CHAPTER 13: Genetics

Foetal death may be due to abnormalities in the ovum itself or due to some disease on the part of the mother, and now and again of the father. The death of the foetus is frequently due to abnormalities in the development of the embryo which are inconsistent with foetal life.

—J. Whitridge Williams (1903)

INTRODUCTION

In Williams' first edition of *Obstetrics*, he rarely referenced inherited conditions that Gregor Mendel had described 50 years earlier. Fast-forward to 2017, when the science of genetics is a major obstetrical discipline.

Genetics is the study of genes, heredity, and the variation of inherited characteristics. Medical genetics deals with the etiology and pathogenesis of human diseases that are at least partially genetic in origin, along with their prediction and prevention. Thus, it is closely linked to *genomics*, which is the study of gene function and interaction. In addition to chromosomal, mendelian, and nonmendelian genetic conditions reviewed in this chapter, medical genetics includes prenatal and preimplantation genetic diagnosis, as well as newborn genetic screening, which are discussed in Chapters 14 and 32, respectively.

Genetic disease is common. Between 2 and 3 percent of newborns have a recognized structural defect. In another 3 percent of individuals, a defect is diagnosed by age 5, and another 8 to 10 percent of persons are discovered by age 18 to have one or more functional or developmental abnormalities. Advances in genomics are used increasingly to provide information regarding susceptibility to genetic diseases, and every indication suggests that this field will reshape prenatal diagnosis.

GENOMICS IN OBSTETRICS

Completed in 2003, the Human Genome Project identified more than 25,000 human genes and led to rapid expansion of genomic research to better understand disease biology (McKusick, 2003). More than 99 percent of our DNA is identical. However, genetic code varies every 200 to 500 base pairs, usually as a single-nucleotide polymorphism. The human genome contains more than 80 million such genetic variants, and understanding their potential role in disease requires not only sophisticated interpretation but also integration of resources (Rehm, 2015).

The National Center for Biotechnology Information (NCBI) maintains genetic and genomic databases that are freely accessible to clinicians and researchers. Several of these databases are particularly useful in obstetrics and maternal-fetal medicine practice. The *GeneReviews* database provides in-depth clinical information for nearly 700 genetic conditions, including diagnostic criteria, management, and genetic counseling considerations (National Center for Biotechnology Information, 2017a). The *Genetic Testing Registry (GTR)* database contains information regarding the benefits and limitations of available tests for a given disorder. It lists more than 48,000 genetic tests and instructions for specimen collection and transport to individual laboratories throughout the world (National Center for Biotechnology Information, 2017b). Another database, *Online Mendelian Inheritance in Man* (OMIM), is a comprehensive catalog of human genes and phenotypes that allows clinicians to search for syndromes based on particular traits or abnormalities. As of early 2017, OMIM included more than 15,000 genes and nearly 5000 mendelian and mitochondrial conditions with a known molecular basis (Johns Hopkins University, 2017). The National Library of Medicine (2017) has also established a database of genetic information intended for patients—one that trainees may find especially helpful—the *Genetics Home Reference (GHR)*. This database contains data on more than 2400 genetic conditions and genes, including resources for families.

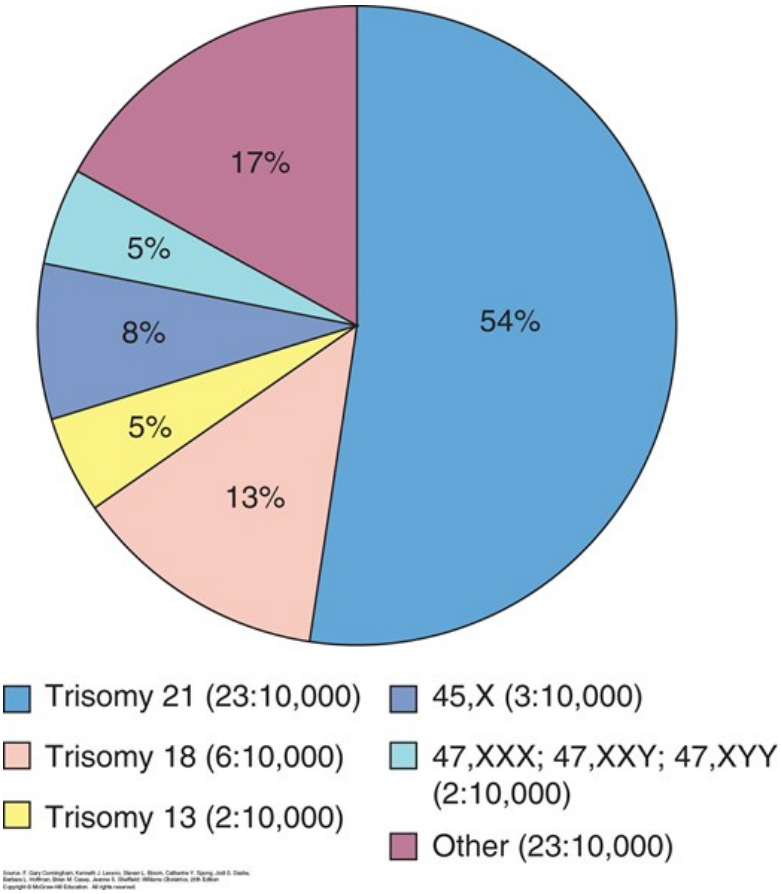
CHROMOSOMAL ABNORMALITIES

Chromosomal abnormalities figure prominently in genetic disease. Aneuploidy accounts for more than 50 percent of first-trimester miscarriages,

approximately 20 percent of second-trimester losses, and 6 to 8 percent of stillbirths and early-childhood deaths (Reddy, 2012; Stevenson, 2004; Wou, 2016). In the European Surveillance of Congenital Anomalies (EUROCAT) network of population-based registries, chromosomal abnormalities were identified in 0.4 percent of births (Wellesley, 2012). Of recognized pregnancies with aneuploidy, trisomy 21 composes just more than half of all cases. Trisomy 18 accounts for almost 15 percent, and trisomy 13 for 5 percent (Fig. 13-1).

FIGURE 13-1

Prevalence and relative proportion of selected chromosomal abnormalities from EUROCAT (European Surveillance of Congenital Anomalies) population-based registries that included >10,000 aneuploid live births, fetal deaths, and pregnancy terminations, 2000–2006. (Data from Wellesley, 2012.)



Standard Nomenclature

Karyotypes are described using the International System for Human Cytogenomic Nomenclature (McGowan-Jordan, 2016). Abnormalities fall into two broad categories—those of *chromosome number*, such as trisomy, and those of *chromosome structure*, such as a deletion or translocation. Each chromosome has a short arm, termed the "p" or petit arm, and a long arm, known as the "q" arm, selected because it is the next letter in the alphabet. The two arms are separated by the centromere.

When reporting a karyotype, the total number of chromosomes is listed first, corresponding to the number of centromeres. This is followed by the sex chromosomes, XX or XY, and then by a description of any structural variation. Specific abnormalities are indicated by standard abbreviations, such as del (deletion) and inv (inversion). The affected region or bands of the p or q arms are then designated, so that the reader will know the exact abnormality location and type. Examples are shown in Table 13-1.

TABLE 13-1

Examples of Karyotype Designations Using the 2016 International System for Human Cytogenetic Nomenclature

Karyotype	Description
46,XX	Normal female chromosome constitution
47,XY,+21	Male with trisomy 21
47,XX,+21/46,XX	Female who is a mosaic of trisomy 21 cells and cells with normal constitution
46,XY,del(4)(p14)	Male with terminal deletion (del) of the short arm of chromosome 4 at band p14
46,XX,dup(5)(p14p15.3)	Female with duplication (dup) of the short arm of chromosome 5 from band p14 to band p15.3
45,XY,der(13;14)(q10;q10)	Male with balanced robertsonian translocation (der) of the long arms of chromosomes 13 and 14—the karyotype now has one normal 13, one normal 14, and the translocation chromosome, reducing the normal 46 chromosome complement to 45
46,XX,t(11;22)(q23;q11.2)	Female with a balanced reciprocal translocation (t) between chromosomes 11 and 22, with breakpoints at 11q23 and 22q11.2
46,XY,inv(3)(p21q13)	Male with inversion (inv) of chromosome 3 that extends from p21 to q13—a pericentric inversion because it includes the centromere
46,X,r(X)(p22.1q27)	Female with one normal X and one ring (r) X chromosome, with the regions distal to p22.1 and q27 deleted from the ring
46,X,i(X)(q10)	Female with one normal X chromosome and an isochromosome (i) of the long arm of the other X
ish 22q11.2(HIRAx2)	FISH of metaphase cells using a probe for the HIRA locus of the 22q11.2 region, with 2 signals identified (no evidence of microdeletion)
ish del(22)(q11.2q11.2)(HIRA-)	FISH of metaphase cells using a probe for the HIRA locus of the 22q11.2 region, with only one signal identified, consistent with the microdeletion
arr[GRCh38] 18p11.32q23(102328_79093443)x3	Microarray analysis (arr), genome build GRCh38, showing a single copy gain on chromosome 18 from band p11.32 to band q23 (essentially the entire chromosome), consistent with trisomy 18
arr[GRCh38] 4q32.2q35.1(163146681_183022312)x1	Microarray analysis (arr), genome build GRCh38, showing a copy loss on the long arm of chromosome 4 at bands q32.2 through q35.1 (19.9 Mb)
arr[GRCh38] 15q11.2q26(23123715_101888908)x2 hmz	SNP microarray analysis (arr), genome build GRCh 38, showing homozygosity for the entire long arm of chromosome 15

FISH = fluorescence in situ hybridization; GRCh38 = Genome Reference Consortium human build 38; HIRA = histone cell cycle regulator; SNP = single nucleotide pleomorphism.

Used with permission from Dr. Kathleen S. Wilson.

Terminology is similar for fluorescence in situ hybridization. Described in Genetic Tests, this technique is used to rapidly identify of a specific chromosome abnormality and verify suspected microdeletion or microduplication syndromes. The report begins with the designation *ish* for in situ

hybridization performed on metaphase cells and *nuc ish* for hybridization performed on interphase nuclei. If no abnormality is identified, this is followed by the probe's specific chromosomal region, such as 22q11.2, and then the name of the probe and the number of signals visualized—for example, HIRAx2. If a deletion is identified, *del* is included before the chromosomal region, and the name of the probe is followed by a minus sign (HIRA-), as shown in Table 13-1. The 22q11.2 microdeletion syndrome is discussed in Abnormalities of Chromosome Structure.

A recent addition to the standard nomenclature is terminology to represent copy number variants identified by *chromosomal microarray analysis*, which is discussed in Chromosomal Microarray Analysis. *Copy number variant* is another term for a microdeletion or microduplication of DNA too small to be visualized with a standard karyotype. The array designation begins with the abbreviation *arr* and the version of the genome build to which the nucleotide designations are aligned, such as GRCh38 for Genome Reference Consortium human build 38. This is followed by the number of the chromosome on which the abnormality is identified, by the p or q arm, and by the specific bands in question. Array reports next include the affected base pair coordinates, thus conveying the exact size and location within the genome for every abnormality identified—including copy number variants of uncertain significance.

Abnormalities of Chromosome Number

The most easily recognized chromosomal abnormalities are numerical. *Aneuploidy* is inheritance of either an extra chromosome—resulting in trisomy, or loss of a chromosome—*monosomy*. These differ from *polyploidy*, which is an abnormal number of haploid chromosome sets, such as triploidy. The estimated incidence of various numerical chromosomal abnormalities is shown in Figure 13-1.

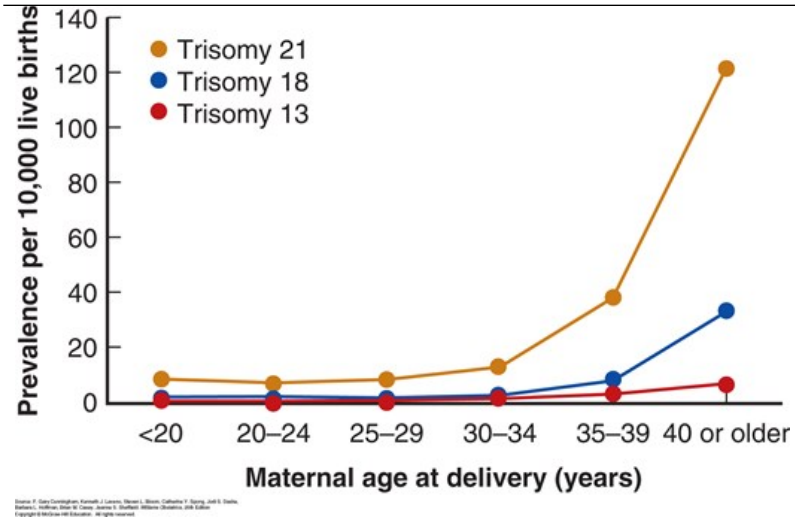
Autosomal Trisomies

These account for approximately half of all chromosomal abnormalities. In most cases, trisomy results from nondisjunction, which is failure of normal chromosomal pairing and separation during meiosis. Nondisjunction may occur if the chromosomes: (1) fail to pair up, (2) pair up properly but separate prematurely, or (3) fail to separate.

The risk of any autosomal trisomy rises steeply with maternal age, particularly after age 35 (Fig. 13-2). Oocytes are suspended in midprophase of meiosis I from birth until ovulation, in some cases for 50 years. Following completion of meiosis at ovulation, nondisjunction results in one gamete having two copies of the affected chromosome, leading to trisomy if fertilized. The other gamete, receiving no copy of the affected chromosome, will be monosomic if fertilized. It is estimated that 10 to 20 percent of oocytes are aneuploid secondary to meiotic errors, compared with 3 to 4 percent of sperm. Although each chromosome pair is equally likely to have a segregation error, it is rare for trisomies other than 21, 18, or 13 to result in a term pregnancy, and most fetuses with trisomies 18 and 13 die before term.

FIGURE 13-2

Prevalence of autosomal trisomies according to maternal age found in population-based birth defects surveillance programs in the United States, 2006–2010, which included live births, stillbirths, and pregnancy terminations. (Data from Mai, 2013. Redrawn with permission from Dashe JS: Aneuploidy screening. Obstet Gynecol 128(1):181, 2016.)



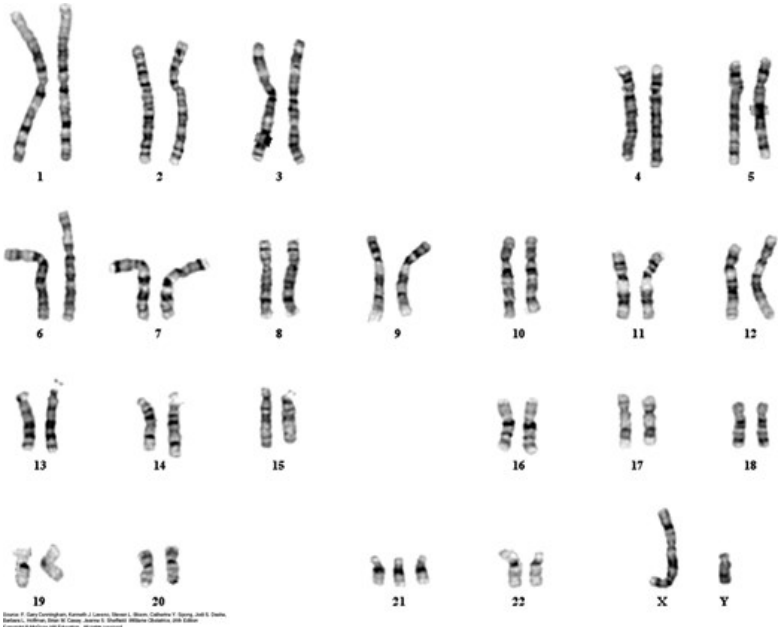
After a pregnancy with an autosomal trisomy, the risk for any autosomal trisomy in a future pregnancy approximates 1 percent until the woman's age-related risk exceeds this. Accordingly, prenatal diagnosis with chorionic villus sampling or amniocentesis is offered in these subsequent pregnancies (Chap. 14, Technique). Parental chromosomal studies are not indicated unless the affected pregnancy was caused by an unbalanced translocation or other structural rearrangement.

Trisomy 21—Down Syndrome

In 1866, J. L. H. Down described a group of intellectually disabled children with distinctive physical features. Nearly 100 years later, Lejeune (1959) demonstrated that Down syndrome is caused by an autosomal trisomy (Fig. 13-3). Trisomy 21 causes 95 percent of Down syndrome cases, whereas 3 to 4 percent of cases are due to a robertsonian translocation, described later (Isochromosomes). The remaining 1 to 2 percent results from an isochromosome or from mosaicism. The nondisjunction that yields trisomy 21 occurs during meiosis I in approximately 75 percent of cases, and the remaining events are during meiosis II.

FIGURE 13-3

Abnormal male karyotype with trisomy 21, consistent with Down syndrome (47,XY,+21). (Used with permission from Dr. Frederick Elder.)



Down syndrome is the most common nonlethal trisomy. Its approximate prevalence is 1 in 500 recognized pregnancies. However, fetal losses and

pregnancy terminations yield an estimated prevalence of 13.5 in 10,000 births in the United States—1 per 740 (Mai, 2013; Parker, 2010). The fetal death rate beyond 20 weeks' gestation approximates 5 percent (Loane, 2013). Coinciding with the older maternal age distribution during the past four decades, the prevalence of Down syndrome has risen approximately 33 percent (Loane, 2013; Parker, 2010; Shin, 2009).

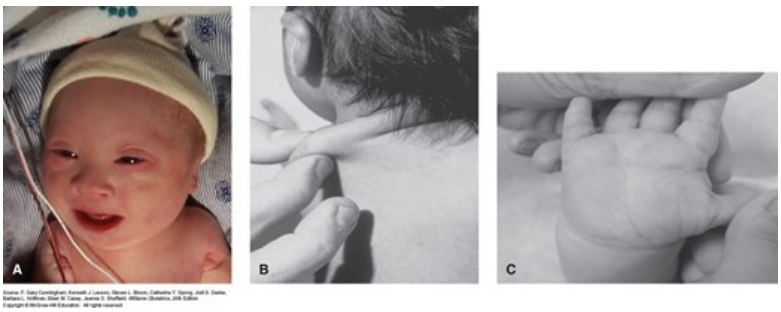
Notably, adult women with Down syndrome are fertile, and a third of their offspring will have Down syndrome (Scharrer, 1975). Males with Down syndrome are almost always sterile because of markedly reduced spermatogenesis.

Approximately 30 percent of second-trimester fetuses with Down syndrome have a major malformation that can be identified sonographically (Hussamy, 2017; Vintzileos, 1995). As discussed in Chapter 14 (Sonographic Screening), when both major anomalies and minor aneuploidy markers are considered, an estimated 50 to 60 percent of Down syndrome pregnancies can be detected sonographically (American College of Obstetricians and Gynecologists, 2016d). Approximately half of liveborn neonates with Down syndrome are found to have cardiac defects, particularly ventricular septal defects and endocardial cushion defects (Figs. 10-29 and 10-30) (Bergstrom, 2016; Freeman, 2008). Gastrointestinal abnormalities are identified in 12 percent and include esophageal atresia, Hirschsprung disease, and duodenal atresia (Fig. 10-38) (Bull, 2011).

Characteristic features of Down syndrome are shown in Figure 13-4. Typical findings include brachycephaly; epicanthal folds and up-slanting palpebral fissures; Brushfield spots, which are grayish spots on the periphery of the iris; a flat nasal bridge; and hypotonia. Infants often have loose skin at the nape of the neck, short fingers, a single palmar crease, hypoplasia of the middle phalanx of the fifth finger, and a prominent space or "sandal-toe gap" between the first and second toes. Some of these findings are prenatal sonographic markers for Down syndrome, reviewed in Chapter 14 (Sonographic Screening).

FIGURE 13-4

Trisomy 21—Down syndrome. **A.** Characteristic facial appearance. **B.** Redundant nuchal tissue. **C.** Single transverse palmar crease. (Used with permission from Dr. Charles P. Read and Dr. Lewis Waber.)



Health problems common in children with Down syndrome include hearing loss in 75 percent, severe optical refractive errors in 50 percent, cataracts in 15 percent, obstructive sleep apnea in 60 percent, thyroid disease in 15 percent, and a higher incidence of leukemia (Bull, 2011). The degree of mental impairment is usually mild to moderate, with an average intelligence quotient (IQ) score of 35 to 70. Social skills in affected children are often higher than predicted by their IQ scores.

Data suggest that approximately 95 percent of liveborn infants with Down syndrome survive the first year. The 10-year survival rate is at least 90 percent overall and is 99 percent if major malformations are absent (Rankin, 2012; Vendola, 2010). Several organizations offer education and support for prospective parents faced with prenatal diagnosis of Down syndrome. These include the March of Dimes, National Down Syndrome Congress (www.ndsccenter.org), and National Down Syndrome Society (www.ndss.org).

Trisomy 18—Edwards Syndrome

The association between this constellation of abnormalities and an autosomal trisomy was first described by Edwards (1960). In population-based series, prevalence of trisomy 18 approximates 1 in 2000 recognized pregnancies—including abortuses, stillbirths, and live births, and approximately 1 in 6600 liveborn neonates (Loane, 2013; Parker, 2010). The difference in prevalence is explained by the high in-utero lethality of the condition and the termination of many affected pregnancies. Perhaps not surprisingly, survival of liveborn neonates is likewise bleak. More than half die within the first week, and the 1-year survival rate approximates only 2 percent (Tennant, 2010; Vendola, 2010). The syndrome is three- to fourfold more common in females (Lin, 2006; Rosa, 2011). Unlike Down and Patau syndromes, which involve acrocentric chromosomes and thus may stem from a robertsonian

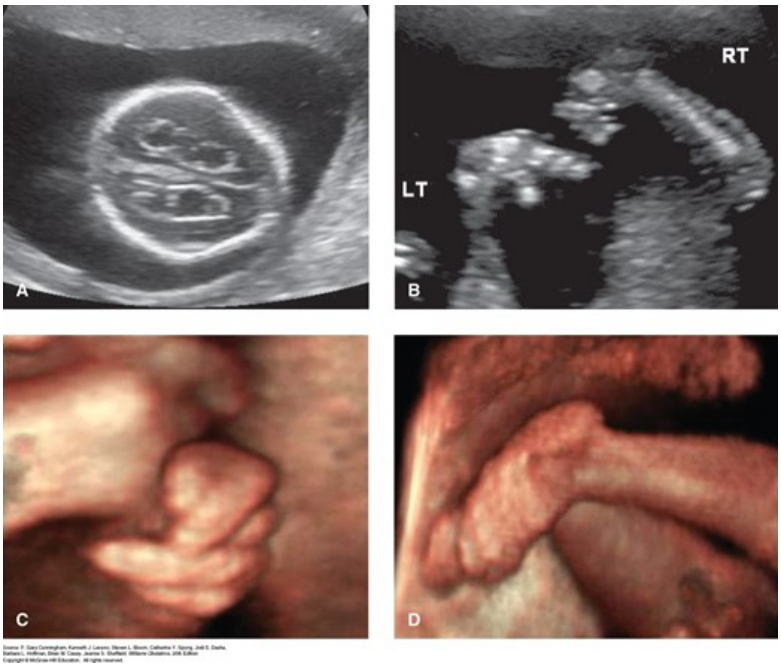
translocation, Edwards syndrome uncommonly results from a chromosomal rearrangement.

Virtually every organ system can be affected by trisomy 18. Common major anomalies include heart defects in more than 90 percent—particularly ventricular septal defects, as well as cerebellar vermian agenesis, myelomeningocele, diaphragmatic hernia, omphalocele, imperforate anus, and renal anomalies such as horseshoe kidney (Lin, 2006; Rosa, 2011; Yeo, 2003). Sonographic images of several of these are shown in Chapter 10.

Cranial and extremity abnormalities are also frequent and include a prominent occiput, posteriorly rotated and malformed ears, micrognathia, clenched hands with overlapping digits, radial aplasia with hyperflexion of the wrists, and rockerbottom or clubbed feet (Fig. 13-5). A "strawberry-shaped" cranium is noted in approximately 40 percent of cases, abnormally wide cavum septum pellucidum in more than 90 percent, and choroid plexus cysts in up to 50 percent (Abele, 2013; Yeo, 2003). Importantly, isolated choroid plexus cysts are not associated with trisomy 18. These cysts only raise the risk for trisomy 18 if fetal structural abnormalities or an abnormal aneuploidy screening test result is also present (Reddy, 2014).

FIGURE 13-5

Trisomy 18—Edwards Syndrome. **A.** This transventricular sonographic view shows fetal choroid plexus cysts and an angulated "strawberry-shaped" skull. **B.** Radial clubhand is manifested as a single forearm bone (radius), with the hands in a fixed, hyperflexed position at right angles to the forearms. **C.** This three-dimensional (3-D) sonographic image shows the characteristic hand position of clenched fists with overlapping digits. **D.** 3-D sonographic image displays a rockerbottom foot.



Pregnancies with trisomy 18 that reach the third trimester often develop fetal-growth restriction, and the mean birthweight is <2500 g (Lin, 2006; Rosa, 2011). Because abnormal fetal heart rate tracings are common during labor, mode of delivery and management of heart rate abnormalities should be discussed in advance. In older reports, more than half of undiagnosed fetuses underwent cesarean delivery for "fetal distress" (Schneider, 1981).

Trisomy 13—Patau Syndrome

This constellation of fetal abnormalities and their association with an autosomal trisomy was first described by Patau and colleagues (1960). The prevalence of trisomy 13 approximates 1 in 12,000 live births and 1 in 5000 recognized pregnancies, which includes abortuses and stillbirths (Loane, 2013; Parker, 2010). As with trisomy 18, trisomy 13 is highly lethal, and most affected fetuses are lost or terminated.

Approximately 80 percent of pregnancies with Patau syndrome result from trisomy 13. The remainder is caused by a robertsonian translocation involving chromosome 13. The most frequent structural chromosomal rearrangement is a translocation between chromosomes 13 and 14, der(13;14)(q10;q10). This translocation is carried by approximately 1 in 1300 individuals, although the risk of an affected liveborn neonate is less than 2 percent (Nussbaum, 2007).

Trisomy 13 is associated with abnormalities of virtually every organ system. One characteristic finding is holoprosencephaly (Fig. 10-15). This is present in approximately two thirds of cases and may be accompanied by microcephaly, hypotelorism, and nasal abnormalities that range from a single nostril to a proboscis. Cardiac defects are found in up to 90 percent of fetuses with trisomy 13 (Shipp, 2002). Other abnormalities that suggest trisomy 13 include neural-tube defects—particularly cephalocele, microphthalmia, cleft lip-palate, omphalocele, cystic renal dysplasia, polydactyly, rockerbottom feet, and areas of skin aplasia (Lin, 2007). For the fetus or newborn with a cephalocele, cystic kidneys, and polydactyly, the differential diagnosis includes trisomy 13 and the autosomal-recessive Meckel-Gruber syndrome. Sonographic images of several of these are shown in Chapter 10 (Neural-Tube Defects).

Few fetuses with trisomy 13 survive until birth. Of those that do, the 1-week survival rate approximates 40 percent, and 1-year survival rate is only about 3 percent (Tennant, 2010; Vendola, 2010). Counseling regarding prenatal diagnosis and management options is similar to that described for trisomy 18.

Unlike other aneuploidies, fetal trisomy 13 confers risk to the pregnant woman. Hyperplacentosis and preeclampsia develop in up to half of pregnancies with trisomy 13 carried beyond the second trimester (Tuohy, 1992). Chromosome 13 contains the gene for soluble fms-like tyrosine kinase-1 (sFlt-1), which is an antiangiogenic protein associated with preeclampsia (Chap. 40, Endothelial Cell Injury). Investigators have documented overexpression of the sflt-1 protein by trisomic 13 placentas and in serum of women with preeclampsia (Bdolah, 2006; Silasi, 2011).

Other Trisomies

In the absence of mosaicism, discussed later (Chromosomal Mosaicism), other autosomal trisomies rarely yield a liveborn neonate. Case of live births with trisomy 9 and with trisomy 22 have been noted (Kannan, 2009; Tinkle, 2003). Trisomy 16 is the most common trisomy found with first-trimester losses and accounts for 16 percent of these losses. However, it is not identified later in gestation. Trisomy 1 has never been reported.

Monosomy

Nondisjunction creates an equal number of nullisomic and disomic gametes. As a rule, missing chromosomal material is more devastating than extra chromosomal material, and almost all monosomic conceptuses are lost before implantation. The one exception is monosomy for the X chromosome (45,X), Turner syndrome, which is discussed subsequently. Despite the strong association between maternal age and trisomy, maternal age and monosomy are not linked.

Polyploidy

This is an abnormal number of complete haploid chromosomal sets. Polyploidy accounts for approximately 20 percent of spontaneous abortions but is rare in later gestations.

Triploid pregnancies have three haploid sets—69 chromosomes. One parent must contribute two sets, and the phenotypic presentation differs according to the parent of origin. With *diandric triploidy*, also known as type I triploidy, the extra chromosomal set is paternal, resulting from fertilization of one egg by two sperm or by a single diploid—and thus abnormal—sperm. Diandric triploidy produces a partial molar pregnancy, discussed in Chapter 20 (Epidemiology and Risk Factors). Diandric triploidy accounts for most triploid conceptions, but their first-trimester loss rate is extremely high. As a result, two thirds of triploid pregnancies identified beyond the first trimester are caused instead by *digynic triploidy* (Jauniaux, 1999). With a digynic triploid pregnancy, also known as type II triploidy, the extra chromosomal set is maternal, and the egg fails to undergo the first or second meiotic division before fertilization. Digynic triploid placentas do not develop molar changes. However, the fetus usually displays asymmetrical growth restriction.

The prevalence of recognized triploid pregnancies approximates 1 in 5000 pregnancies (Zalel, 2016). Triploidy is a lethal aneuploidy, and more than 90 percent of fetuses with either the diandric or digynic form have multiple structural anomalies. These include central nervous system defects—particularly involving the posterior fossa, as well as cardiac, renal, and extremity anomalies (Jauniaux, 1999; Zalel, 2016). Counseling, prenatal diagnosis, and delivery options are similar to those for trisomies 18 and 13. The recurrence risk for a woman whose triploid fetus survived past the first trimester is 1 to 1.5 percent, and thus prenatal diagnosis is offered in future pregnancies (Gardner, 1996).

Tetraploid pregnancies have four haploid sets of chromosomes, resulting in either 92,XXXX or 92,XXYY. This suggests a postzygotic failure to complete

an early cleavage division. The conceptus invariably succumbs, and the recurrence risk is minimal.

Sex Chromosome Abnormalities

45, X—Turner Syndrome

First described by Turner (1938), this syndrome later was found to be caused by monosomy X (Ford, 1959). The prevalence of Turner syndrome is approximately 1 in 2500 liveborn girls (Cragan, 2009; Dolk, 2010). The missing X chromosome is paternally derived in 80 percent of cases (Cockwell, 1991; Hassold, 1990). Screening for Turner syndrome with cell-free DNA is discussed in Chapter 14 (Cell-Free DNA Screening).

Turner syndrome is the only monosomy compatible with life, but it is also the most common aneuploidy in first-trimester losses, accounting for 20 percent. This is explained by the wide range in phenotype. Approximately 98 percent of affected conceptuses are so abnormal that they abort early in the first trimester. Of the remainder, many manifest large, septated cystic hygromas in the late first or early second trimester (Fig. 10-22). When cystic hygromas are accompanied by hydrops fetalis, fetuses nearly always die in utero (Chap 15, Hydrops Fetalis). Less than 1 percent of pregnancies with Turner syndrome yield a liveborn neonate. And, only half of these actually have monosomy X. Approximately a fourth have mosaicism, such as 45,X/46,XX or 45,X/46,XY, and another 15 percent have isochromosome X, that is, 46,X,i(Xq)(Milunsky, 2004; Nussbaum, 2007).

Abnormalities associated with Turner syndrome include left-sided cardiac defects—such as coarctation of the aorta, hypoplastic left heart syndrome, or bicuspid aortic valve—in 30 to 50 percent; renal anomalies, particularly horseshoe kidney; and hypothyroidism. Other features are short stature, broad chest with widely spaced nipples, congenital lymphedema—puffiness over the dorsum of hands and feet, and a “webbed” posterior neck resulting from cystic hygromas. Intelligence scores are generally in the normal range, but affected individuals are at risk for difficulties with visual-spatial organization, nonverbal problem solving, and interpretation of social cues (Jones, 2006). Growth hormone is typically administered in childhood to ameliorate short stature (Kappelgaard, 2011). More than 90 percent have ovarian dysgenesis and require estrogen repletion starting just before adolescence. An exception is mosaicism involving the Y chromosome, as this confers risk for germ cell neoplasm—regardless of whether the child is phenotypically male or female. Accordingly, eventual prophylactic bilateral gonadectomy is indicated (Cools, 2011; Schorge, 2016).

47,XXX

Approximately 1 in 1000 female newborns has an additional X chromosome—47,XXX. The extra X is maternally derived in more than 90 percent of cases (Milunsky, 2004). Affected infants do not have a characteristic appearance, and in the past most children did not come to attention until school age. However, the incidence of 47,XXX is weakly associated with maternal age, and cell-free DNA screening has resulted in increased diagnoses (Table 14-5). Frequent features include tall stature, hypertelorism, epicanthal folds, kyphoscoliosis, clinodactyly, and hypotonia (Tartaglia, 2010; Wigby, 2016). More than a third are diagnosed with a learning disability, half have attention deficit disorder, and overall cognitive scores are in the low-average range. No specific pattern of malformations has been described, but genitourinary problems and seizure disorders are more common (Wigby, 2016). Pubertal development is unaffected. Primary ovarian insufficiency has been reported (Holland, 2001). Because of variable presentation and subtle abnormal findings, it is estimated that this diagnosis is ascertained clinically in only 10 percent of affected children.

Females with two or more extra X chromosomes—48,XXXX or 49,XXXXX—are likely to have physical abnormalities apparent at birth. These abnormal X complements are associated with intellectual disability. For both males and females, the IQ score is lower with each additional X chromosome.

47,XXY—Klinefelter Syndrome

This is the most common sex chromosome abnormality and found in approximately 1 in 600 male infants. The additional X chromosome is maternally or paternally derived with equal propensity (Jacobs, 1995; Lowe, 2001). The association with either advanced maternal or paternal age is weak (Milunsky, 2004).

Like 47,XXX, newborns with 47,XXY usually appear phenotypically normal and do not have a higher incidence of anomalies. As children, boys are typically taller than average and have normal prepubertal development. However, they have gonadal dysgenesis, do not undergo normal virilization, and require testosterone supplementation beginning in adolescence. They may develop gynecomastia. IQ scores usually lie in the average to low-average range, and many have delays in language development and reading (Boada, 2009; Girardin, 2011).

47,XYY

This aneuploidy occurs in approximately 1 in 1000 male newborns. As with 47,XXX and XXY individuals, affected boys tend to be tall. A third have macrocephaly, nearly two thirds demonstrate hypotonia, and tremors are also common (Bardsley, 2013). Rates of major anomalies are not elevated, although hypertelorism and clinodactyly may be identified in more than half. Pubertal development is normal, and fertility is unimpaired. Affected children carry risks for oral and written language impairments, attention deficit disorder is diagnosed in more than half, and the rate of autism spectrum disorder is also increased (Bardsley, 2013; Ross, 2009). Intelligence scores generally lie in the normal range.

Males with more than two Y chromosomes—48,XYYY—or with both additional X and Y chromosomes—48,XXYY or 49,XXXYY—are more likely to have congenital abnormalities, medical problems, and intellectual disability (Tartaglia, 2011).

Abnormalities of Chromosome Structure

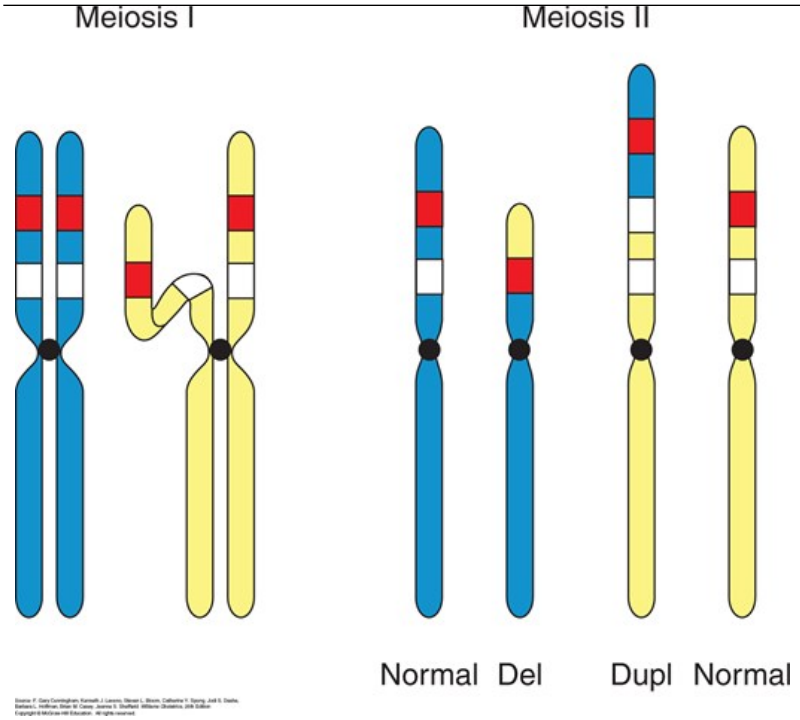
Structural chromosomal abnormalities include deletions, duplications, translocations, isochromosomes, inversions, ring chromosomes, and mosaicism (see Table 13-1). Their overall birth prevalence approximates 0.3 percent (Nussbaum, 2007). Identification of a structural chromosomal abnormality raises two primary questions. First, what phenotypic abnormalities or later developmental abnormalities are associated with this finding? Second, is evaluation of parental karyotype indicated—specifically, are the parents at increased risk of carrying this abnormality? If so, what is their risk of having future affected offspring?

Deletions and Duplications

A chromosomal deletion indicates that a portion of a chromosome is missing, whereas a duplication means that a portion has been included twice. Most deletions and duplications occur during meiosis and result from malalignment or mismatching during the pairing of homologous chromosomes. The misaligned segment may then be deleted, or if the mismatch remains when the two chromosomes recombine, it may result in a deletion in one chromosome and duplication in the other (Fig. 13-6). When a deletion or duplication is identified in a fetus or infant, parental karyotyping should be offered, because if either parent carries a balanced translocation, the recurrence risk in subsequent pregnancies is significantly increased. Deletions involving DNA segments large enough to be seen with standard cytogenetic karyotyping are identified in approximately 1 in 7000 births (Nussbaum, 2007). Common deletions may be referred to by eponyms—for example, del 5p is called *cri du chat syndrome*.

FIGURE 13-6

A mismatch during pairing of homologous chromosomes may lead to a deletion in one chromosome and a duplication in the other. Del = deletion; Dupl = duplication.



Microdeletions and Microduplications

These chromosomal deletions or duplications—smaller than 3 to 5 million base pairs—are too small to be detected with standard karyotyping. However, prenatal chromosomal microarray analysis (CMA), described later (Chromosomal Microarray Analysis), permits identification of syndromes associated with these microdeletion or duplications. When CMA is used, the region of DNA that is missing or duplicated is termed a *genomic copy number variant.* Despite the relatively small size, a microdeletion or duplication may involve a stretch of DNA that contains multiple genes—causing a *contiguous gene syndrome*, which can encompass serious but unrelated phenotypic abnormalities (Schmickel, 1986). In some cases, a microduplication may involve the exact DNA region that causes a recognized microdeletion syndrome (Table 13-2). When a specific microdeletion syndrome is suspected clinically, it is confirmed using either CMA or fluorescence in situ hybridization.

TABLE 13-2

Selected Microdeletion Syndromes

Syndrome	Prevalence	Location	Features
Alagille	1:70,000	20p12.2	Cholestasis (paucity of intrahepatic bile ducts), cardiac disease, skeletal disease, ocular abnormalities, dysmorphic facies
Angelman	1:12,000 to 1:20,000	15q11.2–q13 (maternal genes)	Dysmorphic facies—"happy puppet" appearance, mental retardation, ataxia, hypotonia, seizures
Cri-du-chat	1:20,000 to 1:50,000	5p15.2–15.3	Abnormal laryngeal development with "cat-like" cry, hypotonia, mental retardation
Kallmann syndrome	1:10,000 to 1:86,000	Xp22.3	Hypogonadotropic hypogonadism, anosmia
Langer-Giedion	Rare	8q23.3	Trichorhinophalangeal syndrome, dysmorphic facies, sparse hair, redundant skin, mental retardation
Miller-Dieker	Rare	17p13.3	Neuronal migration abnormalities with lissencephaly, microcephaly, dysmorphic facies
Prader-Willi	1:10,000 to 1:30,000	15q11.2–q13 (paternal genes)	Obesity, hypotonia, mental retardation, hypogonadotropic hypogonadism, small hands and feet
Retinoblastoma	1:280,000	13q14.2	Retinoblastoma, retinoma (benign neoplasm), non-retinal (second primary) tumors
Rubenstein-Taybi	1:100,000 to 1:125,000	16p13.3	Dysmorphic facies, broad thumbs/toes, mental retardation, increased tumor risk
Smith-Magenis	1:15,000 to 1:25,000	17p11.2	Dysmorphic facies, speech delay, hearing loss, sleep disturbances, self-destructive behaviors
Velocardiofacial/DiGeorge/Shprintzen	1:4,000	22q11.2	Conotruncal cardiac defects, cleft palate, velopharyngeal incompetence, thymic and parathyroid abnormalities, developmental delay
WAGR	1:500,000	11p13	Wilms tumor, aniridia, genitourinary anomalies (including ambiguous genitalia), mental retardation
Williams-Beuren	1:7500 to 1:10,000	7q11.23	Dysmorphic facies, dental malformation, mental retardation, aortic and peripheral pulmonary artery stenosis
Wolf-Hirschhorn	1:20,000 to 1:50,000	4p16.3	Dysmorphic facies, delayed growth and development, cleft lip/palate, coloboma, cardiac septal defects
X-linked ichthyosis	1:6,000	Xp22.3	Steroid sulfatase deficiency, corneal opacities

Prevalence reflects live births.

Data from National Library of Medicine, 2017; Johns Hopkins University, 2017.

22q11.2 Microdeletion Syndrome

This syndrome is also known as DiGeorge syndrome, Shprintzen syndrome, and velocardiofacial syndrome. It is the most common microdeletion, with a prevalence of 1 in 3000 to 6000 births. Although inherited in an autosomal dominant fashion, more than 90 percent of cases arise from de novo mutations. The full deletion includes 3 million base pairs, encompasses 40 genes, may include 180 different features, and thus poses some counseling challenges (Shprintzen, 2008). Features can vary widely, even among affected family members. Previously, different constellations of features were thought to characterize the DiGeorge and Shprintzen phenotypes, but it is now accepted that they represent the same microdeletion (McDonald-McGinn, 2015).

In approximately 75 percent of affected individuals, associated abnormalities include conotruncal cardiac anomalies, such as tetralogy of Fallot, truncus arteriosus, interrupted aortic arch, and ventricular septal defects (McDonald-McGinn, 2015). Immune deficiency, such as T-cell lymphopenia, also develops in approximately 75 percent. More than 70 percent have velopharyngeal insufficiency or cleft palate. Learning disabilities, autism spectrum disorder, and intellectual disability are also common. Other manifestations include hypocalcemia, renal anomalies, esophageal dysmotility, hearing loss, behavioral disorders, and psychiatric illness—particularly schizophrenia. Short palpebral fissures, bulbous nasal tip, micrognathia, short philtrum, and small or posteriorly rotated ears are characteristic facial features.

Chromosomal Translocations

These are DNA rearrangements in which a segment of DNA breaks away from one chromosome and attaches to another. The rearranged chromosomes are called derivative (der) chromosomes. There are two types—reciprocal and robertsonian translocations.

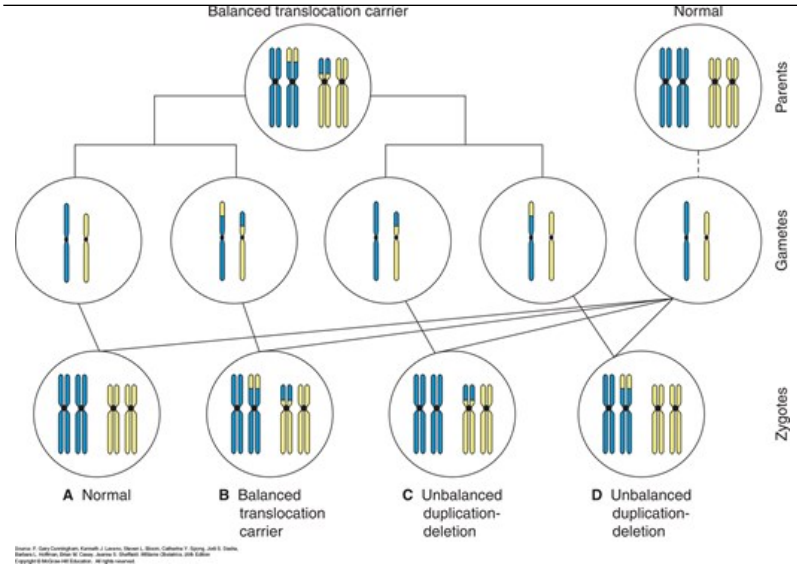
Reciprocal Translocations

A double-segment or reciprocal translocation begins when breaks occur in two different chromosomes. The broken fragments are then exchanged, so that each affected chromosome contains a fragment of the other. If no chromosomal material is gained or lost in this process, the translocation is considered balanced. The prevalence of reciprocal translocations approximates 1 in 600 births (Nussbaum, 2007). Although the balanced translocation carrier is usually normal phenotypically, repositioning of specific genes within chromosomal segments can cause abnormalities. The risk of a major structural or developmental abnormality in an apparent balanced translocation carrier is approximately 6 percent. Interestingly, using CMA technology, up to 20 percent of individuals who appear to have a balanced translocation are found instead to have missing or redundant DNA segments (Manning, 2010).

Balanced translocation carriers are at risk to produce unbalanced gametes, resulting in abnormal offspring. As shown in Figure 13-7, if an oocyte or sperm contains a translocated chromosome, fertilization results in an unbalanced translocation—monosomy for part of one affected chromosome and trisomy for part of the other. The observed risk of a specific translocation can often be estimated by a genetic counselor. In general, translocation carriers identified after the birth of an abnormal child have a 5- to 30-percent risk of producing liveborn offspring with an unbalanced translocation. Carriers identified for other reasons, for example, during an infertility evaluation, have only a 5-percent risk. This is likely because the gametes are so abnormal that conceptions are nonviable.

FIGURE 13-7

A carrier of a balanced translocation may produce offspring who are also carriers of the balanced rearrangement **(B)**, offspring with unbalanced translocations **(C, D)**, or offspring with normal chromosomal complements **(A)**.



Robertsonian Translocations

These involve only *acrocentric* chromosomes, which are chromosomes 13, 14, 15, 21, and 22. The acrocentric chromosomes have extremely short p arms. In a robertsonian translocation, the q arms of two acrocentric chromosomes fuse at one centromere to form a derivative chromosome. The other centromere and both sets of p arms are lost. Because the number of centromeres determines the chromosome count, a robertsonian translocation carrier has only 45 chromosomes. Fortunately, the p arms of the acrocentric chromosomes—the satellite regions—contain redundant copies of genes that code for ribosomal RNA. As these are present in multiple copies on other acrocentric chromosomes, their loss does not affect the translocation carrier, who is usually phenotypically normal. However, when the derivative chromosome is paired during fertilization with a haploid chromosome from the partner, resulting offspring will be trisomic for that chromosome.

Robertsonian translocations are found in 1 in 1000 individuals. The incidence of abnormal offspring approximates 15 percent if a robertsonian translocation is carried by the mother and 2 percent if carried by the father. Robertsonian translocations are not a major cause of miscarriage and are found in fewer than 5 percent of couples with recurrent pregnancy loss. When a fetus or child is found to have a translocation trisomy, both parents should be offered karyotype analysis. If neither parent is a carrier, the recurrence risk is extremely low.

Balanced robertsonian carriers have reproductive difficulties for a number of reasons. If the fused chromosomes are homologous, that is, from the same chromosome pair, the carrier can produce only unbalanced gametes. Each egg or sperm contains either both copies of the translocated chromosome, which would result in trisomy if fertilized, or no copy, which would result in monosomy. If the fused chromosomes are nonhomologous, four of the six possible gametes would be abnormal. The most common robertsonian translocation is der(13;14)(q10;q10), which accounts for up to 20 percent of cases of Patau syndrome (Trisomy 18—Edwards Syndrome).

Isochromosomes

These abnormal chromosomes are composed of either two q arms or two p arms of one chromosome fused together. Isochromosomes are thought to arise when the centromere breaks transversely instead of longitudinally during meiosis II or mitosis. They can also result from a meiotic error in a chromosome with a robertsonian translocation. An isochromosome containing the q arms of an acrocentric chromosome behaves like a homologous robertsonian translocation, and such a carrier can produce only abnormal unbalanced gametes. When an isochromosome involves nonacrocentric chromosomes, with p arms containing important genetic material, the fusion and abnormal centromere break results in two isochromosomes. One is composed of both p arms, and one is composed of both q arms. It is likely that one of these isochromosomes would be lost during cell division, resulting in the deletion of all the genes located on the lost arm. Thus, a carrier is usually phenotypically abnormal and produces abnormal gametes. The most common isochromosome involves the long arm of the X chromosome, i(Xq), which is the etiology of 15 percent of cases of Turner syndrome.

Chromosomal Inversions

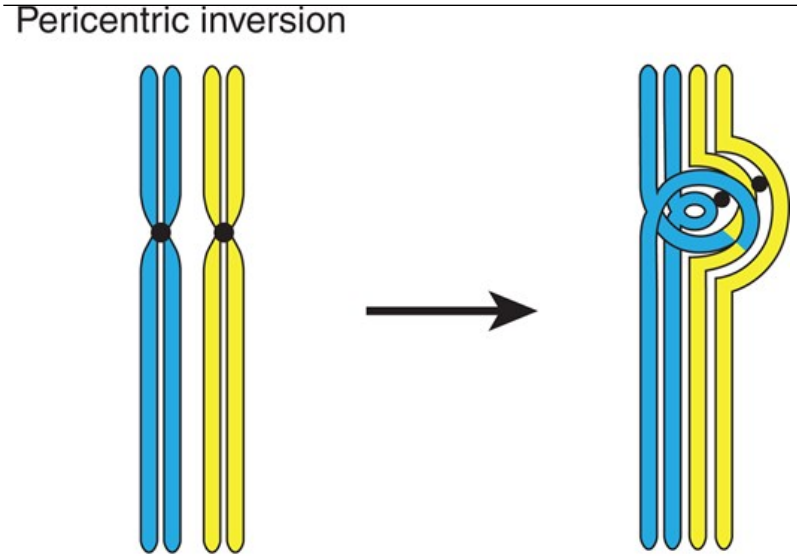
When there are two breaks in the same chromosome, and the intervening genetic material is inverted before the breaks are repaired, the result is a chromosomal inversion. Although no genetic material is lost or duplicated, the rearrangement may alter gene function. There are two types—pericentric and paracentric inversions.

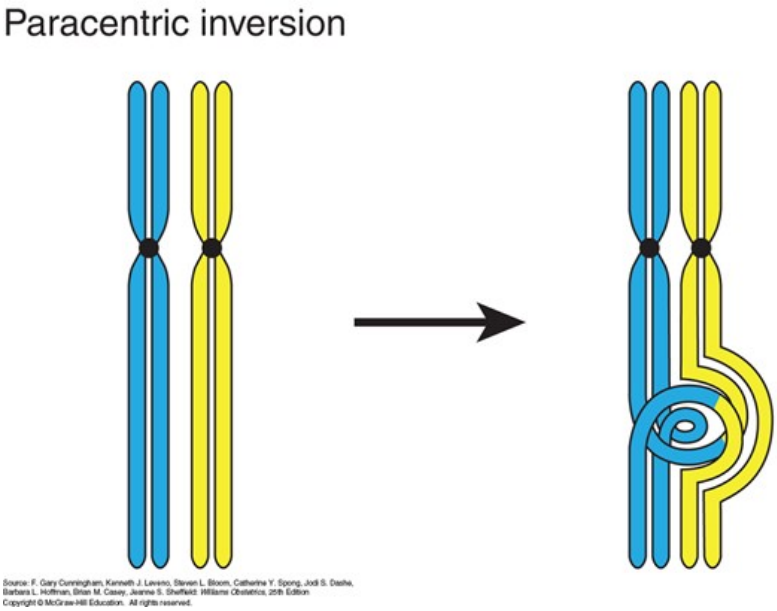
Pericentric Inversion

This results from breaks in both the p and q arms of a chromosome, such that the inverted material includes the centromere (Fig. 13-8). A pericentric inversion causes problems in chromosomal alignment during meiosis and confers significant risk for the carrier to produce abnormal gametes and abnormal offspring. In general, the observed risk of abnormal offspring in a pericentric inversion carrier is 5 to 10 percent if ascertainment was made after the birth of an abnormal child. But the risk is only 1 to 3 percent if prompted by another indication. An important exception is a pericentric inversion on chromosome 9. This is inv(9)(p11q12), which is a normal variant present in approximately 1 percent of the population.

FIGURE 13-8

Mechanism of meiosis in the setting of either pericentric inversion (one involving the centromere) or paracentric inversion (not involving the centromere). Individuals with pericentric inversions are at increased risk for producing offspring with a duplication/deletion. Those with paracentric inversions are at increased risk for early pregnancy loss.





Paracentric Inversion

If there are two breaks within one arm of a chromosome—either p or q—the inverted material does not include the centromere, and the inversion is paracentric (see Fig. 13-8). The carrier makes either normal balanced gametes or gametes that are so abnormal as to preclude fertilization. Thus, although infertility may be a problem, the risk of having an abnormal offspring is extremely low.

Ring Chromosome

If there are deletions at each end of the same chromosome, the ends may come together to form a ring chromosome. The telomere regions, which are the ends of a chromosome, contain specialized nucleoprotein complexes that stabilize the chromosome. If just the telomeres are lost, all necessary genetic material is retained, and the carrier is essentially balanced. If a deletion extends more proximally than the telomere, the carrier is likely to be phenotypically abnormal. An example of this is a ring X chromosome, which may result in Turner syndrome.

Chromosomal Mosaicism

A mosaic individual has two or more cytogenetically distinct cell lines that are derived from a single zygote. Phenotypic expression of mosaicism depends on several factors, including whether the cytogenetically abnormal cells involve the fetus, part of the fetus, just the placenta, or some combination. Of amnionic fluid cultures, mosaicism is found in approximately 0.3 percent but may not reflect the fetal chromosomal complement (Carey, 2014). When the abnormal cells are present in only a single flask of amnionic fluid, the finding is likely pseudomosaicism, caused by cell-culture artifact (Bui, 1984; Hsu, 1984). When abnormal cells involve multiple cultures, however, true mosaicism is more likely, and further testing may be warranted. A second cell line is verified in 60 to 70 percent of these fetuses (Hsu, 1984; Worton, 1984).

Confined Placental Mosaicism

With chorionic villus sampling, studies demonstrate that up to 2 percent of placentas are mosaic, with the mosaicism confined to the placenta in most of these cases (Baffero, 2012; Henderson, 1996). Amniocentesis should be offered. In a series of more than 1000 pregnancies with mosaicism found from chorionic villus sampling, subsequent amniocentesis identified true fetal mosaicism in 13 percent. Uniparental disomy, discussed later (Imprinting), was found in 2 percent, and the remainder resulted from confined placental mosaicism (Malvestiti, 2015). If mosaicism is detected for a chromosome known to contain imprinted genes—such as chromosomes 6, 7, 11, 14, or 15—testing for uniparental disomy should be considered, as there may be fetal consequences (Grati, 2014a).

Although outcomes with confined placental mosaicism are generally good, fetal-growth restriction is more common, and the stillbirth risk is also higher (Reddy, 2009). Fetal-growth restriction may stem from impaired functioning of the aneuploid placental cells (Baffero, 2012). Placental mosaicism for trisomy 16 confers a particularly poor prognosis.

Gonadal Mosaicism

Mosaicism confined to the gonads likely arises from a mitotic error in cells destined to become the gonad, resulting in a population of abnormal germ cells. Because spermatogonia and oogonia divide throughout fetal life, and spermatogonia continue to divide throughout adulthood, gonadal mosaicism may also follow a meiotic error in previously normal germ cells. Gonadal mosaicism can account for de novo diseases in the offspring of normal parents. Autosomal dominant examples are achondroplasia and osteogenesis imperfecta, and X-linked ones include Duchenne muscular dystrophy. Gonadal mosaicism also explains the 6-percent recurrence risk after the birth of a child with a disease caused by a “new” mutation.

MODES OF INHERITANCE

A monogenic or *mendelian* disorder is caused by a mutation or alteration in a single locus or gene in one or both members of a gene pair. Types of mendelian inheritance include autosomal dominant, autosomal recessive, X-linked, and Y-linked. Other monogenic inheritance patterns, described subsequently, include mitochondrial inheritance, uniparental disomy, imprinting, and trinucleotide repeat expansion—also termed anticipation. By age 25, approximately 0.4 percent of the population exhibits an abnormality attributed to a monogenic disorder, and 2 percent will have at least one such disorder during their lifetime (Table 13-3).

TABLE 13-3

Selected Monogenic (Mendelian) Disorders

Autosomal Dominant
Achondroplasia
Acute intermittent porphyria
Adult polycystic kidney disease
Antithrombin III deficiency
BRCA1 and BRCA2 breast and/or ovarian cancer
Ehlers-Danlos syndrome
Familial adenomatous polyposis
Familial hypercholesterolemia
Hereditary hemorrhagic telangiectasia
Hereditary spherocytosis
Huntington disease
Hypertrophic obstructive cardiomyopathy
Long QT syndrome
Marfan syndrome
Myotonic dystrophy
Neurofibromatosis type 1 and 2
Tuberous sclerosis
von Willebrand disease
Autosomal Recessive
α_1-Antitrypsin deficiency
Congenital adrenal hyperplasia
Cystic fibrosis
Gaucher disease
Hemochromatosis
Homocystinuria
Phenylketonuria
Sickle-cell anemia
Tay-Sachs disease
Thalassemia syndromes
Wilson disease
X-Linked
Androgen insensitivity syndrome
Chronic granulomatous disease
Color blindness
Fabry disease
Fragile X syndrome
Glucose-6-phosphate deficiency
Hemophilia A and B
Hypophosphatemic rickets
Muscular dystrophy—Duchenne and Becker
Ocular albinism type 1 and 2

Relationship between Phenotype and Genotype

When considering inheritance, it is the phenotype that is dominant or recessive, not the genotype. With a dominant disease, the normal gene may direct the production of normal protein, but the phenotype is abnormal because it is determined by protein produced by the abnormal gene. With a recessive disease, a heterozygous carrier may produce detectable levels of an abnormal gene product but have no features of the condition because the phenotype is directed by the product of the normal co-gene. For example, erythrocytes from carriers of sickle-cell anemia contain approximately 30 percent hemoglobin S, but because the other 70 percent is hemoglobin A, these cells do not usually sickle in vitro.

Heterogeneity

Genetic heterogeneity explains how different genetic mechanisms can result in the same phenotype. *Locus heterogeneity* indicates that a specific disease phenotype can be caused by mutations in different genetic loci. It also explains why some diseases appear to follow more than one type of inheritance. An example is retinitis pigmentosa, which may develop following mutations in at least 35 different genes or loci and may result in autosomal dominant, autosomal recessive, or X-linked forms.

Allelic heterogeneity describes how different mutations of the same gene may affect presentation of a particular disease. For example, although only one gene has been associated with cystic fibrosis—the *cystic fibrosis conductance transmembrane regulator* gene—more than 2000 mutations in this gene have been described and result in variable disease severity (Chaps. 14 and 51, Cystic Fibrosis and Sarcoidosis).

Phenotypic heterogeneity explains how different disease states can arise from different mutations in the same gene. As an example, mutations in the *fibroblast growth factor receptor 3 (FGFR3)* gene may result in several different skeletal disorders, including achondroplasia and thanatophoric dysplasia, both of which are discussed in Chapter 10 (Skeletal Abnormalities).

Autosomal Dominant Inheritance

If only one copy of a gene pair determines the phenotype, that gene is considered to be dominant. Carriers have a 50-percent chance of passing on the affected gene with each conception. A gene with a dominant mutation generally specifies the phenotype in preference to the normal gene. That said, not all individuals will necessarily manifest an autosomal dominant condition the same way. Factors that affect the phenotype of an autosomal dominant condition include penetrance, expressivity, and occasionally, presence of codominant genes.

Penetrance

This characteristic describes whether or not a dominant gene is expressed at all. A gene with recognizable phenotypic expression in all individuals is 100-percent penetrant, whereas penetrance is incomplete if some carriers express the gene but some do not. This may be quantitatively expressed—for example, a gene that is expressed in some way in 80 percent of individuals who have that gene is 80-percent penetrant. Importantly, incomplete penetrance explains why some autosomal dominant diseases may appear to “skip” generations.

Expressivity

Individuals with the same autosomal dominant trait may manifest the condition differently, even within the same family. Genes with variable expressivity can produce disease manifestations that range from mild to severe. Examples include neurofibromatosis, tuberous sclerosis, and adult polycystic kidney disease.

Codominant Genes

If two different alleles in a gene pair are both expressed in the phenotype, they are considered to be codominant. Blood type, for example, is determined by expression of dominant A and B red-cell antigens that can be expressed simultaneously. Another example of codominance is the group of genes responsible for hemoglobin production. An individual with one gene directing production of hemoglobin S and the other directing production of hemoglobin C will produce both S and C hemoglobin (Chap. 56, Pregnancy and Sickle-Cell Syndromes).

Advanced Paternal Age

Paternal age older than 40 is associated with increased risk for spontaneous genetic mutations, particularly single base substitutions. This may result in offspring with new autosomal dominant disorders or X-linked carrier states. In particular, advanced paternal age has been associated with mutations in the *fibroblast growth factor receptor 2 (FGFR2)* gene, which may cause craniosynostosis syndromes such as Apert, Crouzon, and Pfeiffer syndromes; mutations in the *FGFR3* gene, which may result in achondroplasia and thanatophoric dysplasia; and mutations in the *RET proto-oncogene,* which may cause multiple endocrine neoplasia syndromes (Jung, 2003; Toriello, 2008). Using whole genome sequencing, described later (Whole Genome Sequencing and Whole Exome Sequencing), Kong and associates (2012) also demonstrated that paternal age contributes to a rise in the rate of single-nucleotide polymorphisms among offspring. This rate is approximately two mutations for each year of paternal age. Because individual autosomal dominant disorders are uncommon, the actual risk for any specific condition is low, and no screening or testing is specifically recommended.

Advanced paternal age has also been associated with a slightly greater risk for fetal Down syndrome and for isolated structural abnormalities (Grewal, 2012; Toriello, 2008; Yang, 2007). It is not generally considered to pose an elevated risk for other aneuploidies, probably because the aneuploid sperm cannot fertilize an egg.

Autosomal Recessive Inheritance

Recessive diseases develop only when both gene copies are abnormal. Many enzyme deficiency diseases display autosomal recessive inheritance, and enzyme activity in the carrier is usually about half of normal. Unless carriers are screened for a specific disease, such as cystic fibrosis, they usually are recognized only after the birth of an affected child or the diagnosis of an affected family member (Chap. 14, Cystic Fibrosis). If a couple has a child with an autosomal recessive disease, the recurrence risk is 25 percent for each subsequent pregnancy. Thus, 1/4 of offspring will be homozygous normal, 2/4 will be heterozygous carriers, and 1/4 will be homozygous abnormal. In other words, three of four children will be phenotypically normal, and 2/3 of phenotypically normal siblings are actually carriers.

A heterozygous carrier of a recessive condition is only at risk to have affected children if his or her partner is heterozygous or homozygous for the disease. Genes for rare autosomal recessive conditions have low prevalence in the general population. Thus, the likelihood that a partner will be a gene carrier is small, unless there is consanguinity or the partner is a member of an at-risk group. Heterozygous carriers are usually undetectable clinically but may have biochemical test abnormalities that can be used for carrier screening. Other recessive conditions can be identified only by molecular genetic testing (Chap. 14, Carrier Screening for Genetic Disorders).

Inborn Errors of Metabolism

Most of these autosomal recessive diseases result from absence of a crucial enzyme, leading to incomplete metabolism of proteins, lipids, or carbohydrates. The metabolic intermediates that build up are toxic to various tissues and may result in intellectual disability or other abnormalities.

Phenylketonuria

Also known as phenylalanine hydroxylase (PAH) deficiency, this autosomal recessive disease is caused by mutations in the *PAH* gene. PAH metabolizes phenylalanine to tyrosine, and homozygotes have diminished or absent enzyme activity. This leads to abnormally high levels of phenylalanine, resulting in progressive intellectual impairment, autism, seizures, motor deficits, and neuropsychological abnormalities (Blau, 2010). Because phenylalanine competitively inhibits tyrosine hydroxylase—which is essential for melanin production, affected individuals also have hair, eye, and skin hypopigmentation. More than 500 *PAH* gene mutations have been characterized, and the carrier frequency is 1 in 60, such that the disease affects approximately 1 in 15,000 newborns (American College of Obstetricians and Gynecologists, 2017c). Prompt diagnosis and restriction of dietary phenylalanine beginning early in infancy are essential to prevent neurological damage, and all states mandate newborn screening for phenylketonuria (PKU).

Phenylalanine restriction alone would result in inadequate protein consumption, and phenylalanine-free amino acid-based supplementation is required. Also, in 2007, a synthetic form of the PAH cofactor tetrahydrobiopterin (sapropterin) was approved for PKU treatment. Approximately 25 to 50 percent of affected individuals are sapropterin-responsive and may experience a significant decline in phenylalanine levels and improvement in neuropsychiatric symptoms (Vockley, 2014). Lifelong maintenance of phenylalanine concentrations in the range of 2 to 6 mg/dL (120 to 360 µmol/L) is necessary to prevent worsening neurocognitive and psychiatric problems (American College of Obstetricians and Gynecologists, 2017c). Fortunately, even those who have previously discontinued therapy may experience improved neuropsychological function with treatment.

During pregnancy, women with PKU whose phenylalanine levels remain above the recommended range are at risk to have otherwise normal (heterozygous) offspring who sustain in utero damage as a result of being exposed to toxic phenylalanine concentrations. Phenylalanine is actively transported to the fetus. Hyperphenylalaninemia raises the risk for miscarriage and for PKU embryopathy, characterized by intellectual disability, microcephaly, seizures, growth impairment, and cardiac anomalies. Among women on unrestricted diets, the risk to have a child with intellectual disability exceeds 90 percent, microcephaly occurs in more than 70 percent, and as many as 1 in 6 children have cardiac defects (Lenke, 1980). The Maternal Phenylketonuria Collaborative Study, which included 572 pregnancies followed more than 18 years, reported that maintenance of serum phenylalanine levels in the recommended range between 2 and 6 mg/dL significantly reduced the fetal abnormality risk and resulted in childhood IQ scores in the normal range (Koch, 2003; Platt, 2000). Preconceptional counseling and consultation with providers from experienced PKU centers is recommended.

Consanguinity

Two individuals are considered consanguineous if they have at least one recent ancestor in common. Although uncommon in Western countries, more than 1 billion people are estimated to live in countries in which 20 to 50 percent of marriages are consanguineous (Romeo, 2014). In medical genetics, a union is consanguineous if between second cousins or closer relatives. First-degree relatives share half of their genes, second-degree relatives share a fourth, and third-degree relatives—first cousins—share one eighth. Because of the potential for shared deleterious genes, consanguinity confers an increased risk to have offspring with otherwise rare autosomal recessive diseases or multifactorial disorders. In population-based series, first cousins are reported to have a twofold risk for congenital anomalies (Sheridan, 2013; Stoltenberg, 1997). Consanguinity also is associated with a greater rate of stillbirth (Kapurubandara, 2016). Because CMA performed using a single-nucleotide polymorphism platform may identify consanguinity, it is important that preprocedural counseling include this possibility.

Incest is defined as a sexual relationship between first-degree relatives such as parent-child or brother-sister and is universally illegal. Progeny of such unions carry the highest risk of abnormal outcomes, and older studies reported that up to 40 percent of offspring were abnormal as a result of recessive and multifactorial disorders (Baird, 1982; Freire-Maia, 1984).

X-Linked and Y-Linked Inheritance

Most X-linked diseases are recessive. Common examples include color blindness, hemophilia A and B, and Duchenne and Becker muscular dystrophy. Males with an X-linked recessive gene are usually affected by the disease it causes, because they lack a second X chromosome to express the normal dominant gene. A male with an X-linked disease cannot have affected sons because they cannot receive his X chromosome. When a woman carries a gene causing an X-linked recessive condition, each of her sons has a 50-percent risk of being affected, and each daughter has a 50-percent chance of being a carrier.

Women with an X-linked recessive gene are generally unaffected by the disease it causes. In some cases, however, the random inactivation of one X chromosome in each cell—termed lyonization—is skewed, and female carriers may have features of the condition. For example, approximately 10 percent of female carriers of hemophilia A will have factor VIII levels less than 30 percent of normal, and a similar proportion of female hemophilia B carriers have factor IX levels less than 30 percent. Levels below these thresholds confer a greater risk for abnormal bleeding when affected women give birth (Plug, 2006). Indeed, even with higher levels, carriers are reported to be at increased risk for bleeding complications (Olsson, 2014). Similarly, female carriers of Duchenne or Becker muscular dystrophy carry an elevated risk for cardiomyopathy, and periodic evaluation for cardiac dysfunction and neuromuscular disorders is recommended (American Academy of Pediatrics, 2008).

X-linked dominant disorders mainly affect females, because they tend to be lethal in males. Two examples are vitamin D-resistant rickets and incontinentia pigmenti. One exception is fragile X syndrome, which is discussed subsequently.

The prevalence of Y-linked chromosomal disorders is low. This chromosome carries genes important for sex determination and various cellular functions related to spermatogenesis and bone development. Deletion of genes on the long arm of Y results in severe spermatogenic defects, whereas genes at the tip of the short arm are critical for chromosomal pairing during meiosis and for fertility.

Mitochondrial Inheritance

Human cells contain hundreds of mitochondria, each with its own genome and associated replication system. Oocytes contain approximately 100,000

mitochondria. Sperm hold only about 100, and these are destroyed after fertilization. Each mitochondrion has multiple copies of a 16.5-kb circular DNA molecule that contains 37 genes. Mitochondrial DNA encodes peptides required for oxidative phosphorylation and encodes ribosomal and transfer RNAs.

Mitochondria are inherited exclusively from the mother. Thus, although males and females both can be affected by a mitochondrial disorder, transmission is only through the mother. When a cell replicates, mitochondrial DNA sorts randomly into each of the daughter cells, a process termed replicative segregation. A consequence of replicative segregation is that any mitochondrial mutation will be propagated randomly into the daughter cells. Because each cell holds multiple copies of mitochondrial DNA, the mitochondrion may contain only normal or only abnormal DNA, termed *homoplasmy.* Alternatively, it may contain both normal and mutated DNA, namely *heteroplasmy.* If a heteroplasmic oocyte is fertilized, the relative proportion of mutated DNA may affect whether the individual manifests a given mitochondrial disease. It is not possible to predict the potential degree of heteroplasmy among offspring, and this poses challenges for genetic counseling.

As of 2016, 33 mitochondrial diseases or conditions with known molecular basis were described in Online Mendelian Inheritance in Man (Johns Hopkins University, 2017). Examples include myoclonic epilepsy with ragged red fibers (MERRF), Leber optic atrophy, Kearns-Sayre syndrome, Leigh syndrome, several forms of mitochondrial myopathy and cardiomyopathy, and susceptibility to chloramphenicol toxicity.

DNA Triplet Repeat Expansion—Anticipation

Mendel's first law is that genes are passed unchanged from parent to progeny, and barring new mutations, this is true for many genes or traits. However, certain genes are unstable, and their size, and thus function, may be altered during parent-to-child transmission. This is manifested clinically by anticipation—a phenomenon in which disease symptoms seem to be more severe and to appear at an earlier age in each successive generation. Examples of some DNA triplet (trinucleotide) repeat diseases are shown in Table 13-4.

TABLE 13-4

Some Disorders Caused by DNA Triplet Repeat Expansion

Dentatorubral-pallidoluysian atrophy
Fragile X syndrome
Friedreich ataxia
Huntington disease
Spinal and bulbar muscular atrophy
Myotonic dystrophies
Spinocerebellar ataxias

Fragile X Syndrome

This is the most common inherited form of intellectual disability and affects approximately 1 in 3600 males and 1 in 4000 to 6000 females (American College of Obstetricians and Gynecologists, 2017a). Fragile X syndrome is caused by expansion of a repeated trinucleotide DNA segment—cytosine-guanine-guanine (CGG)—at chromosome Xq27.3. When the CGG repeat number reaches a critical size—the full mutation—the *fragile X mental retardation 1 (FMR1)* gene becomes methylated. Methylation inactivates the gene, which halts expression of FMR1 protein. This protein is most abundant in nerve cells and is essential for normal cognitive development.

Although transmission of the syndrome is X-linked, both the sex of the affected individual and the number of CGG repeats determine the degree of clinical normalcy or impairment. Intellectual disability is generally more severe in males, in whom average IQ scores are 35 to 45 (Nelson, 1995). Affected individuals may have speech and language problems and attention-deficit/hyperactivity disorder. Fragile X syndrome is also the most common known cause of autism or "autistic like" behavior. Associated phenotypic abnormalities become more prominent with age and include a narrow face with large jaw, prominent ears, connective tissue abnormalities, and macroorchidism in postpubertal males. Clinically, four groups have been described (American College of Obstetricians and Gynecologists, 2017a):

- Full mutation—more than 200 repeats
- Premutation—55 to 200 repeats
- Intermediate—45 to 54 repeats
- Unaffected—fewer than 45 repeats

Full mutations are expressed (penetrant) in all males and many females. When a full mutation is present, males typically have significant cognitive and behavioral abnormalities and phenotypic features. In females, random X-inactivation, however, results in variable expression, and the disability may be much less severe. With rare exception, the parent of origin of repeat expansion that leads to a full mutation is female (Monaghan, 2013).

For individuals with a premutation, evaluation and counseling are more complex. A female with the fragile X premutation is at risk to have offspring with the full mutation, depending on the repeat number. The risk of a full mutation in an offspring is 5 percent or less if the CGG repeat number is <70 but exceeds 95 percent with 100 to 200 CGG repeats (Nolin, 2003). Expansion is extremely unlikely in a male premutation carrier, but all of his daughters will carry the premutation. Among women with no risk factors, approximately 1 in 250 carries a fragile X premutation, and the risk approximates 1 in 90 in those with a family history of intellectual disability (Cronister, 2008). Premutation carriers may themselves experience significant health consequences. Males with the premutation are at increased risk for the fragile X tremor ataxia syndrome (FXTAS). This syndrome is characterized by memory loss, executive function deficits, anxiety, and dementia (Monaghan, 2013). Females are at risk for FXTAS as well, although less so. They also have a 20-percent risk for fragile X-associated primary ovarian insufficiency.

The American College of Obstetricians and Gynecologists (2016c, 2017a) recommends carrier screening for women with a family history of fragile X syndrome; individuals with unexplained intellectual disability, developmental delay, or autism; and women with primary ovarian insufficiency. Prenatal diagnosis can be accomplished by amniocentesis or chorionic villus sampling. Specimens obtained by either can define the CGG repeat number, although chorionic villus sampling may not accurately determine *FMR1* gene methylation status.

Imprinting

This term describes some genes that are inherited but not expressed, depending on whether they are inherited from the mother or father. Thus, the resulting phenotype varies according to the parent of origin. Imprinting affects gene expression by epigenetic control, which modifies genetic structure using methods other than altering the underlying nucleotide sequence. For example, methyl group addition may alter gene expression and thereby affect the phenotype without changing the genotype. Importantly, the effect may be reversed in a subsequent generation, because a female who inherits an imprinted gene from her father will pass it in her oocytes with a maternal—rather than paternal—imprint, and vice versa.

Selected diseases that can involve imprinting are shown in Table 13-5. A useful example includes two very different diseases that affect the same region of DNA. First, *Prader-Willi syndrome* is characterized by obesity and hyperphagia; short stature; small hands, feet, and external genitalia; and mild mental retardation. In more than 70 percent of cases, Prader-Willi syndrome is caused by microdeletion or disruption for the paternal 15q11.2-q13. The remaining cases are due to maternal uniparental disomy or due to maternal gene imprinting with the paternal gene inactivated.

TABLE 13-5

Some Disorders That Can Involve Imprinting

Disorder	Chromosomal Region	Parental Origin
Angelman	15q11.2-q13	Maternal
Beckwith-Wiedemann	11p15.5	Paternal
Myoclonus-dystonia	7q21	Maternal
Prader-Willi	15q11.2-q13	Paternal
Pseudohypoparathyroidism	20q13.2	Variable
Russell-Silver syndrome	7p11.2	Maternal

Data from Online Mendelian Inheritance in Man (Johns Hopkins University, 2017.)

In contrast, *Angelman syndrome* includes severe intellectual disability; normal stature and weight; absent speech; seizure disorder; ataxia and jerky arm movements; and paroxysms of inappropriate laughter. In approximately 70 percent of cases, Angelman syndrome is caused by microdeletion for the maternal 15q11.2-q13. In 2 percent, the syndrome is caused by paternal uniparental disomy, and another 2 to 3 percent is due to paternal gene imprinting with the maternal genes inactivated.

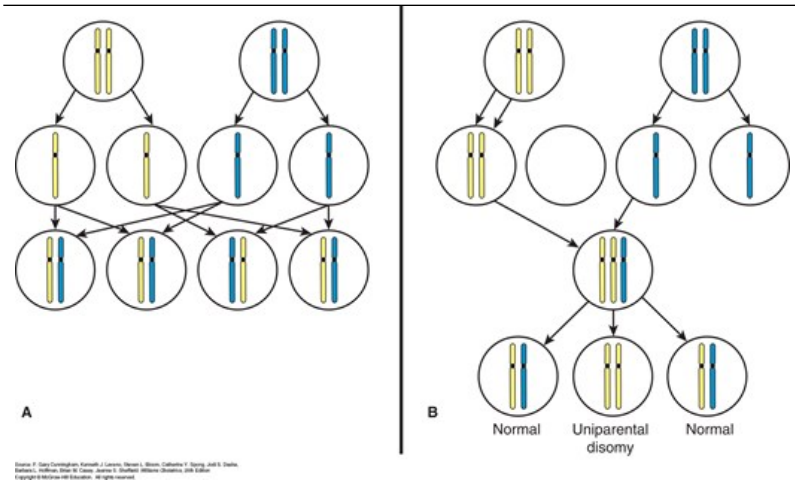
There are other examples of imprinting important to obstetrics. Complete hydatidiform mole, with a paternally derived diploid chromosomal complement, is characterized by abundant placental growth with no fetal structures (Chap. 20, Epidemiology and Risk Factors). Conversely, an ovarian teratoma, with a maternally derived diploid chromosomal complement, is characterized by the growth of various fetal but no placental tissues (Porter, 1993).

Uniparental Disomy

This occurs when both members of a chromosome pair are inherited from the same parent. Often, uniparental disomy does not have clinical consequences. Although both copies are inherited from one parent, they are not identical. However, if chromosomes 6, 7, 11, 14, or 15 are involved, offspring are at increased risk for an abnormality because of parent-of-origin differences in gene expression (Shaffer, 2001). Several genetic mechanisms may cause uniparental disomy, the most common of which is trisomic rescue, shown in Figure 13-9. After a nondisjunction event produces a trisomic conceptus, one of the three homologues may be lost. This will result in uniparental disomy for that chromosome in approximately one third of cases.

FIGURE 13-9

Mechanism of uniparental disomy arising from trisomic “rescue.” **A.** In normal meiosis, one member of each pair of homologous chromosomes is inherited from each parent. **B.** If nondisjunction results in a trisomic conceptus, one homologue is sometimes lost. In a third of cases, loss of one homologue leads to uniparental disomy.



Isodisomy is the unique situation in which an individual receives two *identical* copies of one chromosome in a pair from one parent. This mechanism explains some cases of cystic fibrosis, in which only one parent is a carrier but the fetus inherits two copies of the same abnormal chromosome from that parent (Spence, 1988; Spotila, 1992). It also has been implicated in abnormal growth related to placental mosaicism.

Multifactorial Inheritance

Traits or diseases are considered to have multifactorial inheritance if they are determined by the combination of multiple genes and environmental factors (Table 13-6). Polygenic traits are determined by the combined effects of more than one gene. Most congenital and acquired conditions, as well as common traits, display multifactorial inheritance. Examples include malformations such as clefts and neural-tube defects, diseases such as diabetes and heart disease, and features or traits such as head size or height. Abnormalities that display multifactorial inheritance tend to recur in families, but not according to a mendelian pattern. If a couple has had a child with a multifactorial birth defect, their empirical risk to have another affected child is 3 to 5 percent. This risk declines exponentially with successively more distant relationships.

TABLE 13-6

Characteristics of Multifactorial Diseases

There is a genetic contribution:
No mendelian pattern of inheritance
No evidence of single-gene disorder
Nongenetic factors are also involved in disease causation:
Lack of penetrance despite predisposing genotype
Monozygotic twins may be discordant
Familial aggregation may be present:
Relatives are more likely to have disease-predisposing alleles
Expression more common among close relatives:
Becomes less common in less closely related relatives—fewer predisposing alleles
Greater concordance in monozygotic than dizygotic twins

Adapted from Nussbaum, 2007.

Multifactorial traits that have a normal distribution in the population are termed continuously variable. A measurement that is more than two standard deviations above or below the population mean is considered abnormal. Continuously variable traits tend to be less extreme in the offspring of affected individuals, because of the statistical principle of regression to the mean.

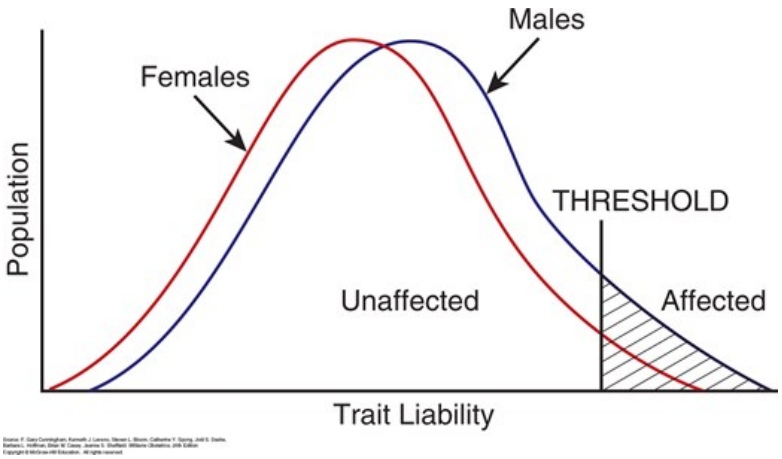
Threshold Traits

Some multifactorial traits do not appear until a threshold is exceeded. Genetic and environmental factors that create propensity or liability for the trait are themselves normally distributed, and only individuals at the extreme of the distribution exceed the threshold and exhibit the trait or defect. Phenotypic abnormality is thus an all-or-none phenomenon. Examples include cleft lip-palate and pyloric stenosis.

Certain threshold traits have a clear male or female predominance. If an individual of the less common gender has the characteristic or defect, the recurrence risk is greater in his or her offspring (Fig. 13-10). An example is pyloric stenosis, which is approximately four times more common in males (Krogh, 2012). A female with pyloric stenosis has likely inherited more predisposing genetic factors than are necessary to produce the defect in a male, and the recurrence risk for her children or siblings is thus higher than the expected 3 to 5 percent. Her male siblings or male offspring would have the highest liability because they not only will inherit more than the usual number of predisposing genes but also are the more susceptible gender.

FIGURE 13-10

Schematic example of a threshold trait, such as pyloric stenosis, which has a predilection for males. Each gender is normally distributed, but at the same threshold, more males than females will develop the condition.



The recurrence risk for threshold traits is also greater if the defect is severe. For example, the recurrence risk after the birth of a child with bilateral cleft lip and palate is approximately 8 percent, but it is only about 4 percent following a child with unilateral cleft lip alone.

Cardiac Defects

Structural cardiac anomalies are the most common birth defects, with a birth prevalence of 8 cases per 1000. More than 100 genes believed to be involved in cardiovascular morphogenesis have been identified, including those directing production of various proteins, protein receptors, and transcription factors (Olson, 2006; Weismann, 2007).

The risk of having a child with a cardiac anomaly is approximately 5 to 6 percent if the mother has the defect and 2 to 3 percent if the father has the defect (Burn, 1998). Selected left-sided lesions, including hypoplastic left heart syndrome, coarctation of the aorta, and bicuspid aortic valve, may have recurrence risks four- to sixfold higher (Lin, 1988; Lupton, 2002; Nora, 1988). Observed recurrence risks for specific cardiac malformations are listed in Table 49-4.

Neural-Tube Defects

These disorders are also classic examples of multifactorial inheritance. Development of neural-tube defects (NTDs) may be influenced by hyperthermia, hyperglycemia, teratogen exposure, ethnicity, family history, fetal gender, and various genes. Selected risks are more strongly associated with the specific defect location. Hyperthermia has been linked with anencephaly risk; pregestational diabetes with cranial and cervical-thoracic defects; and valproic acid exposure with lumbosacral defects (Becerra, 1990; Hunter, 1984; Lindhout, 1992). Sonographic features of NTDs are described in Chapter 10 (Neural-Tube Defects), their prevention with folic acid is discussed in Chapter 9 (Pragmatic Nutritional Surveillance), and fetal

therapy for myelomeningocele is reviewed in Chapter 16 (Open Fetal Surgery).

More than 50 years ago, Hibbard and Smithells (1965) postulated that abnormal folate metabolism was responsible for many NTDs. For a woman with a prior affected child, the recurrence risk of 3 to 5 percent is decreased by at least 70 percent—and potentially by as much as 85 to 90 percent—with periconceptional oral folic acid supplementation at a dosage of 4 mg/d (Grosse, 2007; MRC Vitamin Study Research Group, 1991). However, most NTD cases do not occur in the setting of maternal folic acid deficiency, and it has become clear that the gene-nutrient interactions underlying folate-responsive NTDs are complex. The NTD risk may be affected by genetic variation in folate transport or accumulation, impaired folate utilization via secondary nutrient deficiencies such as vitamin B_{12} or choline deficiency, and genetic variation in activity of folate-dependent metabolic enzymes (Beaudin, 2009).

GENETIC TESTS

All pregnant women should have the option of prenatal aneuploidy *screening* and prenatal genetic *diagnosis* (American College of Obstetricians and Gynecologists, 2016b). Aneuploidy screening may be performed with serum analyte-based screening or with a DNA-based screen, namely, cell-free DNA found in the maternal circulation. Prenatal genetic screening of the parents also aids carrier status determination in at-risk individuals (Chap. 14, Carrier Screening for Genetic Disorders).

For prenatal genetic diagnosis, the most commonly used tests are cytogenetic analysis (karyotyping), fluorescence in situ hybridization (FISH), and chromosomal microarray analysis. Testing may be performed on amnionic fluid or chorionic villi. In selected circumstances, whole genome or whole exome sequencing may be considered, but these are not recommended for routine use. To diagnose a specific disease whose genetic basis is known, DNA-based tests are often employed, typically using polymerase chain reaction (PCR) for rapid amplification of DNA sequences.

Cytogenetic Analysis

Karyotype analysis is commonly performed to test for chromosomal abnormalities. Any tissue containing dividing cells or cells that can be stimulated to divide is suitable for cytogenetic analysis. Karyotyping detects numerical abnormalities, that is, aneuploidy. It also identifies balanced or unbalanced structural rearrangements of at least 5 to 10 megabases in size. Karyotyping has diagnostic accuracy exceeding 99 percent.

The dividing cells are arrested in metaphase, and their chromosomes are stained to reveal light and dark bands. The most commonly used technique is Giemsa staining, which yields the G-bands shown in Figure 13-3. Each chromosome has a unique banding pattern that permits its identification and detection of deleted, duplicated, or rearranged segments. The accuracy of cytogenetic analysis rises with the number of bands produced. High-resolution metaphase banding routinely yields 450 to 550 visible bands per haploid chromosome set. Banding of prophase chromosomes generally yields 850 bands.

Because only dividing cells can be evaluated, the rapidity with which results are obtained correlates with the rapidity of cell growth in culture. Amnionic fluid, which contains epithelial cells, gastrointestinal mucosal cells, and amniocytes, usually yields results in 7 to 10 days. Fetal blood cells may provide results in 36 to 48 hours but are rarely needed (Chap. 14, Fetal Blood Sampling). If fetal skin fibroblasts are evaluated postmortem, stimulation of cell growth can be more difficult, and cytogenetic analysis may take 2 to 3 weeks (Chap. 35, Laboratory Evaluation).

Fluorescence In Situ Hybridization

This technique may be used for rapid identification of a specific chromosome abnormality and for verification of suspected microdeletion or duplication syndromes, such as the 22q11.2 microdeletion described earlier (Abnormalities of Chromosome Structure). Because of its 1- to 2-day turnaround time, FISH is often selected for cases in which findings may alter pregnancy management. To perform FISH, cells are fixed onto a glass slide, and fluorescent-labeled probes are hybridized to the fixed chromosomes (Figs. 13-11 and 13-12). Each probe is a DNA sequence that is complementary to a region of the chromosome or gene being investigated. If the DNA sequence is present, hybridization is detected as a bright signal visible by microscopy. The number of signals indicates the number of chromosomes or genes of that type in the cell being analyzed. Findings are probe-specific. Namely, FISH does not provide information on the entire chromosomal complement but merely the chromosomal or gene region of interest.

FIGURE 13-11

Steps in fluorescence in situ hybridization (FISH).

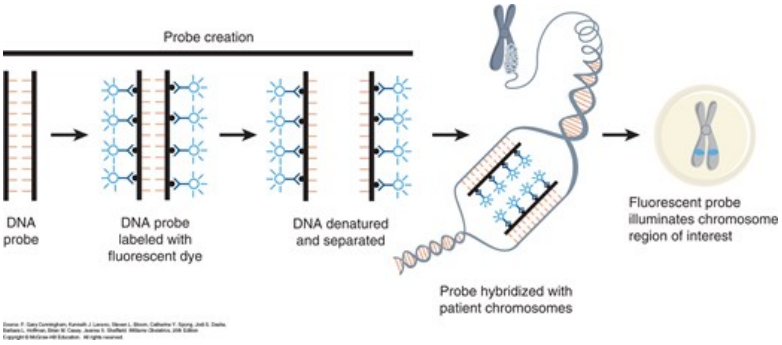
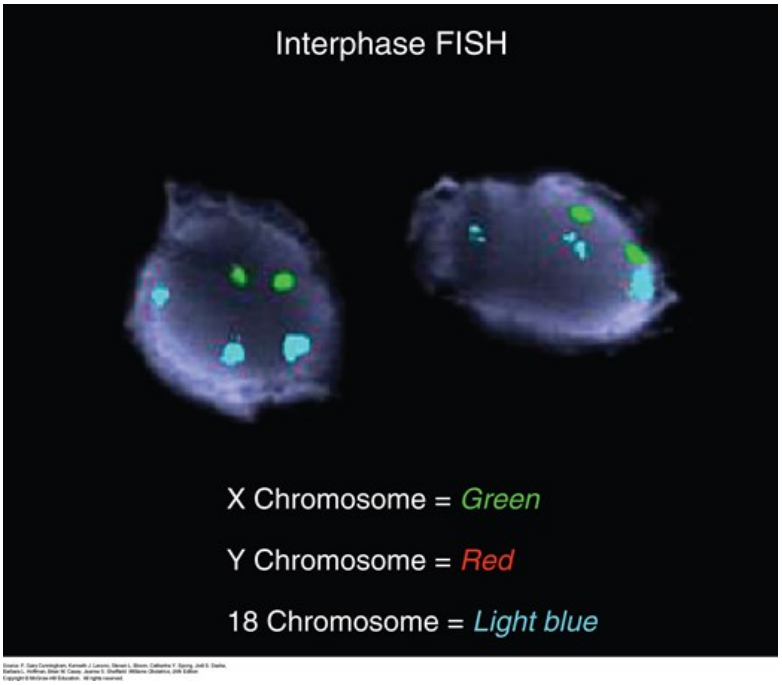


FIGURE 13-12

Interphase fluorescence in situ hybridization (FISH) using α-satellite probes for chromosomes 18, X, and Y. In this case, the three light blue signals, two green signals, and absence of red signals indicate that this is a female fetus with trisomy 18. (Used with permission from Dr. Frederick Elder.)



The most common prenatal application of FISH involves testing interphase chromosomes with DNA sequences specific to chromosomes 21, 18, 13, X, and Y. Figure 13-12 shows an example of interphase FISH using α-satellite probes for chromosomes 18, X, and Y to confirm trisomy 18. In a review of more than 45,000 samples, the concordance between FISH analysis and standard cytogenetic karyotyping was 99.8 percent (Tepperberg, 2001). The American College of Obstetricians and Gynecologists (2016b) recommends that clinical decision-making based on FISH incorporate clinical information consistent with the suspected diagnosis, such as an abnormal aneuploidy screening test result or sonographic finding, or incorporate a confirmatory diagnostic test such as karyotyping or CMA.

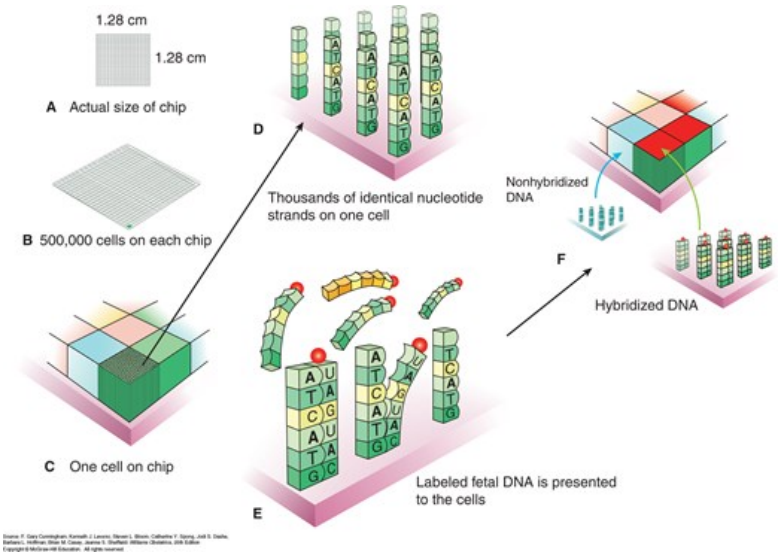
Chromosomal Microarray Analysis

This test is 100 times more sensitive than standard karyotyping and detects microduplications and microdeletions as small as 50 to 100 kilobases. Direct CMA can yield results in 3 to 5 days, whereas if cultured cells are required, results may take 10 to 14 days (American College of Obstetricians and Gynecologists, 2016b). Microarrays use either a comparative genomic hybridization (CGH) platform, a single-nucleotide polymorphism (SNP) platform, or a combination of the two. The CGH microarray platform compares test specimen DNA with a normal control sample. Shown in Figure 13-13, the CGH chip contains reference DNA fragments of known sequence—oligonucleotides. Fetal DNA from the amniocentesis or chorionic villus sampling

specimen is labeled with a fluorescent dye and then hybridized to the DNA on the chip. Normal control DNA is labeled with a different probe and also hybridized to the chip. Then, the intensity of the fluorescent signals from the two samples is compared. With an SNP array, the chip contains known DNA sequence variants—single-nucleotide polymorphisms. When fetal DNA is labeled and hybridized to the chip, the fluorescent signal intensity indicates copy number variation.

FIGURE 13-13

Chromosomal microarray analysis. **A.** Actual microarray chip size. **B.** Each chip contains thousands of cells (squares). **C & D.** Each cell contains thousands of identical oligonucleotides on its surface, and each cell is unique in its nucleotide content. **E.** During genetic analysis, a mixture containing tagged fetal DNA is presented to the chip. Complementary DNA sequences bind. **F.** If a laser is shined on the chip, DNA sequences that have bound will glow. This identifies a matching sequence. (Modified with permission from Doody KJ: Treatment of the infertile couple. In Hoffman BL, Schorge JO, Schaffer JI, et al (eds): Williams Gynecology, 2nd ed. New York, McGraw-Hill, 2012.)



Both types of platforms detect aneuploidy, unbalanced translocations, and microdeletions and microduplications. Neither type of array platform currently detects balanced chromosomal rearrangements. For this reason, couples with recurrent pregnancy loss should be offered karyotyping as the first-line test (Society for Maternal-Fetal Medicine, 2016). In addition, SNP arrays are able to identify triploidy and can detect *absence of heterozygosity.* The latter can occur with uniparental disomy when both copies of a chromosome are inherited from one parent. Further, absence of heterozygosity may occur when there is consanguinity, and counseling prior to performance of an SNP array should include this possibility.

Arrays may be genome-wide or may be targeted to known genetic syndromes. Genome-wide arrays are typically used in research settings, for example, to identify novel microdeletion syndromes in individuals with intellectual disability (Slavotinek, 2008). Targeted arrays are generally preferred prenatally because the likelihood of detecting a *copy number variant of uncertain clinical significance* is lower. In a systematic review, Hillman and colleagues (2013) identified copy number variants of uncertain significance in 1 to 2 percent of prenatal specimens. Not unexpectedly, this may be a source of significant distress to families, even with comprehensive pretest counseling.

Clinical Applications

In pregnancies at increased risk for autosomal trisomy based on aneuploidy screening, karyotyping or FISH plus karyotyping should be offered, and CMA should be made available (American College of Obstetricians and Gynecologists, 2016b). When the karyotype is normal, CMA has identified clinically relevant copy number variants in approximately 6.5 percent of pregnancies with fetal abnormalities and in 1 to 2 percent with no obvious fetal abnormality (Callaway, 2013). The American College of Obstetricians and Gynecologists (2016b) and the Society for Maternal-Fetal Medicine (2016) recommend that CMA be offered as a first-tier test when fetal structural abnormalities are identified, replacing fetal karyotyping in these cases. If a particular anomaly that strongly suggests a specific aneuploidy is identified, such as an endocardial cushion defect (trisomy 21) or alobar holoprosencephaly (trisomy 13), karyotyping or FISH may be offered as the initial test. It is recommended that genetic counseling include information about the benefits and limitations of both CMA and karyotyping, and that each be made available to women who elect prenatal diagnosis (Society for

Maternal-Fetal Medicine, 2016). CMA may identify instances of autosomal dominant genetic disorders that have not yet manifested in an affected parent, and it may also identify instances of nonpaternity.

For stillbirth evaluation, CMA is more likely than standard karyotyping to provide a genetic diagnosis, in part because it does not require dividing cells. The Stillbirth Collaborative Research Network found that when karyotyping was uninformative, approximately 6 percent of cases had either aneuploidy or a pathogenic copy number variant identified with CMA (Reddy, 2012). Overall, CMA yields results nearly 25 percent more often than standard karyotyping alone.

Whole Genome Sequencing and Whole Exome Sequencing

Most fetuses with structural abnormalities have a normal karyotype and a normal CMA result. Whole genome sequencing (WGS) is a technique for analyzing the entire genome. Whole exome sequencing (WES) analyzes just the DNA coding regions, which account for approximately 1 percent of the genome. These next-generation sequencing tools are increasingly used in the postnatal setting to evaluate suspected genetic syndromes and intellectual disability. The American College of Medical Genetics Board of Directors (2012) states that WGS and WES may be considered for evaluation of the fetus with a likely genetic disorder in which CMA has failed to arrive at a diagnosis. The American College of Obstetricians and Gynecologists (2016a) suggests that this be in only selected circumstances, for example, with recurrent or lethal anomalies in which other approaches have been noninformative. Importantly, WGS and WES have significant limitations in their current form, including turnaround times that may be prohibitively long and a high rate of variants of uncertain significance (American College of Medical Genetics, 2012; Atwal, 2014). As a result, the clinical utility of this promising technology for prenatal cases is currently limited.

Fetal DNA in the Maternal Circulation

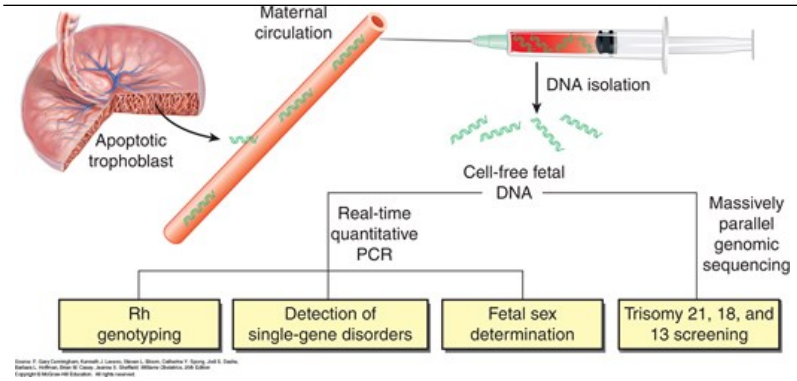
Fetal cells are present in maternal blood at a very low concentration, only 2 to 6 cells per milliliter (Bianchi, 2006). Sometimes, intact fetal cells may persist in the maternal circulation for decades following delivery. Persistent fetal cells may engraft in the mother and result in *microchimerism*, which has been implicated in maternal autoimmune diseases such as scleroderma, systemic lupus erythematosus, and Hashimoto thyroiditis. For prenatal diagnosis, the use of intact fetal cells from maternal blood is limited by low cell concentration, cell persistence into successive pregnancies, and difficulties in distinguishing fetal from maternal cells. In these cases, however, cell-free DNA overcomes these limitations.

Cell-Free DNA

These DNA fragments are derived from maternal cells and from apoptotic placental trophoblast cells—although DNA from the latter is often termed "fetal." Cell-free DNA can be reliably detected in maternal blood after 9 to 10 weeks' gestation (American College of Obstetricians and Gynecologists, 2017b). The proportion of cell-free DNA that is placental is called the fetal fraction, and it composes approximately 10 percent of the total circulating cell-free DNA in maternal plasma. Unlike intact fetal cells, cell-free DNA is cleared within minutes from maternal blood. In research settings, cell-free DNA has been used to detect numerous single-gene disorders transmitted through paternally inherited alleles. These include myotonic dystrophy, achondroplasia, Huntington disease, congenital adrenal hyperplasia, cystic fibrosis, and α-thalassemia (Wright, 2009). Clinical applications of cell-free DNA are aneuploidy screening, fetal sex determination, and Rh D genotyping (Fig. 13-14).

FIGURE 13-14

Cell-free DNA is actually derived from apoptotic trophoblast. The DNA is isolated from maternal plasma, and real-time quantitative polymerase chain reaction (PCR) may be used to target specific regions or sequences. This may be used for Rh D genotyping, detection of paternally inherited single-gene disorders, or fetal sex determination. Screening for autosomal trisomies and sex chromosomal aneuploidies is performed using whole-genome sequencing, chromosome selective or targeted sequencing, and analysis of single nucleotide polymorphisms.



Aneuploidy Screening

Several different types of assays are used to screen for fetal autosomal trisomies and sex chromosomal aneuploidies. These include whole-genome sequencing, which is also called massively parallel or shotgun sequencing; chromosome selective or targeted sequencing; and analysis of SNPs (American College of Obstetricians and Gynecologists, 2016a,b). By simultaneously sequencing millions of DNA fragments, investigators can identify whether the proportion or ratio of fragments from one chromosome is higher than expected. Sequences of fetal DNA are specific to individual chromosomes. Thus, samples from women with a Down syndrome fetus have a larger proportion of DNA sequences from chromosome 21.

The screening performance of cell-free DNA is excellent. In a metaanalysis of 37 studies of largely high-risk pregnancies, the pooled sensitivity to detect Down syndrome was 99 percent, and to identify trisomies 18 and 13, 96 and 91 percent, respectively. For each, the specificity was 99.9 percent (Gil, 2015). The false-positive rate is cumulative for each aneuploidy for which screening is performed, but it is usually below 1 percent. As a result, cell-free DNA screening is recommended as a screening option in those at greater risk for fetal autosomal trisomy (American College of Obstetricians and Gynecologists, 2017b; Society for Maternal-Fetal Medicine, 2015).

Unfortunately, cell-free DNA screens do not yield a result in 4 to 8 percent of cases. This may be due to assay failure, high assay variance, or low fetal fraction (Norton, 2012; Pergament, 2014; Quezada, 2015). Such pregnancies carry a greater risk for fetal aneuploidy. In addition, results may not reflect the fetal DNA complement but rather may indicate confined placental mosaicism, early demise of an aneuploid cotwin, maternal mosaicism, or rarely occult maternal malignancy (Bianchi, 2015; Curnow, 2015; Grati, 2014b; Wang, 2014). Recommendations for counseling are discussed in Chapter 14 (Cell-Free DNA for Secondary Screening).

Fetal Sex Determination

From the standpoint of genetic disease, fetal sex determination may be clinically useful if the fetus is at risk for an X-linked disorder. It may also be beneficial if the fetus is at risk for congenital adrenal hyperplasia because maternal corticosteroid therapy may be avoided if the fetus is male (Chap. 16, Congenital Adrenal Hyperplasia). In a metaanalysis of more than 6000 pregnancies by Devaney and associates (2011), the sensitivity of cell-free DNA testing for fetal sex determination approximated 95 percent between 7 and 12 weeks' gestation and improved to 99 percent after 20 weeks. The test specificity was 99 percent at both time periods, suggesting that cell-free fetal DNA is a reasonable alternative to invasive testing in selected cases.

Rh D Genotype Evaluation

In a predominantly white population, nearly 40 percent of fetuses of Rh D-negative women are themselves Rh D negative. Fetal Rh D genotype assessment from maternal blood can eliminate administration of anti-D immune globulin in these pregnancies, thereby reducing cost and potential risk. With Rh D alloimmunization, early identification of an Rh D-negative fetus might avoid unnecessary middle cerebral artery Doppler assessment or amniocentesis. Evaluation using cell-free DNA is done using real-time PCR to target several exons of the *RHD* gene. These are typically exons 4, 5, and 7.

Rh D-genotyping is performed routinely with cell-free DNA in Denmark and the Netherlands (Clausen, 2012; de Haas, 2016). In a population-based study of more than 25,000 Rh D-negative women screened at 27 weeks, the false-negative rate—in which Rh D-negative status was missed—was only 0.03 percent. The false-positive rate—in which Rh immune globulin would be given unnecessarily—was less than 1 percent (de Haas, 2016). Similar results were reported from the United Kingdom, although the false-negative rate was higher in the first trimester (Chitty, 2014). Investigators concluded that false-negative screening results might increase the alloimmunization risk, but by less than 1 case per million births (Chitty, 2014). Rh D

alloimmunization is discussed in Chapter 15 (Red Cell Alloimmunization).

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