation sequencing in the context of whole *genome* sequencing.

There are large numbers of deletion and duplication CNVs in the human genome. Thus many detected genetic abnormalities, unless associated with well-known clinical phenotypes, may require *parental investigations* because a detected CNV that is inherited could be benign or an incidental polymorphic variant often described as variants of uncertain significance (VUS). A **de novo** abnormality (i.e., one found only in the child and not the parents) is often significant if it is associated with an abnormal phenotype found only in the child and if it involves genes with important functions.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

Table 99.2 Some Abbreviations Used for Description of Chromosomes and Their Abnormalities

ABBREVIATION	MEANING	EXAMPLE	CONDITION
XX	Female	46,XX	Normal female karyotype
XY	Male	46,XY	Normal male karyotype
[##]	Number [#] of cells	46,XY[12]/47,XXY[10]	Number of cells in each clone, typically inside brackets Mosaicism in Klinefelter syndrome with 12 normal cells and 10 cells with an extra X chromosome
cen	Centromere		
del	Deletion	46,XY,del(5p)	Male with deletion of chromosome 5 short arm
der	Derivative	46,XX,der(2),t(2p12;7q13)	Female with a structurally rearranged chromosome 2 that resulted from a translocation between chromosomes 2 (short arm) and 7 (long arm)
dup	Duplication	46,XY,dup(15)(q11-q13)	Male with interstitial duplication in the long arm of chromosome 15 in the Prader-Willi/Angelman syndrome region
ins	Insertion	46,XY,ins(3)(p13q21q26)	Male with an insertion within chromosome 3 A piece between q21 and q26 has reinserted on p13
inv	Inversion	46,XY,inv(2)(p21q31)	Male with pericentric inversion of chromosome 2 with breakpoints at bands p21 and q31
ish	Metaphase FISH	46,XX.ish del(7)(q11.23q11.23)	Female with deletion in the Williams syndrome region detected by in situ hybridization
nuc ish	Interphase FISH	nuc ish(DXZ1 × 3)	Interphase in situ hybridization showing three signals for the X chromosome centromeric region
mar	Marker	47,XY,+mar	Male with extra, unidentified chromosome material
mos	Mosaic	mos 45,X[14]/46,XX[16]	Turner syndrome mosaicism (analysis of 30 cells showed that 14 cells were 45,X and 16 cells were 46,XX)
p	Short arm	46,XY,del(5)(p12)	Male with a deletion on the short arm of chromosome 5, band p12 (short nomenclature)
q	Long arm	46,XY,del(5)(q14)	Male with a deletion on the long arm of chromosome 5, band 14
r	Ring chromosome	46,X,r(X)(p21q27)	Female with one normal X chromosome and a ring X chromosome
t	Translocation	t(2;8)(q33;q24.1)	Interchange of material between chromosomes 2 and 8 with breakpoints at bands 2q33 and 8q24.1
ter	Terminal	46,XY,del(5)(p12-pter)	Male with a deletion of chromosome 5 between p12 and the end of the short arm (long nomenclature)
/	Slash	45,X/46,XY	Separate lines or clones Mosaicism for monosomy X and a male cell line
+	Gain of	47,XX,+21	Female with trisomy 21
−	Loss of	45,XY,−21	Male with monosomy 21

FISH, Fluorescence in situ hybridization.

99.2 Abnormalities of Chromosome Number

Carlos A. Bacino and Brendan Lee

ANEUPLOIDY AND POLYPLOIDY

Typical human cells contain 46 chromosomes, which is a multiple of 23 chromosomes. Haploid cells contain 23 chromosomes (n=23, typically in the ovum or sperm). If a cell's chromosomes are an exact multiple of 23 (46, 69, 92 in humans), those cells are referred to as **euploid**. **Polyploid** cells are euploid cells with more than the normal **diploid** number of 46 (2n) chromosomes, such as 3n, 4n. Polyploid conceptions are usually not viable, but the presence of mosaicism with a karyotypically normal cell line can allow survival. **Mosaicism** is an abnormality defined as the presence of two or more different cell lines in a single individual. **Polyploidy** is a common abnormality seen in first-trimester pregnancy losses.

Triploid cells are those with three haploid sets of chromosomes (3n) and are only viable in a mosaic form. Nonmosaic triploid infants can be liveborn but typically die shortly after birth. **Triploidy** is often the result of fertilization of an egg by two sperm (dispermy). Failure of one of the meiotic divisions, resulting in a diploid egg or sperm, can also result in triploidy. The phenotype of a triploid conception depends on the origin of the extra chromosome set. If the extra set is of **paternal** origin, it results in a partial *hydatidiform mole* (excessive placental growth) with poor embryonic development, but triploid conceptions that have an extra set of **maternal** chromosomes result in severe embryonic restriction with a small, fibrotic placenta (insufficient placental development) that is typically spontaneously aborted.

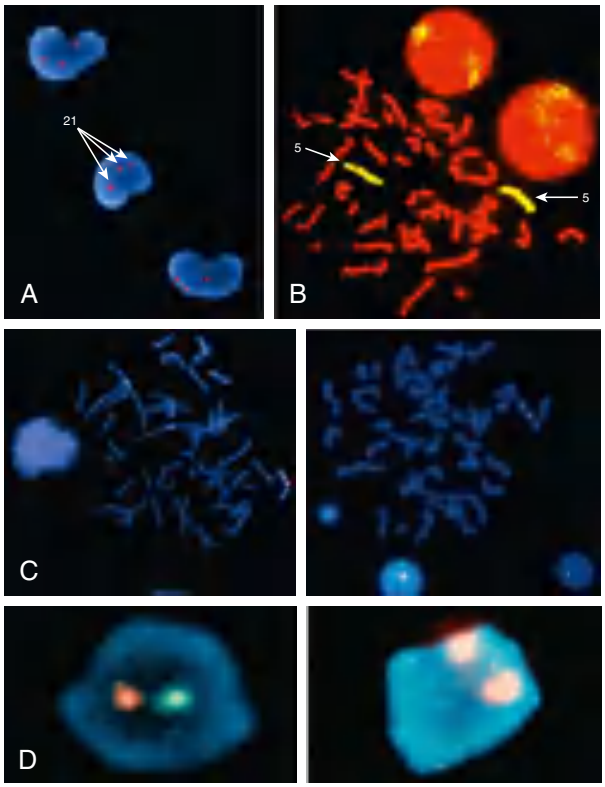


Fig. 99.4 A, Fluorescence in situ hybridization (FISH) analysis of interphase peripheral blood cells from a patient with Down syndrome using a chromosome 21–specific probe. The three *red signals* mark the presence of three chromosomes 21. B, FISH analysis of a metaphase chromosome spread from a clinically normal individual using a whole chromosome paint specific for chromosome 5. Both chromosome 5s are completely labeled *(yellow)* along their entire length. C, FISH on metaphase cells using a unique sequence probe that hybridizes to the elastin gene on chromosome 7q11.23, inside the Williams syndrome critical region. The elastin probe is labeled in *red*, and a control probe on chromosome 7 is labeled in *green*. The *left* image shows normal hybridization to chromosome 7, with two signals for the elastin region and two for the control probe. The *right* image shows a normal chromosome on the right with control and elastin signals and a deleted chromosome 7 on the left, evidenced by a single signal for the control probe. This image corresponds to a patient with a Williams syndrome region deletion. D, FISH in interphase cells using DNA probes that hybridize to repetitive α-satellite sequences in the pericentromeric region for the sex chromosomes. *Left,* interphase cells with two signals, one labeled in *red* for the X chromosome and *green* for the Y chromosome, consistent with a normal male chromosome complement. *Right,* interphase cell showing two *red* signals for the X chromosome, compatible with a normal female chromosome complement.

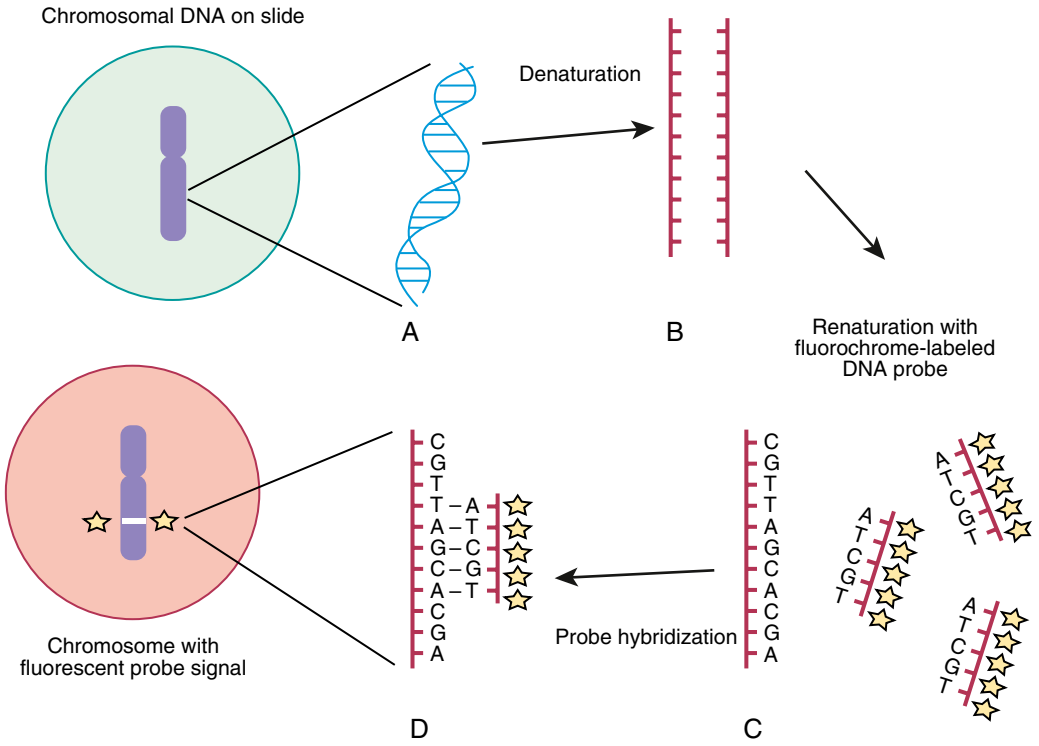


Fig. 99.5 FISH involves denaturation of double-stranded DNA as present in metaphase chromosomes or interphase nuclei on cytogenetic slide preparations (A) into single-stranded DNA (B). The slide-bound (in situ) DNA is then renatured or reannealed in the presence of excess copies of a single-stranded, fluorochrome-labeled DNA base-pair sequence or probe (C). The probe anneals or "hybridizes" to sites of complementary DNA sequence (D) within the chromosomal genome. Probe signal is visualized and imaged on the chromosome by fluorescent microscopy. *(From Lin RL, Cherry AM, Bangs CD, et al. FISHing for answers: the use of molecular cytogenetic techniques in adolescent medicine practice. In: Hyme HE, Greydanus D, eds, Genetic Disorders in Adolescents: State of the art reviews. Adolescent medicine. Philadelphia: Hanley and Belfus; 2002: pp. 305–313.)*

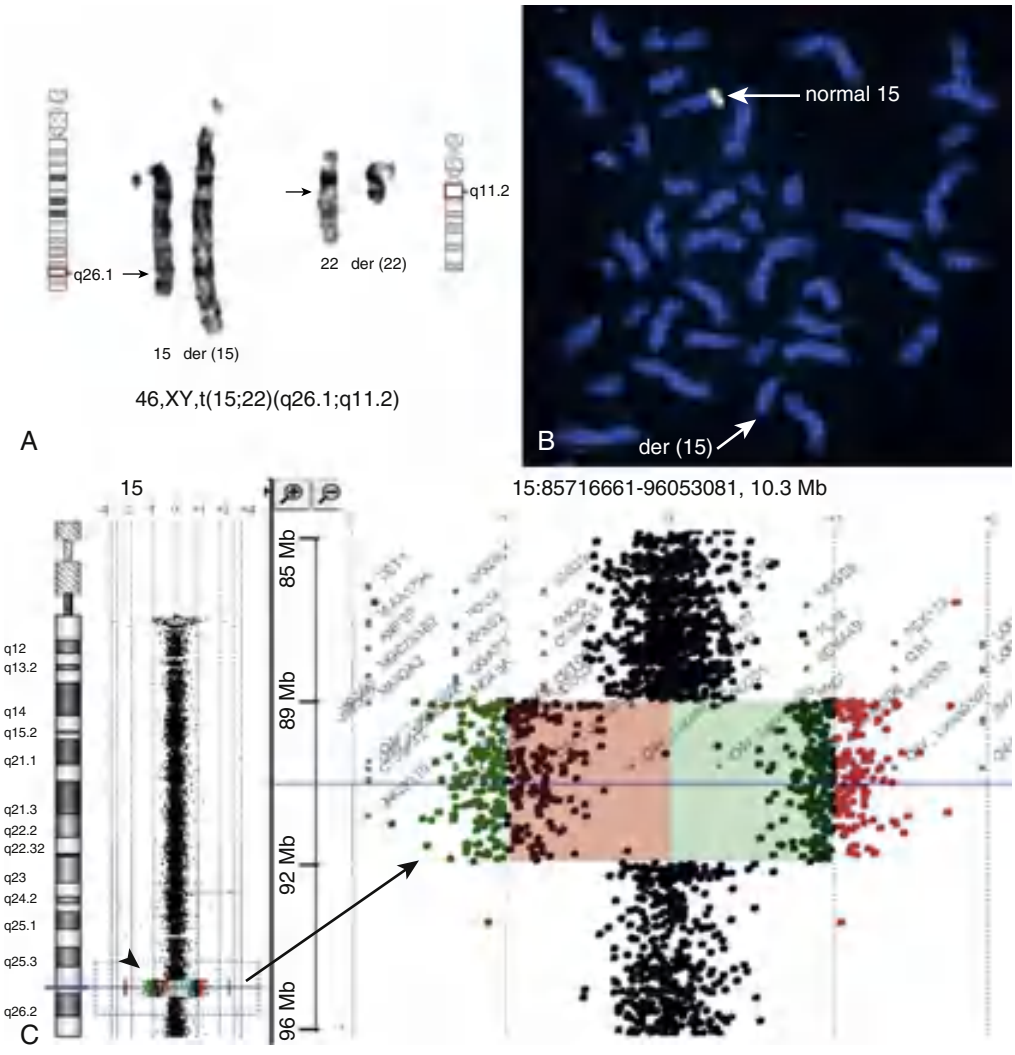


Fig. 99.6 Example of a cryptic microdeletion at a translocation breakpoint of an apparently balanced translocation in a patient with developmental delay (dd) and growth defect. **A,** Partial karyotype shows t(15;22)(q26.1;q11.2). **B,** Fluorescence in situ hybridization (FISH) with clones 2O19 *(top arrow)* and 354M14 *(bottom arrow)* at 15q26.1; *arrows* indicate signals only present on the normal chromosome 15, suggesting a deletion on the der(15). **C,** Two-color array comparative genomic hybridization (aCGH) with dye swap with 244 K oligo probes; *arrowhead* indicates a 3.3-Mbp deletion at chromosome 15q26.1-q26.2; *arrow* points to the close-up view of the deletion. *(From Li MM, Andersson HC. Clinical application of microarray-based molecular cytogenetics: an emerging new era of genomic medicine. J Pediatr. 2009;155:311–317, with permission of the authors and publisher.)*

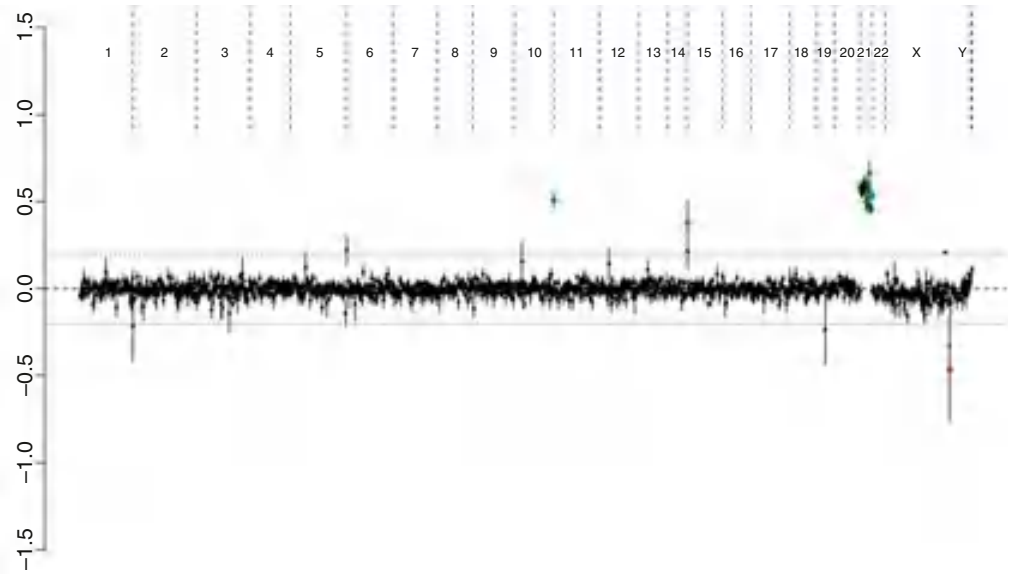


Fig. 99.7 Array comparative genomichybridization (aCGH) in a female patient with Down syndrome. Each *black dot* represents a piece of DNA segment specific for different chromosome location. Most of the dots displayed between the 0.0 and 0.2 axis are considered within normal range. Exceptions are often a result of polymorphic variations. A group of dots colored in *green* clusters on chromosome 21 and above 0.5. These represent a *gain* in copy number of DNA segments for chromosome 21, as seen in Down syndrome and consistent with trisomy 21.

Table 99.3 Chromosomal Trisomies and Their Clinical Findings*

SYNDROME	INCIDENCE	CLINICAL MANIFESTATIONS
Trisomy 13, Patau syndrome	1/10,000 births	Cleft lip often midline; flexed fingers with postaxial polydactyly; ocular hypotelorism, bulbous nose; low-set, malformed ears; microcephaly; cerebral malformation, especially holoprosencephaly; microphthalmia, cardiac malformations; scalp defects; hypoplastic or absent ribs; visceral and genital anomalies Early lethality in most cases, with a median survival of 12 days; ~80% die by 1 year; 10-year survival ~13%; survivors have significant neurodevelopmental delay
Trisomy 18, Edwards syndrome	1/6,000 births	Low birthweight, closed fists with index finger overlapping the third digit and the fifth digit overlapping the fourth, narrow hips with limited abduction, short sternum, rocker-bottom feet, microcephaly, prominent occiput, micrognathia, cardiac and renal malformations, intellectual disability ~88% of children die in the first year; 10-year survival ~10%; survivors have significant neurodevelopmental delay
Trisomy 8, mosaicism	1/20,000 births	Long face; high, prominent forehead; wide, upturned nose; thick, everted lower lip; microretrognathia; low-set ears; high-arched, sometimes cleft, palate; osteoarticular anomalies common (camptodactyly of second through fifth digits, small patella); deep plantar and palmar creases; moderate intellectual disability

*For trisomy 21, see Chapter 57.

Abnormal cells that do not contain a multiple of haploid number of chromosomes are termed **aneuploid** cells. **Aneuploidy** is the most common and clinically significant type of human chromosome abnormality, occurring in at least 3–4% of all clinically recognized pregnancies. **Monosomies** occur when only one, instead of the normal pair (two), of a given chromosome is present in an otherwise diploid cell. In humans, most autosomal monosomies appear to be lethal early in development, and survival is possible in **mosaic forms** or by means of chromosome rescue (restoration of the normal number by duplication of single monosomic chromosome, also known as monosomy rescue). An exception to this rule is monosomy for the X chromosome (45,X), seen in Turner syndrome; nonetheless, the majority of 45,X conceptuses are believed to be lost early in pregnancy for as yet unexplained reasons.

The most common cause of aneuploidy is **nondisjunction**, the failure of chromosomes to disjoin normally during meiosis (see Fig. 99.1). Nondisjunction can occur during meiosis I or II or during mitosis, although maternal meiosis I is the most common nondisjunction in aneuploidies (e.g., Down syndrome, trisomy 18). After meiotic nondisjunction, the resulting gamete either lacks a chromosome or has two copies instead of one normal copy, resulting in a monosomic or trisomic zygote, respectively.

Trisomy is characterized by the presence of three chromosomes, instead of the normal pair (two), of any particular chromosome. Trisomy is the most common form of aneuploidy. Trisomy can occur in all cells, or it may be mosaic. Most individuals with a trisomy exhibit a consistent and specific phenotype depending on the chromosome involved.

FISH is a technique that can be used for rapid diagnosis in the prenatal detection of common fetal aneuploidies, including chromosomes 13, 18, and 21, as well as sex chromosomes (see Fig. 99.4C and D). Direct detection of cell-free fetal DNA (trophoblastic or placental origin) in maternal plasma for fetal trisomy detection is a safe and highly effective screening test for fetal aneuploidy. The most common numerical abnormalities in liveborn children include trisomy 21 (Down syndrome) (see Chapter 57); trisomy 18 (Edwards syndrome); trisomy 13 (Patau syndrome); and sex chromosomal aneuploidies, such as Turner syndrome (usually 45,X), Klinefelter syndrome (47,XXY), 47,XXX, and 47,XYY. By far the most common type of trisomy in liveborn infants is trisomy 21 (47,XX,+21 or 47,XY,+21) (see Table 99.1). Trisomy 18 and trisomy 13 are relatively less common and are associated with a characteristic set of congenital anomalies and severe intellectual disability (Table 99.3). The occurrence of trisomy 21 and other trisomies increases with advanced maternal age (≥35 years). Because of this increased risk, women who are ≥35 years old at delivery should be offered genetic counseling and prenatal diagnosis (including serum screening, ultrasonography, and cell-free DNA detection, amniocentesis, or chorionic villus sampling; see Chapter 117).

99.3 Abnormalities of Chromosome Structure

Carlos A. Bacino and Brendan Lee

TRANSLOCATIONS

Translocations, which involve the transfer of material from one chromosome to another, occur with a frequency of 1 in 500 liveborn human infants. They may be inherited from a carrier parent or appear de novo, with no other affected family member. Translocations are usually reciprocal or Robertsonian, involving two chromosomes (Fig. 99.8).

Reciprocal translocations are the result of breaks in nonhomologous chromosomes, with reciprocal exchange of the broken segments. Carriers of a reciprocal translocation are usually phenotypically normal but are at an increased risk for miscarriage caused by transmission of unbalanced reciprocal translocations and for bearing chromosomally abnormal offspring. Unbalanced translocations are the result of abnormalities in the segregation or crossover of the translocation carrier chromosomes in the germ cells.

Robertsonian translocations involve two acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22) that fuse near the centromeric region with a subsequent loss of the short arms. Because the short arms of all five pairs of acrocentric chromosomes have multiple copies of genes encoding for ribosomal RNA, loss of the short arms of acrocentric chromosomes has no deleterious effect. The resulting karyotype has only 45 chromosomes, including the translocated chromosome, which consists of the long arms of the two fused chromosomes. Carriers of Robertsonian translocations are usually phenotypically normal. However, they are at increased risk for miscarriage and unbalanced translocations in phenotypically abnormal offspring.

In some rare instances, translocations can involve three or more chromosomes, as seen in complex rearrangements. Another less

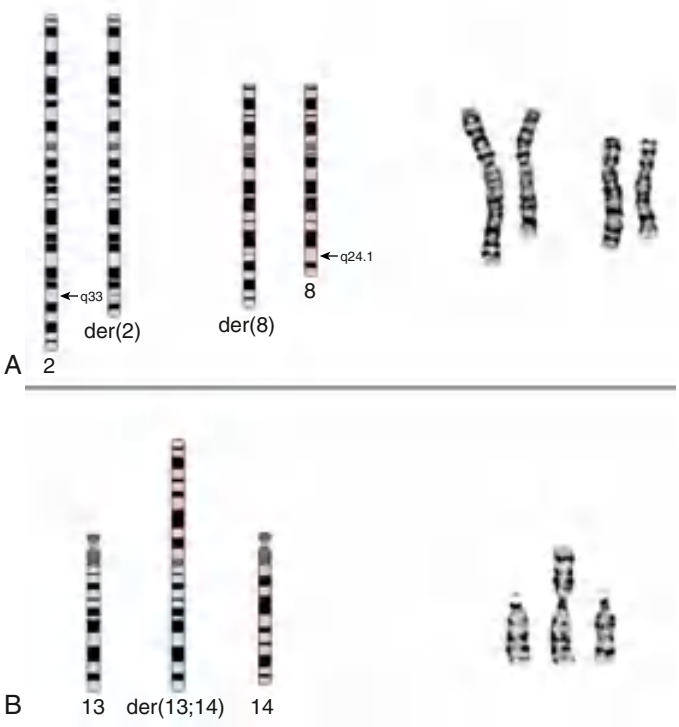


Fig. 99.8 A, Schematic diagram *(left)* and partial G-banded karyotype *(right)* of a reciprocal translocation between chromosome two *(blue)* and chromosome eight *(pink)*. The breakpoints are on the long *(q)* arm of both chromosomes at bands 2q33 and 8q24.1, with the reciprocal exchange of material between the derivative *(der)* chromosomes 2 and 8. This translocation is balanced, with no net gain or loss of material. The nomenclature for this exchange is t(2;8)(q33:q24.1). B, Schematic diagram *(left)* and partial G-banded karyotype *(right)* of a Robertsonian translocation between chromosomes 13 *(blue)* and 14 *(pink)*. The breakpoints are at the centromere (band q10) of both chromosomes, with fusion of the long arms into a single derivative chromosome and loss of the short *(p)* arm material. The nomenclature for this exchange is der(13;14)(q10;q10).

common type is the insertional translocation. **Insertional translocations** result from a piece of chromosome material that breaks away and later is reinserted within the same chromosome at a different site or inserted into another chromosome.

INVERSIONS

An inversion requires that a single chromosome breaks at two points; the broken piece is then inverted and joined into the same chromosome. Inversions occur in 1 in 100 live births. There are two types of inversions, pericentric and paracentric. In **pericentric inversions** the breaks are in the two opposite arms of the chromosome and include the centromere. They are usually discovered because they change the position of the centromere. The breaks in **paracentric inversions** occur in only one arm. Carriers of inversions are usually phenotypically normal, but they are at increased risk for miscarriages, typically in paracentric inversions, and chromosomally abnormal offspring in pericentric inversions.

DELETIONS AND DUPLICATIONS

Deletions involve loss of chromosome material and, depending on their location, can be classified as **terminal** (at the end of chromosomes) or **interstitial** (within the arm of a chromosome). They may be isolated or may occur along with a duplication of another chromosome segment. The latter typically occurs in unbalanced reciprocal chromosomal translocation secondary to abnormal crossover or segregation in a translocation or inversion carrier.

A carrier of a deletion is monosomic for the genetic information of the missing segment. Deletions are usually associated with intellectual disability and malformations. The most commonly observed deletions in routine chromosome preparations include 1p–, 4p–, 5p–, 9p–, 11p–, 13q–, 18p–, 18q–, and 21q– (Table 99.4 and Fig. 99.9), all distal or terminal deletions of the short or the long arms of chromosomes. Deletions may be observed in routine chromosome preparations, and deletions and translocations larger than 5-10 Mbp are usually visible microscopically.

High-resolution banding techniques, FISH, and molecular studies such as CMA can reveal deletions that are too small to be seen in ordinary or routine chromosome spreads (see Fig. 99.7). **Microdeletions** involve loss of small chromosome regions, the largest of which are detectable only with prophase chromosome studies and molecular methods. For submicroscopic deletions, the missing piece can only be detected using molecular methodologies such as DNA-based studies (e.g., CMA, FISH). The presence of extra genetic material from the same chromosome is referred to as **duplication**. Duplications can also

Table 99.4 Common Deletions and Their Clinical Manifestations

DELETION	CLINICAL ABNORMALITIES
4p–	Wolf-Hirschhorn syndrome. The main features are a typical "Greek helmet" facies secondary to ocular hypertelorism, prominent glabella, frontal bossing, microcephaly, dolichocephaly, hypoplasia of the orbits, ptosis, strabismus, nystagmus, bilateral epicanthic folds, cleft lip and palate, beaked nose with prominent bridge, hypospadias, cardiac malformations, and intellectual disability.
5p–	Cri du chat syndrome. The main features are hypotonia, short stature, characteristic shrill cry in the first few weeks of life (also called cat's cry syndrome), microcephaly with protruding metopic suture, hypertelorism, bilateral epicanthic folds, high arched palate, wide and flat nasal bridge, and intellectual disability.
9p–	The main features are craniofacial dysmorphic features with trigonocephaly, slanted palpebral fissures, discrete exophthalmos secondary to supraorbital hypoplasia, arched eyebrows, flat and wide nasal bridge, short neck with low hairline, genital anomalies, long fingers and toes with extra flexion creases, cardiac malformations, and intellectual disability.
13q–	The main features are low birthweight, failure to thrive, microcephaly, and severe intellectual disability. Facial features include high, wide nasal bridge; hypertelorism; ptosis; and micrognathia. Ocular malformations are common (retinoblastoma). The hands have hypoplastic or absent thumbs and syndactyly.
18p–	A few patients (15%) are severely affected and have cephalic and ocular malformations: holoprosencephaly, cleft lip and palate, ptosis, epicanthal folds, and varying degrees of intellectual disability. Most (80%) have only minor malformations and mild intellectual disability.
18q–	The main features are growth deficiency and hypotonia with a "froglike" position with the legs flexed, externally rotated, and in hyperabduction. The face is characteristic, with depressed midface and apparent protrusion of the mandible, deep-set eyes, short upper lip, and everted lower lip ("carplike" mouth); antihelix of the ears is very prominent. Varying degrees of intellectual disability and belligerent personality are present. Myelination abnormalities occur in the central nervous system.

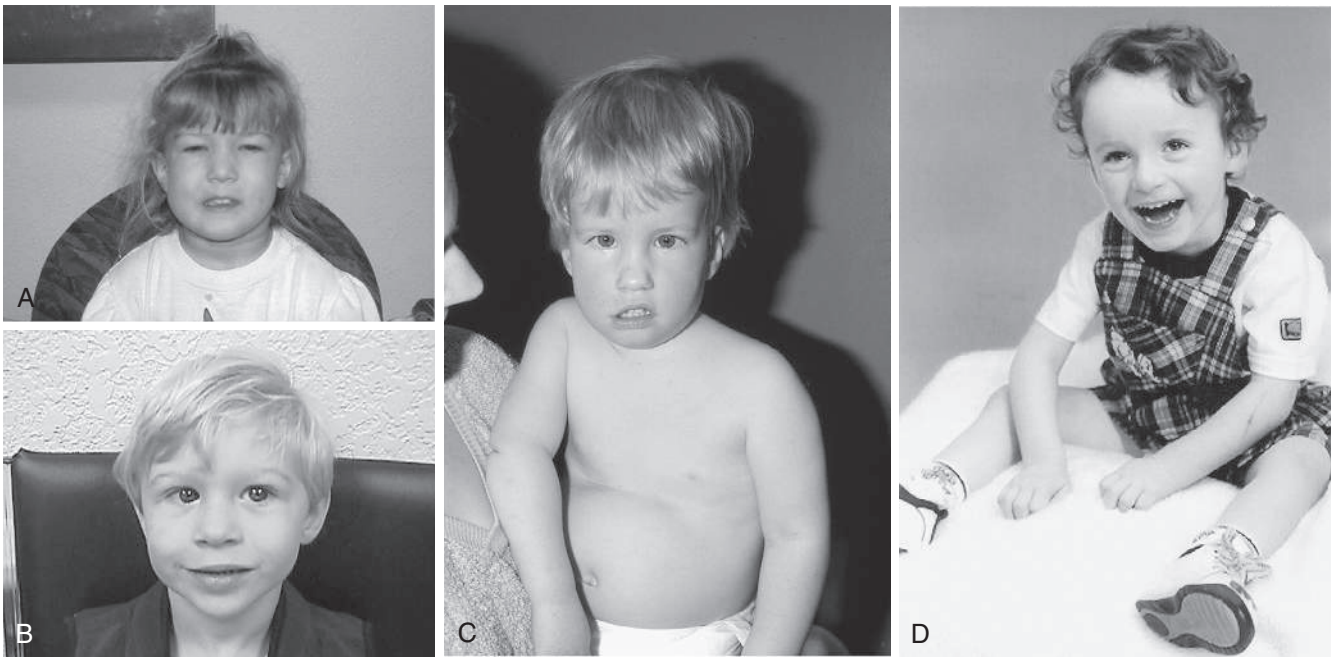


Fig. 99.9 **A,** Child with velocardiofacial syndrome (deletion 22q11.2). **B,** Child with Williams syndrome (deletion 7q11.23). **C,** Child with Prader-Willi syndrome (deletion 15q11-13). **D,** Child with Angelman syndrome (deletion 15q11-13). *(From Lin RL, Cherry AM, Bangs CD, et al. FISHing for answers: the use of molecular cytogenetic techniques in adolescent medicine practice. In: Hyme HE, Greydanus D, eds, Genetic Disorders in Adolescents: state of the art reviews. Adolescent medicine. Philadelphia: Hanley and Belfus; 2002: pp. 305–313.)*

Table 99.5 Microdeletion and Contiguous Gene Syndromes and Their Clinical Manifestations

DELETION	SYNDROME	CLINICAL MANIFESTATIONS
1p36	1p deletion	Growth restriction, dysmorphic features with midface hypoplasia, straight thin eyebrows, pointy chin, sensorineural hearing loss, progressive cardiomyopathy, hypothyroidism, seizures, intellectual disability
5q35	Sotos (50% are deletions of *NSD1* gene in Asians but only 6% in Whites)	Overgrowth, macrocephaly, prominent forehead, prominence of extraaxial fluid spaces on brain imaging, large hands and feet, hypotonia, clumsiness, mental disabilities
6p25	Axenfeld-Rieger	Axenfeld-Rieger malformation, hearing loss, congenital heart defects, dental anomalies, developmental delays, facial dysmorphism
7q11.23	Williams	Round face with full cheeks and lips, long philtrum, stellate pattern in iris, strabismus, supravalvular aortic stenosis and other cardiac malformations, varying degrees of intellectual disability, friendly personality
8p11	8p11	Kallmann syndrome type 2 (hypogonadotropic hypogonadism and anosmia), spherocytosis (deletions of ankyrin 1), multiple congenital anomalies, intellectual disability
8q24.1-q24.13	Langer-Giedion or trichorhinophalangeal type II	Sparse hair, multiple cone-shaped epiphyses, multiple cartilaginous exostoses, bulbous nasal tip, thickened alar cartilage, upturned nares, prominent philtrum, large protruding ears, mild intellectual disability
9q22	Gorlin	Multiple basal cell carcinomas, odontogenic keratocysts, palmoplantar pits, calcification falx cerebri
9q34	9q34 deletion	Distinct face with synophrys, anteverted nares, tented upper lip, protruding tongue, midface hypoplasia, conotruncal heart defects, intellectual disability
10p12-p13	DiGeorge type 2	Many of the DiGeorge type 1 and velocardiofacial type 1 features (conotruncal defects, immunodeficiency, hypoparathyroidism, dysmorphic features)
11p11.2	Potocki-Shaffer	Multiple exostoses, parietal foramina, craniosynostosis, facial dysmorphism, syndactyly, intellectual disability
11p13	WAGR	Hypernephroma (Wilms tumor), aniridia, male genital hypoplasia of varying degrees, gonadoblastoma, long face, upward-slanting palpebral fissures, ptosis, beaked nose, low-set poorly formed auricles, intellectual disability (retardation)
11q24.1-11qter	Jacobsen	Growth restriction, intellectual disability, cardiac and digit anomalies, thrombocytopenia
15q11-q13 (paternal)	Prader-Willi	Severe hypotonia and feeding difficulties at birth, voracious appetite and obesity in infancy, short stature (responsive to growth hormone), small hands and feet, hypogonadism, intellectual disability

Continued

Table 99.5 Microdeletion and Contiguous Gene Syndromes and Their Clinical Manifestations—cont'd

DELETION	SYNDROME	CLINICAL MANIFESTATIONS
15q11-q13 (maternal)	Angelman	Hypotonia, feeding difficulties, gastroesophageal reflux, fair hair and skin, midface hypoplasia, prognathism, seizures, tremors, ataxia, sleep disturbances, inappropriate laughter, poor or absent speech, severe intellectual disability
16p13.3	Rubinstein-Taybi	Microcephaly, ptosis, beaked nose with low-lying philtrum, broad thumbs and large toes, intellectual disability
17p11.2	Smith-Magenis	Brachycephaly, midfacial hypoplasia, prognathism, myopia, cleft palate, short stature, severe behavioral problems, intellectual disability
17p13.3	Miller-Dieker	Microcephaly, lissencephaly, pachygyria, narrow forehead, hypoplastic male external genitals, growth restriction, seizures, profound intellectual disability
20p12	Alagille	Bile duct paucity with cholestasis; heart defects, particularly pulmonary artery stenosis; ocular abnormalities (posterior embryotoxon); skeletal defects such as butterfly vertebrae; long nose
22q11.2	Velocardiofacial-DiGeorge	Conotruncal cardiac anomalies, cleft palate, velopharyngeal incompetence, hypoplasia or agenesis of thymus and parathyroid glands, hypocalcemia, hypoplasia of auricle, learning disabilities, psychiatric disorders
22q13.3 deletion		Hypotonia, developmental delay, normal or accelerated growth, severe expressive language deficits, autistic behavior
Xp21.2-p21.3		Duchenne muscular dystrophy, retinitis pigmentosa, adrenal hypoplasia, intellectual disability, glycerol kinase deficiency
Xp22.2-p22.3		Ichthyosis, Kallmann syndrome, intellectual disability, chondrodysplasia punctata
Xp22.3	MLS	*M*icrophthalmia, *l*inear skin defects, poikiloderma, congenital heart defects, seizures, intellectual disability

Table 99.6 Microduplications and Their Clinical Manifestations

DUPLICATION CHROMOSOME REGION	DISEASE REGION	CLINICAL FEATURES
1q21.1		Macrocephaly, DD, learning disabilities
3q29		Mild to moderate DD, ID, microcephaly
7q11.23	Williams syndrome	DD and severe expressive language disorder, autistic features, subtle dysmorphisms
15q13.3	Prader-Willi/Angelman syndrome	DD, ID, autistic features in duplications of maternal origin
15q24		Growth restriction, DD, microcephaly, digital anomalies, hypospadias, connective tissue abnormalities
16p11.2		FTT, severe DD, short stature, GH deficiency, dysmorphic features
17p11.2	Potocki-Lupski syndrome	Hypotonia, cardiovascular anomalies, FTT, DD, verbal apraxia, autism, anxiety
17q21.31		Severe DD, microcephaly, short and broad digits, dysmorphic features
22q11.2	Velocardiofacial-DiGeorge syndrome	Cardiovascular defects, velopharyngeal insufficiency
Xq28	*MECP2* gene (Rett syndrome)	In males: infantile hypotonia, immune deficiency, dysmorphic features, DD, speech delay, autistic behavior, regression in childhood

DD, Developmental delay; FTT, failure to thrive; GH, growth hormone; ID, intellectual disability.

be sporadic or result from abnormal segregation in translocation or inversion carriers.

Microdeletions and microduplications usually involve regions that include several genes, so the affected individuals can have a distinctive phenotype depending on the number of genes involved. When such a deletion involves more than a single gene, the condition is referred to as a **contiguous gene deletion syndrome** (Table 99.5). With the advent of chromosome microarray, a large number of microduplications, have been uncovered. Many of those **microduplication syndromes** are the reciprocal duplications of the known deletions or microdeletion counterparts and have distinctive clinical features (Table 99.6).

Subtelomeric regions are often involved in chromosome rearrangements that cannot be visualized using routine cytogenetics. *Telomeres,* which are the distal ends of the chromosomes, are gene-rich regions. The distal repetitive sequence structure of telomeres is essentially common to all chromosomes, but proximal to these are unique regions known as *subtelomeres,* which typically are involved in deletions and other chromosome rearrangements. Small subtelomeric deletions, duplications, or rearrangements (translocations, inversions) may be relatively common in children with nonspecific intellectual disability and minor anomalies. Subtelomeric rearrangements have been found in 3–7% of children with moderate to severe intellectual disability and 0.5% of those with mild intellectual disability and can be detected by CMA studies.

Pathogenic variants affecting telomere function and length have been associated with dyskeratosis congenita and other aplastic anemia syndromes, as well as pulmonary or hepatic fibrosis. Both the subtelomeric rearrangements and the microdeletion and microduplication syndromes are typically diagnosed by molecular techniques like CMA and multiple ligation-dependent probe amplification (MLPA) studies. CMA can detect 14–18% of abnormalities in patients who previously had normal cytogenetic studies.

INSERTIONS

Insertions occur when a piece of a chromosome broken at two points is incorporated into a break of a chromosome in another location. A total of three breakpoints are then required, and they can occur between two or within one chromosome. A form of nonreciprocal translocation, insertions are rare. Insertion carriers are at risk of having offspring with deletions or duplications of the inserted segment.

ISOCHROMOSOMES

Isochromosomes consist of two copies of the same chromosome arm joined through a single centromere and forming mirror images of one another. The most commonly reported autosomal isochromosomes tend to involve chromosomes with small arms. Some of the more common chromosome arms involved in this formation include 5p, 8p, 9p, 12p, 18p, and 18q. There is also a common isochromosome abnormality seen in long arm of the X chromosome and associated with Turner syndrome. Individuals who have one isochromosome X within 46 chromosomes are monosomic for genes in the lost short arm and trisomic for the genes present in the long arm of the X chromosome.

MARKER AND RING CHROMOSOMES

Marker chromosomes are rare and are usually chromosome fragments that are too small to be identified by conventional cytogenetics; they usually occur in addition to the normal complement of 46 chromosomes. Most are sporadic (70%); mosaicism is often (50%) noted because of the mitotic instability of the marker chromosomes. The incidence in newborn infants is 1 in 3,300, and the incidence in persons with intellectual disability is 1 in 300. The associated phenotype ranges from normal to severely abnormal, depending on the amount of chromosome material and number of genes included in the fragment.

Ring chromosomes, which are found for all human chromosomes, are rare. A ring chromosome is formed when both ends of a chromosome are deleted and the ends are then joined to form a ring. Depending on the amount of chromosome material that is lacking or in excess (if the ring has duplicated chromosomal material), a patient with a ring chromosome can appear normal or can have different degrees of intellectual disability and/or multiple congenital anomalies.

Marker and ring chromosomes can be found in the cells of solid tumors of children. Some of these markers can be the result of tumor-specific rearrangements, such as translocations, deletions, and duplications, ultimately leading to gene fusions and tumor gene amplifications.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

99.4 Sex Chromosome Aneuploidy

Carlos A. Bacino and Brendan Lee

About 1 in 400 males and 1 in 650 females have some form of sex chromosome abnormality. Considered together, sex chromosome abnormalities are the most common chromosome abnormalities seen in liveborn infants, children, and adults. Sex chromosome abnormalities can be either structural or numerical and can be present in all cells or in a mosaic form. Those affected with these abnormalities might have few or no physical or developmental problems (Table 99.7).

TURNER SYNDROME

Turner syndrome is a condition characterized by complete or partial monosomy of the X chromosome and defined by a combination of phenotypic features (see Table 99.7 and Table 99.8). Half the patients with Turner syndrome have a 45,X chromosome complement. The other half exhibit mosaicism and varied structural abnormalities of the X or Y chromosome. Maternal age is not a predisposing factor for children with 45,X. Turner syndrome occurs in approximately 1

Table 99.7 Sex Chromosome Abnormalities

DISORDER	KARYOTYPE	APPROXIMATE INCIDENCE
Klinefelter syndrome	47,XXY	1/580 males
	48,XXXY	1/50,000-1/80,000 male births
	Other (48,XXYY; 49,XXXYY; mosaics)	
XYY syndrome	47,XYY	1/800-1,000 males
Other X or Y chromosome abnormalities		1/1,500 males
XX males	46,XX	1/20,000 males
Turner syndrome	45,X	1/2,500-1/5,000 females
	Variants and mosaics	
Trisomy X	47,XXX	1/1,000 females
	48,XXXX and 49,XXXXX	Rare
Other X chromosome abnormalities		1/3,000 females
XY females	46,XY	1/20,000 females

Table 99.8 Signs Associated with Turner Syndrome

Short stature
Congenital lymphedema
Horseshoe kidneys
Patella dislocation
Increased carrying angle of elbow (cubitus valgus)
Madelung deformity (chondrodysplasia of distal radial epiphysis)
Congenital hip dislocation
Scoliosis
Widespread nipples
Shield chest
Redundant nuchal skin (in utero cystic hygroma)
Low posterior hairline
Coarctation of aorta
Bicuspid aortic valve
Cardiac conduction abnormalities
Hypoplastic left heart syndrome and other left-sided heart abnormalities
Gonadal dysgenesis (infertility, primary amenorrhea)
Gonadoblastoma (increased risk if Y chromosome material is present)
Learning disabilities (nonverbal perceptual motor and visuospatial skills) (in 70%)
Developmental delay (in 10%)
Social awkwardness
Hypothyroidism (acquired in 15–30%)
Type 2 diabetes mellitus (insulin resistance)
Strabismus
Cataracts
Red-green color blindness (as in males)
Recurrent otitis media
Sensorineural hearing loss
Inflammatory bowel disease
Celiac disease (increased incidence)

in 5,000 female live births. In 75% of patients, the lost sex chromosome is of paternal origin (whether an X or a Y). 45,X is one of the chromosome abnormalities most often associated with spontaneous pregnancy losses. It has been estimated that 95–99% of 45,X conceptions are miscarried.

Clinical findings in the newborns can include small size for gestational age, webbing of the neck, protruding ears, and lymphedema of the hands and feet, although many newborns are phenotypically normal (Fig. 99.10). Older children and adults have short stature and exhibit variable dysmorphic features. Congenital heart defects (40%) and structural renal anomalies (60%) are common. The most common heart defects are bicuspid aortic valves, coarctation of the aorta, aortic stenosis, and mitral valve prolapse. The gonads are generally streaks of fibrous tissue **(gonadal dysgenesis)** (see Chapter 626). There is primary amenorrhea and lack of secondary sex characteristics. These children should receive regular endocrinologic testing.

Patients with **45,X/46,XY mosaicism** can have Turner syndrome, although this form of mosaicism can also be associated with male pseudohermaphroditism, male or female genitalia in association with mixed gonadal dysgenesis, or a normal male phenotype. This variant is estimated to represent approximately 6% of patients with mosaic Turner syndrome. Some of the patients with Turner syndrome phenotype and a Y cell line exhibit masculinization. Phenotypic females with 45,X/46,XY mosaicism have a 15–30% risk of developing **gonadoblastoma**. The risk for the patients with a male phenotype and external testes is not so high, but tumor surveillance is nevertheless recommended. The American Academy of Pediatrics (AAP) has recommended the use of FISH analysis to look for Y chromosome mosaicism in all 45,X patients. If Y chromosome material is identified, laparoscopic gonadectomy is recommended.

Noonan syndrome (see Chapter 101.1) shares many clinical features with Turner syndrome and was formerly called *pseudo-Turner syndrome*, although it is an autosomal dominant disorder resulting from pathogenic variants in several genes involved in the RAS-MAPK pathway. In contrast to Turner syndrome, Noonan syndrome affects both sexes and has a different pattern of congenital heart disease, typically involving right-sided heart lesions.

KLINEFELTER SYNDROME

Persons with Klinefelter syndrome are phenotypically male. This syndrome is the most common cause of hypogonadism and infertility in males and the most common sex chromosome aneuploidy in humans (see Chapter 623). Eighty percent of children with Klinefelter syndrome have a male karyotype with an extra chromosome X-47,XXY. The remaining 20% have multiple sex chromosome aneuploidies (48,XXXY; 48,XXYY; 49,XXXXY), mosaicism (46,XY/47,XXY), or structurally abnormal X chromosomes; the greater the aneuploidy, the more severe the mental impairment and dysmorphism. The prevalence of 47,XXY is 1 in 580 liveborn males. Errors in paternal nondisjunction in meiosis I account for ~50% of the cases.

47,XYY

The incidence of 47,XYY is approximately 1 in 800-1,000 males, with many cases remaining undiagnosed, because most affected individuals have a normal appearance and normal fertility. The extra Y is the result of nondisjunction at paternal meiosis II. Those with this abnormality have normal intelligence but are at risk for learning disabilities. Behavioral abnormalities, including hyperactive behavior, pervasive developmental disorder, and aggressive behavior, have been reported. Early reports that assigned stigmata of criminality to this disorder have long been disproved.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

99.5 Mosaicism

Carlos A. Bacino and Brendan Lee

Mosaicism describes an individual or tissue that contains ≥2 genetically different cell lines typically derived from a single zygote. Genetic differences can result from mitotic nondisjunction (see Fig. 99.1; Table 99.9) or sporadic variant. Study of placental tissue from chorionic villus samples collected at or before the 10th week of gestation has shown that ≥2% of all conceptions are mosaic for a chromosome abnormality. With the exception of chromosomes 13, 18, and 21, complete autosomal trisomies are usually nonviable, and the contribution of a normal cell line might allow these other trisomic conceptions to survive to term. Depending on the point at which the new cell line arises during early embryogenesis, mosaicism may be present in some tissues but not in others. **Germline mosaicism**, which refers to the presence of mosaicism in the germ cells of the gonad, may be associated with an increased risk for recurrence of an affected child if the germ cells are affected with a chromosomal abnormality or with a specific gene pathogenic variant.

PALLISTER-KILLIAN SYNDROME

Pallister-Killian syndrome is characterized by coarse facies (prominent full cheeks), abnormal ear lobes, localized alopecia (sparse hair

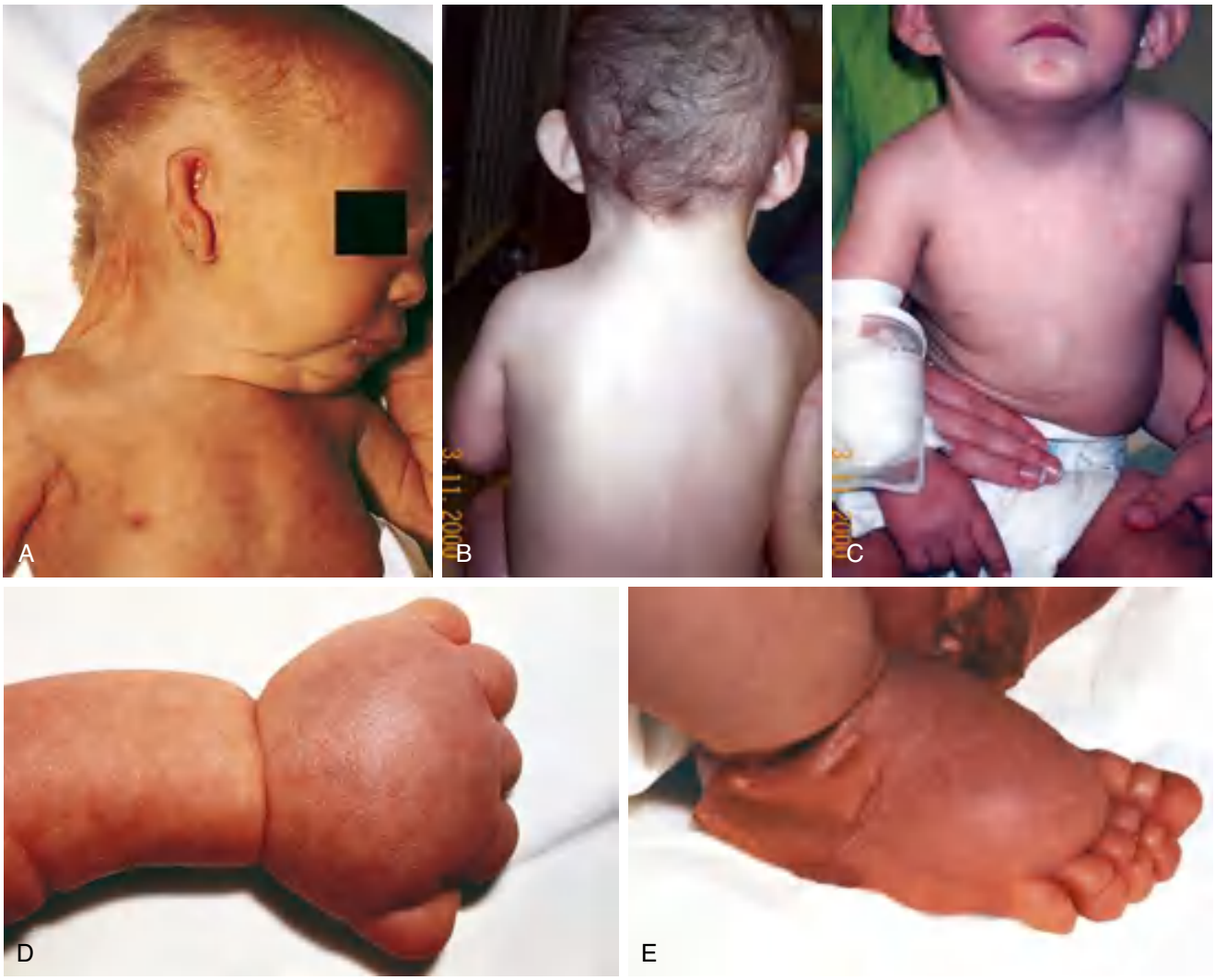


Fig. 99.10 Physical manifestations associated with Turner syndrome. **A,** This newborn shows a webbed neck with low hairline, shield chest with widespread nipples, abnormal ears, and micrognathia. **B,** The low-set posterior hairline can be better appreciated in this older child, who also has protruding ears. **C,** In this frontal view, mild webbing of the neck and small, widely spaced nipples are evident, along with a midline scar from prior cardiac surgery. The ears are low-set and prominent, protruding forward. **D** and **E,** The newborn shown in **A** also has prominent lymphedema of the hands and feet. *(From Madan-Khetarpal S, Arnold G. Genetic disorders and dysmorphic conditions. In: Zitelli BJ, McIntire SC, Nowalk AJ, eds, Zitelli and Davis' Atlas of Pediatric Physical Diagnosis, 6th ed. Philadelphia: Elsevier; 2012: Fig. 1.25.)*

in the temporal regions), pigmentary skin anomalies, diaphragmatic hernia, cardiovascular anomalies, supernumerary nipples, seizures, and profound intellectual disability. The syndrome is caused by mosaicism for an isochromosome of the short chromosome of 12 (12p). The presence of the isochromosome 12p in cells gives four functional copies for the short arm of chromosome 12 in the affected cells. The isochromosome 12p is preferentially cultured from fibroblasts that can be readily obtained from a skin punch biopsy and is seldom present in lymphocytes. A CMA obtained from a buccal swab can be another tool to detect this disorder. The abnormalities seen in affected persons probably reflect the presence of abnormal cells during early embryogenesis.

HYPOMELANOSIS OF ITO

Hypomelanosis of Ito is characterized by unilateral or bilateral macular hypo- or hyperpigmented whorls, streaks, and patches (see Chapter 694). Sometimes these pigmentary defects follow the lines of Blaschko that represent areas of early epidermal cell migration. Hair and tooth anomalies are common. Abnormalities of the eyes, musculoskeletal system (growth asymmetry, syndactyly, polydactyly, clinodactyly), and central nervous system (microcephaly, seizures, intellectual disability) may also be present. Patients with hypomelanosis of Ito might have two genetically distinct cell lines. The mosaic chromosome anomalies that have been observed involve both autosomes and sex chromosomes and have been demonstrated in about

Table 99.9 Other Rare Mosaic Aneuploidy Syndromes

DISORDER	KARYOTYPE	CLINICAL MANIFESTATIONS
Trisomy 8	47,XX/XY,+8	Variable growth and intellectual deficiency The majority of patients are mosaic Deep palmar and plantar furrows Joint contractures
Trisomy 9	47,XX/XY,+9	The majority of patients are mosaic Craniofacial (high forehead, microphthalmia, low-set malformed ears, bulbous nose) Skeletal (joint contractures) Heart defects (60%)
Trisomy 16	47,XX/XY,+16	The most commonly observed autosomal aneuploidy in spontaneous abortion Recurrence risk negligible
Tetrasomy 12p	46,XX[12]/46,XX,+i(12p)[8] (mosaicism for an isochromosome 12p)	Pallister-Killian syndrome Sparse anterior scalp hair (more so temporal region), eyebrows, and eyelashes; prominent forehead; full cheeks; long philtrum with thin upper lip and cupid-bow configuration; polydactyly; streaks of hyper- and hypopigmentation

50% of clinically affected patients. The mosaicism might not be visible in lymphocyte-derived chromosome studies; it is more likely to be found when chromosomes are analyzed from skin fibroblasts. The distinct cell lines might not always be caused by observable chromosomal anomalies but might result from single-gene pathogenic variants or other mechanisms.

99.6 Chromosome Instability Syndromes

Carlos A. Bacino and Brendan Lee

Chromosome instability syndromes, formerly known as *chromosome breakage syndromes,* are characterized by an increased risk of malignancy and specific phenotypes. They display autosomal recessive inheritance and have an increased frequency of chromosome breakage and/or rearrangement, either spontaneous or induced. Chromosome instability syndromes result from specific defects in DNA repair, cell cycle control, and apoptosis. The resulting chromosomal instability leads to the increased risk of developing neoplasms. The classic chromosome instability syndromes are Fanconi anemia, ataxia telangiectasia, Nijmegen syndrome, ICF (immunodeficiency, centromere instability, facial anomalies) syndrome, Roberts syndrome, and Bloom syndrome (Table 99.10).

99.7 Uniparental Disomy and Imprinting

Carlos A. Bacino and Brendan Lee

UNIPARENTAL DISOMY

Uniparental disomy **(UPD)** occurs when both chromosomes of a pair or areas from one chromosome in any individual have been inherited from a single parent. UPD can be of two types, uniparental *iso*disomy or uniparental *hetero*disomy. **Uniparental isodisomy** means that both chromosomes or chromosomal regions are identical (typically the result of monosomy rescue by duplication). **Uniparental heterodisomy** means that the two chromosomes are different members of a pair, both of which were still inherited from one parent. This results from a trisomy that is later reduced to disomy, leaving two copies from one parent. The phenotypic result of UPD varies according to the chromosome involved, the parent who contributed the chromosomes, and whether it is isodisomy or heterodisomy. Three types of phenotypic effects are seen in UPD: those related to imprinted genes (i.e., the absence of a gene that is normally expressed only when inherited from a parent of a specific sex), those related to the uncovering of autosomal recessive disorders, and those related to a vestigial aneuploidy producing mosaicism (see Chapter 97).

In **uniparental isodisomy**, both chromosomes or regions (and thus the genes) in the pair are identical. This is particularly important when the parent is a carrier of an autosomal recessive disorder. If the offspring of a carrier parent has UPD with isodisomy for a chromosome that carries an abnormal gene, the abnormal gene will be present in two copies, and the phenotype will be that of the autosomal recessive disorder; the child has an autosomal recessive disorder even though only one parent is a carrier of that recessive disorder. It is estimated that all humans carry approximately 20 abnormal autosomal recessive genes. Some autosomal recessive disorders, such as spinal muscular atrophy, cystic fibrosis, cartilage-hair hypoplasia, α- and β-thalassemias, and Bloom syndrome, have been reported in cases of UPD. The possibility of uniparental isodisomy should also be considered when a person is affected with >1 recessive disorder because the abnormal genes for both disorders could be carried on the same isodisomic chromosome. Uniparental isodisomy is a *rare* cause of recessively inherited disorders. Uniparental isodisomies can also be detected by SNP microarrays.

Maternal UPD involving chromosomes 2, 7, 14, and 15 and **paternal UPD** involving chromosomes 6, 11, 15, and 20 are associated with phenotypic abnormalities of growth and behavior. UPD of maternal chromosome 7 is associated with Russell-Silver syndrome with intrauterine growth restriction. These phenotypic effects may be related to imprinting (see later) (Fig. 99.11).

Although microdeletions cause the majority of cases, UPD for chromosome 15 is seen in some instances of Prader-Willi syndrome and Angelman syndrome. In **Prader-Willi syndrome**, approximately 25–29% of cases have maternal UPD (missing the paternal chromosome 15) (Fig. 99.12). In **Angelman syndrome**, paternal UPD of chromosome 15 is only observed in approximately 5% of the cases (missing the maternal chromosome 15). The phenotype for Prader-Willi syndrome and Angelman syndrome in cases of UPD is thought to result from the lack of specific parental contributions from chromosome 15. In Prader-Willi syndrome the paternal contribution is missing, and the maternal contribution is missing in Angelman syndrome. Prader-Willi syndrome may be caused by paternal deficiency of a cluster of small nucleolar RNAs (snoRNAs).

Table 99.10 Chromosome Instability Syndromes

SYNDROME	LABORATORY FINDINGS AND GENES INVOLVED	CLINICAL FINDINGS
Fanconi anemia (FA)	Chromosome breakage induced by diepoxybutane and mitomycin C There are least 21 FA genes, most are autosomal recessive, only one is X linked	Short stature, microcephaly in 1/3 of cases, radial ray defects including thumb anomalies, pancytopenia, skeletal anomalies, renal anomalies, café-au-lait macules, ear abnormalities and hearing loss
Ataxia telangiectasia	Chromosome instability with rearrangements between chromosomes 7 and 14 in lymphocytes often involving the T-cell receptors in those chromosomes Decreased IgA levels Pathogenic variants in the *ATM* gene. Autosomal recessive	Progressive cerebellar ataxia with later development of conjunctival telangiectasias, choreoathetosis, and dystonia Sinopulmonary infections Predisposition to malignancies like B-cell lymphomas, T-cell leukemias and solid tumors
Nijmegen syndrome	Translocations involving chromosomes 7 and 14 in up to 50% of cells Pathogenic variants in the *NBN* gene (autosomal recessive)	IUGR, short stature, progressive microcephaly, and intellectual disability, recurrent sinopulmonary infections Susceptibility to malignancies before age 20 years like T- and B-cell lymphomas, medulloblastomas, gliomas, rhabdomyosarcoma
ICF (immunodeficiency, centromere instability, facial anomalies) syndrome, types I and II	Pericentromeric chromosomal instability on PHA-stimulated metaphases in chromosomes 1, 9, and 16 Decrease of IgG and IgE in type I, IgM and IgE in type II Low T cells, like NK cells. Pathogenic variants in *DNMT3B* and ZBTB24 genes in types I and II, respectively Autosomal recessive	Immunodeficiency, recurrent infections, intellectual disability, facial dysmorphic features
Roberts-SC phocomelia syndrome	Premature chromosome separation with chromatid/centromere repulsion in prophase or early metaphase easily detected using C-banding Pathogenic variants in *ESCO2* gene Autosomal recessive	Prenatal growth deficiency, absent or hypoplastic arms typically symmetric with reduction of digits' length including thumbs that are usually more affected than lower extremities, bilateral cleft lip and palate, dysmorphic features, cardiac anomalies, renal anomalies, and intellectual disability
Bloom syndrome	Marked chromosomal increase of sister chromatid exchange in the presence of BrdU and increased spontaneous chromosome breakage Decreased IgG, IgA, IgM Pathogenic variants in the *RECQL3* gene. Autosomal recessive	Severe prenatal and postnatal growth deficiency, microcephaly (average intelligence), sensitivity to sunlight (butterfly distribution over the face), cafe-au-lait macules, insulin resistance, predisposition to malignancies: lymphomas, leukemias, squamous cell carcinoma and solid tumors Hypersensitivity to chemotherapy
Werner syndrome	"Variegated translocation mosaicism": chromosomal aberrations, including translocations, inversions, and deletions Telomere loss Pathogenic variants in *RECQL2* Autosomal recessive	Short stature, premature aging appearance, and predisposition to malignancies Loss and graying of hair, hoarseness, scleroderma-like changes in the 20s Cataracts, type II diabetes, osteoporosis, hypogonadism in the 30s Neoplasms in >40% of cases including sarcomas, melanomas, and thyroid cancer Myocardial infarction and malignancies are cause of early death

IUGR, Intrauterine growth restriction; PHA, phytohemagglutinin; NK, natural killer.

These findings suggest that there are differences in function of certain regions of chromosome 15, depending on whether it is inherited from the mother or from the father. Angelman syndrome is caused by absent function of the maternal gene *UBE3A* and can be the result of maternal deletion, maternal *UBE3A* pathogenic variant, paternal UPD, and abnormalities in the maternal imprinting center on chromosome 15q11-13 region.

UPD most frequently arises when a pregnancy starts off as a **trisomic conception** followed by **trisomy rescue.** Because most trisomies are lethal, the fetus can only survive if a cell line loses one of the extra chromosomes to revert to the disomic state. One third of the time, the disomic cell line is uniparental. This is the typical mechanism for Prader-Willi syndrome, and it is often associated with advanced maternal age. The embryo starts off as trisomy 15 secondary to maternal meiosis I nondisjunction, followed by random loss of the paternal chromosome. In this case the disomic cell line becomes the more viable one and outgrows the trisomic cell line. When mosaic trisomy is found at prenatal diagnosis, care should be taken to determine whether UPD has resulted and whether the chromosome involved is one of the disomies known to be associated with phenotypic abnormalities (i.e., chromosome involved in UPD containing imprinted genes). There must always be concern that some residual cells that are trisomic are present in some tissues, leading to malformations or dysfunction. The

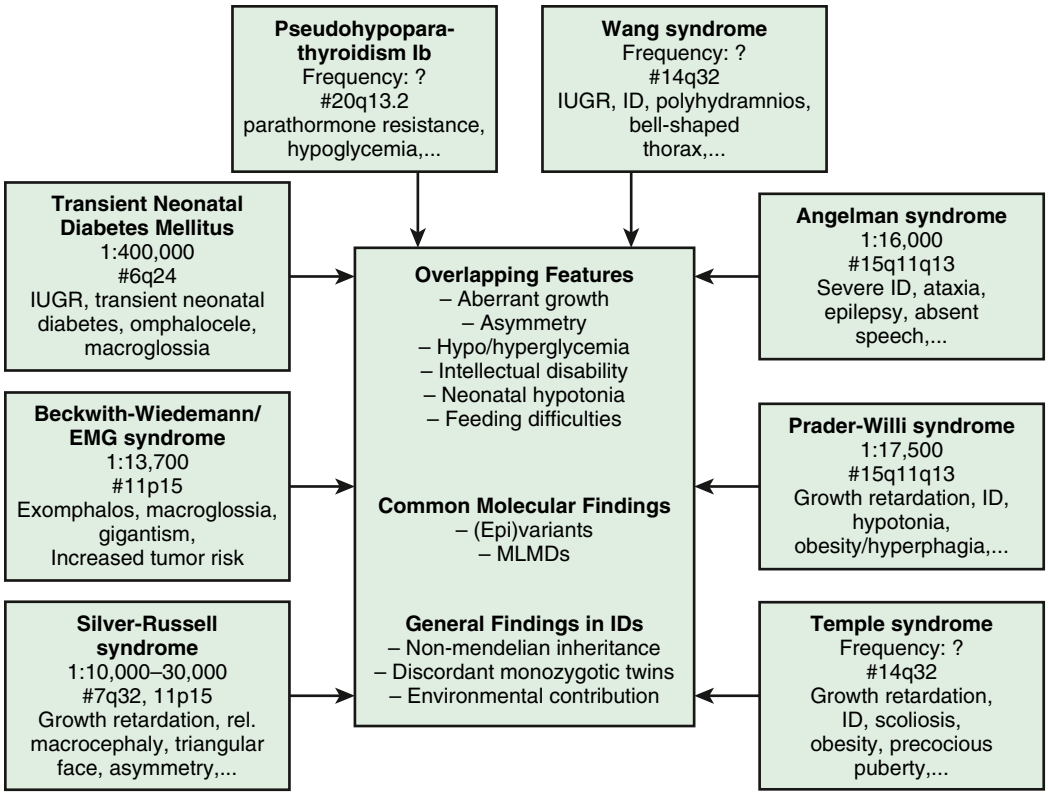


Fig. 99.11 Overview of the common clinical and molecular findings in the eight known imprinting disorders, including specific features, frequencies, and major chromosomal localization. #6q24, Chromosome 6q24; EMG, exomphalos-macroglossia-gigantism; IUGR, intrauterine growth restriction; ID, intellectual disability; MLMDs, multilocus methylation defects. *(From Eggermann T, Elbracht M, Schröder C, et al. Congenital imprinting disorders: a novel mechanism linking seemingly unrelated disorders. J Pediatr. 2013;163:1204.)*

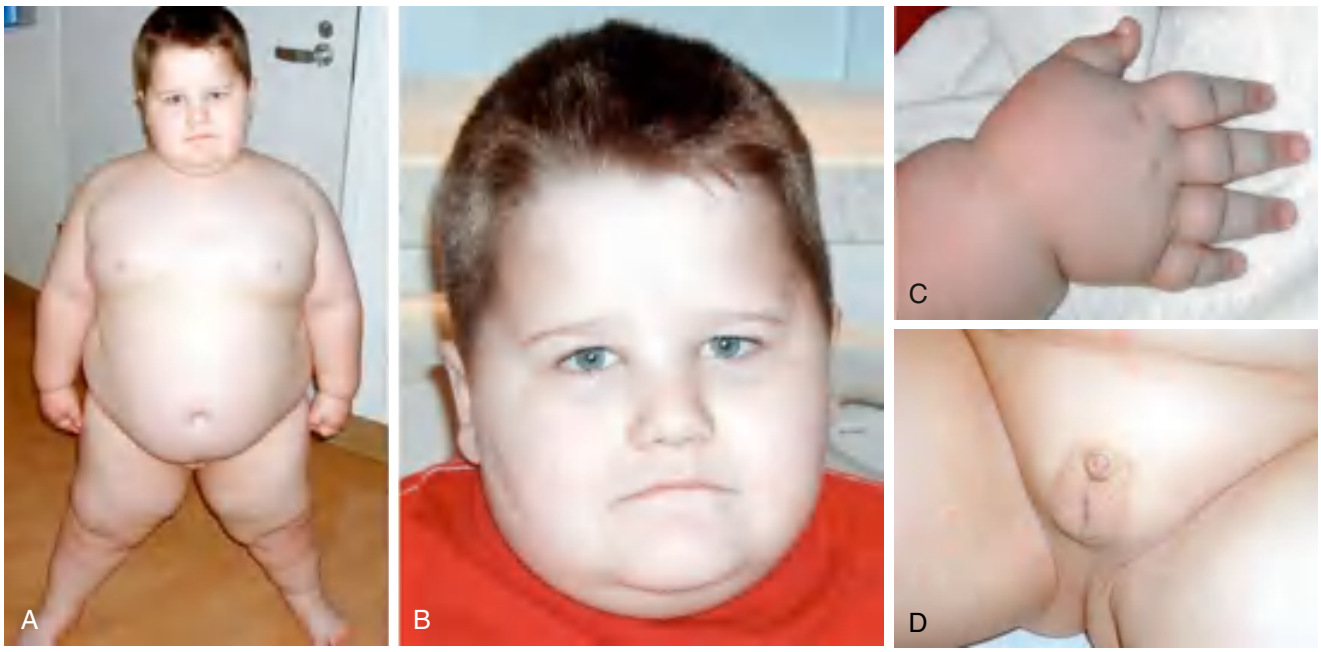


Fig. 99.12 Prader-Willi phenotype. A and B, Individual showing morbid obesity with facial features as shown. C, Upper extremities are notable for small hands relative to body size. D, External genitalia after laparoscopic orchiopexy at 13 months. Parental informed consent, as approved by the Baylor College of Medicine Institutional Review Board, was obtained to publish the photographs. *(From Sahoo T, del Gaudio D, German JR, et al. Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. Nat Genet. 2008;40:719–721.)*

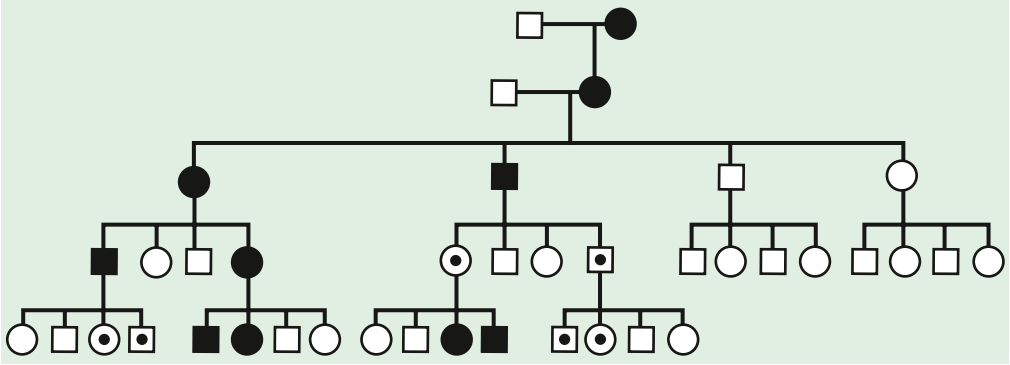


Fig. 99.13 In this hypothetical pedigree suggestive of imprinting, phenotypic effects occur only when the mutated gene is transmitted from the mother, but not when it is transmitted from the father, that is, maternal deficiency. Equal numbers of males and females can be affected and not affected phenotypically in each generation. A nonmanifesting transmitter gives a clue to the sex of the parent who passes the expressed genetic information; that is, in maternal deficiency disorders (also termed *paternal imprinting*), there are "skipped" nonmanifesting females. This is theoretical, because in most clinical scenarios of maternal deficiency, such as Angelman syndrome, affected persons do not reproduce.

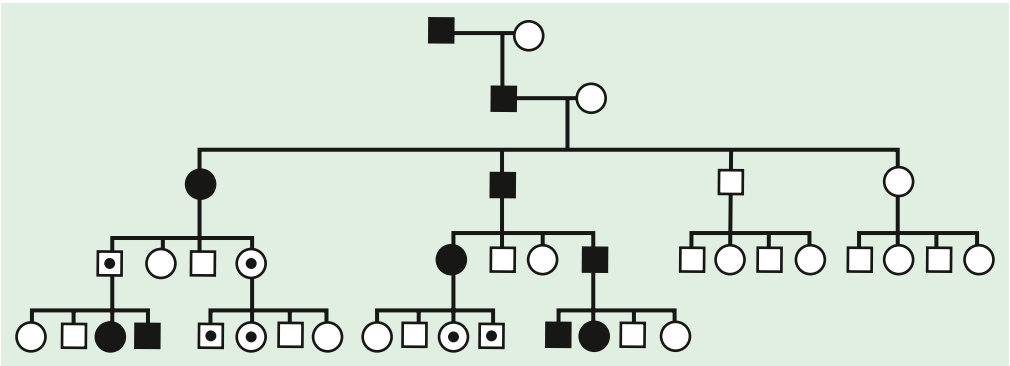


Fig. 99.14 In theoretical pedigrees suggestive of paternal deficiency (maternal imprinting), phenotypic effects occur only when the mutated gene is transmitted from the father but not when transmitted from the mother. Equal numbers of males and females can be affected and not affected phenotypically in each generation. In a theoretical situation, a nonmanifesting transmitter gives a clue to the sex of the parent who passes on the expressed genetic information; that is, in paternal deficiency (also known as *maternal imprinting*), there are "skipped" nonmanifesting males. In real-life clinical cases of Prader-Willi syndrome, affected persons do not reproduce.

Table 99.11 Consensus Diagnostic Criteria for Prader-Willi Syndrome

	MAJOR CRITERIA (1 POINT EACH)	MINOR CRITERIA (1/2 POINT EACH)
1	Neonatal/infantile hypotonia	Decreased fetal movement and infantile lethargy
2	Feeding problems and failure to thrive as an infant	Typical behavior problems
3	Weight gain at 1-6 years; obesity; hyperphagia	Sleep apnea
4	Characteristic dysmorphic facial features	Short stature for family by 15 years
5	Small genitalia; pubertal delay and insufficiency	Hypopigmentation for the family
6	Developmental delay/intellectual disability	Small hands and feet for height
7		Narrow hands, straight ulnar border
8		Esotropia, myopia
9		Thick, viscous saliva
10		Speech articulation defects
11		Skin picking

From Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med.* 2012;14(1):15. Table 2.

Table 99.12 Nutritional Phases in Prader-Willi Syndrome

PHASE	MEDIAN AGES	CLINICAL CHARACTERISTICS
0	Prenatal to birth	Decreased fetal movements and lower birthweight than siblings
1a	0-9 mo	Hypotonia with difficulty feeding and decreased appetite
1b	9-25 mo	Improved feeding and appetite and growing appropriately
2a	2.1-4.5 yr	Weight increasing without appetite increase or excess calories
2b	4.5-8 yr	Increased appetite and calories, but can feel full
3	8 yr to adulthood	Hyperphagic, rarely feels full
4	Adulthood	Appetite is no longer insatiable

Modified from Miller JL, Lynn CH, Driscoll DC, et al. Nutritional phases in Prader-Willi syndrome. *Am J Med Genet A.* 2011;155A:1040–1049.

Table 99.13 Molecular Mechanisms Causing Prader-Willi and Angelman Syndromes

	PRADER-WILLI SYNDROME	ANGELMAN SYNDROME
15q11-q13 deletion	~70% (paternal)	~70% (maternal)
Uniparental disomy	~30% (maternal)	~5% (paternal)
Single-gene pathogenic variants	None detected	*UBE3A* gene encoding the E6-AP ubiquitin-protein ligase (11% of total but mostly in familial cases)
Imprinting center pathogenic variants	5%	1%
Unidentified	<1%	10–15%

Data from Nicholls RD, Knepper JL. Genome organization, function and imprinting in Prader-Willi and Angelman syndromes. *Annu Rev Genomics Hum Genet.* 2001;2:153–175; and Horsthemke B, Buiting K. Imprinting defects on human chromosome 15. *Cytogenet Genome Res.* 2006;113:292–299.

presence of aggregates of trisomic cells might account for the spectrum of abnormalities seen in persons with some UPDs.

IMPRINTING

Genomic imprinting occurs when the phenotypic expression of a gene depends on the *parent of origin* for certain genes or chromosome regions. Whether the genetic material is expressed or not depends on the sex of the parent from whom it was derived. Genomic imprinting can be suspected in some cases on the basis of a pedigree. In these pedigrees the disease is always transmitted from the same sex and could be passed on silently for several generations by the opposite sex (Figs. 99.13 and 99.14). Imprinting probably occurs in many different parts of the human genome and is thought to be particularly important in gene expression related to development, growth, cancer, and behavior; >60 genes have been classified as imprintable. Imprinting disorders may arise from UPD, deletions or duplications, epigenetic aberrant methylation patterns, or single nucleotide pathogenic variants in a specific gene.

A classic example of imprinting disorder is seen in **Prader-Willi syndrome** and **Angelman syndrome**, two very different clinical conditions. These syndromes are both commonly caused by a deletion of the same region in the proximal long arm of chromosome 15. A deletion of the paternally derived chromosome causes Prader-Willi syndrome, in which the maternally derived copy is still intact, for which some of the imprinted genes within this region normally remain silent. Prader-Willi syndrome can be diagnosed clinically (Table 99.11) and confirmed with genetic testing. Additional clinical features and issues of weight gain are noted in Table 99.12. The weight gain is difficult to control, but treatment with growth hormone has resulted in improvements in height, lean body mass, decreased adipose tissue, and improvement in cognitive function.

A maternal deletion of the same region as in Prader-Willi syndrome causes Angelman syndrome, leaving intact the paternal copy that in this case has genes that are also normally silent. In other situations, UPD can lead to the same diagnosis (Table 99.13). Many other disorders are associated with this type of parent-of-origin effect, as in some cases of Beckwith-Wiedemann syndrome (see Chapter 598.1), Russell-Silver syndrome, and neonatal diabetes.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

Chapter 100

Dysmorphology, Phenotyping, and Sequences

Anne M. Slavotinek

Dysmorphology is the study of differences in human form and the mechanisms that cause them. It has been estimated that 1 in 40 newborns, or 2.5%, have a recognizable birth defect or pattern of malformations at birth; ~50% of these newborns have a single, isolated malformation, whereas in the other half, multiple malformations are present. About 20–30% of infant deaths and 30–50% of deaths after the neonatal period are caused by congenital abnormalities (http://www.marchofdimes.com/peristats/). In 2021, birth defects accounted for ~1 in 5 infant deaths in the United States, with a rate of 108.2 deaths per 100,000 live births, which was higher than other causes of mortality, such as preterm/low birthweight (80.4/100,000), sudden infant death syndrome (39.8/100,000), maternal complications of pregnancy (33.4/100,000), and unintentional injury (33.5/100,000).

CLASSIFICATION OF BIRTH DEFECTS

Birth defects can be subdivided into isolated (single) defects or multiple defects, typically described as multiple congenital anomalies (MCAs). An isolated primary defect can be classified, according to the presumed cause of the defect, as a malformation, dysplasia, deformation, or disruption (Table 100.1 and Fig. 100.1). Most birth defects are malformations, a term used interchangeably with anomalies. A malformation is a structural defect arising from a *localized* error in morphogenesis that results in the abnormal formation of a tissue or organ. Dysplasia refers to the *abnormal organization of cells* into tissues. Malformations and dysplasias can both affect the intrinsic structure of tissues and organs. In contrast, a deformation is an *alteration* in the shape or form of a structure or organ that has previously developed or differentiated normally. A disruption is a defect resulting from the *destruction* of a structure that had formed normally before the insult.

Most human disorders with altered morphogenesis display multiple anomalies or malformations rather than an isolated birth defect. When several malformations coexist in a single individual, they can be classified as a syndrome, sequence, or an association. A **syndrome** is defined as a pattern comprising multiple anomalies that are related by pathophysiology, resulting from a single, defined etiology. **Sequences** consist of multiple anomalies that are caused by a single event, although the sequence itself can have different etiologies. An **association** refers to a nonrandom grouping of anomalies in which there is an unclear, or unknown, relationship among the anomalies, such that they do not fit the criteria for a syndrome or sequence.

Malformations and Dysplasias

Human anomalies and dysplasias can be caused by pathogenic gene variants, chromosome aberrations and copy number variants, environmental factors, or interactions between genetic and environmental factors (Table 100.2). Some malformations are caused by deleterious sequence variants in single genes, whereas other anomalies arise because of deleterious sequence variants in multiple genes acting in combination, termed *digenic* or *oligogenic* inheritance. In the past, it was thought that malformations were caused by monogenic pathogenic variants in 7.5% of patients; chromosomal anomalies in 6%; multigenic pathogenic variants in 20%; and known environmental factors, such as maternal diseases, infections, and teratogens, in 6–7% (Table 100.3). In the remaining 60–70% of patients, malformations were classified as having an unknown etiology. Currently, the percentages have increased for all of the possible genetic etiologies of malformations due to improved cytogenetic and molecular genetic methods, including microarrays and single nucleotide polymorphism (SNP) arrays for detecting copy number variants, and next-generation sequencing (NGS) technologies, such as exome or genome sequencing, for identifying novel genes and pathogenic sequence variants.

Many developmental abnormalities caused by *pathogenic sequence variants* in a single gene display characteristic of mendelian patterns of inheritance such as autosomal dominant, autosomal recessive, and X-linked inheritance. Genes that cause birth defects or MCA syndromes are often transcription factors, part of evolutionarily conserved signal transduction pathways, or regulatory proteins required for key developmental events (see Chapters 101 and 102 and Table 100.2).

In addition, syndromes with MCA can be caused by chromosomal aberrations or copy number variants and teratogens (see Tables 100.2 and 100.3). **Down syndrome** typically results from an extra copy of an entire chromosome 21 or, less frequently, an extra copy of the Down syndrome critical region on chromosome 21. Chromosome 21 is a small chromosome that contains an estimated 250 genes, and thus individuals with Down syndrome typically have an increased dosage

Table 100.1 Mechanisms, Terminology, and Definitions of Dysmorphology

TERMINOLOGY	DEFINITION	EXAMPLE
Sequence	Single error in morphogenesis that results in a series of subsequent defects	Pierre-Robin sequence, in which a small jaw results in glossoptosis and cleft palate 22q11 deletion sequence of primary fourth brachial arch and third and fourth pharyngeal pouch defects, leading to aplasia or hypoplasia of the thymus and parathyroid glands, aortic arch anomalies, and micrognathia
22q11 deletion	Mechanical (uterine) force that alters structure of intrinsically normal tissue	Oligohydramnios produces deformations by in utero compression of limbs (e.g., dislocated hips, equinovarus foot deformity), crumpled ears, or small thorax
Disruption sequence	In utero tissue destruction after a period of normal morphogenesis	Amnionic membrane rupture sequence, leading to amputation of fingers/toes, tissue fibrosis, and tissue bands
Dysplasia sequence	Atypical organization of cells into tissues or organs	Neurocutaneous melanosis sequence, with atypical migration of melanocyte precursor cells from the neural crest to the periphery, manifesting as melanocytic hamartomas of skin and meninges
Malformation syndrome	Appearance of multiple malformations in unrelated tissues that have a known, unifying cause	Trisomy 21 Teratogens Numerous multiple congenital anomaly syndromes as described above

From Kliegman RM, Greenbaum LA, Lye PS, eds. *Practical Strategies in Pediatric Diagnosis and Therapy,* 2nd ed. Philadelphia: Elsevier; 2004.

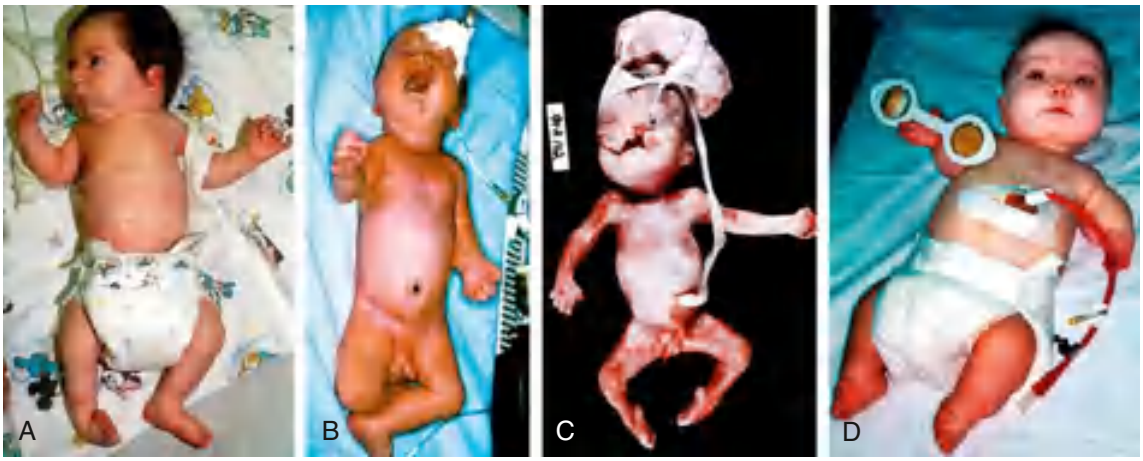


Fig. 100.1 Four major types of problems in morphogenesis: malformation, deformation, disruption, and dysplasia. **A,** Infant with campomelic dysplasia syndrome, which results in multiple anomalies caused by a pathogenic variant in *SOX9*. **B,** Infant with oligohydramnios deformation sequence caused by premature rupture of membranes from 17 weeks' gestation until birth at 36 weeks; the infant was delivered from a persistent transverse lie. **C,** Fetus with early amnion rupture sequence with attachment of the placenta to the head and resultant disruption of craniofacial structures with distal limb contractures. **D,** Infant with diastrophic dysplasia caused by biallelic pathogenic variants in a sulfate transporter protein. *(From Graham Jr JM. Smith's Recognizable Patterns of Human Deformation, 3rd ed. Philadelphia: Saunders; 2007, Fig. 1-1, p. 4.)*

for the genes encoded by this chromosome that causes their physical differences (see Chapter 57).

Neural tube defects (NTDs) are an example of a birth defect that typically displays multifactorial inheritance. NTDs and other birth defects, such as cleft lip and palate, can recur in families, but inheritance for the majority of affected individuals does not follow a straightforward, mendelian inheritance pattern, and multiple genes and environmental factors acting together likely contribute to the pathogenesis (see Table 100.2). Many of the genes involved in NTDs are unknown, so one cannot predict with certainty the mode of inheritance or a precise recurrence risk for an individual case. Empirical recurrence risks can be provided on the basis of population studies and the presence of single or multiple family members with the same malformation. However, one important gene/environment interaction has been identified for NTDs (see Chapter 631.1). **Folic acid deficiency** is associated with NTDs and can result from a combination of dietary factors and increased utilization during pregnancy. A common variant in the gene for an enzyme in the folate recycling pathway, 5,10-methylene-tetrahydrofolate reductase *(MTHFR)*, that makes this enzyme less stable, may also be important in folic acid status. Several teratogenic causes of birth defects have been described (see Tables 100.2 and 100.3). **Ethanol** causes a recognizable pattern of anomalies that is variably called fetal alcohol syndrome (FAS), fetal alcohol spectrum disorder (FASD), or fetal alcohol effects (FAE) (see Chapter 146). Children who were exposed to ethanol during the pregnancy can display microcephaly, developmental delays, hyperactivity, and facial anomalies. Ethanol, which is toxic to the developing central nervous system (CNS), causes cell death in developing neurons.

Deformations

Many deformations involve the musculoskeletal system (Fig. 100.2). Fetal movement is required for the proper development of the musculoskeletal system, and restriction of fetal movement can result in musculoskeletal deformations, such as clubfoot, or talipes. Two major intrinsic etiologies of deformations are **primary neuromuscular disorders** and **oligohydramnios**, or decreased amniotic fluid, which can be caused by fetal renal defects. Both primary neuromuscular disorders and oligohydramnios can compromise fetal movements. The major extrinsic causes of deformations are those that result in fetal crowding and restriction of fetal movement. Examples of extrinsic causes include oligohydramnios resulting from chronic leakage of amniotic fluid, and abnormal shape of the amniotic cavity. When a fetus is in the breech position (Fig. 100.3), the incidence of deformations is increased 10-fold. The shape of the amniotic cavity also has a profound effect on the shape of the fetus and is influenced by many factors, including uterine shape and the volume of amniotic fluid (Fig. 100.4).

It is important to determine whether deformations result from intrinsic or extrinsic causes. Most children with deformations from *extrinsic* causes are otherwise completely normal, and their prognosis is usually excellent. Correction typically occurs spontaneously. Deformations caused by *intrinsic* factors, such as multiple joint contractures resulting from CNS or peripheral nervous system defects, have a different prognosis and may be much more significant for the child (Fig. 100.5).

Disruptions

Disruptions are caused by destruction of a previously normally formed organ or body part. At least two mechanisms are known to produce disruptions. One involves entanglement followed by tearing apart, or amputation, of a normally developed structure, usually a digit or limb, by strands of amnion floating within amniotic fluid that are termed amniotic bands (Fig. 100.6). The other mechanism involves interruption to the blood supply to a developing body part, which can lead to infarction, necrosis, and resorption of structures distal to the insult. If interruption to the blood supply occurs early in gestation, the disruptive defect typically involves **atresia,** or absence of a body part. Genetic factors have been considered to play a minor role in the pathogenesis of disruptions, as most occur as sporadic events in otherwise healthy individuals, but little is known regarding etiology. The prognosis for a disruptive defect is determined entirely by the extent and location of the tissue loss.

Multiple Anomalies: Sequences, Associations, and Syndromes

The pattern of multiple anomalies that occurs when a single primary defect in early development produces multiple anomalies because of a cascade of secondary and tertiary developmental effects is called a *sequence* (Fig. 100.7). When evaluating a child with MCAs, the physician must differentiate between multiple anomalies that are caused by a single localized error in morphogenesis (a sequence) from syndromes with multiple anomalies. In the former, recurrence risk counseling for the multiple anomalies depends entirely on the risk of recurrence for the single, localized malformation. **Pierre-Robin sequence** is a pattern of multiple anomalies produced by mandibular hypoplasia. Because the tongue is relatively large for the oral cavity, it drops back (glossoptosis), blocking closure of the posterior palatal shelves and causing a U-shaped cleft palate. There are numerous causes of mandibular hypoplasia, all of which can result in the characteristic features of Pierre-Robin sequence.

VATER association was first defined as the nonrandom occurrence of a combination of congenital anomalies comprising vertebral defects,

Table 100.2 Examples of Malformations with Distinct Causes, Clinical Features, and Pathogenesis

DISORDER	CAUSE/INHERITANCE	SELECTED CLINICAL FEATURES	PATHOGENESIS
Spondylocostal dysostosis syndrome	Mendelian; autosomal recessive	Abnormal vertebral and rib segmentation	Deleterious sequence variants in *DLL3* and other genes
Rubinstein-Taybi syndrome	Autosomal dominant	Intellectual disability Broad thumbs and halluces; valgus deviation of these digits Hypoplastic maxillae Prominent nose and columella Congenital heart disease	Deleterious sequence variants in *CBP* and *EP300*
X-linked lissencephaly	X-linked	Male: severe intellectual disability, seizures Female: variable	Deleterious sequence variants in *DCX*
Aniridia	Autosomal dominant	Absent iris or iris/foveal hypoplasia	Deleterious sequence variants in *PAX6*
Waardenburg syndrome, type I	Autosomal dominant	Deafness White forelock Wide-spaced eyes Iris heterochromia and/or pale skin pigmentation	Deleterious sequence variants in *PAX3*
Holoprosencephaly	Loss of function or haploinsufficiency for multiple genes	Microcephaly Cyclopia Single central incisor	*SHH,* multiple other genes
Velocardiofacial syndrome	Microdeletion 22q11.2	Congenital heart disease, including conotruncal defects Cleft palate T-cell defects Facial anomalies	*TBX1* haploinsufficiency/ pathogenic variants; haploinsufficiency for other genes in the deleted interval also contributes to the phenotype
Down syndrome	Additional copy of chromosome 21 (trisomy 21)	Intellectual disability Characteristic facial anomalies Congenital heart disease Increased risk of leukemia Alzheimer disease	Increase in dosage of an estimated 250 genes on chromosome 21
Neural tube defects	Multifactorial	Meningomyelocele	Defects in folate sensitive enzymes or folic acid uptake
Fetal alcohol syndrome	Teratogenic	Microcephaly Developmental delay Facial anomalies Behavioral abnormalities	Ethanol toxicity to developing brain
Retinoic acid embryopathy	Teratogenic	Microtia Congenital heart disease	Isotretinoin effects on neural crest and branchial arch development

anal atresia, tracheoesophageal fistula (TEF) with esophageal atresia, radial anomalies, and renal dysplasia. The association was expanded to include cardiac anomalies (C) and limb anomalies (L), leading to the longer acronym, **VACTERL**. Although there are still no definitively established diagnostic criteria, most clinicians consider that a minimum of three of the seven component features must be present for the diagnosis. In addition, the designation of VATER/VACTERL association is typically one of exclusion; *growth and developmental delays as well as facial anomalies are atypical and should prompt the consideration of alternative diagnoses.* Anorectal defects and TEF are considered more characteristic of VATER/VACTERL association than some of the other components, such as cardiac anomalies. A single umbilical artery is a common finding. The estimated incidence of VATER/VACTERL has ranged from 1 in 10,000 to 1 in 40,000 infants. VACTERL with hydrocephalus, also known as VACTERL-H, is a separate condition.

VATER/VACTERL association has so far defied the identification of a simple mendelian etiology. Many cases are sporadic, although there are rare reports of familial inheritance and first-degree relatives with clinical findings in the component systems of VATER/VACTERL association. Hedgehog signaling has been postulated to be important, as mouse models with defective Hedgehog and/or ciliary signaling manifest the clinical findings observed in VATER/VACTERL. However, cytogenetic studies have not demonstrated chromosome aberrations or copy number variants. Exome sequencing (ES) has not identified a single causative gene or gene family, although nonrecurrent variants have been described in mendelian disease genes, most typically in patients with renal involvement. Clinical overlap has led to the identification of pathogenic variants in the *SALL1*, *SALL4,* and *MID1* genes in patients with anorectal malformations.

Pathogenic variants in several autosomal recessive genes in the **kynurenine pathway**, including *KYNU*, *HAAO,* and *NADSYN1*, have been associated with a spectrum of anomalies that overlap with VATER/VACTERL association, including vertebral defects, cardiac anomalies, renal malformations, and limb defects of variable severity. Biallelic variants in these genes perturb the synthesis of nicotinamide adenine dinucleotide (NAD) synthesis; these conditions have been grouped together as **congenital NAD deficiency disorders.** The overall contribution of these genes to the pathogenesis of VATER/VACTERL association appears to be small in terms of the entire group of patients but remains an important diagnostic consideration. Interestingly, studies in mice with biallelic

Table 100.3 Causes of Congenital Malformations

MONOGENIC
X-linked hydrocephalus
Achondroplasia
Ectodermal dysplasia
Apert syndrome
Treacher Collins syndrome
CHROMOSOMAL ABERRATIONS AND COPY NUMBER VARIANTS
Trisomy 21, 18, 13
XO, XXY
Deletions 4p−, 5p−, 7q−, 13q−, 18p−, 18q−, 22q−
Prader-Willi syndrome (70% of affected patients have deletion of chromosome 15q11.2-q13)
MATERNAL INFECTION
Intrauterine infections (e.g., herpes simplex virus, cytomegalovirus, varicella-zoster virus, rubella virus, Zika virus, toxoplasmosis)
MATERNAL ILLNESS
Diabetes mellitus
Phenylketonuria
Hyperthermia
UTERINE ENVIRONMENT
Deformation
Uterine pressure, oligohydramnios: clubfoot, torticollis, congenital hip dislocation, pulmonary hypoplasia, seventh nerve palsy
Disruption
Amniotic bands, congenital amputations, gastroschisis, porencephaly, intestinal atresia
Twinning
ENVIRONMENTAL AGENTS
Polychlorinated biphenyls
Herbicides
Mercury
Alcohol
MEDICATIONS
Thalidomide
Diethylstilbestrol
Phenytoin
Warfarin
Cytotoxic drugs
Paroxetine
Angiotensin-converting enzyme inhibitors
Isotretinoin (vitamin A)
D-Penicillamine
Valproic acid
Mycophenolate mofetil
UNKNOWN ETIOLOGIES
Neural tube defects, such as anencephaly and spina bifida
Cleft lip/palate
Pyloric stenosis
SPORADIC SEQUENCE COMPLEXES
VATER/VACTERL sequence (vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula with esophageal atresia, radial and renal anomalies)
Pierre-Robin sequence
NUTRITIONAL
Neural tube defects due to low folic acid

From Behrman RE, Kliegman RM, eds. *Nelson's Essentials of Pediatrics*, 4th ed. Philadelphia: Saunders; 2002.

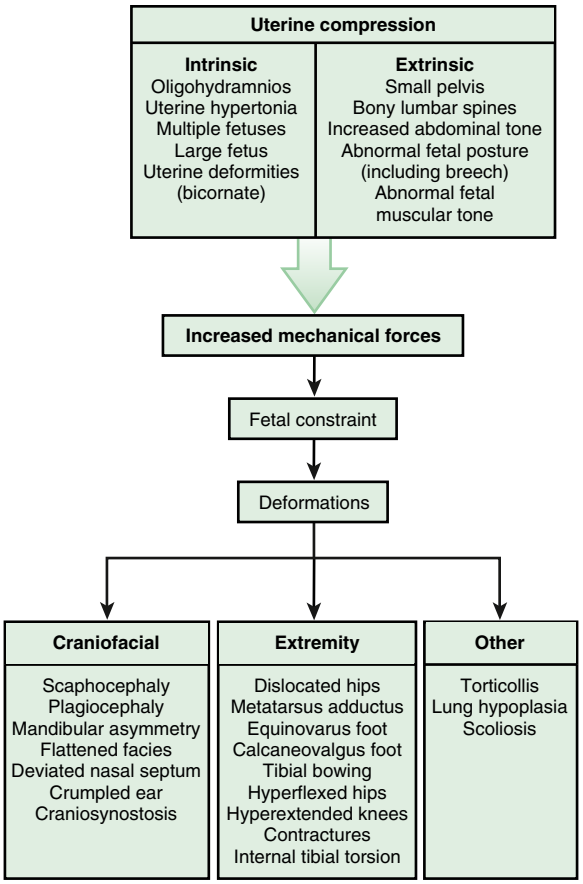


Fig. 100.2 Deformation abnormalities resulting from uterine compression. *(From Kliegman RM, Jenson HB, Marcdante KJ, et al., eds.* Nelson Essentials of Pediatrics, *5th ed. Philadelphia: Saunders; 2005.)*

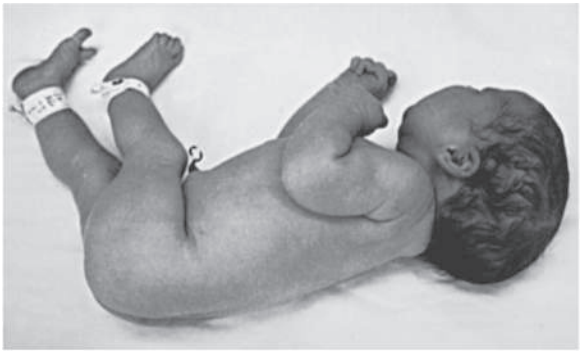


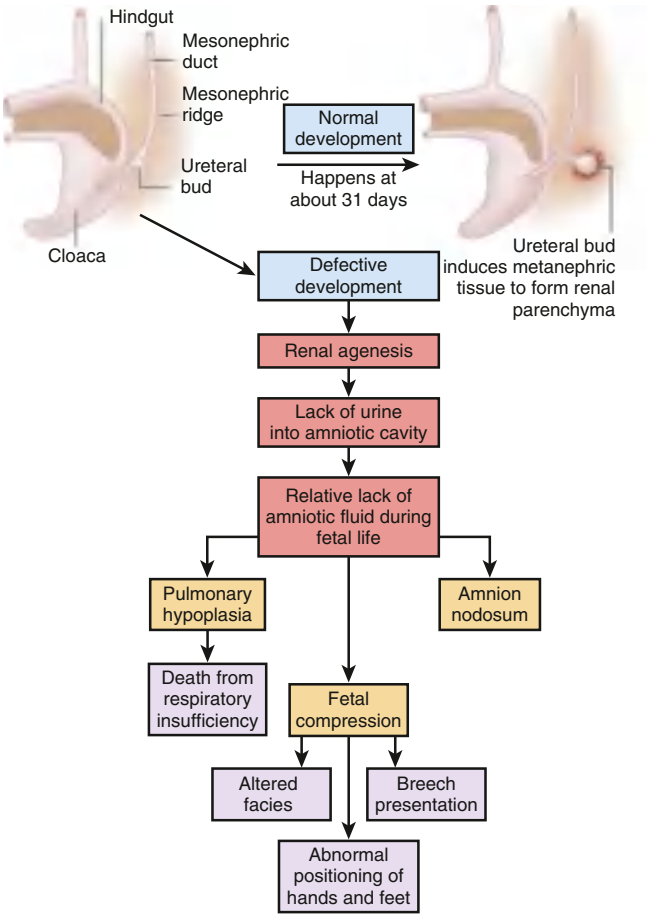
Fig. 100.3 Breech deformation sequence.

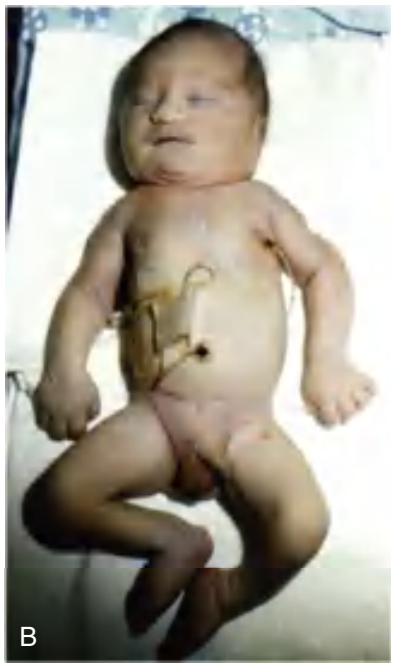
loss-of-function variants in *Haao* and *Kynu* that were provided with niacin in their diet showed that the vitamin can overcome the metabolic block associated with loss-of-function for these genes. Niacin enters the NAD biosynthesis pathway downstream to *Haao* and *Kynu*, and this finding implies that the availability of niacin and other components of the kynurenine pathway can influence NAD levels during pregnancy and consequently the clinical severity resulting from the variants affecting some of the genes involved in NAD synthesis. These conditions outline the importance of considering the influence of environmental factors on the phenotype caused by genetic variation.

MOLECULAR MECHANISMS OF MALFORMATIONS

Inborn Errors of Development

Genes that cause malformation syndromes, as well as genes whose expression is disrupted by environmental agents or teratogens, can participate in numerous cellular processes, including signal transduction, transcription, or the regulation of genes and proteins required for key developmental events. When anomalies are viewed as alterations resulting from disturbances to important developmental pathways, this provides a molecular framework for understanding the birth defects (see Chapter 101).





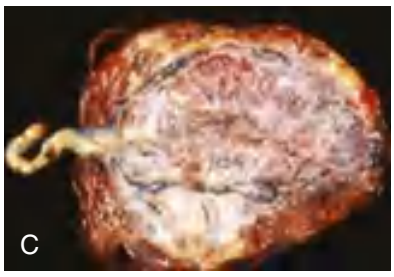
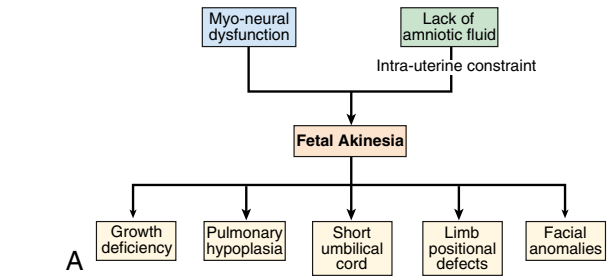


Fig. 100.4 A, Consequences of renal agenesis. B, Multiple deformational defects. C, Defects in amnion nodosum; brown-yellow granules from vernix have been ribbed into defects of the amniotic surface. *(From Jones KL, Jones MC, Del Campo M, eds. Smith's Recognizable Patterns of Human Malformation, 7th ed. Philadelphia: Elsevier; 2013: p. 821.)*



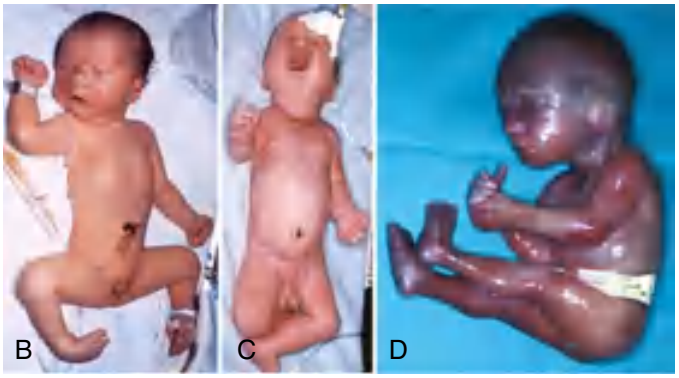


Fig. 100.5 A, Diagram demonstrating the etiologically heterogeneous phenotype that results from fetal akinesia. B, Infant born with myotonic dystrophy to a mother with the same condition. He had multiple joint contractures with thin bones and respiratory insufficiency. C, Infant immobilized in a transverse lie after amnion rupture at 26 weeks. D, Fetus with bilateral renal agenesis resulting in oligohydramnios. *(From Graham JL. Smith's Recognizable Patterns of Human Malformation, 3rd ed. Philadelphia: Elsevier; 2007: Fig. 47-2.)*

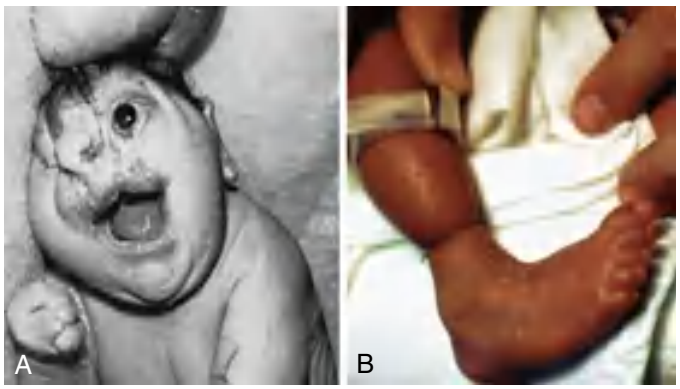


Fig. 100.6 A, Amniotic band disruption sequence. B, Bands constricting the ankle leading to deformational defects and amputations. *(From Jones KJ. Smith's Recognizable Patterns of Human Malformation, 6th ed. Philadelphia: Saunders; 2006.)*

It is important to consider MCA syndromes as members of a group based on shared or overlapping clinical findings and related causative genes that act in the same developmental or metabolic pathway. See Chapter 101 for the Sonic Hedgehog disorders, RASopathies, craniosynostoses, and chromatin regulatory disorders as examples; disruption of specific steps in this pathway result in a variety of related developmental disorders and anomalies (see Fig. 100.2).

Cytogenetic Aberrations and Chromosomal Imbalance

Cytogenetic imbalances resulting from an additional copy of a whole human chromosome can result in characteristic and recognizable

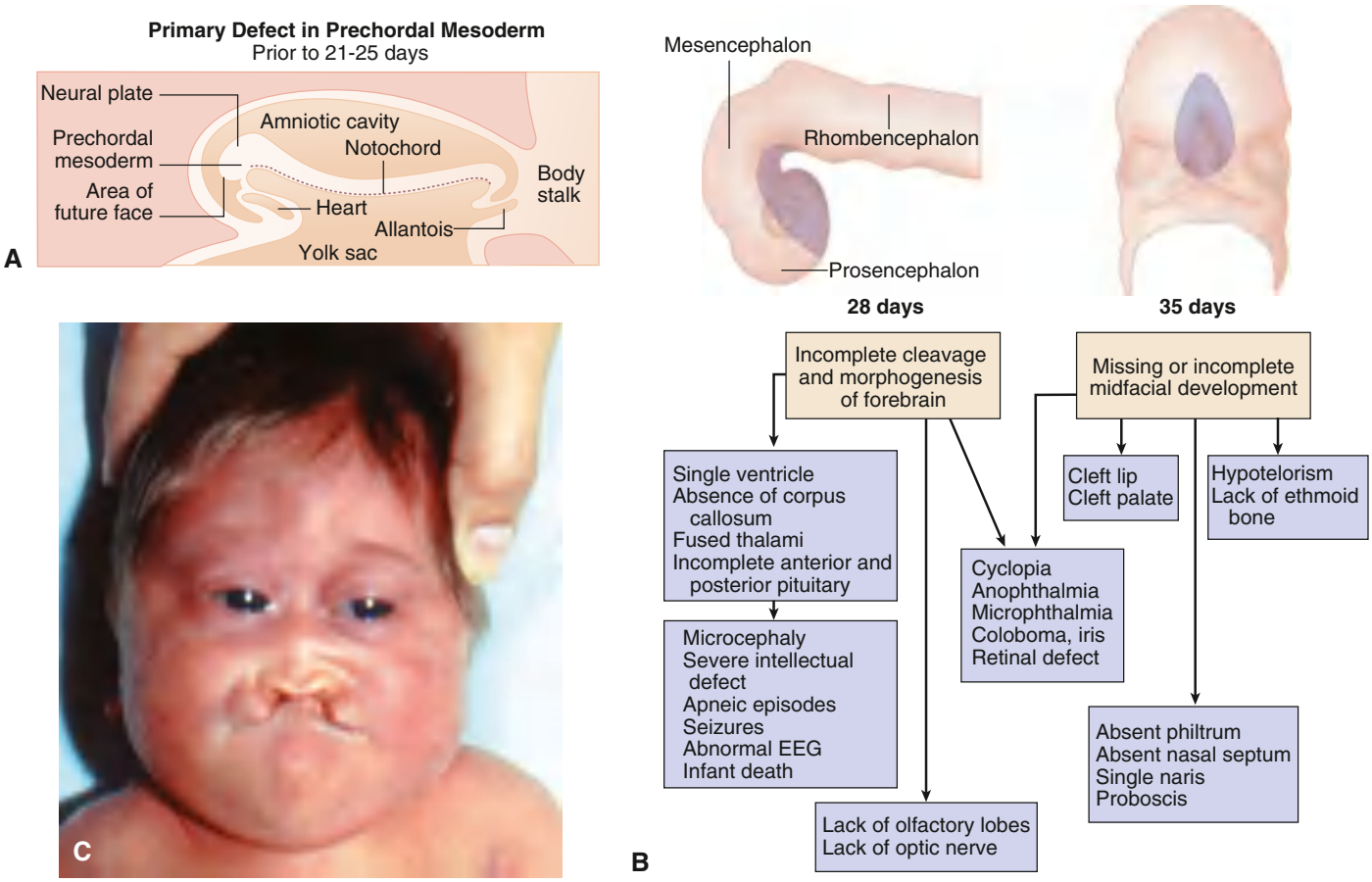


Fig. 100.7 Holoprosencephaly sequence. **A,** Schematic longitudinal section of 21-day embryo. **B,** Developmental pathogenesis of the sequence. **C,** Affected individual. *(From Jones KL, Jones MC, Del Campo M, eds.* Smith's Recognizable Patterns of Human Malformation*, 7th ed. Philadelphia: Elsevier; 2013: pp. 802–803.)*

syndromes. An additional copy of chromosome 21 results in **Down syndrome** (see Chapter 57); loss of one of the X chromosomes results in **Turner syndrome** (see Chapter 99.2 for discussion of syndromes with whole chromosomal imbalances). With the advent of high-resolution cytogenetic techniques, such as fluorescence in situ hybridization (FISH), array comparative genomic hybridization (array CGH), and SNP arrays, it has become straightforward to identify **submicroscopic chromosome deletions and duplications**. Several recurrent chromosome deletions and duplications have been identified as the cause of characteristic and recognizable syndromes (see Table 100.6 and Chapter 99), including **Williams syndrome** (deletion of chromosome 7q11.23), **Miller-Dieker syndrome** (deletion of chromosome 17p13.3), **Smith-Magenis syndrome** (deletion of chromosome 17p11.2), and 22q11 deletion syndrome (deletion of chromosome 22q11.2, also known as **velocardiofacial syndrome**). Collectively, chromosome microarrays (CMAs) and genetic sequencing have made it possible to uncover rare microdeletions, microduplications, and single nucleotide pathogenic variants associated with birth defects, intellectual disability, and neuropsychiatric disorders. The sensitivity and specificity of microarrays made them a standard starting point for the evaluation of a child with MCAs and intellectual disabilities, but exome, genome, and gene panel testing have been preferred due to higher diagnostic yield. It is important to note that unaffected individuals may carry small microdeletions and microduplications as part of familial variation. Therefore it is important to compare copy number variants that are identified in children with birth defects or developmental delays with their parents' chromosome analyses and with databases of normal variants detected in individuals without such birth defects.

APPROACH TO THE CHILD WITH FACIAL ANOMALIES

One approach to the child with facial anomalies is the *pattern recognition* approach, which compares the physical differences in the patient against a broad and memorized (or computerized) knowledge of human disorders. This approach can be appropriate for experienced dysmorphologists and clinical geneticists. In conjunction, a systematic *genetic mechanism* approach can also be effective for clinicians who are not dysmorphology experts. By gathering and analyzing the clinical data, the general pediatrician can diagnose the patient in a straightforward case or initiate a referral to an appropriate specialist.

Medical History

The history for a patient with birth defects includes elements related to etiologic factors. The family history, or *pedigree*, is necessary to assess the inheritance pattern, or lack thereof, for a disorder. For disorders that have a simple mendelian inheritance pattern, recognition of the mode of inheritance can be critical for narrowing the differential diagnosis, then prioritizing common genes with the appropriate inheritance pattern that could cause the patient's clinical features. Many common birth defects have a complex, or multifactorial, genetic etiology, such as isolated cleft palate and spina bifida or NTDs. The recognition of a close relative affected with a birth defect similar to the proband's findings can be useful. Typically, a three-generation pedigree is sufficient for this purpose (see Chapter 97).

The perinatal history is also an essential component of the history. It includes the pregnancy history of the mother, which can be useful for recognition of recurrent miscarriages that may be indicative of a chromosomal disorder, factors that may relate to deformations or disruptions such as oligohydramnios, and maternal exposures to teratogenic drugs or chemicals, including isotretinoin and ethanol, that are potential causes of microcephaly.

Another component of the history that is often useful is the natural history of the **phenotype**. MCA syndromes caused by chromosomal aberrations and single-gene disorders are frequently *static*, meaning that, although the patients can experience new complications over

Table 100.4 Definitions of Common Clinical Signs of Syndromes with Facial Anomalies

SIGN	DEFINITION
Brachycephaly	A condition in which head shape is shortened from front to back along the sagittal plane; typically the back of the skull (occiput) and face are flatter than normal.
Brachydactyly	Short digits.
Brushfield spots	Speckled white spots or rings about two-thirds of the distance to the periphery of the iris of the eye.
Camptodactyly	Permanent flexion of one or more fingers that can be associated with missing interphalangeal creases.
Clinodactyly	A medial or lateral curving of the fingers or toes; usually refers to incurving of the fifth finger.
Hypoplastic or small nail	A small nail on a digit.
Low-set ears	This designation is made when the helix meets the cranium at a level below a horizontal plane that is an extension of a line through both inner canthi.
Melia	A suffix meaning "limb" (e.g., amelia, missing limb; brachymelia, short limb).
Wide-set eyes	Increased distance between the center of the pupils of the two eyes; can be measured as an *increased interpupillary distance* (IPD).
Plagiocephaly	A condition in which head shape is asymmetric in the sagittal or coronal plane; can result from asymmetry in cranial suture closure, asymmetry of brain growth, or deformation of the skull.
Posterior hair whorl	A single hair whorl occurs to the right or left of midline and is within 2 cm anterior to the posterior fontanel in 95% of cases.
Postaxial polydactyly	Extra finger or toe present on the lateral side of the hand or foot.
Preaxial polydactyly	Extra finger or toe present on the medial side of the hand or foot.
Prominent lateral palatine ridges	Relative overgrowth of the lateral palatine ridges that can be caused by a deficit of tongue thrust into the hard palate.
Scaphocephaly	A condition in which the head is elongated from front to back in the sagittal plane; most normal skulls are scaphocephalic; also termed *dolichocephaly*.
Shawl scrotum	The scrotal skin joins around the superior aspect of the penis and represents a mild deficit in full migration of the labial-scrotal folds.
Short palpebral fissures	Decreased horizontal distance of the eyelid folds based on measurements from the inner canthus to the outer canthus.
Syndactyly	Incomplete separation of the fingers or toes. It most commonly occurs between the third and fourth fingers and between the second and third toes.
Synophrys	Eyebrows that meet in the midline.
Telecanthus	Lateral displacement of the inner canthi. The inner canthal distance (ICD) is increased, but the IPD is normal.
Widow's peak	V-shaped midline, downward projection of the scalp hair in the frontal region. It represents an upper forehead intersection of the bilateral fields of periocular hair growth suppression. A widow's peak can occur together with wide-spaced eyes.

time, the phenotype is typically not progressive. In contrast, disorders that cause facial anomalies because of metabolic perturbations, for example, Hunter syndrome or Sanfilippo syndrome, can be mild or may not be apparent at birth, but can progress, causing deterioration of patient status over time.

Physical Examination

The physical examination is very important for identifying anomalies that can aid a genetic diagnosis. The essential element of the physical evaluation is an assessment of the patient's clinical findings, with the clinician performing an organized evaluation of the size and formation of various body structures. Familiarity with the terminology typically used to describe anomalies is helpful (Table 100.4). The size and shape of the head is relevant; for example, many children with Down syndrome have mild microcephaly and a shortened anteroposterior dimension of skull, termed brachycephaly. Eye position, shape, and the slant of the palpebral fissures are useful signs for many disorders. Reference standards are available with which physical measurements (e.g., interpupillary distance) can be compared. It can be useful to categorize anomalies as "major" or "minor" birth defects. Major defects either cause significant dysfunction or require surgical correction (see Table 100.5), and minor defects neither cause significant dysfunction nor require surgical correction (e.g., clinodactyly) (Table 100.6 and Fig. 100.8). By cataloging physical parameters, the clinician may be able to identify a characteristic pattern of anomalies and recognize the diagnosis. Facial recognition software has been helpful in identifying the etiology of patients with facial dysmorphology (Fig. 100.9).

Imaging Studies

Imaging studies can be critical in diagnosing an underlying genetic etiology. If short stature or disproportionate stature (e.g., long trunk and short limbs) is noted, a full skeletal survey with radiographs should be performed. The skeletal survey can detect anomalies in bone number, structure, and formation that can be used to narrow the differential diagnosis. When there are abnormal neurologic signs or symptoms, such as hypotonia, and an abnormal head size, such as microcephaly, brain imaging can be indicated. Other studies and examinations, such as echocardiography, renal ultrasonography, and hearing and ophthalmology evaluation, can also be useful to identify additional major or minor anomalies that may serve as diagnostic clues.

Table 100.5 Major Malformations*

NEUROLOGIC
Severe hydrocephalus
Lissencephaly
Schizencephaly
Megalencephaly
Neural tube defect
Meningomyelocele
Encephalocele

CARDIOVASCULAR
Various congenital heart malformations
Cardiomyopathy
Genetic arrhythmia syndromes

GENITOURINARY
Ambiguous genitalia
Kidney malformations
Urachal defects

RESPIRATORY
Congenital pulmonary airway malformation
Tracheoesophageal fistula

ABDOMINAL WALL
Gastroschisis
Omphalocele

CRANIOFACIAL
Craniosynostosis
Facial cleft
Cleft lip and palate
Structural eye defect
Coloboma
Aniridia
Structural ear defects
Microtia
Aplasia of the auditory canal

LIMB
Amelia
Split/hand foot malformation

*Not an inclusive list.
From Basel D. Dysmorphology in a genomic era. *Clin Perinatol.* 2020;47:15–23. Table 1.

Table 100.6 Minor Malformations*

CRANIOFACIAL
Large fontanel
Flat or low nasal bridge
Saddle nose, upturned nose
Micrognathia
Cutis aplasia of the scalp

EYE
Palpebral fissures
Telecanthus or epicanthus
Up or down slanting
Hypertelorism
Brushfield spots

EAR
Posteriorly rotated pinna
Lack of helical fold
Preauricular with or without auricular skin tags
Small pinna
Auricular (preauricular) pit or sinus
Folding of helix
Darwinian tubercle
Crushed (crinkled) ear
Asymmetric ear sizes
Low-set ears

SKIN
Dimpling over the bones
Capillary hemangioma (face/posterior neck)
Dermal melanosis (African, Asian)
Sacral dimple
Pigmented nevi
Redundant skin folds
Cutis marmorata
Café-au-lait macules

HAND
Simian crease
Bridged upper palmar creases
Fifth finger clinodactyly
Joint hypermobility (hyperextension of thumb)
Cutaneous syndactyly
Polydactyly
Short, broad thumb
Narrow or hyperconvex nails
Hypoplastic nails
Camptodactyly
Short fourth metacarpal

FOOT
Syndactyly of second/third toe
Asymmetric toe length
Clinodactyly of second toe
Overlapping toes
Nail hypoplasia
Wide gap between hallux and second toe
Deep plantar crease between hallux and second toe

OTHER
Mild calcaneovalgus
Hydrocele
Shawl scrotum
Hypospadias
Hypoplasia of labia majora
Supernumerary nipples
Undescended testes
Tongue tie

*Not an inclusive list.
From Basel D. Dysmorphology in a genomic era. *Clin Perinatol.* 2020;47:15–23. Table 2.

Diagnosis

The examining physician should gather data on the patient's pedigree and perinatal and pediatric history and should have an appreciation for the natural history of the clinical findings. At this point, the physician has examined the child, identified atypical physical features, and obtained appropriate imaging studies.

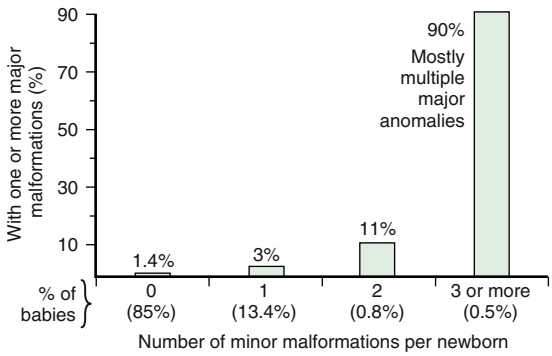


Fig. 100.8 Frequency of major malformations in relation to the number of minor anomalies detected in a given newborn baby. *(From Jones KJ. Smith's Recognizable Patterns of Human Malformation, 6th ed. Philadelphia: Saunders; 2006.)*

The clinician should now attempt to organize the findings to elucidate potential developmental processes. An assessment based on **specificity** can be helpful for this process. If a child has multiple findings, such as a patent ductus arteriosus (PDA), mild growth restriction, mild microcephaly, and holoprosencephaly, micropenis, and ptosis, a selection of the rarer or pathognomonic findings may be prioritized. The PDA, ptosis, mild growth restriction, and mild microcephaly are considered *nonspecific findings*, as they are present in many disorders or often present as isolated features that are not part of a syndrome. However, holoprosencephaly and micropenis are present in fewer syndromes and are not considered part of normal variation. The clinician can therefore search for disorders that include both holoprosencephaly and micropenis. The search can be performed manually using the features index of a textbook such as *Smith's Recognizable Patterns of Human Malformation* or a computerized database such as Online Mendelian Inheritance in Man (OMIM). Searching for both holoprosencephaly and micropenis returns a list of diagnostic possibilities, and the physician can then return to the patient to examine for additional features of the leading possible candidate disorders. Appropriate genetic testing can then be undertaken to confirm the clinician's hypothesis and verify the diagnosis. Alternatively, broad genetic testing can be undertaken if the patient's clinical findings are nonspecific.

Laboratory Studies and Genetic Testing

The laboratory evaluation of a child with anomalies can be critical to reach or confirm the correct diagnosis, particularly for metabolic conditions. Array CGH and SNP arrays enable the detection of chromosome abnormalities and copy number variants and, in the case of SNP arrays, evaluation for loss of heterozygosity. Chromosome deletion syndromes may also be identified with specific and sensitive FISH analysis, although this technology has been largely replaced by chromosome arrays and NGS sequencing. Cytogenetic studies with Giemsa-banded (G-banded) chromosome analysis are still useful for the diagnosis of Down syndrome and balanced translocations (Table 100.7). These tests are sensitive methods for the detection of cytogenetic alterations associated with birth defects and MCAs. Cytogenetic alterations can also be detected by whole genome sequencing that is becoming increasingly utilized.

Molecular testing for deleterious sequence variants that cause pleiotropic malformation syndromes is also available for many disorders as clinical testing. In most cases, however, such testing should not be performed indiscriminately; instead, it should be ordered thoughtfully after a differential diagnosis has been considered. *NGS with ES and whole genome sequencing has led to the identification of innumerable novel genes and revolutionized the testing that is available for patients and families with intellectual disability, birth defects, and other genetic diseases.* A strong suspicion of a genetic diagnosis warrants consideration of testing to confirm the diagnosis, facilitate patient treatment and anticipatory guidance, clarify recurrence risks, and enable carrier testing for additional family members. Single genes can still be tested by *Sanger sequencing* that targets single or multiple exons. However,

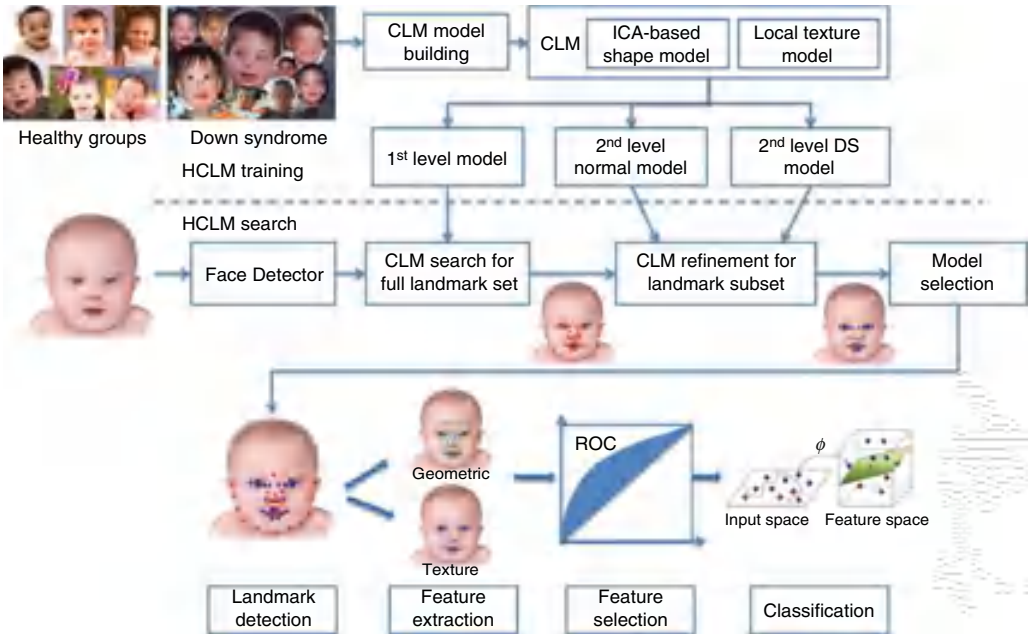


Fig. 100.9 The Framework of Facial Recognition for Genetic Syndrome Detection. ICA, Independent component analysis; CLM, constrained local model; HCLM, hierarchical CLM; DS, Down syndrome; ROC, receiver operating characterstics. *(From Zhao Q, Okada K, Rosenbaum K, et al. Digital facial dysmorphology for genetic screening: hierarchical constrained local model using ICA.* Med Image Anal. *2014;18:699–710. Fig. 2.)*

Table 100.7 Chromosomal Deletion Syndromes

CONDITION	BRIEF DESCRIPTION	PROBE
Williams syndrome	Proportionate short stature, mild-moderate to severe intellectual disability, friendly personality, stellate pattern of iris pigmentation, supravalvular aortic stenosis, wide mouth with full lips	7q11
WAGR syndrome	Wilms tumor, aniridia, growth delay, intellectual disability, and genitourinary anomalies	11p13
Prader-Willi syndrome Angelman syndrome	Distinct syndromes with common or overlapping areas of deletion; phenotype depends on gender of the parent of origin of the deletion Prader-Willi syndrome: hypotonia in infancy, short stature, obesity, mild-moderate and occasionally severe intellectual disability, small hands and feet (caused by paternal deletion of 15q11-13 or maternal uniparental disomy for chromosome 15) Angelman syndrome: severe intellectual disability, absence of speech, ataxia, tremulous movements, large mouth, frequent drooling (caused by maternal deletion of chromosome 15q11-13 or paternal uniparental disomy)	15q11
Smith-Magenis syndrome	Brachycephaly, prognathism, self-destructive behavior, wrist biting, pulling out nails, head banging, indifference to pain, intellectual disability, hyperactivity	17p11.2
Miller-Dieker syndrome	Microcephaly, narrow temples, hypotonia/hypertonia, abnormal posturing, seizures, severe to profound intellectual disability, poor growth, lissencephaly and other brain abnormalities on CT or MRI	17p13
22q11 deletion syndrome	Cleft palate, congenital heart disease, learning/behavior problems, long face, prominent nose, limb hypotonia, slender hands with tapering fingers, T-cell deficiency, immunoglobulin deficiency	22q11

WAGR, Wilms tumor, aniridia, genitourinary anomalies, and mental retardation.
From Kliegman RM, Lye PS, et al., eds. *Nelson Pediatric Symptom-Based Diagnosis*, Philadelphia: Elsevier; 2018: Table 25-10.

for diagnoses that have substantial genetic heterogeneity (e.g., hearing loss), *panel testing* often using NGS, in which multiple relevant genes can be interrogated for single nucleotide variants and gene deletions and duplications, is more expeditious than single-gene testing. Panel tests also frequently have the advantage of providing high coverage for the genes on the panel compared with the coverage for the same genes that can be obtained by ES. However, in situations with diagnostic uncertainty, such as the investigation of a child with intellectual disability and facial anomalies for which there is no clearly recognizable pattern, **exome** or **genome sequencing (GS)** may be most useful as a broad testing approach. ES examines approximately 200,000 exons, or the 1–2% of the DNA that comprises the coding regions of the genome. ES is typically performed with a *trio* approach, in which the patient and both biological parents are tested simultaneously, so that the inheritance pattern, or segregation, of deleterious sequence variants can be determined, thus simplifying analysis. Trio sequencing has resulted in higher diagnostic yields than proband-only sequencing and can approach 30–40% for indications such as intellectual disability. In contrast, GS examines the entire DNA content, including noncoding regions, and increasingly enables analysis for cytogenetic rearrangements in addition to copy number loss or gain. ES and GS are applicable to a wide range of birth defects and genetic diseases and can discover causative variants in known or novel genes associated with a particular condition.

Management and Counseling

Management and genetic counseling are essential aspects of the approach to a patient with a genetic disorder. For example, children with Down syndrome have a high incidence of hypothyroidism, and children with achondroplasia have a high incidence of cervicomedullary

junction abnormalities and monitoring for both is appropriate. One of the many benefits of an early and accurate diagnosis is that **anticipatory guidance and medical monitoring** of patients for syndrome-specific medical risks can improve their quality of life and prolong it. When a diagnosis is made, the treating physicians can access published information on the natural history and management of the disorder through the medical literature, genetics reference texts, and databases. Providing access to disorder-specific patient support groups can also provide immense benefit to patients and families.

The second major benefit of an accurate diagnosis is that it provides data for **recurrence risk** estimates. Genetic disorders may have direct effects on only one member of the family, but the diagnosis of the condition can have implications for the entire family. One or both parents may be carriers, and siblings may be carriers or may want to know their genetic status when they reach their reproductive years. Recurrence risk provision is an important component of genetic counseling and should be included in all evaluations for families affected with birth defects or other inherited disorders (see Chapter 98).

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

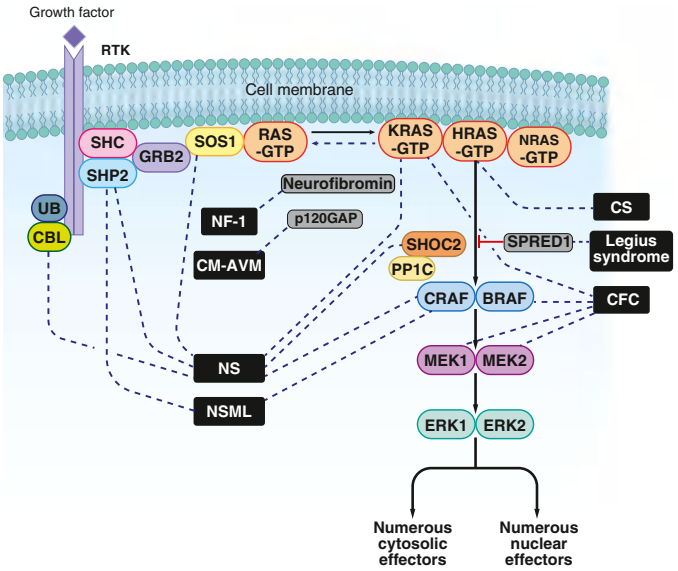


Fig. 101.1 The RAS/MAPK signal transduction pathway. The MAPK signaling pathway of protein kinases is critically involved in cellular proliferation, differentiation, motility, apoptosis, and senescence. The RASopathies are patterns of anomalies caused by pathogenic variants in genes that encode components or regulators of the RAS/MAPK pathway (indicated by *dashed lines*). These disorders include neurofibromatosis type one (NF1), Noonan syndrome (NS), Noonan syndrome with multiple lentigines (NSML), capillary malformation–arteriovenous malformation syndrome (CM-AVM), Costello syndrome (CS), cardiofaciocutaneous syndrome (CFC), and Legius syndrome. RAS/MAPK, RAS protein family/mitogen-activated protein kinase. (*From Rauen KA. The RASopathies. Annu Rev Genom Hum Genet. 2013;14:355–369.*)

Chapter 101

Signaling Pathway Disorders

Chad R. Haldeman-Englert and Anne M. Slavotinek

An increasing number of defined molecular genetic etiologies explain many multiple congenital anomaly (MCA) syndromes. Several themes have emerged: (1) many clinical disorders that were believed to represent a single entity have multiple genetic etiologies, often within genes that are functionally related; (2) many disorders believed to be distinct have common underlying genetic bases; and (3) teratogens and genetic disorders with similar clinical features can often be understood in the context of shared underlying pathology.

It is useful to consider MCA syndromes as members of a group of disorders based on shared or overlapping clinical findings and related causative genes that act in the same developmental or metabolic pathway.

101.1 RAS/MAPK Pathway

Chad R. Haldeman-Englert and Anne M. Slavotinek

The RASopathies comprise a clinically defined group of conditions caused by germline pathogenic variants in genes encoding the components or regulators of the renin-angiotensin system (RAS)/mitogen-activated protein kinase (MAPK) pathway. This pathway is one of the most important signaling pathways in human development (Fig. 101.1). Signaling is initiated when one of several growth factors or cytokines binds extracellularly to a transmembrane receptor tyrosine kinase (RTK), which transmits the signal to activate of one of several intracellular RAS proteins, such as HRAS, KRAS, or NRAS. Phosphorylation then occurs in a stepwise manner to one or more RAF proteins (ARAF, BRAF, and/or CRAF), and subsequently to MEK1 and/or MEK2, and then ERK1 and/or ERK2. Activation of ERK1/2 has downstream targets including transcription factors, membrane proteins, and other protein kinases that direct cellular growth and differentiation.

More than 20 genes implicated in RAS/MAPK signaling have been implicated in human disorders, most with some degree of clinical overlap. Common RASopathies include **Noonan syndrome (NS),** NS with multiple lentigines, Costello syndrome, cardiofaciocutaneous (CFC) syndrome, neurofibromatosis type one (NF1), Legius syndrome, and capillary malformation–arteriovenous malformation syndrome (Table 101.1).

NS represents the canonical RASopathy and is an autosomal dominant disorder resulting from pathogenic variants in several genes in the RAS/MAPK pathway. NS occurs in an estimated 1 in 1,000-2,500 live births. Approximately 30–75% of the cases are familial, and the condition typically exhibits autosomal dominant inheritance, although the *LZTR1* gene can be associated with autosomal dominant or autosomal recessive inheritance. Pathogenic variants in RAS/MAPK genes are found in ~70% of individuals with NS, with missense variants activating the *PTPN11* gene that encodes the nonreceptor protein tyrosine phosphatase SHP-2; this is observed in 50% of affected individuals. Pathogenic variants involving *SOS1* occur in 10–13% of affected individuals; variants in *RAF1* and *RIT1* each occur in 5% of patients, and variants in *KRAS*, *BRAF*, *MAP2K1*, *LZTR1*, and *NRAS* and duplications of the 12q24 region containing the *PTPN11* gene are rare. The strong clinical overlap between NS and other RASopathies, such as CFC syndrome, is due to the involvement of the same pathway and often identical genes in pathogenesis.

Common features in NS comprise short stature, a characteristic pattern of facial anomalies including ptosis, wide-spaced eyes with down-slanting palpebral fissures, epicanthal folds, anomalous and low-set ears and micrognathia, congenital heart disease, chest deformities with pectus excavatum and/or pectus carinatum, a short or wide neck with a low posterior hairline, and cryptorchidism

Table 101.1 Genes and Features of the RAS/MAPK Pathway

SYNDROME	RAS/MAPK PATHWAY GENE	PROTEIN	PROTEIN FUNCTION	CLINICAL PHENOTYPE
Noonan syndrome	*PTPN11* *SOS1* *RAF1* *KRAS* *NRAS* *SHOC2* *CBL* *RIT1*	SHP2 SOS1 CRAF KRAS NRAS SHOC2 CBL RIT1	Phosphatase RasGEF Kinase GTPase GTPase Scaffolding E3 ubiquitin ligase GTPase	Craniofacial dysmorphic features, including a broad forehead, hypertelorism, down-slanting palpebral fissures, ptosis, a high-arched palate, and low-set, posteriorly rotated ears; congenital heart defects; short stature; undescended testicles; ophthalmologic abnormalities; bleeding disorders; normal neurocognitive function or mild impairment; predisposition to cancer
Noonan syndrome with multiple lentigines	*PTPN11* *RAF1*	SHP2 RAF1/CRAF	Phosphatase Kinase	Same as Noonan syndrome, but with possible development of multiple skin lentigines as individuals age; unclear predisposition to cancer
Capillary malformation–arteriovenous malformation	*RASA1*	p120-RasGAP	RasGAP	Multifocal capillary malformations, which may be associated with arteriovenous malformations and fistulae; unclear predisposition to cancer
Costello syndrome	*HRAS*	HRAS	GTPase	Craniofacial features similar to those of Noonan syndrome but potentially more coarse; congenital heart defects; failure to thrive; short stature; ophthalmologic abnormalities; multiple skin manifestations, including papilloma; normal neurocognitive function or mild impairment; hypotonia; predisposition to cancer
Cardiofaciocutaneous syndrome	*BRAF* *MAP2K1* *MAP2K2* *KRAS*	BRAF MEK1 MEK2 KRAS	Kinase Kinase Kinase GTPase	Craniofacial features similar to those of Noonan syndrome; congenital heart defects; failure to thrive; short stature; ophthalmologic abnormalities; multiple skin manifestations, including progressive formation of nevi; normal neurocognitive function or mild impairment; hypotonia; unclear predisposition to cancer
Neurofibromatosis type 1	*NF1*	Neurofibromin	RasGAP	Café-au-lait maculae; intertriginous freckling; neurofibromas and plexiform neurofibromas; iris Lisch nodules; osseous dysplasia; optic pathway glioma; normal neurocognitive function or mild impairment; predisposition to other cancers
Legius syndrome	*SPRED1*	SPRED1	SPROUTY-related, EVH1 domain–containing protein 1	Café-au-lait maculae; intertriginous freckling; macrocephaly; normal neurocognitive function or mild impairment; no apparent predisposition to cancer

Modified from Rauen KA. The RASopathies. *Annu Rev Genom Hum Genet*. 2013;14:355–369. Table 3.

(Table 101.2 and Fig. 101.2). The pattern of congenital heart disease typically involves right-sided lesions, most frequently presenting with pulmonary valvular stenosis and hypertrophic cardiomyopathy, but atrial and ventricular septal defects, branch pulmonary artery stenosis, tetralogy of Fallot, and coarctation of the aorta have all been described. Children with NS can manifest developmental delays in association with hypotonia and may have challenges with articulation and coordination. High-frequency sensorineural hearing loss is common and should be considered in patients with language delays. Hepatosplenomegaly, low levels of clotting factors XI and XII, and primary lymphatic anomalies have also been noted; patients may rarely develop acute lymphoblastic leukemia or juvenile myelomonocytic leukemia (JMML). Puberty may be delayed, and an adult height that reaches the lower limit of the typical range can be achieved by the end of the second decade. Other distinctive phenotypes include NS-like disorder with loose anagen hair caused by *SHOC2* variants and NS-like disorder with or without JMML caused by *CBL* variants.

DIAGNOSIS

Initial suspicion of a RASopathy generally depends on recognition of one or more characteristic features associated with the various conditions. Genetic testing includes a panel of genes including those in the RAS/MAPK pathway. Multigene panel testing is preferred over single-gene analysis due to the many overlapping symptoms seen in RASopathy disorders. Genetic testing can often be performed on blood, saliva, or a buccal swab. If a gene in the RAS/MAPK pathway is found to have a pathogenic or likely pathogenic variant, this would provide molecular confirmation of the clinical diagnosis.

TREATMENT

Management of NS is noted in Table 101.3. Human growth hormone will improve growth velocity in many individuals with NS and short stature; it is recommended for those who fall below the third percentile for height. MEK inhibitors, such as trametinib, have been used to treat refractory lymphedema or chylous effusions and possibly severe cardiac hypertrophy.

Table 101.2	Clinical Findings Associated with Noonan Syndrome
Short stature	
Failure to thrive (use of specific Noonan syndrome growth curves is recommended)	
Tall forehead	
Epicanthal folds	
Ptosis	
Blue-green irides	
Wide-spaced eyes	
Low nasal bridge, upturned nose	
Down-slanting palpebral fissures	
Low-set and posteriorly rotated ears	
Dental malocclusion	
Low posterior hairline	
Pectus excavatum	
Pectus carinatum superiorly	
Scoliosis	
Pigmented villonodular synovitis (polyarticular)	
Cubitus valgus	
Pulmonary valve stenosis (dysplastic valve)	
Hypertrophic cardiomyopathy	
Atrial septal defect, ventricular septal defect	
Lymphedema	
Nevi, lentigines, café-au-lait spots	
Cryptorchidism	
Small penis	
Delayed puberty	
Bleeding disorders, including thrombocytopenia and coagulation factor deficiencies	
Leukemia, myeloproliferative disorders, other malignancies	
Cognitive delay	

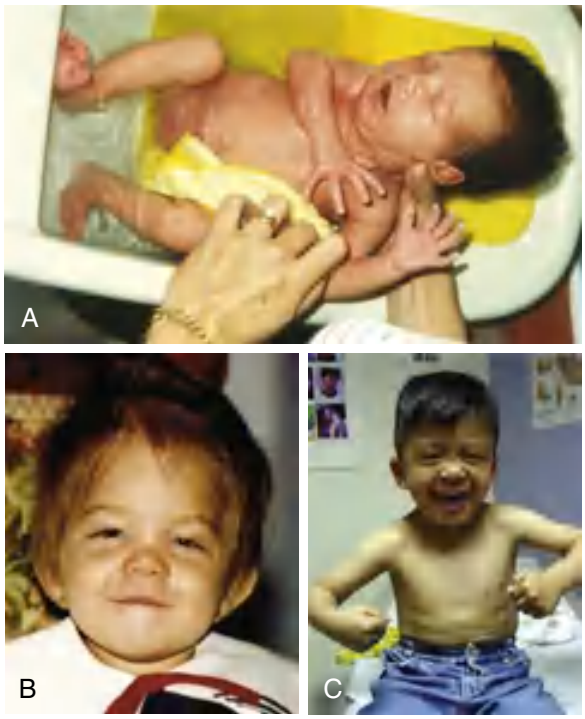


Fig. 101.2 Noonan syndrome. **A**, Newborn. **B**, Toddler. **C**, Affected male. Note the down-slanting palpebral fissures, low-set ears, elevated left shoulder secondary to scoliosis, and wide-spaced nipples. (*From Jones KL, Jones MC, Del Campo M, eds. Smith's Recognizable Patterns of Human Malformation, 8th ed. Philadelphia: Elsevier; 2022: Fig. 1AC, p. 150 and Fig. 2A, p. 151; **A** and **B** courtesy Dr. Jacqueline Noonan, University of Kentucky, Lexington, Kentucky.*)

NEUROFIBROMATOSIS

NF1 is a multisystem disorder that primarily abstractly involves the skin and peripheral nervous system (see Chapter 636.1). NF1 is associated with marked clinical variability. NF1 is inherited in an autosomal dominant manner. The disorder is caused by pathogenic variants in the *NF1* gene, which encodes neurofibromin and functions as a regulator of RAS signaling (see Table 101.1 and Fig. 101.1).

LEGIUS SYNDROME

Legius syndrome is a RASopathy caused by pathogenic variants in *SPRED1,* which encodes an HRAS regulator. Individuals with Legius syndrome present with typical multiple café-au-lait macules inherited as an autosomal dominant trait but do not develop the serious medical complications of NF1.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

101.2 Sonic Hedgehog Pathway

Chad R. Haldeman-Englert

The Hedgehog signaling pathway plays numerous tissue-specific critical roles during embryogenesis and postnatal life. Disruption of specific steps in this pathway result in a variety of related developmental disorders and anomalies (Fig. 101.3). Activation of this pathway in the child or adult may lead to abnormal cellular proliferation and cancer. Three genes, Sonic (SHH), Indian (IHH), and Desert (DHH), comprise the hedgehog family, with SHH being the most widely expressed. These genes encode secreted signaling molecules important in cellular differentiation, proliferation, and survival. Secreted SHH acts as a *ligand* for specific cellular receptors to have a signal transduced by other components of the pathway to regulate downstream gene transcription. The SHH ligand is expressed in the embryo in regions important for development of the brain, face, limbs, and the gut.

Deleterious sequence variants in *SHH* can cause **holoprosencephaly** (see Fig. 100.7), a variably severe, midline defect associated with clinical effects ranging from cyclopia to a single maxillary incisor with close spacing of the ocular orbits. The SHH protein is processed by proteolytic cleavage to an active N-terminal form, which is then further modified by the addition of cholesterol. The steroidal alkaloid cyclopamine exerts a teratogenic effect by inhibiting cholesterol modification of SHH and can result in holoprosencephaly in sheep. In humans, a defect in cholesterol biosynthesis involving the recessive delta-7-dehydrocholesterol reductase gene *(DHCR7)* results in **Smith-Lemli-Opitz syndrome** (SLOS) (see Chapter 106).

In addition to microcephaly and holoprosencephaly, patients with SLOS (see Fig. 101.3) display syndactyly, classically of the second and third toes, postaxial polydactyly of the hands and feet, an upturned or anteverted nose, ptosis, and cryptorchidism.

The cholesterol-modified active form of SHH binds to its transmembrane receptor Patched (PTCH1). This SHH-PTCH1 complex then inhibits the activity of the transmembrane protein Smoothened (SMO). Because SMO normally acts to suppress downstream targets, the GLI family of transcription factors, inhibition of SMO by PTCH1 results in activation of GLI1, GLI2, and GLI3. Pathogenic variants resulting in activation of *SMO* can be oncogenic, particularly in basal cell carcinomas and medulloblastomas. In addition, *PTCH1* and its orthologue, *PTCH2*, act as tumor suppressors, and somatic, inactivating sequence variants can be associated with loss of tumor suppressor function. Relatedly, germline inactivating variants in *PTCH1* result in **Gorlin syndrome** (see Fig. 101.3), an autosomal dominant disorder characterized by a broad face, dental anomalies, rib defects and shortened

Table 101.3 Management of Noonan Syndrome

	AT DIAGNOSIS	AFTER DIAGNOSIS	IF SYMPTOMATIC
General	Complete physical and neurologic examination; medical genetics consultation to confirm diagnosis, consider molecular genetic testing and genetic counseling	Yearly complete physical and neurologic examination; return to geneticist if genotype negative or for multisystem assessment; genetic counseling at adolescence or when a young adult	Orchiopexy by age 1 year for cryptorchidism; if lymphoedema, refer to specialty clinic; brain and cervical spine MRI if intracranial pressure increases; electroencephalogram and referral to neurologist if seizures suspected
Developmental	Multidisciplinary developmental assessment	Developmental screening yearly for children age 5-18 years	Neuropsychologist testing if screening abnormal; referral to early intervention if delays detected before age 3 years; individual education plan for children age 5-18 years with delays
Dental	First dental assessment between age 1 year and 2 years	Yearly dental assessment	—
Growth and feeding	Plot growth on curves for Noonan syndrome	Plot growth on curves for Noonan syndrome 3 times per year until age 3 years, then yearly	Refer to gastroenterologist for feeding problems or recurrent vomiting, or if evidence of growth failure without comorbid cause exists; thyroid function tests if signs or symptoms of hypothyroidism
Cardiovascular	Cardiac examination, electrocardiogram, echocardiogram	Follow up on the basis of initial findings. If initial assessment normal, repeat every 5 years	—
Ophthalmologic	Baseline eye examination Baseline audiology examination	Repeat every 2 years, sooner if indicated	—
Audiologic	Baseline audiology examination	Repeat if recurrent otitis media or speech delay	Refer to ear, nose, and throat specialist for recurrent otitis media or serous otitis; hearing aids or classroom interventions for hearing loss
Hematologic	Complete blood cell count with differential, and prothrombin time or activated partial thromboplastin time	Repeat complete blood cell count with differential and prothrombin time or activated partial thromboplastin time if age 6-12 months at initial screen Preoperatively: complete blood cell count with differential and prothrombin time or activated partial thromboplastin time, second tier (in consultation with hematologist) factor IX, XI, and XII concentrations, von Willebrand factor, platelet aggregation	Prothrombin time or activated partial thromboplastin time if bleeding abnormal or persistent, refer to hematologist; complete blood cell count with differential for splenomegaly; complete blood cell count with differential and liver function studies for hepatosplenomegaly
Renal	Kidney ultrasound	—	—
Skeletal	Clinical assessment of spine with radiology if indicated by examination	Repeat spinal examination yearly through adolescence; radiology and referral to orthopaedic specialist if abnormal	—

From Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. *Lancet*. 2013;381:333–342.

metacarpals; basal cell nevi that can undergo malignant transformation; and an increased risk of cancers, including medulloblastomas and rhabdomyosarcomas. *GLI1* amplification has been found in several human tumors, including glioblastoma, osteosarcoma, rhabdomyosarcoma, and B-cell lymphomas. Likewise, pathogenic variants in *GLI3* can cause **Greig cephalopolysyndactyly syndrome** (GCPS), **Pallister-Hall syndrome** (PHS), postaxial polydactyly type A (and A/B), and preaxial polydactyly type IV (see Fig. 101.3). GCPS consists of wide-spaced eyes, syndactyly, additional digits on the radial border of the hand or inner aspect of the foot, termed preaxial polydactyly, and broad thumbs and halluces. PHS is an autosomal dominant disorder characterized by postaxial polydactyly, syndactyly, hypothalamic hamartomas, imperforate anus, and occasionally holoprosencephaly. GLI3 binds to CBP, the protein that is haploinsufficient in **Rubinstein-Taybi syndrome**.

Disorders that are caused by pathogenic variants in genes that function together in a developmental pathway typically have overlapping clinical manifestations. The overlapping features result from the embryonic tissues in which SHH is important for development, including the brain, face, limbs, and gut as previously noted. Brain defects are present in holoprosencephaly (see Fig. 100.7), SLOS, and PHS. Facial anomalies are found in holoprosencephaly, SLOS, Gorlin syndrome, GCPS, and PHS. Limb defects occur in SLOS, Gorlin syndrome, GCPS, PHS, and the polydactyly syndromes. Overexpression, or activating sequence variants, affecting the SHH pathway results in cancer,

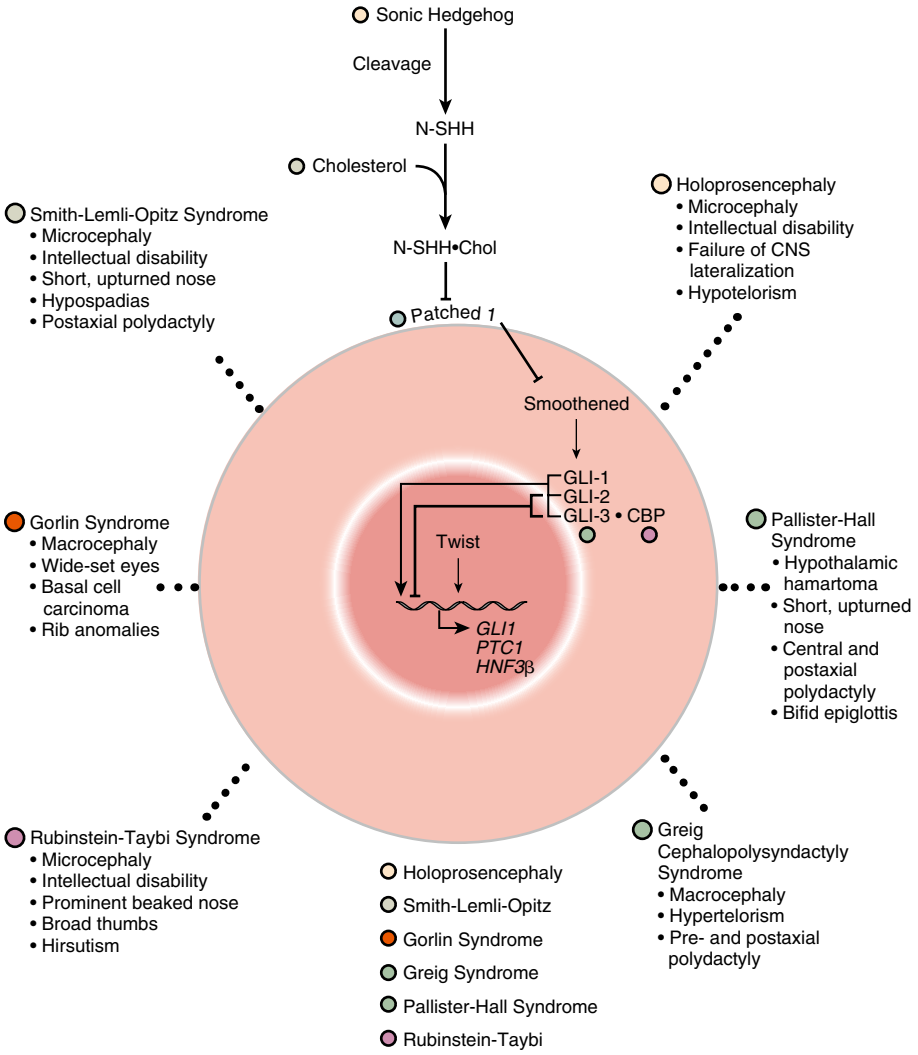


Fig. 101.3 Signaling components and clinical features of the sonic hedgehog (SHH) signaling pathway. Deleterious sequence variants in genes that function together in a developmental pathway typically have overlapping clinical manifestations. Several components of the SHH pathway have been identified and their relationships elucidated. Pathogenic variants in several members of this pathway result in phenotypes with facial anomalies, as seen in holoprosencephaly, Smith-Lemli-Opitz syndrome, Gorlin syndrome, Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, and Rubinstein-Taybi syndrome. CNS, Central nervous system.

including basal cell carcinomas, medulloblastomas, glioblastomas, and rhabdomyosarcomas.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

101.3 Ciliopathies

Chad R. Haldeman-Englert

Cilia are microtubule-containing organelles that project from the surface of most human cells. Three types of cilia exist in humans: motile cilia, nodal cilia, and primary cilia. Motile cilia typically contain a sweeping motion and play important roles in the respiratory epithelium (see Chapter 455 for discussion of structure of cilia and their role in primary ciliary dyskinesia and respiratory disorders). Nodal cilia play a very brief role during embryonic development, and their whirling flow in the Henson's node during gastrulation is central in determining the left-right body axis. Defects in this process manifest as alterations in body orientation, including situs inversus and heterotaxy (see Chapter 480.11).

Primary cilia are nonmotile and are present in nearly all cells. The membrane of each cilium is continuous with the plasma membrane. There are unique differences in its membrane that allow cilia to detect changes in the extracellular environment to function as mechanoreceptors, osmosensors, and chemosensors and convey this information via various intracellular signaling pathways to regulate diverse cellular processes (proliferation, polarity, nerve growth, differentiation, and tissue maintenance). These pathways may include Hedgehog (HH), G-protein–coupled receptors (GPCRs), Wingless and Int-1 (WNT), RTKs, and transforming growth factor β (TGF-β).

Pathogenic changes to genes that control the function and signaling within the cilia give rise to conditions called *ciliopathies*. Given the extent of cilia located throughout the human body, symptoms associated with ciliopathies can be variable and extensive. Defects can occur to many different organs during fetal development through adulthood. Organ systems generally affected in ciliopathies include the brain, eyes, liver, kidneys, and skeleton (Fig. 101.4). There are many well-described ciliopathy effects, including retinitis pigmentosa, hearing loss, infertility, primary ciliary dyskinesia, polycystic kidney disease, and nephronophthisis, as well as a number of syndromes including Joubert, Bardet-Biedl, Meckel-Gruber, and orofaciodigital syndromes (Table 101.4).

The diagnosis of a ciliopathy is generally based on recognition of the clinical features followed by either targeted gene testing, a multigene panel, or exome/genome sequencing. If a diagnosis is established with molecular testing, additional studies may be indicated to evaluate for additional medical and developmental concerns. Surveillance for potential changes to the kidneys, liver, and eyes should be routinely performed.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

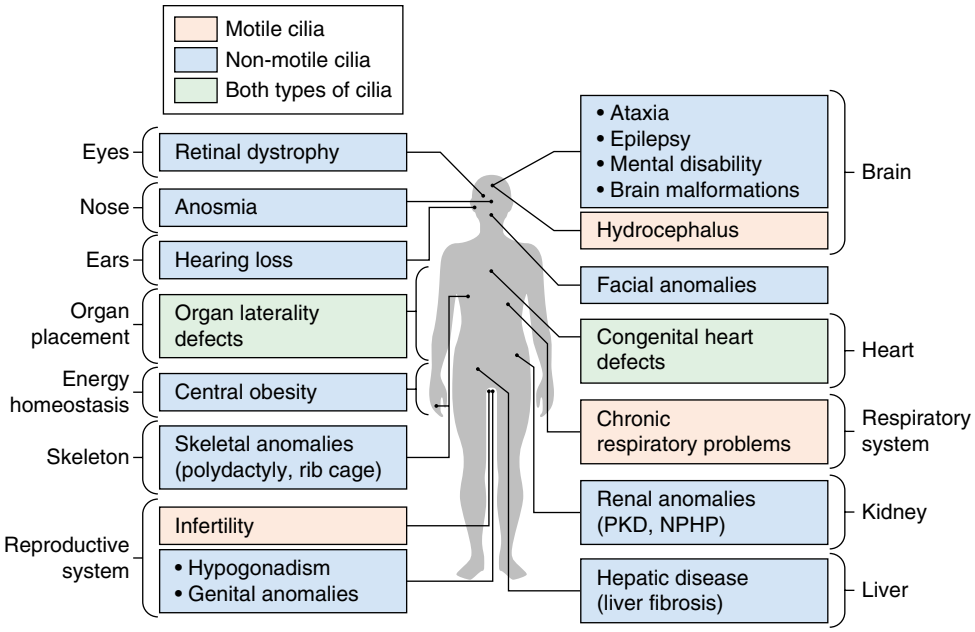


Fig. 101.4 Ciliopathies of motile and/or nonmotile cilia encompass most human organ systems. Different organ systems or tissues are affected in diverse ciliopathies, with the most common phenotypic manifestations for each organ. Ciliopathies caused primarily by defects in motile cilia are in *orange,* those in nonmotile (primary) cilia are in *blue,* and those associated with defects in nodal cilia are in *green.* NPHP, Nephronophthisis; PKD, polycystic kidney disease. (*Modified from Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. Nat Rev Molec Cell. 2017;18:533–547. Fig. 2.*)

Table 101.4 Childhood Diseases and Syndromes Associated with Motile and Primary/Sensory Ciliopathies

PEDIATRIC CILIOPATHY	CLINICAL MANIFESTATIONS	SELECTED GENE(S)
MOTOR		
Primary ciliary dyskinesia	Chronic bronchitis, rhinosinusitis, otitis media, laterality defects, infertility, CHD	*DNAI1, DNAH5, DNAH11, DNAI2, KTU, TXNDC3, LRRC50, RSPH9, RSPH4A, CCDC40, CCDC39*
PRIMARY/SENSORY		
Autosomal recessive polycystic kidney disease	RFD, CHF	*PKHD1*
Nephronophthisis	RFD, interstitial nephritis, CHF, RP	*NPHP1-8, ALMS1, CEP290*
Bardet-Biedl syndrome	Obesity, polydactyly, ID, RP, renal anomalies, anosmia, CHD	*BBS1-12, MKS1, MKS3, CEP290*
Meckel-Gruber syndrome	RFD, polydactyly, ID, CNS anomalies, CHD, cleft lip, cleft palate	*MKS1-6, CC2D2A, CEP290, TMEM216*
Joubert syndrome	CNS anomalies, ID, ataxia, RP, polydactyly, cleft lip, cleft palate	*NPHP1, JBTS1, JBTS3, JBTS4, CORS2, AHI1, CEP290, TMEM216*
Alström syndrome	Obesity, RP, DM, hypothyroidism, hypogonadism, skeletal dysplasia, cardiomyopathy, pulmonary fibrosis	*ALMS1*
Orofaciodigital syndrome type I	Polydactyly, syndactyly, cleft lip, cleft palate, CNS anomalies, ID, RFD	*OFD1*
Ellis van Creveld syndrome	Chondrodystrophy, polydactyly, ectodermal dysplasia, CHD	*EVC, EVC2*
Jeune asphyxiating thoracic dystrophy	Narrow thorax, RFD, RP, dwarfism, polydactyly	*IFT80*
Sensenbrenner syndrome	Dolichocephaly, ectodermal dysplasia, dental dysplasia, narrow thorax, RFD, CHD	*IFT122, IFT43, WDR35*
Short rib–polydactyly syndromes	Narrow thorax, short limb dwarfism, polydactyly, renal dysplasia	*WDR35, DYNC2H1, NEK1*

CHD, Congenital heart disease; CHF, congenital hepatic fibrosis; CNS, central nervous system; DM, diabetes mellitus; ID, intellectual disabilities; RFD, renal fibrocystic disease; RP, retinitis pigmentosa.

From Ferkol TW, Leigh MW. Ciliopathies: the central role of cilia in a spectrum of pediatric disorders. *J Pediatr.* 2012;160:366–371.

101.4 Craniosynostoses

Chad R. Haldeman-Englert

To permit appropriate brain growth, the cranial sutures do not completely fuse until adulthood. Growth of the skull occurs perpendicular to the direction of each suture. Craniosynostosis arises when one or more cranial sutures prematurely ossify, which usually becomes apparent between the third trimester of pregnancy and the first year of life (see Chapter 631.10). The shape of the skull is often the clue to the affected suture (Fig. 101.5). Most patients with craniosynostosis do not have additional syndromic features; this may depend on the sutures involved. Patients with bicoronal or multisuture craniosynostosis often have an associated genetic syndrome, whereas patients with isolated sagittal craniosynostosis do not have features of an identifiable genetic cause.

Craniosynostosis can result from reduced intrinsic factors (poor brain growth), increased extrinsic forces (primiparity, multiple pregnancy, fetal position, high birth weight), or genetic abnormalities leading to uncontrolled cranial bone growth. Genes commonly associated with syndromic craniosynostosis include three fibroblast growth factor receptor genes *(FGFR1*, *FGFR2*, *FGFR3)*, *TWIST1*, and *EFNB1*. *FGFR1*, *FGFR2*, and *FGFR3* are associated with recognizable conditions such as Crouzon, Pfeiffer, Apert, and Muenke syndromes; *TWIST1* with Saethre-Chotzen syndrome; and *EFNB1* with craniofrontonasal syndrome. Distinguishing between the FGFR-related craniosynostosis syndromes can often be made based on additional features, particularly of the hands and feet (see Chapter 631.10).

Other pathogenic gene variants have been identified in patients with syndromic and nonsyndromic craniosynostosis (Table 101.5). Given the overlap of clinical features of many of the craniosynostosis conditions, appropriate genetic testing for patients with craniosynostosis could include either a multigene panel or exome/genome sequencing. The diagnosis is made based on the genetic abnormality and clinical features.

Management of patients with craniosynostosis is often complex and performed with a multidisciplinary team of various specialists, including audiology, dentistry, genetics, neurosurgery, ophthalmology, orthodontics, otolaryngology, pediatrics, plastic surgery, and speech therapy (see Chapter 631.10).

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

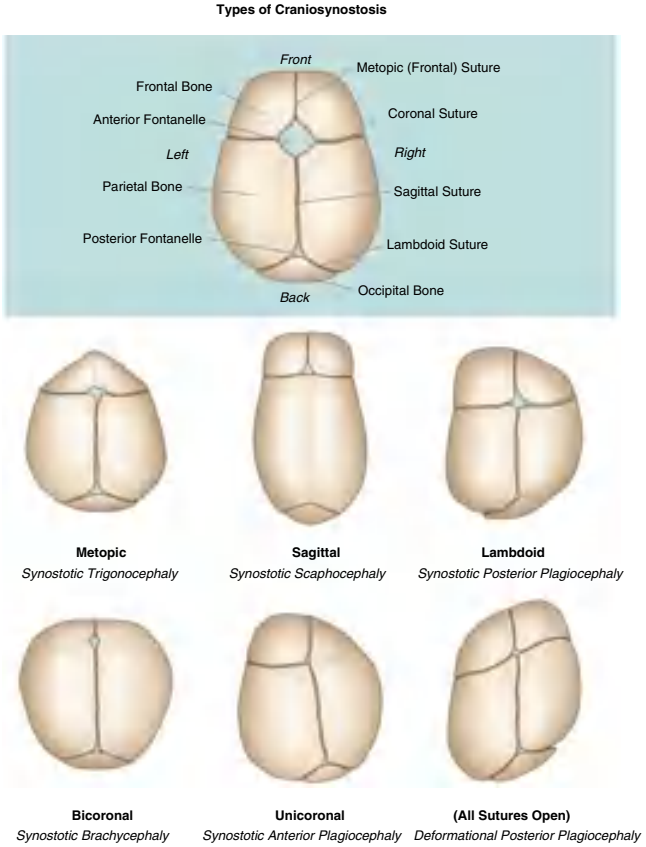


Fig. 101.5 Types of craniosynostosis. (*From Buchanan EP, Xue Y, Xue AS, Olshinka A, Lam S. Multidisciplinary care of craniosynostosis. J Multidiscip Healthcare. 2017;10:263–270. Fig. 1.* Dove Medical Press Ltd.)

Table 101.5 Core Genes For Which Specific Types of Variants Are Associated with Craniosynostosis in More Than Half of Affected Individuals

GENE (MIM#)	INHERITANCE PATTERN	CLINICAL DISORDER (MIM#)	PREVALENCE (%)	TYPICAL SUTURE FUSION	MAJOR PHENOTYPIC FEATURES
ASXL1 (612990)	AD (n)	Bohring-Opitz syndrome (605039)	—	Metopic	Forehead nevus flammeus, ulnar deviation and flexion of wrists and metacarpophalangeal joints, severe intellectual disability
CDC45 (603465)	AR	—	—	Coronal	Thin eyebrows, small ears, variable short stature
COLEC11 (612502)	AR	3MC syndrome 2 (265050)	—	Metopic	Hypertelorism, blepharoptosis, arched eyebrows, cleft lip/palate, hearing loss, radioulnar synostosis, genital and vesicorenal anomalies
EFNB1 (300035)	XLD (male sparing)	Craniofrontonasal syndrome (304110)	0.8	Coronal	Hypertelorism, notched nasal tip, chest anomalies, longitudinal splitting of nails; heterozygous females more severely affected than hemizygous males
ERF (611888)	AD	ERF-related craniosynostosis (600775)	1.1	Multisuture	Exorbitism, midface hypoplasia, Chiari type I malformation, postnatal onset of craniosynostosis
FGFR1 (136350)	AD	Pfeiffer syndrome (101600)	—	Coronal	Mild craniofacial features, broad medially deviated thumbs and halluces, cutaneous syndactyly, specific amino acid substitution p.Pro252Arg
FGFR1	AD (n)	Osteoglophonic dysplasia (166250)	—	Multisuture	Prominent brow ridges, depressed nasal bridge, rhizomelic dwarfism, localized lytic lesions of metaphyses
FGFR2 (176943)	AD (n)	Apert syndrome (101200)	3.6	Coronal, multisuture	Midface hypoplasia, dilated cerebral ventricles, complex syndactyly of the hands and feet
FGFR2	AD (n)	Beare-Stevenson syndrome (123790)	*	Multisuture	Choanal atresia, prominent umbilical stump, furrowed scalp/neck skin, acanthosis nigricans in survivors
FGFR2	AD	Crouzon syndrome (123500)	2.4	Multisuture, coronal, sagittal	Exorbitism, midface hypoplasia, beaked nose ("crouzonoid" facies), clinically normal hands and feet
FGFR2	AD (n)	Pfeiffer syndrome (101600)	0.8	Multisuture	Broad thumbs and halluces; in severe cases, cloverleaf skull, brain anomalies, tracheal sleeve, fused elbows
FGFR2	AD (n)	Bent bone dysplasia (614592)	*	Coronal	Osteopenia, reduced mineralization of the calvaria, bent long bones; perinatal lethal
FGFR3 (134934)	AD	Muenke syndrome (602849)	4.0	Coronal	Defined by specific amino acid substitution p.Pro250Arg; may include sensorineural hearing loss, mild brachydactyly, cone-shaped epiphyses
	AD (n)	Crouzon/acanthosis nigricans (612247)	0.4	Multisuture	Crouzonoid facies, choanal stenosis, hydrocephalus, acanthosis nigricans; specific amino acid substitution p.Ala391Glu
	AD (n)	Thanatophoric dysplasia II (187601)	*	Multisuture	Lethal skeletal dysplasia, micromelic limb shortening, straight femora; specific amino acid substitution p.Lys650Glu
IHH (600726)	AD	Philadelphia craniosynostosis (185900)	—	Sagittal	Cutaneous and osseous syndactyly

Table 101.5 Core Genes For Which Specific Types of Variants Are Associated with Craniosynostosis in More Than Half of Affected Individuals—cont'd

GENE (MIM#)	INHERITANCE PATTERN	CLINICAL DISORDER (MIM#)	PREVALENCE (%)	TYPICAL SUTURE FUSION	MAJOR PHENOTYPIC FEATURES
IL11RA(600939)	AR	Craniosynostosis and dental anomalies (614188)	—	Multisuture	Maxillary hypoplasia, delayed tooth eruption, supernumerary teeth, minor digit abnormalities, conductive hearing loss
MEGF8(604267)	AR	Carpenter syndrome 2 (614796)	—	Metopic	Hypertelorism, arched eyebrows, lateralization defects, brachydactyly, syndactyly, preaxial polydactyly
MSX2(123101)	AD	Boston craniosynostosis (604757)	—	Sagittal, coronal, multisuture	None diagnostic; syndrome defined by specific amino acid substitutions p.Pro148His, p.Pro148Leu
POR(124015)	AR	Antley-Bixler syndrome (201750)	—	Bicoronal, multisuture	Choanal stenosis, radiohumeral synostosis, bowed femora, multiple joint contractures, genital abnormalities; abnormal steroidogenesis
RAB23(606144)	AR	Carpenter syndrome 1 (201000)	—	Multisuture	Obesity, cardiac defects, polysyndactyly, brachydactyly, genu valgum, hypogenitalism, umbilical hernia, learning disability
RUNX2(600211)	AD (n)	—	—	Multisuture	None diagnostic; syndrome defined by specific gene duplication
SKI(164780)	AD (n)	Shprintzen-Goldberg syndrome (182212)	—	Sagittal, multisuture	Hypertelorism, micrognathia, high-arched palate, arachnodactyly, joint contractures, pectus deformity, aortic root aneurysm, mitral valve prolapse, learning disability
TCF12(600480)	AD	TCF12-related craniosynostosis (615314)	1.3	Coronal	Resembles mild Saethre-Chotzen syndrome; diagnosis defined by presence of mutations in the gene, ~50% nonpenetrance
TWIST1(601622)	AD	Saethre-Chotzen syndrome (101400)	3.6	Coronal	Low frontal hairline, hypertelorism, eyelid ptosis, down-slanting palpebral fissures, blocked tear ducts, small ears with prominent crus helicis
WDR35(613602)	AR	Cranioectodermal dysplasia 2 (613610)	—	Sagittal	Facial dysmorphism, narrow thorax, short long bones, brachydactyly, sparse hair, hypoplastic teeth, cystic kidneys, hepatic fibrosis
ZIC1(600470)	AD (n)	ZIC1-related craniosynostosis	0.2	Coronal	Severe learning disability

The prevalence figures are for percent total craniosynostosis cases with specified mutation, from the cohort attending the Craniofacial Unit, Oxford, born between 1998 and 2008 (n = 531) and surgically treated before end of 2013.
*Usually lethal at birth.
AD, Autosomal dominant; AR, autosomal recessive; XLD, X-linked dominant; (n), usually arises by new mutation.
From Twigg SRF, Wilkie AOM. A genetic-pathophysiological framework for craniosynostosis. *Am J Hum Genetics*. 2015;97:359–377. Table 1.

Chapter 102

Chromatin Regulatory Disorders

Kosuke Izumi

Pathogenic variants in genes that encode chromatin proteins that orchestrate gene expression in a range of human tissues frequently cause pediatric developmental disorders that include developmental delay/intellectual disability and facial dysmorphisms.

KABUKI SYNDROME

Kabuki syndrome (MIM: 147920 and 300867) is a genetic disorder characterized by facial features, mild to moderate intellectual disability, growth deficiency, and skeletal anomalies. Kabuki syndrome was originally named "Kabuki make-up syndrome" due to patients' facial features, suggestive of those of Kabuki actors (Fig. 102.1). Approximately 1:32,000 to 1:86,000 individuals are estimated to have Kabuki syndrome.

Molecular Etiology

Heterozygous *KMT2D* pathogenic variants (~90%), and hemizygous (in males) or heterozygous (in females) *KDM6A* pathogenic variants (~10%) cause classic Kabuki syndrome; *KMT2D* encodes a histone methyltransferase and *KDM6A* encodes a histone demethylase. Both of these histone modification enzymes regulate histone codes to orchestrate global gene expression. Most genetic changes causing Kabuki syndrome are due to de novo changes. However, because *KDM6A* locates on the X chromosome, its inheritance pattern can be complex, with most *KDM6A* pathogenic variants being de novo, but maternal transmission of pathogenic *KDM6A* variants has been seen. Kabuki-like syndromes share many clinical features but do not have pathogenic variants in *KMT2D* or *KDM6A*.

Clinical Features of Kabuki Syndrome (Table 102.1)

Physical features: Common features of Kabuki syndrome include long palpebral fissures with eversion of the lateral third of the lower eyelid, arched broad eyebrows, a short columella, depressed nasal tip, large, prominent or cupped ears, and persistent fingertip pads (see Fig. 102.1). Dental anomalies such as widely spaced teeth and hypodontia are commonly seen.

Growth and feeding: Individuals with Kabuki syndrome often have short stature and feeding difficulties. The degree of difficulty with feeding and growth varies, but it is not uncommon for infants or children with Kabuki syndrome to require feeding tubes.

Development and behavior: Global developmental delay is commonly seen in children with Kabuki syndrome, and individuals with Kabuki syndrome typically have mild to moderate intellectual disability. Most individuals speak and ambulate independently. Although infrequent, autism is seen in Kabuki syndrome.

Neurology: Hypotonia is a common feature. Structural brain differences are typically not seen, but individuals with Kabuki syndrome have an ~30% risk for seizures.

Congenital heart disease: Approximately 70% of children with Kabuki syndrome have a structural heart defect. The most common types include coarctation of the aorta and septal defects, although complex congenital heart disease is also seen.

Gastrointestinal: In addition to feeding difficulties, structural anomalies such as anal atresia, congenital diaphragmatic hernia (CDH), and cholestasis can be seen.

Endocrinology: Hypoglycemia or hyperinsulinemia can be seen, and some females with Kabuki syndrome have early premature thelarche.

Ophthalmology: The common everted lower eyelid seen in Kabuki can lead to excessive tearing. Other eye differences can include ptosis, cataracts, corneal differences, blue sclerae, or strabismus.

Ear, nose, and throat: Hearing loss, mainly conductive hearing loss, and more rarely sensorineural hearing loss, is seen in up to 50% of individuals with Kabuki syndrome. Chronic otitis media is a major cause of conductive hearing loss. Individuals with Kabuki syndrome can also have cleft lip and/or palate.

Genitourinary: Kidney and urinary tract disorders occur in more than 25% and can include abnormal renal location, duplicated collecting system, hypospadias and cryptorchidism in males, and hypoplastic labia in females.

Musculoskeletal: Many individuals have joint hypermobility that can manifest with scoliosis. Spinal abnormalities such as sagittal clefts and hemivertebrae can be seen in individuals with Kabuki syndrome.

Immunology and hematology: Frequent and recurrent infections including sinopulmonary infections and otitis media are common in children with Kabuki syndrome. Some can have hypogammaglobulinemia and immune dysfunction, with some needing regular intravenous immunoglobulin injection. Autoimmune disorders such as vitiligo, immune thrombocytopenia, hemolytic anemia, and coagulopathy are also common.

Molecular diagnosis of Kabuki syndrome: This is often made using sequencing and deletion/duplication analyses of *KMT2D* and *KDM6A* following clinical suspicion or by exome sequencing. Often, parental testing is performed to determine whether the identified variant is de novo. In addition, a DNA methylation profile has been identified for Kabuki syndrome caused by *KMT2D* and *KDM6A* variants, and a matching profile can support the diagnosis in the context of uncertain pathogenicity of identified *KMT2D* or *KDM6A* variants.

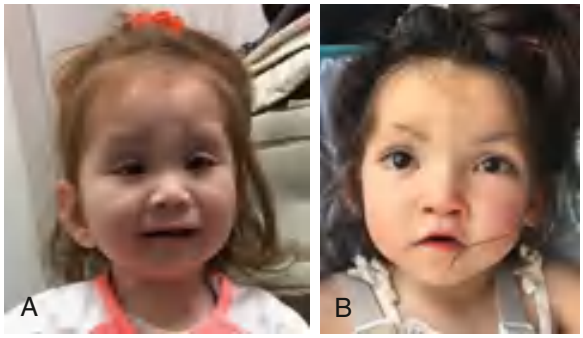


Fig. 102.1 Physical features of Kabuki syndrome. A, Patient with *KMT2D* c.10201C>T, p.Gln3401* pathogenic variant. Dysmorphic features include long and up-slanted palpebral fissures and large prominent ears. B, Patient with *KDM6A* c.3717G>A; p.Trp1239* pathogenic variant. Dysmorphic features include long and up-slanted palpebral fissures.

RUBINSTEIN-TAYBI SYNDROME

Rubinstein-Taybi syndrome (RSTS; MIM 180849 and 613684) is characterized by down-slanted palpebral fissures, broad thumb and first toe, developmental delay/intellectual disability, and short stature (Fig. 102.2). It occurs in ~1 in 125,000 births.

Molecular Etiology

RSTS is caused by heterozygous pathogenic variants in *CREBBP* and *EP300* genes. These genes encode transcriptional co-activators as histone lysine acetyltransferases. RSTS is caused by pathogenic variants, typically de novo in *CREBBP* in 55% and in *EP300* in ~8%. *CREBBP* pathogenic variants tend to result in more typical RSTS features, with *EP300* alterations resulting in a wider, often milder, phenotypic spectrum.

Table 102.1 Kabuki Phenotype Scoring List

CLINICAL FEATURES	POSSIBLE SCORE	FEATURES
Facial features	0-5 points (0-3 features = 1 point; 4-6 = 2 point; 7-9 = 3 point; 10-12 = 4 point; 13-15 = 5 point)	Long palpebral fissures; everted lower eyelids; large dysplastic ears; arched eyebrows, sparse lateral one third; flat nasal tip; abnormal dentition; high/cleft palate; strabismus; blue sclera; micrognathia; ptosis; broad nasal root; oligodontia; thin upper and full lower lip; lip nodules
Limb/extremity features	Up to 1 point (0-1 features = 0 point; 2-4 = 1 point)	Persistent fetal pads; brachy- or clinodactyly; lax joints; hip dislocation
Microcephaly	1 point	
Short stature	1 point	
Heart	1 point	
Kidney	1 point	
Sum	0-10	

From Wang YR, Xu NX, Wang J, Wang XM. Kabuki syndrome: review of the clinical features, diagnosis and epigenetic mechanisms. *World J Pediatr*. 2019;15:528–535. Table 1.

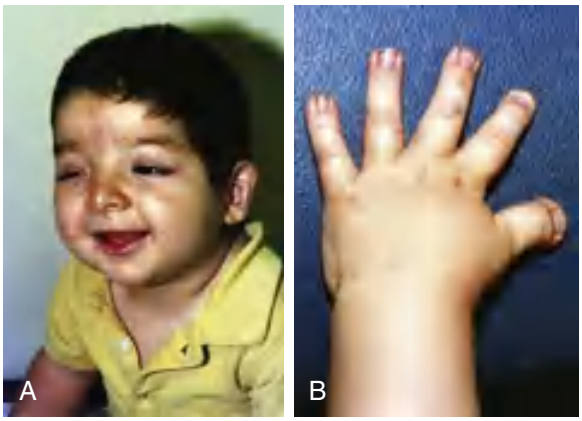


Fig. 102.2 Rubinstein-Taybi syndrome. A, A 21-month-old child. Note the hirsutism, down-slanting palpebral fissures, maxillary hypoplasia, prominent nose with nasal septum extending below alae nasi, and low, posteriorly rotated ears. B, Broad thumbs with radial angulation and persistent fingertip pads. *(A courtesy Dr. Marilyn C. Jones, Children's Hospital, San Diego, California; from Jones KL, Jones MC, Del Campo M, eds.* Smith's Recognizable Patterns of Human Malformation, *8th ed. Philadelphia: Elsevier; 2022, Fig. 1C, p. 108 and Fig. 2C, p. 109.)*

Clinical Features of RSTS

Physical features: Characteristic facial features include arched eyebrows, down-slanting palpebral fissures, low-hanging columella, and grimacing smile. Individuals often have broad thumbs and/or halluces.

Growth: Prenatal growth is usually normal, but most individuals have short stature caused by slow postnatal growth. Obesity may be seen during childhood or adolescence.

Development: Global developmental delay is typical with milder features often seen in individuals with *EP300* pathogenic variants. The average age of walking is 30 months and first words 25 months. Speech delay occurs in 90% of children and some remain nonverbal. Features of autism spectrum disorder, as well as impulsivity, distractibility, mood instability, and stereotypies can be seen.

Intellectual disability: This is in the moderate to severe range is typical for individuals with RSTS, with an IQ range of 25-79. Verbal ability is typically poorer than other skills.

Neurologic: Craniospinal and posterior fossa abnormalities, such as Chiari malformation or cervical cord compression, can be seen as well as seizures or abnormal electroencephalogram (EEG).

Eye findings: These can include refractory errors, ptosis, cataracts, coloboma, nystagmus, strabismus, and glaucoma.

Congenital heart defects: These are seen in ~30% of individuals with RSTS. Common defects include atrial septal defect, ventricular septal defect, patent ductus arteriosus, coarctation of the aorta, pulmonary stenosis, aortic stenosis, and vascular ring.

Respiratory: Obstructive sleep apnea is common.

Genitourinary: Kidney anomalies include hydronephrosis and duplications. Many males have cryptorchidism.

Gastrointestinal: Feeding problems due to gastroesophageal reflux and constipation are common.

Musculoskeletal: Joint laxity, scoliosis, and vertebral abnormalities can be seen. Hypotonia is common.

Molecular diagnosis: This can be made using a gene panel (sequencing and deletion/duplication analyses of *CREBBP* and *EP300*). For individuals with clinical features with atypical RSTS features, exome sequencing is recommended.

CORNELIA DE LANGE SYNDROME

Cornelia de Lange syndrome (CdLS; MIM 122470) is characterized by craniofacial features including synophrys, high-arched eyebrows and thin downturned upper lip, hirsutism, intellectual disability, microcephaly, growth retardation, limb anomalies, such as micromelia, phocomelia, and oligodactyly, and several other systemic abnormalities. Its population incidence is approximately 1 in 50,000.

Molecular Mechanism of CdLS

CdLS is caused by pathogenic variants in genes encoding the structural and regulatory components of the cohesin complex. Variants have been found in *NIPBL*, *HDAC8*, *RAD21*, *SMC1A,* and *SMC3* in CdLS. The cohesin complex is composed of SMC1A, SMC3, RAD21, and other subunits that comprise a ringlike structure that "embraces" chromatin. *NIPBL* encodes a protein that controls cohesin complex genome loading. The SMC3 component of the cohesin complex becomes acetylated once loaded onto the genome and is subsequently deacetylated by HDAC8 for cohesin protein recycling. Pathogenic variants in *BRD4* are an infrequent cause of CdLS. Cohesin has shown to play a key role in transcriptional regulation, and global transcriptional alterations due to cohesin dysfunction are thought to lead to the pathogenesis of CdLS.

NIPBL pathogenic variants are found in approximately 60% of the individuals with CdLS and are more likely to be identified in more severely (or "classically") affected individuals, with loss-of-function variants resulting in a more severe phenotype. Pathogenic variants in *SMC1A*, *SMC3, RAD21*, *HDAC8,* and *BRD4* can be found in 1–5% of the individuals with CdLS. Pathogenic variants in all related genes are typically acquired de novo, although instances of inherited variants from minimally affected mothers have been noted for the X-linked *HDAC8* and *SMC1A* genes.

Fig. 102.3 Physical features of Cornelia de Lange syndrome. **A**, Patient with *NIPBL* c.3100_3106del; p.K1034QfsX7 pathogenic variant. Dysmorphic features include synophrys, long eyelashes, upturned nasal tip with anteverted nares, long philtrum, and micrognathia. Severe reduction defects of upper extremities are also depicted. **B**, Patient with *SMC1A* c.2077C>G; p.R693G mutation. Dysmorphic features include mild synophrys.

Clinical Manifestations of CdLS

Facial features: Include synophrys with highly arched and/or thick eyebrows, long/thick eyelashes, short nasal bridge, upturned nasal tip with anteverted nares, long and/or smooth philtrum, thin vermilion of the upper lip, downturned corners of the mouth, highly arched palate with or without cleft palate, small widely spaced teeth, and micrognathia (Fig. 102.3).

Growth failure: Prenatal-onset growth failure and microcephaly are common.

Ophthalmologic: Common manifestations include ptosis, myopia, and nystagmus.

Otolaryngologic: Sensorineural hearing impairment is common, but conductive hearing loss can be seen.

Cardiovascular: Approximately 50% of individuals with CdLS have congenital heart disease. The most common abnormalities include pulmonic or peripheral pulmonic stenosis, ventricular septal defects, atrial septal defects, coarctation or hypoplastic aortic arch, aortic valve anomaly, tetralogy of Fallot, double-outlet right ventricle, and atrioventricular canal.

Gastrointestinal: Gastroesophageal reflux disease is present in nearly all instances, and some require a feeding tube during infancy/childhood. Other rare gastrointestinal abnormalities include pyloric stenosis (4%), intestinal malrotation (2%), and CDH (1%).

Genitourinary. Renal abnormalities such as vesicoureteral reflux and cryptorchidism are common.

Skeletal: Severe abnormalities of the upper extremities are seen in 25% of individuals with classical CdLS. Upper extremity deficiencies range from severe reduction defects such as complete absence of the forearms to mild fifth finger clinodactyly (see Fig. 102.3). Radioulnar synostosis is also common.

Skin: Hypertrichosis is common, and scalp hair, typically thick, can extend onto the temporal regions.

Developmental delay/intellectual disability: Most individuals with classical CdLS demonstrate global developmental delay. The overall range of IQ levels is broad, from below 30 to 85, with an average IQ of 53. Those affected individuals with classic features are more likely to have severe to profound intellectual disability. Fifty percent of children with CdLS walk by 24 months and 95% by 10 years old. A range of behavioral issues have been reported, and behavior problems are often directly related to frustration from an inability to communicate.

Neurologic: Approximately 25% of individuals with CdLS experience seizures.

Molecular Diagnosis of CdLS: Due to a relatively high frequency of mosaic variants not detected in blood, a next-generation sequencing panel of CdLS genes (*NIPBL, SMC1A, HDAC8, SMC3, RAD21,* and *BRD4*) performed on DNA extracted from buccal cells has been recommended as the most effective way of detecting causal variants. For individuals with atypical CdLS features, exome sequencing is recommended.

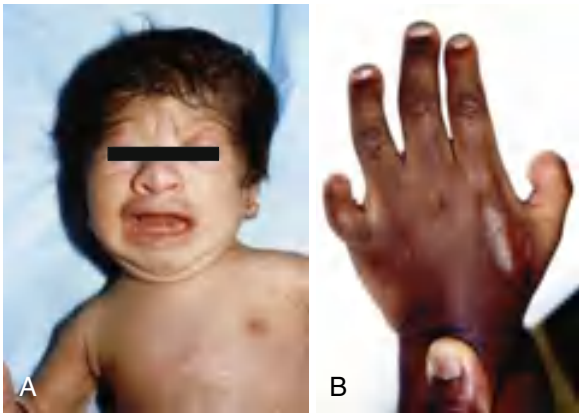


Fig. 102.4 Coffin-Siris syndrome. Note the coarse face and wide mouth with full lips (**A**), and hypoplastic fifth fingernails (**B**). *(From Jones KL, Jones MC, Del Campo M, eds.* Smith's Recognizable Patterns of Human Malformation, *8th ed. Philadelphia: Elsevier; 2022, Fig. 1A and 1D, p. 819.)*

COFFIN-SIRIS SYNDROME

Coffin-Siris syndrome (CSS) is characterized by thick eyebrows, periorbital fullness, wide mouth with full lips, and coarse facial features; fifth fingernail hypoplasia; absence of terminal phalanges; hypertrichosis; developmental delay/intellectual disability; and short stature (Fig. 102.4).

Molecular Mechanism of CSS

CSS is typically caused by de novo heterozygous germline pathogenic variants in genes encoding a component of BAF (BRG1/hBRM-associated factors) complex, which belongs to the SWI/SNF chromatin remodeler complex family. Pathogenic variants have been found in *ARID1A*, *ARID1B*, *SMARCA4*, *SMARCB1*, *SMARCE1,* and *SOX11*. Variants in one of these genes can be found in ~60–70% of the patients with CSS.

Clinical Features of CSS

Facial features: Characteristic facial features of CSS include wide mouth with thick, everted upper and lower lips, broad nasal bridge with broad nasal tip, thick eyebrows, and long eyelashes. Facial features typically coarsen over time.

Growth: Prenatal growth profile tends to be normal, but postnatal weight and height measurements are usually below the 50th percentile.

Development: Global developmental delay is common and variable; children sit around 12 months, walk at around 30 months, and say their first words at 24 months. Most individuals have intellectual disability, typically moderate to severe (IQ range: 40-69). Some individuals with CSS have behavioral concerns, aggression, or autistic features.

Neurologic: The majority of children with CSS have hypotonia. Structural brain malformations such as Dandy-Walker variant, and

agenesis of the corpus callosum may be seen. Seizures occur in up to 50% of individuals. Hearing impairment can occur.

Ophthalmology: Visual impairment is common, and ocular abnormalities include ptosis, strabismus and myopia.

Gastrointestinal: Feeding issues and slow growth are common.

Genitourinary: Renal malformation such as horseshoe kidney, and genitourinary malformation such as cryptorchidism and hypospadias have been noted.

Musculoskeletal: Fifth digit nail and distal phalanx hypoplasia or aplasia, a diagnostic criterion in the pre-exome era, is commonly seen. Brachydactyly of the fifth finger, joint laxity, and scoliosis are also frequent.

Cardiac: Congenital heart defects can be seen and include ventricular septal defects, atrial septal defects, and tetralogy of Fallot.

Skin: Hirsutism and hypertrichosis are typical.

Molecular Diagnosis of CSS

For those whom clinical diagnosis of CSS is strongly suspected, CSS gene sequencing and deletion/duplication panel is recommended. For those with atypical clinical features, comprehensive genetic testing such as exome sequencing is warranted.

OTHER CHROMATIN DISORDERS

An increasing number of genetic disorders, due to mutations in genes encoding chromatin transcriptional regulatory proteins, have been identified. These disorders include CHARGE syndrome due to *CHD7* pathogenic variants, Wiedemann-Steiner syndrome due to *KMT2A* pathogenic variants, Arboleda-Tham syndrome due to *KAT6A* pathogenic variants, and Say-Barber-Biesecker/Young-Simpson syndrome due to *KAT6B* pathogenic variants. CHD7 is a chromatin remodeler protein with a chromodomain histone-modification recognition motif. *KAT6A* and *KAT6B* encode histone modification enzymes. Common manifestations of genetic diagnoses due to these chromatin remodelers and histone modification enzyme defects include developmental delay, hypotonia, and facial dysmorphisms. Some of the clinical features enable astute clinicians to distinguish between typical cases of these syndromes. However, in their atypical presentations, a clinical diagnosis may not be feasible. Exome sequencing is recommended in any children with facial dysmorphisms as well as developmental delay to detect possible chromatin disorders.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.

Chapter 103
Genetics of Common Disorders

Neil A. Hanchard and Brendan Lee

Common pediatric diseases, like many common adult-onset diseases, are usually multifactorial. The combination of many genes and environmental factors contribute to a complex sequence of events leading to disease. The complexity of the combination of contributing factors increases the challenge of finding genetic variants that cause disease. Genetic tools include public databases of genetic variants and the human haplotype map. Genotyping has permitted very large numbers of common genetic variants (those with population frequencies >5%) to be efficiently tested in large numbers of patients. Genetic sequencing technologies are being used to investigate the role of rare coding sequence variants in common diseases. The incorporation of these tools into large, well-designed population studies has developed into the field of **genetic epidemiology**.

103.1 Major Genetic Approaches to the Study of Common Pediatric Disorders

Neil A. Hanchard and Brendan Lee

Millions of genetic variants are present in every person. Many of these variants have no known impact on health, while others have a measurable influence. Sometimes, sequence changes in a single gene consistently cause a disease, as seen with cystic fibrosis and sickle cell anemia; more often, specific genetic changes are insufficient to determine the manifestation of a specific medical condition by themselves. Rather, these changes can contribute indirectly or partially to the development of disease, and these are best conceptualized as *modifiers* of disease risk. Figure 103.1 demonstrates the relationship between variant frequency and the relative medical impact of the allele. The spectrum of variant impact ranges widely from a slightly increased risk of illness to predetermined, fully expressed disease. Studies aimed at discovering *rare* variants with strong health effects require only small sample populations to achieve statistical significance, whereas those studying *common* variants that contribute partially to the overall effect often require much larger sample sizes because of the small impact of multiple variants.

In many cases, genetic susceptibility is the result of the cumulative risk of many common variants. For common conditions, the genetic predisposition alone is not sufficient to cause disease. Everyone inherits a different degree of disease vulnerability, which is then augmented by exposure to certain environmental or other factors. Figure 103.2 is a model for the contribution of common genetic variants to individual health. One goal of medical genetics is to identify the genes that contribute to initial genetic susceptibility and help prevent the occurrence of disease, either by avoiding inciting environmental factors or by instituting interventions that reduce risk. For persons who cross the threshold of disease, the goal is to better understand the pathogenesis in the hope that this will suggest better approaches to treatment. Common genetic variation can also influence responses to medications and the risk of adverse drug reactions, as well as augments the impact of environmental exposures.

Complex traits may be inherently difficult to study if the precision of diagnosis **(phenotype)** is low, as often occurs with neurobehavioral traits. A starting point in the genetic analysis of a complex trait, therefore, is to obtain evidence in support of a genetic contribution and to estimate the relative strength of genetic and environmental factors. Complex traits typically exhibit familial clustering but are not transmitted in a distinct pattern as seen with classical mendelian autosomal dominant or recessive inheritance. Complex traits often show variation between groups of different ancestries, possibly reflecting the differences in frequency and/or effect of genetic variants within these groups.

Assessing the potential genetic contribution begins by determining whether the trait is seen among related individuals more often than in the general population. A common measure of **familiality** is the first-degree relative risk (usually designated by the symbol λ_s), which is equal to the ratio of the prevalence rate in siblings and/or parents to the prevalence rate in the general population. The λ_s for type 1 diabetes is about 15. The relative strength of genetic and nongenetic risk factors can be estimated by variance components analysis. The **heritability** of a trait is the estimate of the fraction of the total variance contributed by genetic factors (Fig. 103.3).

A minority of cases of common diseases such as diabetes may be caused by single-gene pathogenic variants (mendelian inheritance), chromosomal disorders, and other genomic disorders (see Chapter 629.4). However, these *unique* causes of the disease can provide important insight into the molecular pathways involved. Chromosomal regions with genes that might contribute to disease susceptibility have historically been located with **linkage mapping**, which locates regions

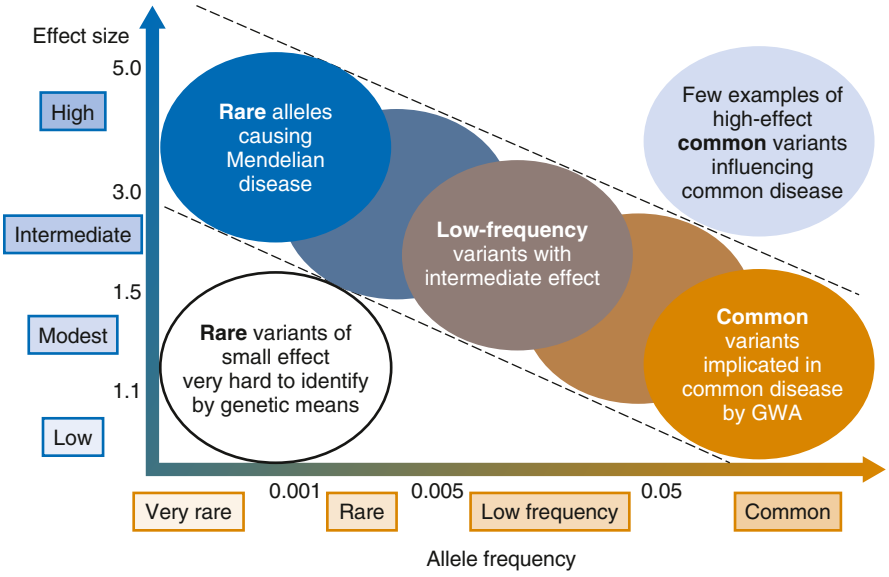


Fig. 103.1 Relationship between allele frequency and relative strength of genetic effect. Alleles with large effect tend to be very rare but can be studied with a small sample size because of the relative ease of allele detection when medical impact is high. Common variants tend to have a modest or low effect on health, requiring large datasets to visualize statistically small effects. The vast majority of disease-associated alleles identified to date have the characteristics shown within the *diagonal dotted lines*. GWA, Genome-wide association. *(Adapted from McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet. 2008;9:356–369.)*

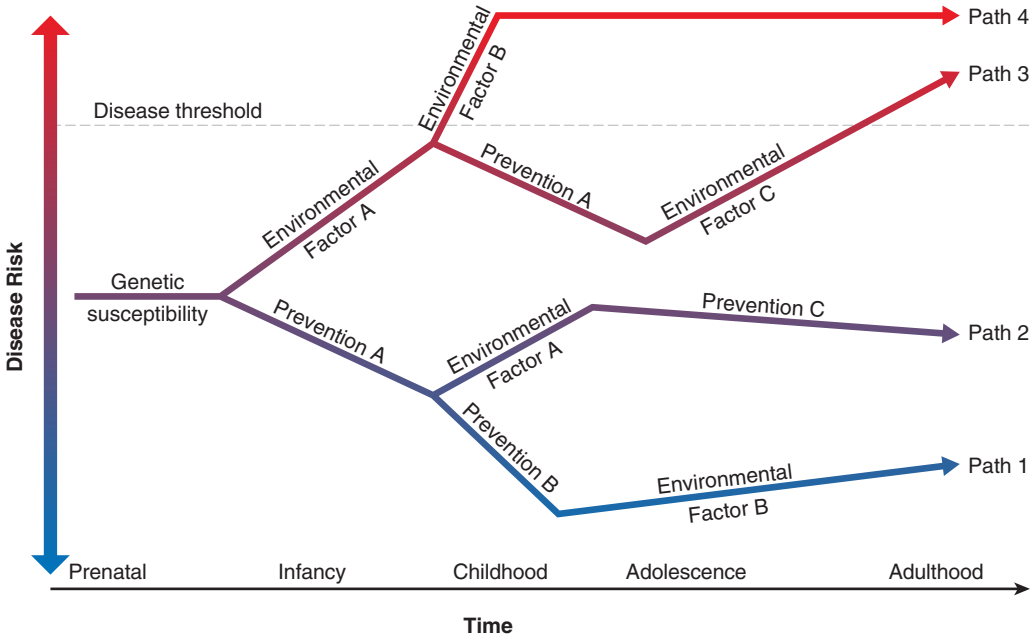


Fig. 103.2 Model for the influence of gene-environment interaction on genetic susceptibility to common diseases. Everyone inherits common variants that determine initial genetic liability for disease risk. For multifactorial disorders, the initial genetic susceptibility is insufficient to produce disease on its own. Over time, exposure to environmental factors increases the likelihood of a disease state. Identifying the gene variants responsible for risk can lead to prevention strategies or treatments.

Phenotypic variance		Genetic variance		Environmental variance		Measurement variance
V_P	=	V_G	+	V_E	+	V_M

$$h^2 = V_G / V_P$$

Heritability

Fig. 103.3 Heritability concept. The phenotypic variance of a particular trait can be partitioned between the contributions of the genetic variance, environmental variance, and measurement variance. This is usually empirically determined. Heritability is defined by the proportion of the phenotypic variance that is accounted for by the genetic variance. One can estimate the heritability from correlation of a quantitative trait between relatives.

of DNA that are inherited in families with the specific disease. In practical terms, however, this has become quite difficult for most complex traits either because of a dearth of families or because the effects of individual genetic loci are weak; thus linkage mapping is seldom used anymore.

Genetic **association studies** are a powerful way of identifying common gene variants (>5% in the population) that each have an effect on the risk of disease. Detection of the usually small to moderate effect of each variant alongside interactions with environmental factors requires well-powered studies that often include thousands of individuals. A number of parallel approaches for analyzing the aggregate effects of rare variants in genes have also been developed. Such rare variant association methods also require large sample sizes because the gene effects also appear to be relatively weak.

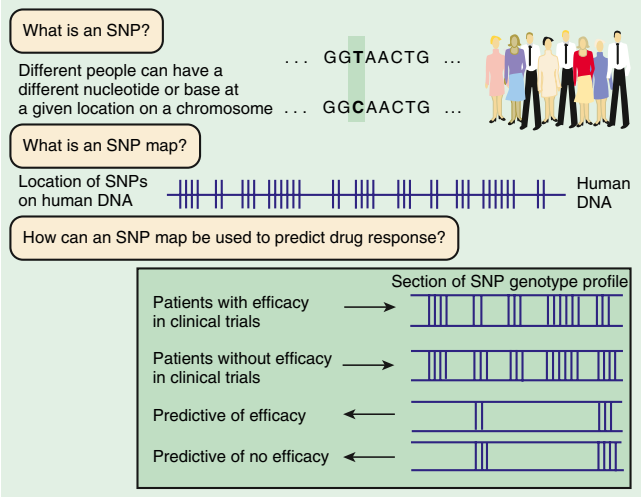


Fig. 103.4 Different combinations of single nucleotide polymorphisms (SNPs) are found in different individuals. The locations of these SNPs can be pinpointed on maps of human genes. Subsequently, they can be used to create profiles that are associated with difference in response to a drug, such as efficacy and nonefficacy. *(Adapted from Roses A. Pharmacogenetics and the practice of medicine. Nature. 2000;405:857–865.)*

Association studies require markers along the DNA that can be **genotyped** (ascertaining the combination of alleles at a locus in a diploid organism), typically with large-scale, high-throughput laboratory techniques. Markers that are typically used are **single nucleotide polymorphisms** (**SNPs**; Fig. 103.4). A sample of the same region of genome from 50 people will reveal that approximately 1 in every 200 bases varies between individuals. Catalogues of SNPs identified in thousands of individuals from multiple populations consist of tens of millions of SNPs across the genome (dbSNP; https://www.ncbi.nlm.nih.gov/snp/). Although most SNPs lack an obvious function, some will alter the amino acid sequence of the protein, and many have been shown to regulate the expression of nearby or distant genes (https://gtexportal.org/home/) or indirectly impact expression by altering the level of surrounding DNA methylation or the ability of proteins to act *(The ENCODE Project Consortium)*. Some of these functional alterations can directly affect susceptibility to disease.

A complex clinical **phenotype** can be defined by the presence or absence of a disease as a **dichotomous trait**, or by selection of a clinically meaningful continuous or **quantitative trait**, such as serum glucose in type 2 diabetes. Although it might not be possible to define subgroups of patients in advance based on disease mechanisms, the more uniform the phenotype, the more likely that a genetic study will be informative. **Locus heterogeneity** refers to the situation in which a similar trait results from alteration of different genes. **Allelic heterogeneity** is when more than one variant in a particular gene can contribute to different diseases. The development of a trait or disease from a nongenetic mechanism results in a **phenocopy**. These three factors often contribute to the difficulty in identifying individual disease susceptibility genes, because they reduce the effective size of the study population and thus require even larger sample sizes.

For genetic variants that directly lead to disease, a person bearing any variant or **allele** (inherited unit, DNA segment, or chromosome) in a gene has a given probability of being affected with a specific gene variant–associated disease. This is called the **penetrance**. Some diseases manifest signs only later in life **(age-related penetrance)**, which could lead to misclassifying children with the disease-producing variant to be classified as unaffected. Single-gene disorders are typically caused by pathogenic variants with relatively high penetrance. In contrast, most common variants have low penetrance because their overall contribution to the disease is small. Many such common variants can contribute to disease risk for a complex trait; for example, human height is influenced by >400 genes.

Ideally, important environmental exposures should be measured and accounted for in a population because there may be a dependent interaction between the environmental factor and specific genetic variant. An example is the likely requirement for a viral infection preceding onset of type 1 diabetes. Although **gene-environment interactions** are strongly suspected to play an important role in common diseases, it is difficult to identify and measure them. Very large studies with uniform collection of information about environmental exposures are rare, but are becoming more common with the expansion of large biorepositories that are integrated with electronic health records. Methods, such as genome-wide analyses of DNA methylation, sometimes referred to as epigenome-wide association studies, have been used to assess evidence for early environmental exposures that influence common diseases in later life. This apparent relationship between the environment and DNA methylation (and related genetic features) is being used to discover and validate other possible gene-environment interactions.

GENOME-WIDE APPROACHES TO ASSOCIATION

For multifactorial common diseases, association analyses have been used to identify disease-related genes. There are two types of association study: **direct association**, in which the risk-altering variant itself is tested to see whether its presence correlates with disease, and **indirect association**, in which markers that are physically close to the biologically important variant are used as proxies. The correlation of markers with other genetic variants in a small region of the genome is called **linkage disequilibrium**. Indirect association is enabled by the construction of linkage disequilibrium maps in continental reference populations (e.g., Europeans, Asians, West Africans) (International HapMap Project). SNPs that proxy most genetic variation have been identified across most of the genome and can be genotyped at low cost using specially designed microarrays that typically include 2-5 million genetic markers. This resolution provides a good proxy of genetic variation in relatively homogenous populations where linkage disequilibrium is generally high (such as Europeans), thus facilitating "genome-wide" testing of variants in those populations.

Three basic study designs are used for association testing:

1. **Case-control design:** the frequency of an allele in an affected group is compared with an unaffected group. This is the mostly commonly used framework for association testing, as it aligns with many more traditional epidemiologic study designs.
2. **Cohort design:** large numbers of people are ascertained and then followed for the onset of any number of diseases. Cohort analysis is very expensive, and there are few true cohort studies. However, investments in genomics of large-scale population-based cohorts in Iceland *(https://www.decode.com/)*, the United Kingdom *(https://www.ukbiobank.ac.uk/)*, and the United States *(https://allofus.nih.gov/)* include hundreds of thousands of individuals all tied to electronic health records and have begun to show unique insights to disease risk and pathogenesis.
3. **Family-based control design:** parents or siblings of an affected individual are used as the controls. Family-based control study designs are somewhat attractive for pediatric diseases because it is usually possible to enroll parents. These studies also solve a major problem in testing for association because the parents are perfectly matched for genetic background; however, family-based study designs are inherently less efficient to recruit than case-control studies and are used less commonly.

The success of any association analysis depends on the design of a well-powered study and an accurately measured trait to avoid phenotypic misclassification. In large, population-based studies, confounding by ethnicity or **population stratification,** could distort results. Variants more common in people from a particular ancestry group could cause an apparent association of a variant with a disease. This association would not be a true association between an allele and a disease, because the association would be confounded by genetic background. Methods for measuring subtle mismatching between cases and controls using many thousands of markers routinely genotyped

in genome-wide association studies allow researchers to account for this effect. The implementation of stringent thresholds for statistical significance replicate associations in other groups have helped make genome-wide association studies robust and reproducible.

Association studies are a powerful tool to find genetic variation that confers risk to an individual; however, the effect of any single genetic variant will be a small contribution to the overall risk and pathogenesis of disease. Hundreds of thousands of genetic associations between a variant or gene and disease, such as the *APOE*ε4 allele with an increased risk of Alzheimer disease, have now been observed for thousands of complex diseases and traits, including autoimmune disease, asthma, and bone density/fractures. Genetic variants that implicate a novel gene in a process drive research into systems and pathways that can alter disease outcome. With increasingly large datasets and advances in statistical analyses, all the genetic variants that impact common disease risk can also be combined to derive a composite genetic risk score. This score can then be integrated with known environmental risk factors to identify individuals at particularly high risk of disease who might require more urgent intervention or monitoring. In adults, this approach is being developed to identify individuals at high risk for coronary heart disease, although concerns about how well genetic risk scores work in different population groups are yet to be overcome.

Low-cost methods for sequencing the complete coding (exomes) *and* full DNA sequence (genomes) of individuals have facilitated comprehensive evaluation of a wider range of genetic variants involved in common diseases. Rare genetic variants, including small insertions or deletions, are important in pediatric diseases such as neurodevelopmental disorders, cardiovascular malformations, and other birth defects. Common traits such as height, obesity, diabetes, and autoimmune diseases are also affected by rare variants. In common severe disorders such as intellectual disability and complex heart malformations, de novo pathogenic variants (i.e., pathogenic variants not present in either parent) play an important role.

Visit Elsevier eBooks+ at eBooks.Health.Elsevier.com for Bibliography.