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This Technology Assessment was developed by the American College of Obstetricians and Gynecologists' Committee on Genetics in collaboration with committee members Neeta L. Vora, MD and Steven J. Ralston, MD.

Modern Genetics in Obstetrics and Gynecology

ABSTRACT: *Knowledge of human genetics has increased dramatically, and obstetrician–gynecologists and other health care providers are increasingly called on to incorporate genetics and genetic testing into medical practice. Advances in our understanding of the molecular basis of inherited disorders have led to the development of DNA-based tests that can be used for prenatal and postnatal diagnosis, carrier testing, and aneuploidy screening. These techniques have allowed for diagnosis of a wide variety of genetic diseases ranging from aneuploidies to single-gene disorders. Once a specific pathogenic variant in a gene has been identified, direct testing for that specific variant is possible; this is the most accurate molecular diagnostic method. Counseling patients about genetic testing and results can be challenging and obstetrician–gynecologists and women's health care providers are encouraged to make use of and refer to genetics professionals when necessary. The correct interpretation of molecular genetic diagnostic testing is highly dependent on an accurate clinical diagnosis, test sensitivity, ethnic variability in variants and disease prevalence,* genetic heterogeneity*, reduced penetrance, and phenotypical variability. As genetics becomes a more integral part of routine medical practice, it is essential that obstetrician–gynecologists and other health care providers be aware of advances in the understanding of genetic disease and the fundamental principles of genetic screening and molecular testing. This technology assessment has been revised to reflect advances in clinical genetics related to obstetrics and gynecology, including epigenetics, sequencing, and testing.*

Introduction

Genetics is the study of heredity and variation of inherited characteristics. Knowledge of human genetics has increased dramatically, and obstetrician–gynecologists and other health care providers are increasingly called on to incorporate genetics and genetic testing into medical practice. The Human Genome Project, the first identification and mapping of the full human DNA sequence, was completed in 2003. This project produced a detailed map of the *genes* (a DNA region that encodes a polypeptide chain or protein), markers, and other landmarks along each chromosome. Current and potential benefits of the Human Genome Project and the field of human *genomics* (the study of the interaction of an individual's genes with each other and with the environment) include the development of genetic screening and diagnostic tests and novel preventive therapies, technologies, and strategies. (See the "Glossary" section for definitions of terms used in genetics and molecular diagnostic testing.) The goal of this document is to provide obstetrician–gynecologists and other women's health care providers with a broad overview of genetic concepts and diagnostic techniques; it has been updated from a previous version to account for recent innovations and discoveries in the field of genetics. Counseling patients about genetic testing and results can be challenging and obstetrician–gynecologists and women's health care providers are

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encouraged to make use of and refer to genetics professionals when necessary. For additional information on genetic counseling, see ACOG Committee Opinion No. 693, *Counseling About Genetic Testing and Communication of Genetic Test Results*.

Organization of the Genome

The DNA in each human nucleus includes approximately 25,000–30,000 genes. If stretched out it would be more than 2 meters in length. The DNA is compactly wrapped around histones to form nucleosomes, which are then organized into solenoid structures and looped around a nonhistone protein scaffold to form *chromatin* (see Fig. 1). As the cell enters prophase, the chromatin condenses until it assumes the familiar structure of metaphase chromosomes. Each chromosome is composed of densely packed nontranscribed DNA near the centromeres, called *heterochromatin*, and less densely packed transcribed DNA called euchromatin (see Fig. 2).

Seventy-five percent of the *genome* is unique, single-copy DNA, and the remainder consists of various classes of repetitive DNA. Almost none of the repetitive DNA and only a small portion of the single-copy DNA code for recognized proteins. The single-copy DNA contains all of the genes necessary to make and sustain the organism. The repetitive DNA sequences are likely important in regulatory functions that have not yet been fully described. These regions also contain unique markers that identify each individual and can be used to study genetic variation between individuals.

Genes, Alleles, and Polymorphisms

A gene is a unique series of four purine and pyrimidine bases that ultimately specifies an amino acid sequence for a polypeptide chain of a protein. The basic gene contains one or more encoding *exons*, which are DNA sequences that will be transcribed into messenger RNA (mRNA), and *introns*, which are noncoding or nontranscribed intervening sequences that separate exons (see Fig. 3). Approximately 1.5% of the genome is composed of exons, collectively called the *exome*. The regions upstream and downstream to the exons are called the 5′-untranslated region and the 3′-untranslated region, respectively. These regions are transcribed, but not translated, and are important in gene regulation.

The processes of *transcription* and *translation* are more complex than previously recognized. The transcription of DNA to mRNA requires *enhancer DNA sequences* and *silencer (repressor) DNA sequences*

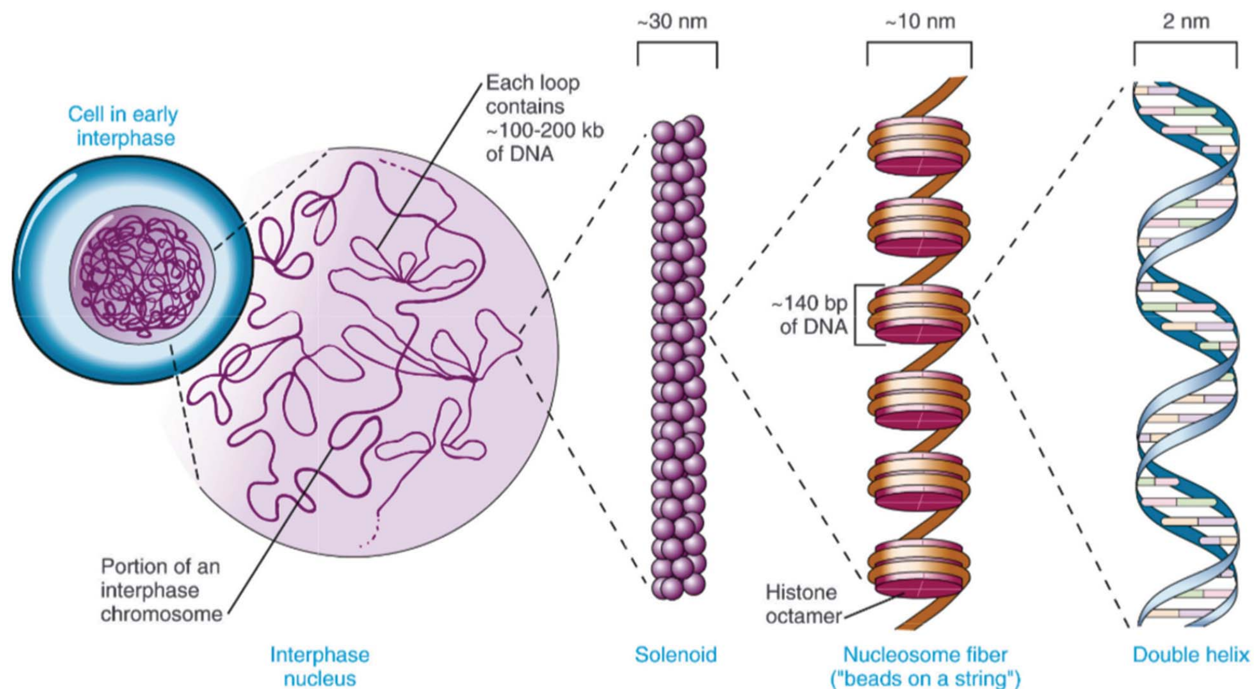

Figure 1. Solenoid structures. Hierarchical levels of chromatin packaging in a human chromosome. (Reprinted from Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson genetics in medicine. 8th ed. Philadelphia [PA]: Elsevier; 2016.)

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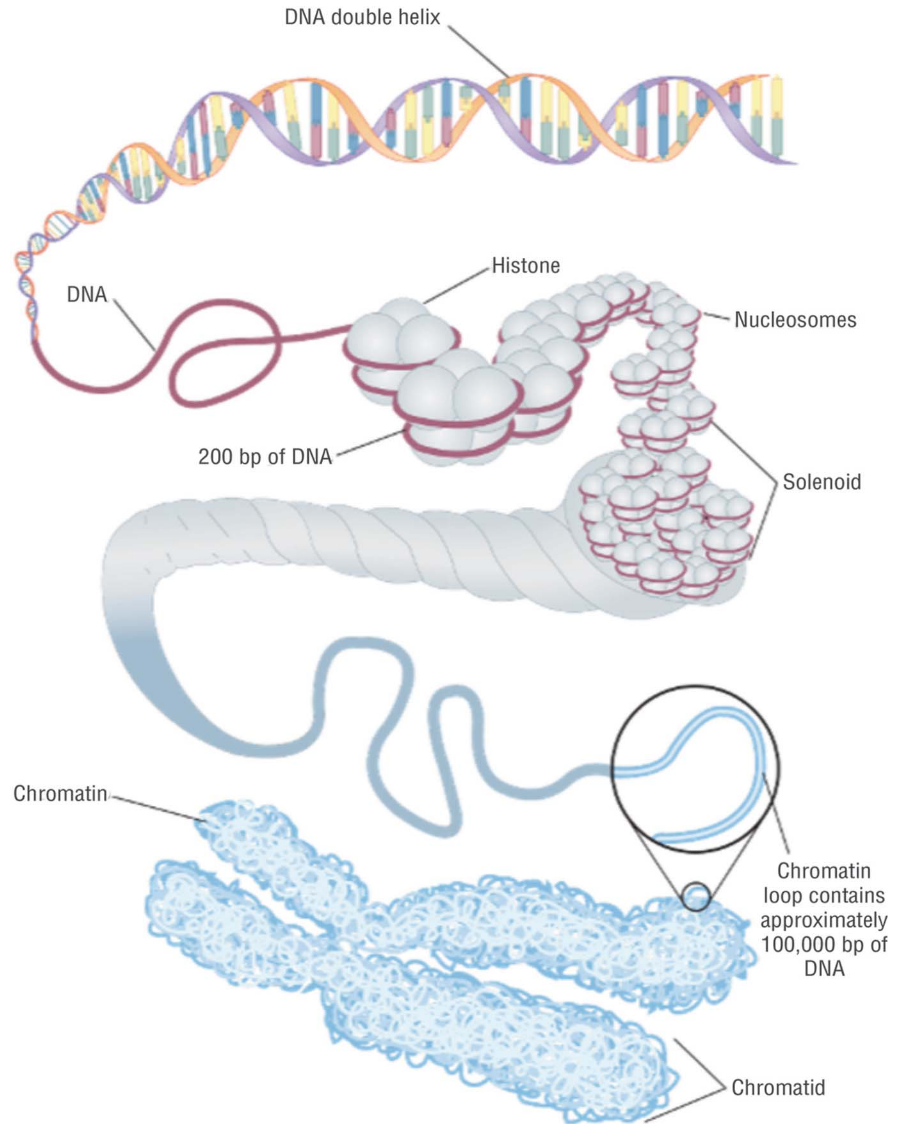

Figure 2. Chromatin. Patterns of DNA coiling. DNA is wound around histones to form nucleosomes. These are organized into solenoids, which in turn make up the chromatin loops. (Reprinted from Jorde LB, Carey JC, Bamshad MJ. Medical genetics. 5th ed. Philadelphia [PA]: Elsevier; 2016.)

located upstream or downstream of a gene that can increase and decrease transcriptional activity of the gene. Transcription is initiated when the enzyme RNA polymerase II binds to the promoter, a DNA sequence upstream of a gene. RNA polymerase moves along a single strand of DNA and forms a complementary strand of mRNA. Once the mRNA is formed, a cap, a chemically modified guanine *nucleotide,* is added to the 5′ end to prevent the mRNA from being degraded, and a chain of 100–200 adenine bases (poly-A tail) is added to the 3′ end. Before the mRNA leaves the nucleus, the introns are excised by a process called splicing. The mature mRNA then moves to the ribosomes for translation. The protein product usually is created in a precursor form that undergoes cleavage to form a smaller active protein or combines with other polypeptides to form a larger functional protein. The protein may be further modified in the Golgi apparatus of the cell by glycosylation. Some genes contain more than one promoter in different parts of the gene or have alternative splice sites, which allow the same gene to encode different protein products.

Gene expression also is controlled by other, so-called *epigenetic* mechanisms, including *DNA methylation* and *histone modification*. Epigenetic processes alter the expression of a gene without changing its underlying DNA sequence. Methylation of the regulatory region prevents gene transcription and effectively turns the gene off. This process serves the important function of controlling tissue-specific gene activation; the expression of genes that are not essential for a specific tissue type is prevented, whereas necessary genes

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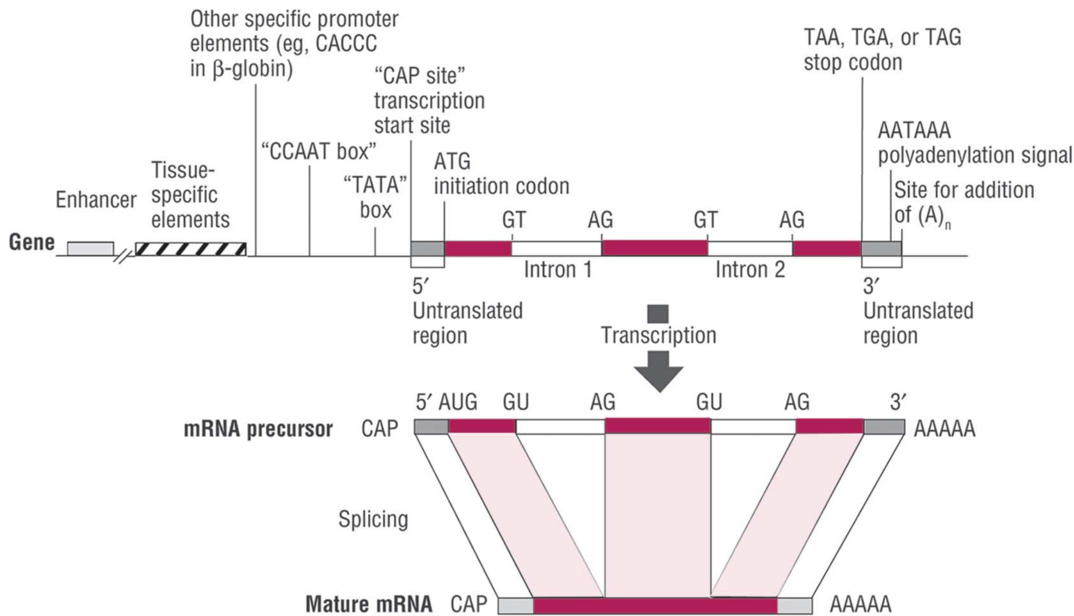

Figure 3. Structure of a typical gene. In this example, the gene has three exons (solid dark regions) and two introns (white regions), a 5′ untranslated region and a 3′ untranslated region. The intron splice donor site (GT) and the splice acceptor site (AG) identify where introns are removed in messenger RNA production. The upstream region (5′) contains an enhancer, tissue specific elements, and promoter elements, such as the CCAAT box and TATA box, which regulate expression. Typical locations for the translation initiation site (ATG), translation stop codons (TAA, TAG, TGA), and the polyadenylation signal (AATAA) are shown. (Reprinted from Gelehrter TD, Collins FS, Ginsburg D. Principles of medical genetics. 2nd ed. Baltimore [MD]: Williams & Wilkins; 1998.)

remain active. For example, the globin genes are methylated in all nonerythroid tissues but are not methylated in reticulocytes, which ensure that only red blood cells produce hemoglobin (Hb). Methylation also allows temporal control of gene expression. *Alleles*, alternate forms of genes, such as those that may be found on the same place on each of the two inherited chromosomes, may demonstrate different patterns of methylation that are not tissue specific depending on parent-of-origin (see also the "Imprinting" and "Epigenetics" sections later in this document). In addition, gene expression can be activated or suppressed through histone modification. Specific enzymes can acetylate, phosphorylate, methylate, or ubiquitinate histones, which will in turn alter gene expression, DNA repair mechanisms, and chromosomal condensation.

Alleles are normal variants within the population (see Table 1). For example, the genes that determine blood type and Rhesus factor (Rh) status have several normal alleles. In contrast, a pathogenic variant (previously referred to as a mutation) is an alteration in the DNA sequence resulting in a change in protein structure or function that may have adverse effects. The American College of Medical Genetics and Genomics has put out a new classification scheme for variants (pathogenic, likely pathogenic, benign, likely benign, uncertain significance) and henceforth, this current terminology will be used in this document (1). The distinction between an allele variant and a pathogenic variant can be blurred; many benign alleles probably result from ancient DNA variants that either did not affect survival or conferred some selective advantage. Pathogenic variants can occur in the germline (eg, gametes) and can be transmitted from one generation

Table 1. Examples of Alleles That Cause Different Blood Types

Genotype	Blood Type	Antibodies Present
I^AI^A	A	Anti-B
I^AI^O	A	Anti-B
I^BI^B	B	Anti-A
I^BI^O	B	Anti-A
I^AI^B	AB	None
I^OI^O	O	Anti-A and anti-B

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to the next or may occur only in the somatic cells and be associated with cancer. Many types of variants exist, such as single purine or pyrimidine base substitutions (*missense* and *truncating/nonsense variants*), *deletions* and *insertions* of one or more bases, and large chromosome deletions and duplications visible on metaphase karyotype.

Most DNA (more than 99%) is identical among all humans. The small differences in our DNA are called *polymorphisms* and generally consist of *single nucleotide polymorphisms*, variations (deletions or duplications) that occur every 200–500 base pairs. Some polymorphisms may modify gene function, but in most cases, polymorphisms are benign variants and do not cause disease (see Box 1). When newly discovered genes are analyzed, it can be difficult to distinguish a normal allele from a polymorphism or a pathogenic variant. For example, more than 100 variations of the *BRCA 1* gene, one of the major genes associated with familial breast and ovarian cancer, have been identified. Currently, it is not known whether all of these variations are actually pathogenic or if some are benign polymorphisms. When an identified DNA change cannot be characterized reliably as benign or pathogenic, it is referred to as a *variant of uncertain significance*.

Mendelian (Single-Gene) Disorder Patterns of Inheritance

In general, a mendelian trait or disease (named for Gregor Mendel, the father of modern genetics) is determined by a single gene. Diseases caused solely by abnormalities in a single gene are relatively rare. The *phenotype* of many single-gene disorders is influenced by modifying genes, or by the independent actions of a combination of additional genes, often with environmental influences.

Box 1. Define Gene, Allele, and Polymorphism

Gene: A unit of heredity responsible for the inheritance of a specific trait that occupies a fixed chromosomal site and corresponds to a sequence of nucleotides along a DNA molecule.

Allele: Alternative form of a gene; a single allele for each locus is inherited from each parent.

Polymorphisms: The occurrence in the same population of more than one allele or genetic marker at the same locus.

Autosomal Dominant

All autosomal genes come in pairs; one copy of the gene is present on each member of a chromosome pair. The influence of each gene in determining the phenotype is described as dominant or recessive. If one of the genes in a pair specifies the phenotype in preference to the other gene, that gene and the trait or disease that it specifies are considered to be dominant. The individual with a dominant gene that causes an *autosomal dominant* trait or disease has a 50% chance of passing on the affected gene with each pregnancy (Fig. 4). One of the hallmarks of autosomal dominant disorders is that they can be inherited by either sex and there is male-to-male transmission.

The phenotype of an individual with a dominant gene pathogenic variant is determined by several factors. One factor is *penetrance*, which indicates whether or not the mutant gene is expressed. If a dominant gene produces a recognizable phenotypic expression in all individuals who carry it, it is said to have complete penetrance. A gene that is not fully expressed in all individuals who have it has incomplete penetrance. For individuals, penetrance is an all-or-nothing phenomenon, but penetrance in a population varies and can be quantified. For example, a phenotype that is expressed in 80% of individuals who carry the gene has 80% penetrance.

Incomplete penetrance may account for some autosomal dominant diseases that appear to "skip" generations. Alternatively, the individual that carries the dominant gene pathogenic variant may have mild phenotypic abnormalities that have been overlooked, the disease may have a late onset, the gene *carrier* may be too young or may have died before the gene's effects were manifested, or phenotypic expression of the dominant gene may require the presence of a second mutated gene or certain epigenetic phenomena. For example, up to 85% of women with a *BRCA 1* pathogenic variant develop breast cancer during their lifetime; therefore, the finding of a *BRCA 1* pathogenic variant indicates a strong predisposition to breast cancer, but it does not indicate which women with the pathogenic variant will develop a malignancy or at what age.

The degree to which a penetrant gene is expressed (the range of phenotypic features) is called *expressivity*. If individuals who carry the affected gene do not all have identical phenotypes, the gene is said to have variable expressivity. Many such genes produce a range of phenotypic features from mild to severe. An example of a disease with variable expressivity is neurofibromatosis, in which some individuals present

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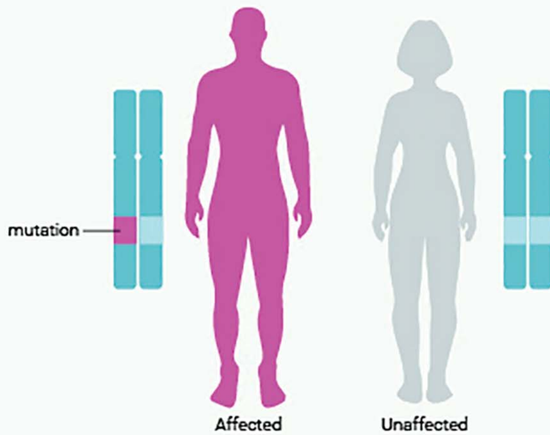

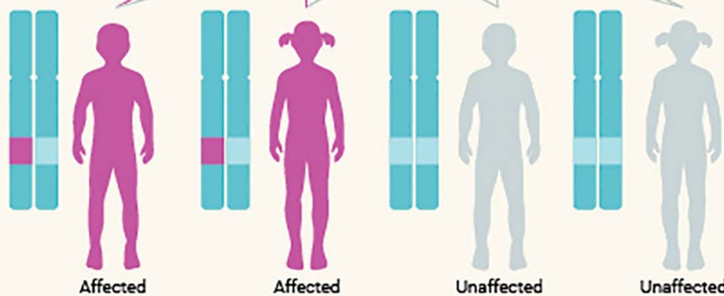

Figure 4. Autosomal dominant inheritance. An individual affected by an autosomal dominant disorder has a 50% chance of passing the mutated gene to each offspring. The chance that an offspring will not inherit the mutated gene also is 50%. (Reprinted from U.S. National Library of Medicine, Genetics Home Reference. If a genetic disorder runs in my family, what are the chances that my children will have the condition? Bethesda [MD]: NLM; 2018. Available at: https://ghr.nlm.nih.gov/primer/inheritance/riskassessment. Retrieved June 22, 2018.)

with skin manifestations but others have central nervous system tumors.

Autosomal Recessive

Autosomal recessive traits or diseases occur only when both copies of the gene in question contain pathogenic variants (Fig. 5). Individuals who have only one abnormal gene (heterozygotes, also known as carriers) may have some phenotypic alteration recognized at the biochemical or cellular level, but only individuals who have two copies of the affected gene (homozygotes) have the disease. Many diseases that involve enzyme deficiency are autosomal recessive. The enzyme level in the carrier of an abnormal recessive gene will be approximately 50% of the normal level, but because enzymes are made in great excess, this reduction usually is not enough to cause a disease. However, the reduced enzyme level can be used for genetic screening purposes. For example, one method to identify carriers of Tay–Sachs disease is by measurement of hexosaminidase A levels. Carriers of recessive conditions that do not produce any physical or quantitative biochemical change in an individual who is a heterozygote can be identified only by molecular methods.

The carrier of a gene that causes an autosomal recessive disease may be recognized after the birth of an affected child, after the diagnosis of an affected family member, or as the result of a genetic screening program. A couple whose child has an autosomal recessive disease has a 25% recurrence risk with each pregnancy. The likelihood that an unaffected sibling of an affected child is a carrier of the gene is two out of three (one fourth of all offspring will be homozygous unaffected, two fourths will be heterozygotes/carriers, and one fourth will be homozygous affected; thus, three of four children will be phenotypically normal and two of these three will be carriers). Carriers will not have affected children unless their partners are gene carriers or are affected. Because

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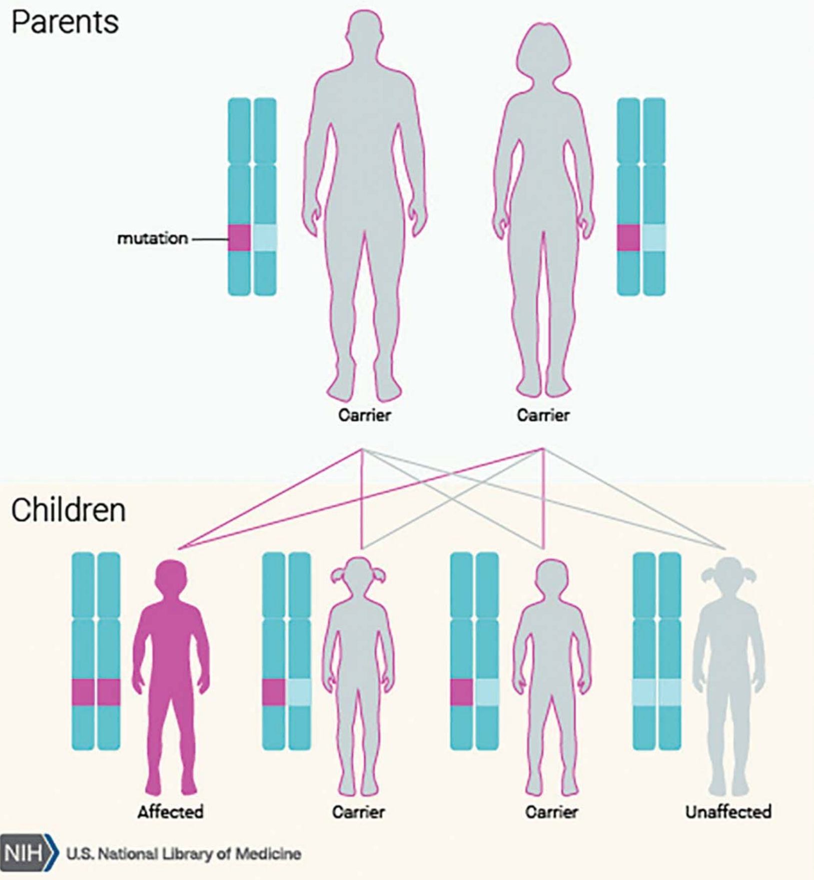

Figure 5. Autosomal recessive inheritance. Two unaffected individuals who each carry one copy of the mutated gene for an autosomal recessive disorder (carriers) have a 25% chance with each pregnancy of having an offspring affected by the disorder. The chance with each pregnancy of having an unaffected offspring who is a carrier of the disorder is 50%, and the chance that an offspring will not have the disorder and will not be a carrier is 25%. (Reprinted from U.S. National Library of Medicine, Genetics Home Reference. If a genetic disorder runs in my family, what are the chances that my children will have the condition? Bethesda [MD]: NLM; 2018. Available at: https://ghr.nlm.nih.gov/primer/inheritance/riskassessment. Retrieved June 22, 2018.)

genes that lead to rare autosomal recessive conditions have a low prevalence in the general population, the chance that an unrelated partner also will carry the same disorder is low. Even in related couples, unless quite closely related, this risk is small (see the "Consanguinity" section). An individual who inherits two different mutated alleles for the same disorder is called a *compound heterozygote*. For example, an individual who inherits a ΔF508 and W1282X pathogenic variant in the gene for cystic fibrosis will have a cystic fibrosis phenotype although the two alleles are not identical.

Co-Dominant

If the genes in a gene pair are different from each other, but both are expressed in the phenotype, they are considered to be *co-dominant*. For example, the genes that determine blood type are co-dominant because an individual is capable of expressing both A and B red cell antigens simultaneously (Fig. 6). Similarly, the genes responsible for the hemoglobinopathies are co-dominant. An individual with one hemoglobin gene that directs the production of sickle hemoglobin (Hb S) and the other gene directs the production of hemoglobin C (Hb C) will produce Hb S and Hb C.

X-Linked

X-linked diseases usually are recessive and primarily affect men because men have only one copy of the X chromosome. Examples of X-linked recessive disorders are color blindness, hemophilia A (classical hemophilia or factor VIII deficiency), and Duchenne muscular dystrophy. Women who carry an X-linked recessive gene generally are unaffected unless the X chromosome that carries the abnormal gene remains active in most cells, a process known as *skewed X-inactivation*.

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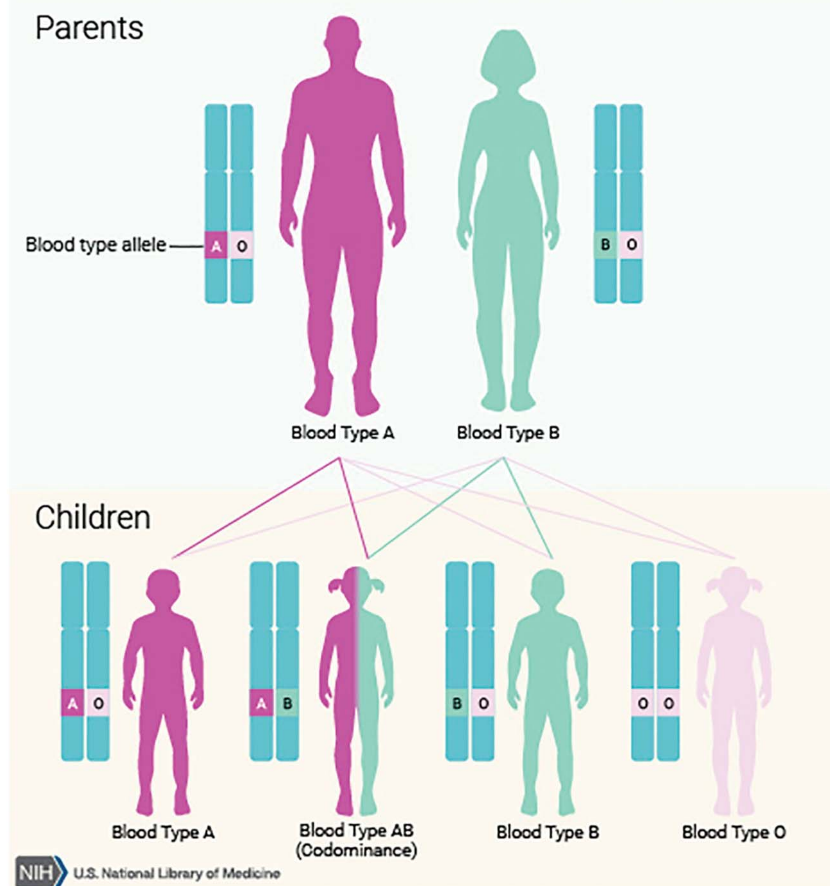

Figure 6. Co-dominant inheritance. In this pattern of inheritance, each parent contributes a different version of a particular gene, and both versions influence the resulting genetic trait. The chance of developing a genetic condition with co-dominant inheritance and the characteristic features of that condition is based on the version of the genes that are passed from the parents to their child. (Reprinted from U.S. National Library of Medicine, Genetics Home Reference. If a genetic disorder runs in my family, what are the chances that my children will have the condition? Bethesda [MD]: NLM; 2018. Available at: https://ghr.nlm.nih.gov/primer/inheritance/riskassessment. Retrieved June 22, 2018.)

When a woman carries a gene causing an X-linked recessive condition, she has a 50% chance of passing on the gene with each pregnancy; each of her sons has a 50% chance of being affected and each of her daughters has a 50% chance of being a carrier (Fig. 7). When a man has an X-linked disease, none of his sons will be affected (because they receive only the Y chromosome from their father), but all of his daughters will be carriers (because they all receive the affected X chromosome from their father). X-linked dominant disorders affect females predominantly because they tend to be lethal in male offspring (eg, incontinentia pigmenti).

Duplication and Deletion Syndromes

A duplication is the presence of an extra copy of a region of a chromosome, whereas a deletion refers to a region of a chromosome that is missing. The carrier of an autosomal deletion is effectively monosomic for the genes in the missing segment, whereas the carrier of a duplication is trisomic for the duplicated genes. Deletions and duplications usually are described by their location (eg, duplication 4p) or by the two chromosomal break points that define the missing or extra segment (eg, 4p15.2→p16.1). If the deletion is a common one, it may be defined by an eponym (eg, 5p– often is referred to as cri-du-chat syndrome). Terminal deletions and duplications are caused by chromosome breakage with loss or gain of the terminal chromosome segment. Interstitial deletions and duplications occur during prophase of meiosis when homologous chromosomes align and cross-overs occur. If the chromosomes are misaligned during this process, the mismatched segment could be deleted or duplicated (see Fig. 8).

When a deletion or duplication is identified in a fetus or child, the parents typically are tested to determine if the deletion or duplication arose de novo in the offspring or if either parent is a carrier or has a translocation that increased the risk of

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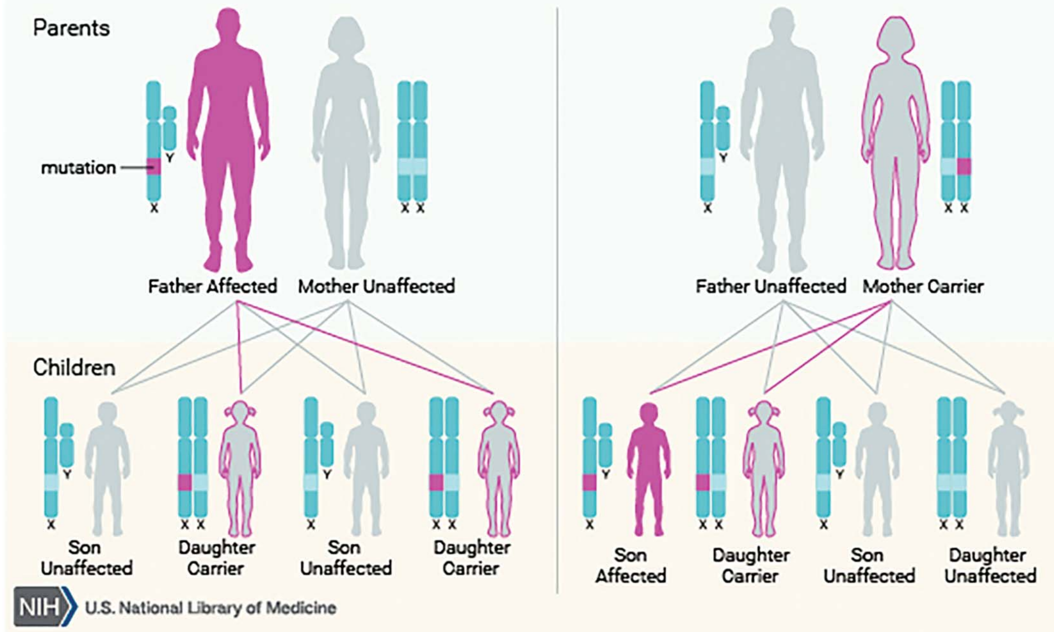

Figure 7. X-linked recessive inheritance. A man with an X-linked recessive disorder will not pass the gene to any male offspring; female offspring will carry one copy of the mutated gene. A woman who is a carrier has a 50 percent chance of having male offspring who are affected and a 50% chance of having female offspring who carry one copy of the mutated gene. (Reprinted from U.S. National Library of Medicine, Genetics Home Reference. If a genetic disorder runs in my family, what are the chances that my children will have the condition? Bethesda [MD]: NLM; 2018. Available at: https://ghr.nlm.nih.gov/primer/inheritance/riskassessment. Retrieved June 22, 2018.)

a duplication or deletion and would increase risk of recurrence in future pregnancies. Deletions and duplications of at least 5–10Mb may be identified by routine or high-resolution cytogenetic analysis (traditional banding *karyotype*). Microdeletions and duplications that are too small to be detected by traditional cytogenetic techniques often can be recognized with molecular techniques, such as *fluorescence* in situ *hybridization* and *microarrays* (see the "Fluorescence In Situ Hybridization" section and ACOG Committee Opinion No. 682, *Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology*).

Chromosome deletions and duplications can result in phenotypic abnormalities with widely varying pathophysiologies because the abnormality may span two or more genes, and genes that are in proximity to one another on a chromosome may have completely unrelated functions and control independent traits. Deletion syndromes usually cause more serious phenotypic and functional abnormalities than duplication syndromes because monosomy generally has more severe consequences than trisomy.

Syndromes caused by a microdeletion that involve genes physically located together in a chromosome segment are called *contiguous gene deletion syndromes*, and their study has yielded a great deal of information about gene location and function. Although deletions can occur in any area of any chromosome, some deletions occur more frequently than would be expected by chance alone. Several common chromosome deletion syndromes, such as DiGeorge syndrome, which most often results from a microdeletion of the long arm of chromosome 22 (del 22q11.2), have been recognized. Affected individuals typically have conotruncal cardiac defects; thymus and parathyroid gland hypoplasia or aplasia; atypical facies, including short palpebral fissures, micrognathia with a short philtrum, and ear anomalies; and learning and speech difficulties. Deletions of the terminal portions of the short arms of chromosomes 4 (4p– or Wolf–Hirschhorn syndrome) and 5 (5p– or cri-du-chat

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Figure 8. Deletion and duplication. (Reprinted from Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson genetics in medicine. 8th ed. Philadelphia [PA]: Elsevier; 2016.)

syndrome) also occur more frequently than would be expected by chance alone. The reason for these observed increases remains unknown.

Nonmendelian Patterns of Inheritance

Mendel's first law states that genes are passed unchanged from parent to progeny. Barring the occurrence of de novo variants, this law applies to many genes or traits, yet not all conditions or diseases are inherited in the classic Mendelian pattern described above. Alternate paths for genetic inheritance are described as follows.

Hereditary Unstable DNA: Trinucleotide Repeats

It is now known that certain genes, such as the gene responsible for fragile X syndrome, are susceptible to *expansion*, and their size and consequently their function may be altered as they are transmitted from parent to child. Generally, these genes contain a region of triplet (trinucleotide) repeats, such as CGG repeats.

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The number of triplet repeats in gametes can increase when meiosis is completed before fertilization, in a process known as *genetic anticipation*, and as a consequence of *triplet repeat expansion*, the gene may then become methylated and turned off resulting in phenotypic abnormalities. This region of excessive triplet repeats is known as *hereditary unstable DNA*. Some triplet regions might expand only during female meiosis (eg, Fragile X) and others may expand during male meiosis (eg, Huntington disease). Some triplet regions are passed from parent to child for many generations without expansion for unknown reasons.

Fragile X syndrome is the most common inherited form of intellectual disability. Transmitted as an X-linked disorder, fragile X syndrome is caused by expansion of a repeated trinucleotide segment that leads to altered transcription of the fragile X mental retardation 1 (*FMR1*) gene. The number of CGG repeats varies among individuals and has been classified into four groups (Table 2). The chance of transmission of a disease-producing, expanded allele to a fetus depends on the sex of the parent and the number of trinucleotide CGG repeats present in the parental gene. Other factors (such as AGG interrupters in fragile X Syndrome) may influence the repeat expansion. A woman who carries a premutation can transmit either her normal or expanded allele; the premutation allele may expand, resulting in the birth of an affected child. The larger the size of the premutation repeat, the more likely the expansion to a fully expanded CGG repeat (2).

Other common triplet repeat disorders include Huntington Disease (a CAG repeat), myotonic dystrophy (a CTG repeat), and Friedreich's ataxia (a GAA repeat). Huntington Disease and myotonic dystrophy are autosomal dominant disorders although Friedreich's Ataxia is autosomal recessive. These disorders all share the same characteristic: a premutation allele may remain unchanged or expand during meiosis leading to pathology (see Fig. 9).

Imprinting

The function of certain genes may vary based on the sex of the parent from whom they were inherited through a process known as *imprinting*. Imprinting is a mechanism for epigenetic control of gene expression; that is, it changes the phenotype without permanently altering the genetic structure. An imprinted gene can be inherited in an inactivated (transcriptionally silent) state as the result of methylation of the promoter region (see Fig. 10). The extent of the imprinting and, thus, the degree of inactivation is determined by the sex of the transmitting parent.

When a gene is inherited in an imprinted or inactivated state, gene function is necessarily directed entirely by the active co-gene inherited from the other parent. It appears that imprinting exerts an effect in part by controlling the dosage of specific genes. Certain important genes appear to be monoallelic: Under normal circumstances, only one member of the gene pair is functional. Most genes, however, are biallelic and have two functioning copies. Only a fraction of human genes are imprinted, and it is likely that these will be identified with more frequency as more information on the human genome is obtained.

Table 2. Full Expansion of Fragile X *CGG* Repeat From Maternal Premutation Allele

Maternal Number of Triplet Repeats (Cytosine–Guanine–Guanine)	Status of Individual	Full Allele Expansion* (%)
Less than 45	Unaffected	
45–54	Intermediate (also called "gray zone")	
55–59	Premutation	4
60–69	Premutation	5
70–79	Premutation	31
80–89	Premutation	58
90–99	Premutation	80
100–200	Premutation	98
More than 200†	Full expansion	100†

*The likelihood of expansion also may be affected by other factors, including the presence of *AGG* interrupters that reduce the risks of expansion.

†Pesso R, Berkenstadt M, Cuckle H, Gak E, Peleg L, Frydman M, et al. Screening for fragile X syndrome in women of reproductive age. Prenat Diagn 2000;20:611–4.

Modified from Nolin SL, Brown WT, Glicksman A, Houck GE Jr, Gargano AD, Sullivan A, et al. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. Am J Hum Genet 2003;72:454–64.

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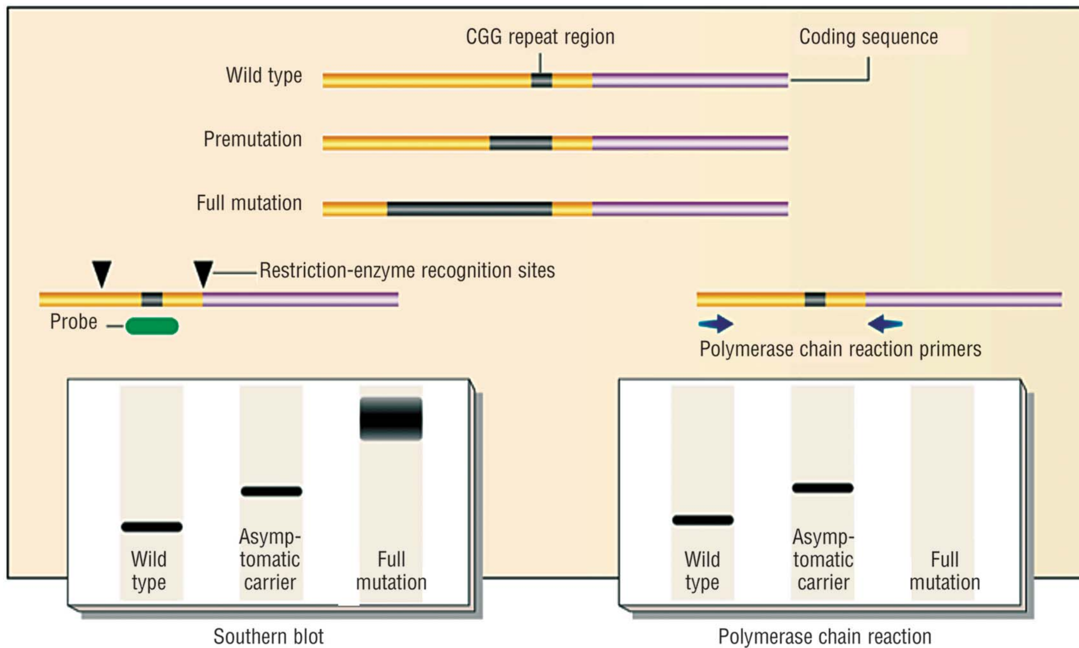

Figure 9. Molecular diagnosis. Trinucleotide repeat analysis by polymerase chain reaction and Southern blot analysis. A region of DNA that contains the CGG repeat is amplified and the product is analyzed on an agarose gel preparation. The DNA fragment from a carrier with a premutation is larger than that from a noncarrier because of the expanded number of repeats. Sometimes the expansion is so large that the DNA fails to amplify, and the number of repeats cannot be detected with polymerase chain reaction. In this case, a Southern blot is performed (left panel) and the DNA from an individual with a full mutation appears as a larger band. (Reprinted from Korf B. Molecular diagnosis. N Engl J Med 1995;332:1499–502.)

Imprinting was first recognized in association with genetic disease. Two phenotypically distinct genetic diseases were discovered in association with the same chromosomal deletion at 15q11–13. If the maternally derived chromosome 15 segment is deleted, the result is Angelman syndrome, which is characterized by normal stature and weight, severe intellectual impairment, absent speech, seizure disorder, ataxia and jerky arm movements, and paroxysms of inappropriate laughter. In contrast, if the paternally derived chromosome 15 segment is deleted, the result is obesity; hyperphagia; short stature; small hands, feet, and external genitalia; and mild intellectual disability—features collectively describing Prader–Willi syndrome. Although the described deletions are one cause of these disorders, a deletion is not required to produce the phenotype. If an individual has two normal intact copies of chromosome 15, but both are inherited from the father (see the "Uniparental Disomy" section), the phenotype is consistent with Angelman syndrome, because the maternal contribution is missing. Likewise, two complete copies of chromosome 15 of maternal origin produces Prader–Willi syndrome (see Fig. 11). Studies of the methylation patterns in individuals with the 15q11–13 deletion have shown that differential methylation (imprinting) is responsible for these observed phenotypic differences.

Several clinical examples from obstetrics illustrate the developmental importance of imprinting. The complete hydatidiform mole, which has an exclusively paternally derived diploid chromosome complement, is characterized by the abundant growth of placental tissue, but no fetal structures. In contrast, exclusively maternally derived, full diploid chromosomal complement results in a benign cystic ovarian teratoma, a tumor of embryonic or fetal tissue with no placenta (3). Triploidy, characterized by a pregnancy with three complete haploid chromosome complements (69 chromosomes), also has two distinct phenotypes based on the parent of origin of the extra chromosome set. If the extra haploid chromosome complement is paternal, it will result in a partial hydatidiform mole and the placenta will be large and cystic. If the extra chromosome complement is maternally derived, the fetus will be anomalous, and the placenta will be extremely small. These observations confirm that the paternally and maternally derived imprinted genes must be present for normal fetal and placental development to occur.

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Figure 10. DNA methylation. (Reprinted from Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson genetics in medicine. 8th ed. Philadelphia [PA]: Elsevier; 2016.)

Uniparental Disomy

Uniparental disomy occurs when both members of a chromosome pair are inherited from the same parent (see Fig. 12). Every gene on both chromosomes in question will have necessarily come from only one parent. Uniparental disomy usually occurs as the result of "correction" of a trisomic zygote by loss of a chromosome. When a trisomic pregnancy loses one of three copies of a chromosome, by chance it may retain two chromosomes transmitted by the same parent; this results in *uniparental heterodisomy* (inheritance of two different homologous chromosomes from one parent). Less frequently, "rescue" of a monosomic pregnancy that has only one chromosome may occur by duplication of the chromosome during mitosis; this will produce a cell with two copies of the same chromosome or *uniparental isodisomy*. Both forms of uniparental disomy can lead to disease states as described in the "Imprinting" section.

Mitochondrial Inheritance

Mitochondrial inheritance involves the inheritance of a trait encoded on the mitochondrial genome. Mitochondrial diseases are a clinically heterogeneous group of rare disorders that result from dysfunction of the mitochondrial respiratory chain. Some mitochondrial disorders affect a single organ (eg, Leber hereditary optic neuropathy), whereas others involve multiple organ systems. The mitochondria are essential for aerobic respiration and, therefore, mitochondrial diseases commonly affect tissues with high energy requirements, including the central nervous system (hearing

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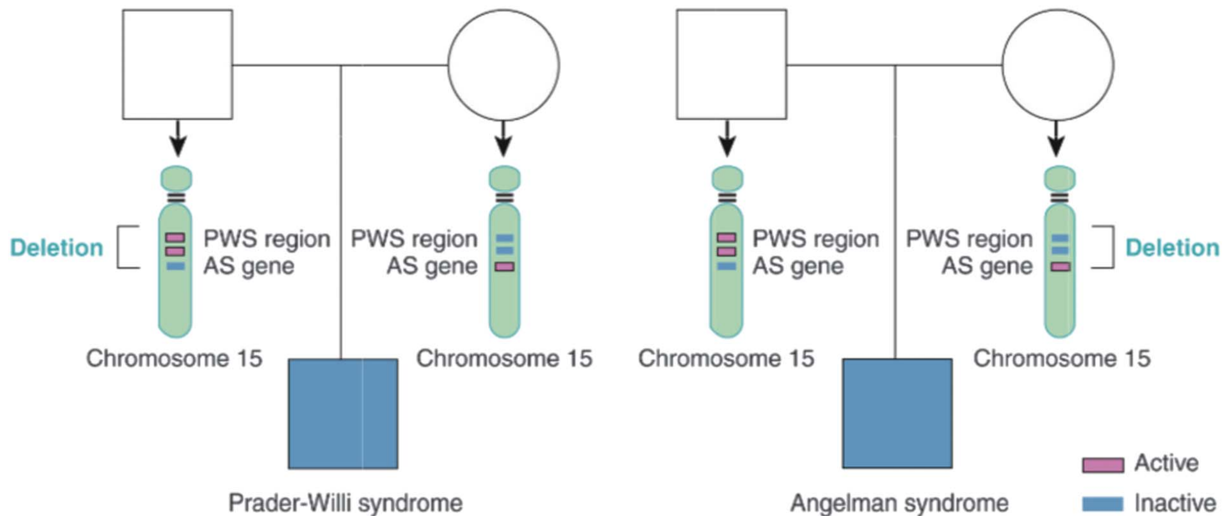

Figure 11. Prader-Willi syndrome from two complete copies of chromosome 15 of maternal origin. (Reprinted from Jorde LB, Carey JC, Bamshad MJ. Medical genetics. 5th ed. Philadelphia [PA]: Elsevier; 2016.)

and vision problems, seizures, encephalopathy, and stroke-like episodes), heart (cardiomyopathy), and muscles (myopathy and respiratory difficulties). The onset of mitochondrial disorders may range from infancy to adulthood.

A mitochondrion contains its own genome and associated replication systems. It divides in a mitotic fashion; there is no meiosis of this DNA even in germ cells and, therefore, there is no chance for recombination. Each human oocyte contains approximately 100,000 mitochondria in the nuclear cytoplasm. In contrast, a sperm contains only about 100 mitochondria and these are selectively eliminated during fertilization and, thus, all mitochondria are maternally inherited.

Although mitochondria have their own genome, the normal function of a mitochondrion is dependent on DNA from the nucleus as well. Genetic defects in either DNA complement can result in disease, but the inheritance pattern depends on the source of the DNA. Nuclear mitochondrial gene defects are inherited in the usual autosomal recessive or dominant patterns. The inheritance of mitochondrial DNA (mtDNA) defects, however, is more complex.

If a mutation occurs in the mtDNA, it may segregate into a daughter cell during cell division and, thus, be propagated into future generations of that cell line. *Mitochondrial heteroplasmy* is the presence within a single cell of normal mitochondria and abnormal mitochondria. The proportion of normal to mutated mtDNA (ie, the mutant load) may vary in different tissues and can even change over time, affecting the expression and severity of disease. Thus, if an oocyte that contains largely mutant mitochondrial DNA is fertilized, the child produced might develop a mitochondrial disease depending on the eventual mutant load in its various tissues. Moreover, a woman who has a heteroplasmic mtDNA mutation may transmit a variable amount of mutated mtDNA to each of her offspring, resulting in considerable clinical variability even among siblings within the same family (4).

Germline Mosaicism

In rare circumstances, some phenotypically normal individuals have *mosaicism*, the presence of two or more populations of cells with different genetic characteristics within one organ or tissue. *Germline mosaicism, also called gonadal mosaicism,* can arise as the result of a mitotic error that occurs in zygotic cells destined to become the gonad. Because spermatogonia and oogonia continue to divide throughout fetal development, gonadal mosaicism also can occur as the result of a premeiotic error in the dividing germ cells of the embryo. Germline mosaicism may explain the occurrence of a "new" (not previously occurring in a family) autosomal dominant mutation causing a disease, such as achondroplasia or osteogenesis imperfecta, or a new X-linked disease such as Duchenne muscular dystrophy. Also, it explains the recurrence of such diseases in more than one offspring in a previously unaffected family. Because of the potential for germline mosaicism, the

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Figure 12. Uniparental disomy. (Reprinted from Jorde LB, Carey JC, Bamshad MJ. Medical genetics. 5th ed. Philadelphia [PA]: Elsevier; 2016.)

empiric recurrence risk after the birth of a child with a disease caused by a new mutation may be higher than expected in the general population. Currently, testing is not available to determine the presence of germline mosaicism.

Polygenic and Multifactorial Inheritance

Polygenic traits result from the combined effects of many genes; multifactorial traits are determined by genetic and environmental factors. It is now believed that most inherited traits are multifactorial or polygenic. Traits determined by single genes and transmitted by strict mendelian inheritance without the contribution of modifier genes are probably relatively rare. Birth defects caused by *multifactorial inheritance* or *polygenic inheritance* are recognized by their tendency to recur in families but not according to a Mendelian inheritance pattern (see Box 2). When counseling couples regarding their offspring's risk of a familial multifactorial trait (eg, congenital heart defects, cleft palate, and neural tube defects), it is important to consider the affected relative's degree of relatedness to the fetus, not the parents. The recurrence risk for first-degree relatives (the fetus's parents or siblings are affected) usually is quoted as 2–3%, but the risk decreases exponentially with successively more distant relationships. Generally, multifactorial traits fall into one of the following three categories: 1) *continuously variable traits*, 2) *threshold traits*, or 3) *complex disorders of adult life*.

Continuously Variable Traits. Continuously variable traits have a normal distribution in the general population. Examples include height and head size. By convention, abnormality is defined as a trait or measurement greater than two SDs above or below the population mean. These are typically measurable or quantitative traits and are believed to result from

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Box 2. Characteristics of Multifactorial Inheritance

- The disorder is familial, but pattern of inheritance is not apparent.
- The risk to first-degree relatives is the square root of the population risk.
- The risk is significantly decreased for second-degree relatives.
- The recurrence risk is increased if more than one family member is affected.
- The risk is increased if the defect is more severe (ie, the recurrence risk for bilateral cleft lip is higher than for unilateral cleft lip).
- If the defect is more common in one sex than in the other sex, the recurrence risk is higher if the affected individual is of the less commonly affected sex.

the individually small effects of many genes combined with environmental factors. Such traits tend to be less extreme in the offspring of affected individuals.

Threshold Traits. Threshold traits do not appear until a certain threshold of liability is exceeded. Factors that create liability to the malformation are assumed to be continuously distributed in the population; only individuals who are at the extreme of this distribution exceed the threshold and have the trait or defect. The phenotypic abnormality is thus an all-or-none phenomenon. Individuals in high-risk families have enough abnormal genes or environmental influences that their liability approaches the threshold. For as-yet-unknown reasons, some factor or factors will cause certain family members to cross the threshold, resulting in the defect. Cleft lip and palate and pyloric stenosis are examples of threshold traits.

Certain threshold traits favor one gender, which indicates that males and females have a different liability threshold. When a family includes an affected individual who is the less frequently affected gender, this indicates that even more abnormal genes or environmental influences than necessary for the development of a disease are present, and the affected individual (and possibly the family) has a more extreme position in the normal distribution of predisposing factors. The affected individual's first-degree relatives (eg, siblings) have an increased liability for that particular trait. For example, pyloric stenosis is more common in males than in females. If a girl is born with pyloric stenosis, this indicates that she or her parents have even more abnormal genes or predisposing factors than usually are necessary to produce pyloric stenosis. After the birth of a girl with pyloric stenosis, the recurrence risk for her siblings or for her future children will be higher than it would be if the child with pyloric stenosis had been male; male siblings or offspring will have the highest liability because they are the susceptible sex.

The recurrence risk for threshold traits also is higher if the defect is severe, again indicating the presence of more abnormal genes or influences. For example, the recurrence risk after the birth of a child with bilateral cleft lip and palate is 8% compared with only 4% after unilateral cleft lip without cleft palate.

Complex Disorders of Adult Life. Complex disorders of adult life are traits in which many genes determine an individual's susceptibility to environmental factors, with disease resulting from the most unfavorable combination. This category includes common adult diseases, such as hypertension and type 2 diabetes mellitus. These disorders usually are familial and behave as threshold traits but with a very strong environmental influence. The genetic mechanisms of many common adult diseases have not yet been elucidated, although several associated genes have been identified.

Other Important Genetic Concepts

Epigenetics

The traditional teaching of genetic inheritance is that, barring a mutation in the DNA sequence itself, genes are passed from parent to offspring undisturbed in their structure and function. Epigenetics describes the process by which genes can be modified biochemically and affect the expression of the genes without changing the actual DNA sequence. Epigenetic mechanisms are what allow a pluripotent stem cell to develop into such disparately functioning cells as hepatocytes and leukocytes even though both use the exact same DNA complement. Several mechanisms have been identified that modify gene expression by altering how genes are made available to the transcription and translation enzymes in cells (see the "Genes" section for more on methylation, histone modifications, and noncoding RNA fragments). In addition, many human disease states, such as obesity, asthma, and diabetes, appear to have epigenetic origins often beginning in fetal development or childhood (Fig. 13). The most important aspect of the epigenetic origin of disease is that some of these modifications in DNA expression may be preventable or even reversible. It also is becoming increasingly clear that some acquired epigenetic changes that predispose to disease

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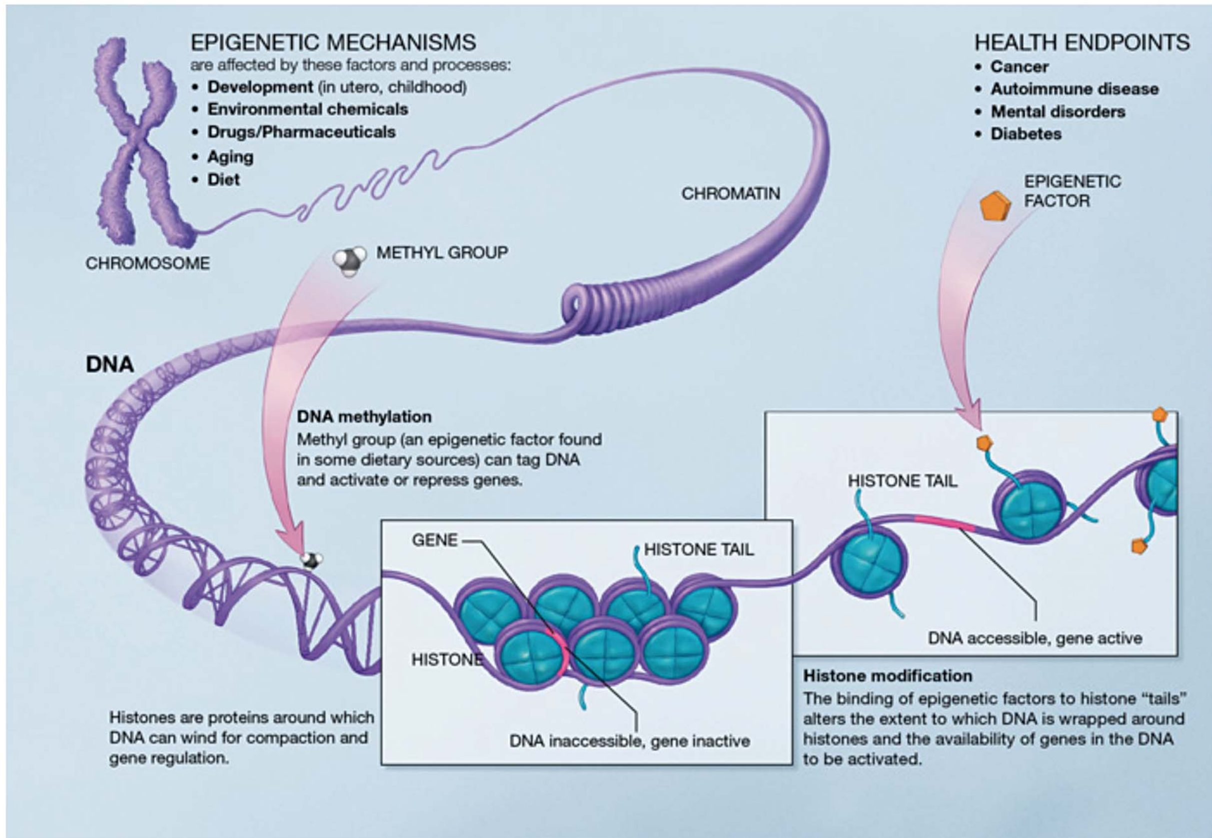

Figure 13. Epigenetics. (Reprinted from National Institutes of Health. A scientific illustration of how epigenetic mechanisms can affect health. Bethesda [MD]: NIH; 2018. Available at: https://commonfund.nih.gov/epigenomics/figure. Retrieved June 22, 2018.)

can be transmitted through the germline from parent to child.

Consanguinity

Consanguinity is typically defined as a union between two individuals who are second cousins or closer in family relationship. In other words, consanguinity refers to the degree of relatedness or genetic sharing between biological parents; the more closely related two parents are by blood, the higher the probability of shared recessive genes being passed to offspring. A detailed family pedigree will usually reveal if significant consanguinity exists. As a rule of thumb, couples who are less closely related than second cousins (ie, couples who share great-grandparents) will have risks similar to the general population. However, complex family trees may need to be interpreted by a genetics professional, and formulas exist to calculate the degree of relatedness between couples and their risk of abnormal offspring. It should be noted, however, that even first cousins have only a small (2–3%) increased risk of birth defects compared with the general population (5).

Molecular Diagnostic Testing

Advances in our understanding of the molecular basis of inherited disorders have led to the development of DNA-based tests that can be used for prenatal and postnatal diagnosis, carrier testing, and aneuploidy screening. These techniques have allowed for diagnosis of a wide variety of genetic diseases ranging from aneuploidies to single gene disorders.

DNA for molecular testing can be obtained from any cell with a nucleus. Sources can be blood, skin, hair, buccal swabs, and tissue blocks from stored pathology specimens. Most diagnostic laboratories prefer blood samples or buccal swabs because of the high yield of genetic material for DNA testing. Cultured amniocytes, chorionic villi, and fetal blood are used for prenatal DNA testing of the fetus. Preimplantation genetic diagnosis of embryos created

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by in vitro fertilization also can be performed with cytogenetic or molecular techniques.

Variant Detection

Once a specific pathogenic variant in a gene has been identified, direct testing for that specific variant is possible; this is the most accurate molecular diagnostic method. Molecular genetic testing usually is performed to identify common pathogenic variants that are well characterized and account for most cases. In some cases, the pathogenic variants are unique to a specific ethnicity. For example, two specific pathogenic variants in the *aspartoacylase* gene account for 97% of the variants that cause Canavan disease in the Ashkenazi Jewish population. For some genetic disorders, the variants are unique to an individual family. Nevertheless, these variants still can be identified by commercial laboratories or academic hospital laboratories. A helpful reference for molecular testing information, whether commercially available or research-based, is provided by GeneTests, a genetic testing resource website available at http://www.genetests.org.

A variety of methods are available for detection of pathogenic variants. The methodology usually is determined by the nature of the specific variant and an ever-increasing array of techniques is available. Only a few of the commonly used methods will be specifically described in this document. Molecular tools such as *Southern blot analysis* and *polymerase chain reaction* (PCR), are among the mainstays of molecular genetic testing and often are used to detect gene deletions associated with disorders, such as Duchenne muscular dystrophy and spinal muscular atrophy. When the region of interest involves large deletions or large repetitive sequences, as in fragile X syndrome and alpha thalassemia, PCR is less reliable and these large deletions or expanded triplet repeat disorders are visualized more easily with Southern blot analysis.

Polymerase Chain Reaction. Polymerase chain reaction allows the exponential amplification of a targeted gene or DNA sequence. It has applications in many clinical scenarios including carrier screening, preimplantation genetic diagnosis, and infectious disease testing. Only minute quantities of DNA, typically 0.1–1 micrograms, are necessary for PCR. DNA can be amplified using very small numbers of cells from readily accessible body fluids, such as urine and saliva, or even a single cell, such as in preimplantation genetic testing. One important prerequisite of PCR is that the sequence of the gene, or at least the borders of the region of DNA to be amplified, must be known.

The PCR procedure has three steps (Fig. 14): 1) DNA is denatured by heating to render it into single strands, 2) the PCR primers (short pieces of DNA exactly complementary to the ends of each piece of the double-stranded DNA to be amplified) anneal to their complementary regions of the DNA, and 3) synthesis of the complementary strand of DNA occurs in the presence of Taq polymerase and nucleotide triphosphates (dATP, dCTP, dGTP, and dTTP). The reaction cycle of denaturation, annealing, and synthesis is repeated 25–30 times to produce millions of copies of DNA in a short period.

Southern Blot Analysis. Southern blot analysis provides a means to study genetic disorders at the DNA level. This procedure, named after its inventor E. M. Southern, separates DNA fragments according to size and allows the identification of specific DNA fragments using labeled DNA probes.

The steps to prepare a Southern blot are shown in Figure 15. First, DNA is extracted from nucleated cells (leukocytes, trophoblasts, or amniocytes) or tissue and digested into small pieces with a restriction enzyme. Then, DNA is loaded into an agarose gel, an electric current is applied, and DNA fragments migrate down the gel according to size (smaller fragments move faster). The DNA contained within the agarose gel is still double stranded and must be denatured by alkali treatment into single-stranded pieces and then transferred to a nitrocellulose or nylon membrane. The membrane contains many thousands of fixed DNA fragments. Specific DNA fragments can be detected with a labeled DNA probe, such as a *complementary DNA probe*, genomic DNA (exons, introns, or regulatory regions), or short pieces of single-stranded DNA (*oligonucleotide primers*), which usually range in size from 20 base pairs to 50 base pairs. The DNA probe will hybridize only with DNA fragments on the blot that are complementary. The DNA fragment is visualized by a variety of methods.

DNA Sequencing

Availability of rapid DNA sequencing was pivotal to the advanced completion of the Human Genome Project and has an important role in forensic medicine, drug discovery, and diagnostics. Knowing the complete sequence of a gene has many advantages especially when it is important for disease prediction, as in *BRCA 1* and *BRCA 2* testing. In such cases, sequencing both strands of the DNA double helix is

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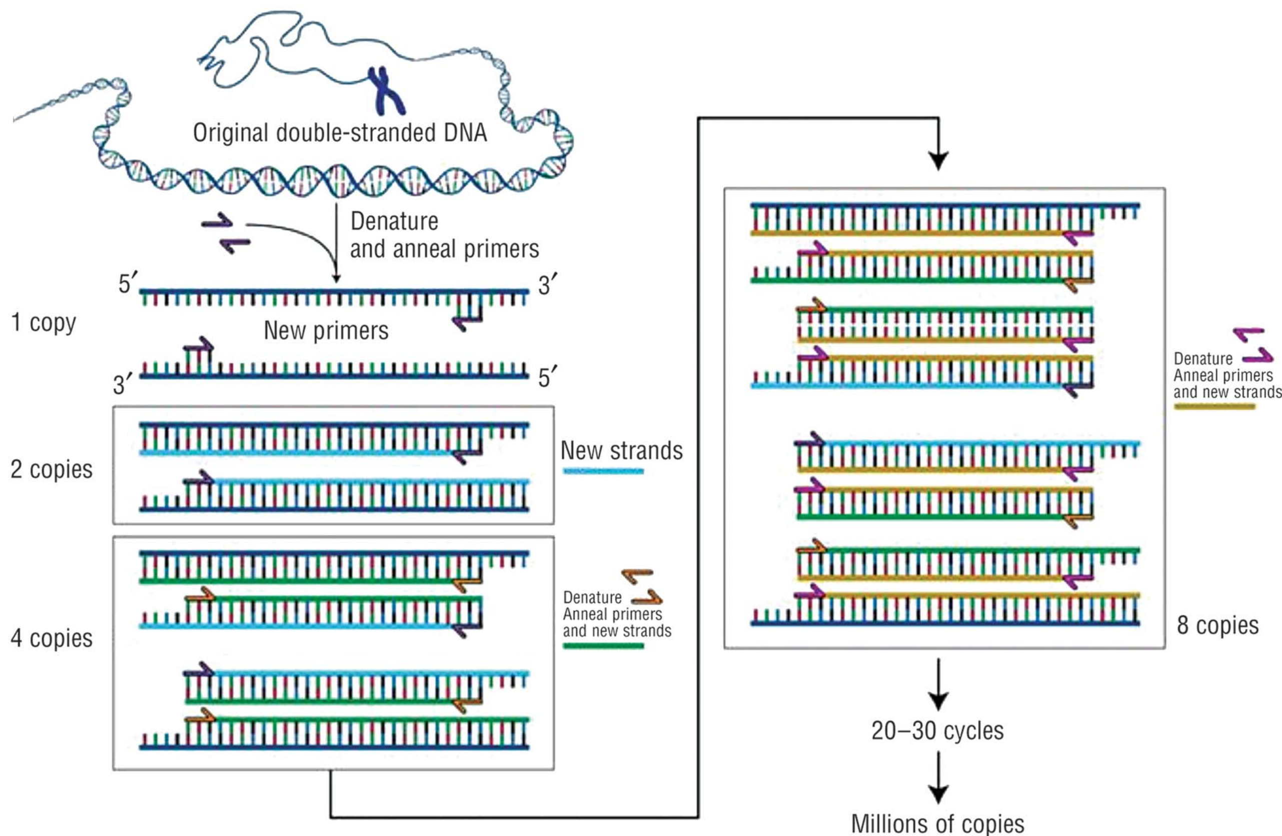

Figure 14. Polymerase chain reaction (PCR). (Reprinted from National Human Genome Research Institute. Polymerase chain reaction [PCR]. Talking Glossary of Genetic Terms. Available at: https://www.genome.gov/glossary/?id=159. Retrieved June 22, 2018.)

necessary to detect and confirm heterozygosity. Sequencing also is used when a disease is strongly suspected, but routine molecular testing does not identify any of the commonly recognized pathogenic variants. Often, variant panels are geared toward specific populations and may not detect pathogenic variants in individuals of ethnic backgrounds in which a given disorder is rare; DNA sequencing can be useful in such instances. Advantages of sequencing should be weighed against important limitations, such as discovery of variants of uncertain significance. Also, in cases of deletion or duplication in one allele, sequencing does not readily detect these changes and important pathogenic copy number variants can be missed.

Recent development of high-throughput sequencing (also known as next-generation sequencing) has resulted in rapidly decreasing costs and expanding applications of DNA sequencing. Although several techniques have been developed, most make the sequencing process parallel by producing thousands or millions of sequences at once. Such next-generation sequencing has allowed development of cell-free DNA screening for fetal aneuploidy, among other applications.

Molecular Cytogenetic Techniques

Chromosomes increasingly are studied with molecular techniques. Such strategies have the advantage of detecting smaller chromosomal aberrations, large enough to have phenotypic consequences but often too small to be seen with routine karyotyping. Commonly used techniques include fluorescence in situ hybridization and single nucleotide polymorphism microarrays.

Fluorescence In Situ Hybridization. Fluorescence in situ hybridization is performed on either metaphase chromosome preparations from cultured lymphocytes, amniocytes, or chorionic villi, or interphase nuclei from blood, tissue, chorionic villi, or amniotic fluid (Fig. 16). After the chromosomes or nuclei are fixed on a microscope slide, they are hybridized with a fluorochrome-labeled DNA probe specific for a particular region of a chromosome. The probe hybridizes to complementary

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Figure 15. Southern blot analysis. (Reprinted from National Human Genome Research Institute. Southern blot. Talking Glossary of Genetic Terms. Available at: https://www.genome.gov/glossary/index.cfm?id=459. Retrieved June 22, 2018.)

DNA sequences that can then be visualized with a fluorescence microscope. Fluorescence in situ hybridization of metaphase chromosomes is used as an adjunct to routine cytogenetic analysis for the detection of submicroscopic chromosome deletions and duplications too small to be detected by conventional cytogenetics. DNA probes have been developed for common deletion and duplication syndromes. Fluorescence in situ hybridization also may be used to identify or confirm the presence of a subtle chromosomal rearrangement (eg, a translocation) or to identify the origin of an extra structurally abnormal chromosome (ie, a marker chromosome). Fluorescence in situ hybridization of interphase nuclei is used prenatally for the detection of common aneuploidies, such as trisomy 21, when rapid assessment is desirable. Fluorescence in situ hybridization also is used for preimplantation genetic diagnosis, when only one to two cells are available for analysis and rapid testing is required.

Microarray Technology. In contrast to the conventional karyotype, which can detect genetic abnormalities that result from changes in the number or structure of chromosomes, microarray analysis can provide information at the submicroscopic level by demonstrating duplications and deletions of DNA throughout the human genome (Fig. 17). DNA microarray technology is a molecular method used to study gene expression.

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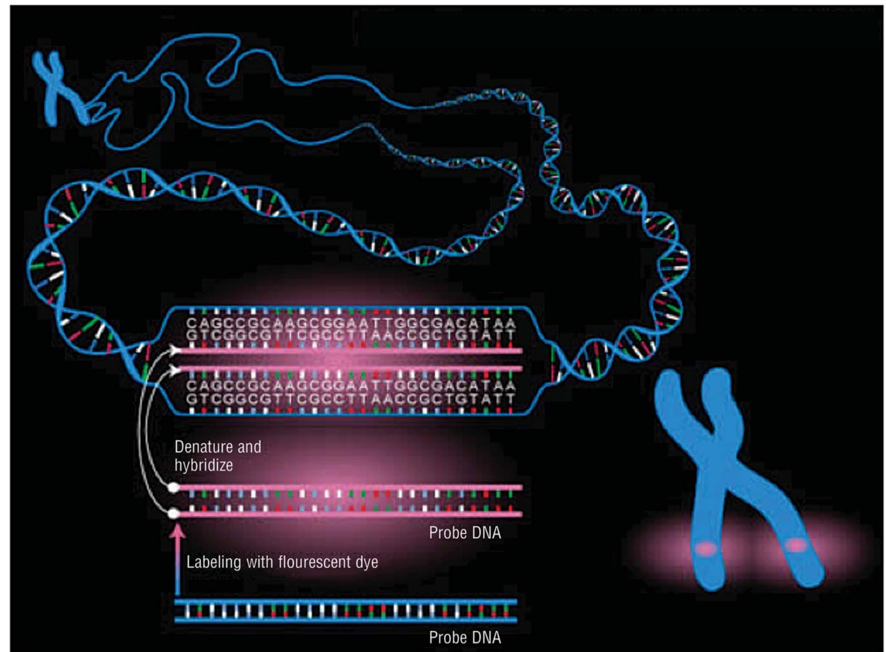

Figure 16. Fluorescence in situ hybridization. (Reprinted from National Human Genome Research Institute. Fluorescence in situ hybridization [FISH]. Talking Glossary of Genetic Terms. Available at: https://www.genome.gov/glossary/index.cfm?id=65. Retrieved June 22, 2018.)

Duplicated or deleted sections of DNA are known as *copy number variants*; the high resolution of chromosomal microarray analysis enables the detection of copy number variants that are 1/100th the size of those identified by current conventional G-banded karyotyping. It can be used to identify cytogenetic changes in tumors, to characterize subtle unbalanced translocations, to identify the origin of marker or supernumerary chromosomes, and to detect intrachromosomal duplications or deletions. For additional information on microarray, see ACOG Committee Opinion No. 682, *Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology*.

Whole-Exome and Whole-Genome Sequencing

State of the art sequencing technologies are being applied in pediatric and adult genetic practice with notably increasing diagnostic yield. Whole-genome sequencing refers to sequencing the entire genome, both the noncoding regions (introns) and coding regions (exons). However, at this time, the ability to interpret the intronic regions is limited, and whole-genome sequencing is not routinely being performed in clinical practice. Yet, whole-exome sequencing, which involves sequencing only the exons or protein-coding regions of the genome, is more frequently used in clinical genetics (6, 7) (Fig. 11). Exons generally have greater clinical relevance and applicability to patient care, and most of our understanding of Mendelian inherited disorders is derived from research on variants in the exome, which comprises only 1% of the human genome. Given that standard genetic testing from amniocentesis or chorionic villus sampling may fail to yield a diagnosis in 20–30% of cases, whole-exome sequencing as a prenatal test may be reasonable in select circumstances, such as in fetuses with multiple anomalies or in cases of recurrent fetal phenotypes with no diagnosis by standard genetic testing (eg, karyotype or microarray) (8). The American College of Medical Genetics and Genomics recommends considering whole-exome sequencing when specific genetic tests available for a phenotype, including targeted sequencing tests, have failed to arrive at a diagnosis in a fetus with multiple congenital anomalies suggestive of a genetic disorder (9).

Whole-exome sequencing is accomplished by using a number of high-throughput sequencing technologies. After isolating DNA from a tissue sample, sequencing machines determine base pair arrangements of many different DNA strands simultaneously. Given the extremely large amount of information obtained from whole-exome sequencing, the subsequent analysis can be difficult and time-consuming; thus, it is often expeditious to test both parents and the fetus at the same time (also known as *trio-sequencing*). Trio-sequencing allows a large number of uninformative variants to be filtered

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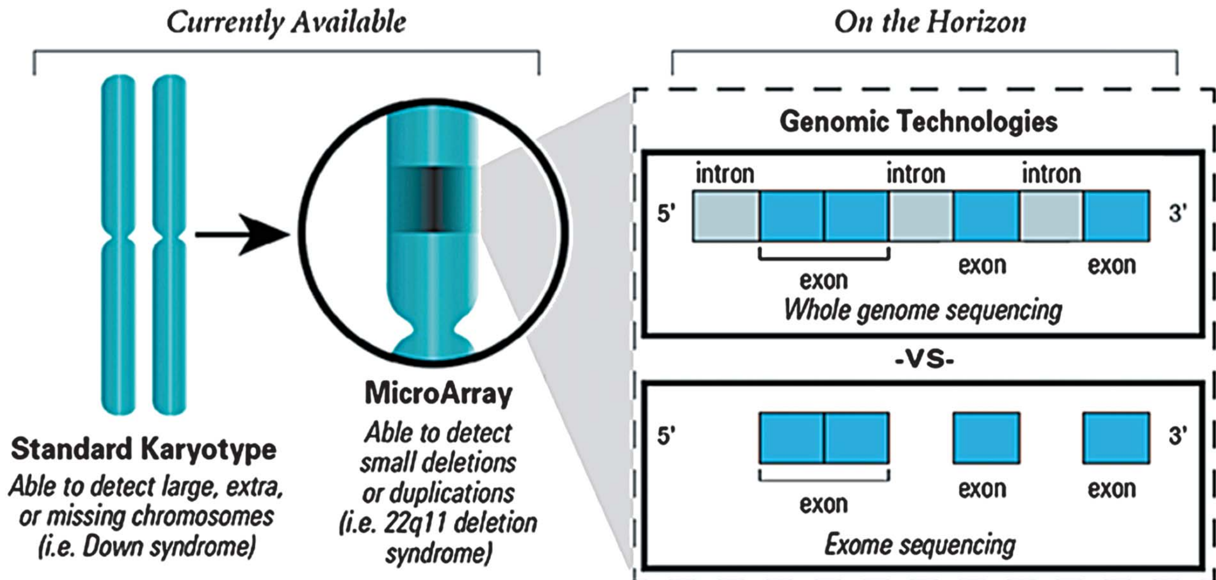

Figure 17. Diagnostic capability of prenatal genetic tests. (Reprinted from Hardisty E, Vora N. Advances in genetic prenatal diagnosis and screening. Current Opinion in Pediatrics. 2014; 26[6]:6348.)

out. Nevertheless, even with trio-based sequencing and complex bioinformatics protocols, turnaround time and difficulty with interpretation of variants may reduce the clinical utility of whole-exome sequencing in the prenatal setting (10). Another challenge with trio-sequencing is the possibility of finding a medically actionable incidental finding in the parental exomes. For example, a parent may be found to have an inherited variant in a cancer gene that is pathogenic but unrelated to the fetal ultrasound findings. Any variants deemed pathogenic or likely pathogenic should also be confirmed using traditional Sanger sequencing because false-positives can occur with whole-exome sequencing.

Other limitations of whole-exome sequencing include its inability to identify trinucleotide repeats, aneuploidy, microdeletions or duplications, and structural variants such as translocations and *inversions*, as well as its inability to sequence the entire exome (most whole-exome screening platforms cover 85–90% of exons), which can result in false-negative test results. In addition, this testing raises a number of ethical concerns, such as problems ensuring equal access (given the high cost of testing) and issues regarding disclosure (eg, the possibility of revealing nonpaternity, consanguinity, and medically actionable incidental findings). The clinical complexities of using these techniques are discussed in ACOG Committee Opinion No. 682.

Performance and Limitations of Molecular Testing

The correct interpretation of molecular genetic diagnostic testing is highly dependent on an accurate clinical diagnosis, test sensitivity, ethnic variability in variants and disease prevalence, *genetic heterogeneity*, reduced penetrance, and phenotypical variability. The accuracy of testing for a specific variant and for the suspected genetic disorder may be decreased if an affected relative is not available to determine if a detectable variant is present, if medical records are not available to confirm the diagnosis, or if paternity is in question.

The sensitivity of molecular screening and diagnostic testing varies because of a number of factors that affect the ability to detect pathogenic variants. Genetic disorders often result from a variety of genetic alterations. For example, more than 1,000 different variants have been reported for *BRCA 1* and *BRCA 2*. In addition, the variant detection rates for many genetic disorders, including neurofibromatosis, cystic fibrosis, and hemophilia, are less than 100%. Therefore, the absence of an identifiable pathogenic variant does not exclude the possibility that an individual may be a carrier of or affected with a given disorder. Furthermore, ethnic differences often exist in detection rates; for example, the sensitivity of the screening test

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for cystic fibrosis varies among different ethnic groups, ranging from less than 50% in those of Asian ancestry to 94% in the Ashkenazi Jewish population. The incidence and carrier risk of some genetic disorders also are dependent on ethnicity and have led to recommendations for carrier screening for specific genetic disorders that may vary by ethnicity. Differences in test sensitivity and the prevalence of pathogenic variants in different ethnic groups should be considered for each individual genetic disorder and discussed with the patient before testing. Disease prevalence and test sensitivity are considerations when recommending molecular-based carrier testing.

Another limitation of molecular testing is genetic heterogeneity. In some cases, more than one gene or chromosomal locus may be responsible for a genetic disorder, a type of genetic heterogeneity known as locus heterogeneity. For example, at least two genes on two different chromosomes have been identified as causing tuberous sclerosis, an autosomal dominant disorder.

Summary

Human genetics and molecular testing are increasingly important in medicine, including obstetric and gynecologic practice. As genetics becomes a more integral part of routine medical practice, it is essential that obstetrician–gynecologists and other health care providers be aware of advances in the understanding of genetic disease and the fundamental principles of genetic screening and molecular testing. As the genetic basis for reproductive disorders, common diseases, and cancer is elucidated with improved molecular technology, genetic testing opportunities are expanding and influencing treatment options and prevention strategies.

For More Information

The American College of Obstetricians and Gynecologists has identified additional resources on topics related to this document that may be helpful for ob-gyns, other health care providers, and patients. You may view these resources at www.acog.org/Genetics.

These resources are for information purposes only and are not meant to be comprehensive. Referral to these resources does not imply the American College of Obstetricians and Gynecologists' endorsement of the organization, the organization's website, or the content of the resource. The resources may change without notice.

Glossary

Allele: Alternative form of a gene; a single allele for each locus is inherited from each parent.

Autosomal Dominant: An allele located on an autosome (nonsex chromosome) that expresses itself phenotypically in the presence of the same or a different allele (ie, in either a homozygous or heterozygous condition).

Autosomal Recessive: An allele located on an autosome that does not express itself phenotypically in the presence of a dominant allele (ie, in a heterozygous condition) and is only phenotypically expressed in a homozygous condition.

Carrier: An individual who does not have any symptoms of a particular genetic disorder but may have one abnormal allele for the gene that is associated with the disorder.

Chromatin: An intranuclear and intrachromosomal complex made up of DNA and histone and nonhistone proteins that condense to form chromosomes during cell division.

Co-Dominant: Alleles that are different from each other but are both expressed in the phenotype.

Complementary DNA Probe: A synthetic DNA sequence that is exactly complementary to messenger RNA, lacking introns and regulatory regions.

Complex Disorders of Adult Life: Complex disorders of adult life are traits in which many genes determine an individual's susceptibility to environmental factors, with disease resulting from the most unfavorable combination.

Compound Heterozygote: Two different mutated recessive alleles for the same disorder.

Consanguinity: A union between two individuals who are second cousins or closer in family relationship.

Contiguous Gene Deletion Syndrome: A syndrome caused by a deletion that involves genes that are physically located together in a chromosome segment.

Continuously Variable Traits: Measurable or quantitative traits that have a normal distribution in the general population and result from the individually small effects of many genes combined with environmental factors.

Copy Number Variants: Duplicated or deleted sections of DNA at least 1,000 base pairs in size that differ from a representative reference genome. Copy number variants can be qualified as pathogenic or benign to clarify clinical relevance.

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Deletion: A copy number variant that is generated by removal of a sequence of DNA with the regions on either side being joined together.

DNA Methylation: A process for control of tissue specific gene expression. Methylation "turns off" the regulatory region of a gene thereby preventing DNA transcription.

Enhancer DNA Sequences: DNA sequences located upstream or downstream of a gene that can increase transcriptional activity of the gene.

Epigenetic: Inherited changes in phenotype or gene expression that are caused by mechanisms other than changes in the underlying DNA sequence (eg, by methylation).

Exome: The part of the genome composed of exons.

Exon: A region of a gene made up of DNA sequences that will be transcribed into messenger RNA.

Expansion: (See Hereditary Unstable DNA).

Expressivity: The degree to which a genotype is expressed in the phenotype (range of phenotypic features).

Fluorescence in situ Hybridization: A procedure for detecting specific nucleic acid sequences in morphologically preserved chromosomes, cells, and tissue sections using fluorescent labeled oligonucleotide probes.

Gene: A unit of heredity responsible for the inheritance of a specific trait that occupies a fixed chromosomal site and corresponds to a sequence of nucleotides along a DNA molecule.

Genetic Anticipation: When the age of onset of a genetic condition occurs earlier and its features become more severe with successive generations. Anticipation is associated with triplet repeat expansion disorders.

Genetic Heterogeneity: When a genetic condition or phenotype is caused by one of many possible allele or locus variants. Allelic heterogeneity refers to multiple different variants within a single gene locus that produce the same phenotype or condition. Locus heterogeneity refers to variants in different gene loci that cause the same phenotype or condition.

Genome: The entire complement of genetic material in a chromosome set.

Genomics: The study of the interaction of an individual's genes with each other and with the environment.

Germline Mosaicism (Gonadal Mosaicism): A condition in which the germline cells develop pathogenic variants during embryonic growth that are not present in the somatic cells. Germline pathogenic variants can result in disease in the offspring, but the parent is not affected and does not have the pathogenic variant in his or her somatic cells.

Hereditary Unstable DNA (Triplet Repeat Expansion): Genes containing a region of triplet codon repeats, such as (CGG)n. The number of triplet repeats can increase during meiosis. If the expansion of repeats reaches a critical number, the gene becomes methylated and is turned off, resulting in phenotypic abnormalities.

Heterochromatin: Chromatin that remains condensed throughout interphase. It contains DNA that is genetically inactive and replicates late in the S phase of the cell cycle.

Histone Modification: Modification to histone proteins after translation that can alter gene expression, such as through acetylation, phosphorylation, methylation, and ubiquitination.

Hybridization: A semiquantitative technique for evaluating the relative abundance of nucleic acid sequences in a mixture or the extent of similarity between homologous sequences.

Imprinting: The phenomenon by which certain genes are expressed in a parent-of-origin specific manner. Imprinted genes are inactive, and thus only the gene inherited from the other parent is expressed.

Intron: The region of a gene that is made up of noncoding DNA sequences and lies between the exons.

Insertion: A copy number variant caused by the presence of an additional sequence of nucleotide pairs in DNA.

Inversion: A copy number variant involving the removal of a DNA sequence, its rotation by 180 degrees, and its reinsertion in the same location.

Karyotype: The chromosome constitution of an individual.

Methylation: (See DNA Methylation).

Missense Variant: A variant that alters a codon so that it encodes a different amino acid.

Mitochondrial Heteroplasmy: The presence of multiple mitochondrial DNA variants within a cell or individual.

Mitochondrial Inheritance: The inheritance of a trait encoded on the mitochondrial genome. Because mitochondria are inherited exclusively from the mother, mitochondrial inheritance is exclusively maternal.

Mosaicism: The presence of two or more populations of cells with different characteristics within one organ or tissue.

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Multifactorial Inheritance: Inheritance of traits that are determined by a combination of genetic and environmental factors.

Nonsense or Truncating Variant: A variant that results in a shortened protein.

Nucleotide: A component of a DNA or RNA molecule composed of a nitrogenous base, one deoxyribose or ribose sugar, and one phosphate group. In DNA, adenine specifically joins to thymine and guanine joins to cytosine. In RNA, uracil replaces thymine.

Oligonucleotide Primer: A short sequence of nucleotides that is hybridized to a DNA or RNA strand using the enzymes DNA polymerase or reverse transcriptase.

Penetrance: The ability of a mutant gene to be expressed in an individual who carries the gene.

Phenotype: Observable physical characteristics of an organism that results from the expression of the genotype and its interaction with the environment.

Polygenic Inheritance: Inheritance of traits that are determined by the combined effects of many genes.

Polymerase Chain Reaction (PCR): A method for enzymatically amplifying a short sequence of DNA through repeated cycles of denaturation, binding with an oligonucleotide primer, and extension of the primers by a DNA polymerase.

Polymorphisms: The occurrence in the same population of more than one allele or genetic marker at the same locus.

Silencer (Repressor) DNA: DNA sequences located upstream or downstream of a gene that can decrease transcriptional activity of the gene.

Single Nucleotide Polymorphism: DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered.

Skewed X-inactivation: Skewed X chromosome inactivation occurs when the inactivation of one X chromosome is favored over the other, leading to an uneven number of cells with each chromosome inactivated.

Southern Blot Analysis: A technique used to detect specific DNA sequences by separating restriction enzyme digested DNA fragments on an electrophoresis gel, transferring (blotting) these fragments from the gel onto a membrane or nitrocellulose filter, followed by hybridization with a labeled probe to a specific DNA sequence.

Threshold Traits: Traits that are not manifested until a certain threshold of liability is exceeded.

Translation: The process of protein synthesis in which RNA codes for specific amino acid sequences.

Transcription: The process of RNA synthesis from a DNA template that is directed by RNA polymerase.

Trio Sequencing: Whole-exome sequencing of a fetus and its biological parents.

Triplet Repeat Expansion: (See Hereditary Unstable DNA).

Uniparental Disomy: Inheritance of two copies of part or all of a chromosome from one parent and no copy from the other parent.

Uniparental Heterodisomy: Inheritance of two homologous chromosomes from one parent.

Uniparental Isodisomy: Inheritance of two identical chromosomes from one parent.

Variant: An alteration of DNA sequences in a gene that results in a heritable change in protein structure or function that frequently has adverse effects.

Variant of Uncertain Significance: Also known by the acronym VUS or VOUS or as variants of uncertain clinical significance (VUCS). Variants of uncertain significance are identified DNA changes that either cannot be characterized reliably as benign or pathogenic at the time of the study because of limited data describing outcomes in association with the changes or that are associated with a variable phenotype (because of incomplete penetrance or variable expressivity).

X-inactivation: A process by which one of the X chromosomes in each somatic cell of females is rendered inactive. This results in a balance in gene expression between the X chromosomal and autosomal genes, which is necessary because males have only one X chromosome.

X-linked: An allele for a trait or disorder that is located on the X chromosome and may be either dominant or recessive.

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