43 Basic genetics and patterns of inheritance

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INTRODUCTION

The field of medical genetics is rapidly expanding. Genetics is becoming increasingly important in the general practice of medicine. Thus, the primary care physician has a responsibility to acquire adequate knowledge of genetics to provide his or her patients with the most up-to-date medical care. In addition to advances in the understanding of the genetic basis for many of the more frequent chromosomal, single-gene, and multiple anomaly syndromes, there is greater knowledge of the role of genetics in common disorders such as hypertension, diabetes, heart disease, cancer, psychiatric disorders, and other medical problems. Medical genetics has a particularly great impact on the practice of obstetrics and on the evaluation and the management of fetal abnormalities, resulting in a need for close working relationships between clinical geneticists and specialists in maternal–fetal medicine. As the growth of information and technology continues, it is anticipated that new forms of genetic therapy, at the somatic cell level and possibly at the germ cell level, will be developed. Some of these treatments may be employed preconceptionally and during pregnancy, requiring the involvement of obstetricians and gynecologists.

The human genome contains three billion base pairs of DNA, packaged into units known as chromosomes and encoding an estimated 20,000 to 25,000 genes (1,2). At the present time, over 12,000 human genes have been mapped and almost 3000 single-gene disorders have been described for which the molecular genetic basis is known (3). The Human Genome Project, begun in the late 1980s and completed in 2003, produced a haploid human genome sequence, which was a composite of the DNA sequence of several individuals. As a result, new genes and new information about the genetic basis for many disorders have been discovered. This has already begun to have a huge impact on the practice of medical genetics and medicine in general.

CYTOGENETICS

Cytogenetics is the study of chromosomes and their abnormalities (4,5). The field of cytogenetics is a relative newcomer in clinical laboratory medicine. It was not until the mid-1950s that chromosome analysis could be done. Soon after it was determined that the normal number of chromosomes in humans is 46, rapid discoveries in the area of human cytogenetics were made. In 1959, LeJeune identified the cytogenetic basis of Down syndrome, and around the same time, sex chromosome abnormalities were found in patients with anomalies of sexual development. Techniques for the analysis of chromosomes have continued to be improved and refined, allowing more subtle abnormalities to be identified. Newer molecular cytogenetic methods have revolutionized the identification and confirmation of microdeletions, microduplications, and cryptic translocations.

Humans have a total of 46 chromosomes, including 22 pairs of numbered chromosomes, or autosomes, and one pair of sex chromosomes. Each chromosome comprises a single length of double-stranded DNA, ranging in size from 50 million to 250 million base pairs. This genetic information is tightly compressed and packaged in the formation of the chromosomes. The DNA molecules are complexed with proteins called histones. The histones are packaged into nucleosomes, which are further compacted into a helical structure known as a solenoid. Finally, the solenoids are wound in a helical fashion and compressed to form the chromatin loops. Chromatin is packaged into chromosomes, which can be viewed microscopically during the metaphase stage of cell division.

Performing cytogenetic analysis has become an integral part of many areas of medical practice, including obstetrics, pediatrics, and oncology. Traditional chromosome analysis first involves obtaining a sample of living tissue. This can be blood, skin, amniotic fluid, products of conception, bone marrow, or any viable solid tissue. Blood is the most frequently analyzed tissue for routine chromosome analysis. For blood, the lymphocytes are isolated. For amniotic fluid, the amniocytes are obtained by spinning down the fluid and removing the cell pellet. For solid tissues, the tissue is minced and/or sonicated. For all tissue types, the cells are cultured in tissue culture for 48 to 72 hours. Cell division is arrested at metaphase by the addition of colcemid. The cells are then harvested and placed on a microscope slide. The cell nuclei are ruptured by adding a hypotonic solution, then stained to show the bands, and images of the metaphase chromosome spreads are analyzed. Each chromosome is studied by looking at the banding pattern to identify not only numerical abnormalities, but also structural problems. Newer computerized technology allows karyotype analysis by digital imaging methods. By convention, the 22 autosome pairs are arranged by size, from the largest to the smallest, in four rows, with the pair of sex chromosomes in the lower right corner (Fig. 1). Ideograms are schematic representations of banding patterns used by cytogeneticists to standardize numbering of specific bands (Fig. 2).

Chromosomes have distinctive structural appearances when viewed microscopically. There are two arms with a central narrowing called the centromere. The shorter arm is termed “p” for “petit,” while the longer arm is denoted “q,” because q comes after p alphabetically. The p arm is always shown at the top on karyotypes. There are three types of chromosomes structurally (Fig. 3). Metacentric chromosomes have p and q arms that are almost equal in length, with the centromere located in the middle. Submetacentric chromosomes have a shorter p arm with the centromere placed closer to the top of the chromosome and acrocentric chromosomes have small p arms called satellites with the centromere located near the top of the chromosome. The ends of chromosomes are called the telomeres.

Figure 1 Normal male karyotype.

Figure 2 Ideogram of human chromosome showing Giemsa banding patterns. Source: From Ref. 6.

Various banding techniques are used to visualize the chromosomes. The most frequently used method is Giemsa banding or G-banding, which results in a specific pattern of dark and light bands on each chromosome. The older method, quinacrine banding, or Q-banding, produces the same dark and light patterns, but requires the use of a fluorescence microscope and is not used routinely. Reverse banding or R-banding, results in the opposite of the dark and light pattern seen with G-banding; this may be used to

better see the ends of the chromosomes. C-banding stains the constitutive heterochromatin, which is near the centromeres and NOR stain visualizes the nucleolar organizing regions of the satellites and stalks of acrocentric chromosomes. For routine karyotype analysis, G-banding is typically used by most laboratories. Routine karyotyping cannot detect gains or losses of cytogenetic material smaller than about 4Mb in size and therefore can miss significant abnormalities.

Figure 3 Human chromosome morphology as classified by variation in chromosome size and centromere position. Source: From Ref. 6.

Figure 4 Example of fluorescence in situ hybridization analysis in a patient with Williams syndrome. On the left panel, an interphase cell is shown with a deletion of one copy of the elastin (ELN) gene within the critical 7q11.23 Williams syndrome region; there is only one red ELN signal and there are two green control signals. On the right panel, a metaphase cell is shown indicating a deletion on chromosome 7 with a missing red signal. Source: Courtesy of Dr. Shashikant Kulkarni.

Fluorescence in situ hybridization (FISH) is a technique in which DNA probes attached to fluorescent dyes are hybridized to specific chromosome regions from patient samples, either interphase cells or metaphase chromosome spreads, most often from lymphocytes or amniocytes. The chromosomes are then visualized by fluorescent microscopy. Probes can be designed to hybridize to whole chromosomes, specific chromosome segments of interest, centromeres, or telomeres. FISH is widely used for the diagnosis of suspected recognizable microdeletion syndromes (Fig. 4). Multicolor FISH is useful for analyzing submicroscopic structural rearrangements undetectable by classic cytogenetic techniques or for identifying marker chromosomes. FISH is also used for rapid screening for aneuploidy including trisomy 13, 18, or 21 and abnormalities of X and Y.

Chromosome microarray analysis using array-based comparative genomic hybridization (aCGH) is a powerful new technique for the detection of genomic copy number alterations, including deletions and duplications (7–9). It was originally developed as a research tool, but is rapidly replacing traditional cytogenetic methods of analysis for the diagnosis of chromosomal defects. With this method, DNA

from the patient's DNA and DNA from a normal control are each labeled with different tags for analysis by fluorescence (Fig. 5). The patient and control DNA are mixed together along with unlabeled human cot-1 DNA to suppress repetitive DNA sequences, and the mix is hybridized to a slide containing thousands of defined DNA probes. The (fluorescence) color ratio along the chromosomes is used to evaluate regions of DNA gain or loss in the patient sample as compared with the control DNA. Clinically available arrays include focused arrays that are designed to identify all microdeletions detectable by FISH, bacterial artificial chromosome arrays that can detect rearrangements greater than 1 Mb, oligonucleotide arrays using DNA probes that can pick up imbalances as small as 20 to 80 kb, and single-nucleotide polymorphism arrays. Diagnosis of alterations in DNA dosage or genomic copy number that are known to be associated with a variety of syndromic and non-syndromic pediatric and adult genetic disorders can be found by aCGH analysis. Copy number variations (CNV) or polymorphisms that are not known to be associated with an abnormal phenotype or are considered to be benign are often detected. In addition, previously undescribed deletions or duplications with unclear clinical

Figure 5 Array comparative genomic hybridization (chromosome microarray) technology. (A) DNA from the patient sample is labeled with a green fluorescence dye and a control DNA is labeled with a red fluorescence dye. The two samples are mixed and co-hybridized to an array of genomic DNA targets on a slide. The ratios of fluorescence intensities are proportional to the ratio of copy numbers of DNA sequences in the patient versus the control samples. Green area indicates duplication and red area indicates deletion. (B) The slides are scanned into image files using a microarray scanner. (C) The output shows hundreds of spots with different ratios of fluorescence intensities. (D) The array profile is analyzed; shown here is a patient profile indicating a deletion of three DNA markers within the neurofibromatosis type 1 critical region on chromosome 17p11.2. Source: From Ref. 7.

significance may be identified, requiring parental analysis to determine inheritance of the abnormality and often making genetic counseling for the family difficult. Another caveat of using chromosome microarray analysis is that it cannot detect balanced chromosome rearrangements such as translocations or inversions if there is no gain or loss of a chromosome segment.

CHROMOSOMAL ABNORMALITIES

Chromosome abnormalities are seen in 1 in 150 to 1 in 170 live births and thus, as a group, contribute significantly to morbidity and mortality among neonates and infants (1). Any baby that has two or more congenital anomalies should have a karyotype performed (Table 1). Some of the more frequent chromosome anomalies will be reviewed, as well as the categories of chromosome abnormalities.

Aneuploidy

The term “aneuploidy” refers to an abnormal total number of chromosomes (10). For example, an individual who has either a missing or extra copy of one of the chromosomes is said to have aneuploidy. Aneuploidy is the most common type of chromosome aberration found in humans and arises by a process known as “nondisjunction.” Nondisjunction occurs when there is a failure of proper distribution of chromosome pairs into daughter cells during meiosis, leading to gametes that have either an extra copy of a given chromosome or a missing copy of that chromosome. Meiosis is the process by

Table 1 Indications for Chromosome Analysis
<ul style="list-style-type: none">• Suspected recognizable chromosome abnormality• Infant with two or more malformations• Mental retardation or developmental delay of unknown etiology, with or without malformations• Relatives of people with known translocation, deletion, or duplication• Stillborn infants with malformations or no known reason for fetal demise• Females with short stature• Males with infertility, small testes, or gynecomastia• Women and their partners who have experienced recurrent miscarriages

which the chromosomes in the gamete are reduced from the diploid state (46 chromosomes) to the haploid state (23 chromosomes). There are two cell divisions in meiosis, called meiosis I and meiosis II. In meiosis I cell division, which is also known as the reduction division stage, two haploid cells are formed from one diploid cell. Meiosis II division results in replication of each haploid cell. Nondisjunction can occur in either meiosis I or meiosis II, although 80% of the time it occurs during meiosis I (11). There is a clear association between advancing maternal age and an increased risk for nondisjunctional events (12). However, regardless of parental ages, it has been shown that the extra number 21 in children with trisomy 21 is of maternal origin in 95% of cases and of paternal origin in only 5% of cases (13). In general, if a zygote

Figure 6 Two-year-old boy with Down syndrome. Note brachycephaly, dysplastic ears, epicanthal folds, and upslanting palpebral fissures.

Figure 7 Karyotype of trisomy 21 karyotype. The arrow indicates extra chromosome 21.

has monosomy for an autosome, early embryogenesis will fail and a spontaneous pregnancy loss will occur, sometimes even before the first missed menstrual period. On the other hand, trisomy for an autosome may result in a longer surviving embryo or fetus, or even a liveborn infant. Trisomy 13, trisomy 18, and trisomy 21 (Down syndrome) are the only autosomal trisomies that can result in viable infants in the non-mosaic state. However, mosaicism for many different autosomal trisomies, that is, the presence of both normal and trisomic cells in one individual, has been described in viable infants. Sex chromosome aneuploidy has, in general, less deleterious effects on the affected individual.

Down Syndrome

Down syndrome, or trisomy 21, is the most common autosomal chromosome abnormality seen in liveborn infants (Fig. 6). It occurs in approximately 1 in 700 newborns. As mentioned above, the risk of nondisjunction increases with advancing maternal age. The American College of Obstetrics and Gynecology has recommended that invasive prenatal

testing for Down syndrome and other trisomies should be offered to all women, regardless of age, and not limited to those over age 35 (14). Down syndrome is caused by the presence of three copies of chromosome 21 (Fig. 7). In 95% of cases, there is straightforward trisomy 21 (denoted 47,XX,+21 in a female and 47,XY,+21 in a male), with an extra 21 in every cell of the body, arising by nondisjunction. About 2% to 3% of patients have mosaicism, with some portion of cells having a normal chromosome complement; mosaicism is caused by postzygotic nondisjunction. The features of Down syndrome may be milder in the mosaic form, depending on what percentage of cells are normal. About 2% of Down syndrome cases are caused by a translocation, usually a Robertsonian translocation involving another acrocentric chromosome, such as the number 14 (14;21) or the number 21 (21;21). Karyotyping on all newborns with clinical features of Down syndrome will identify those who have the translocation form. Approximately 25% of translocation cases are inherited from a parent, with a high risk of recurrence in future pregnancies (15,16). Therefore, chromosome analysis should be done

Figure 8 Newborn infant with trisomy 18, showing dysmorphic facies, flexion contracture of fingers, and rocker bottom feet.

on the parents of all cases of translocation-type Down syndrome. If a woman is a 14;21 Robertsonian translocation carrier, there is a 15% risk of Down syndrome in each pregnancy, as well as a high risk of recurrent spontaneous abortions due to the other possible unbalanced chromosome complements in the offspring. If a man is a carrier, the risk of a liveborn baby with trisomy 21 is only about 5% (15,16).

Individuals with Down syndrome usually have a moderate degree of mental retardation, with IQ in the 50 to 60 range. Some have milder mental retardation, while others fall into the severe range. The degree of mental retardation cannot be determined at birth. Early intervention, with involvement in infant stimulation and parent training programs, can improve the developmental outcome. Most people with Down syndrome are educable and many can be mainstreamed in regular classrooms in school. Increasing numbers of individuals with Down syndrome are able to live semi-independently and hold jobs in supervised settings (17, 18).

There are a number of associated birth defects and medical problems in Down syndrome. Approximately 40% to 50% of babies with trisomy 21 have a congenital heart defect, frequently an atrioventricular canal or septal defect (19). About 12% of these babies have a malformation of the gastrointestinal tract, most often duodenal atresia, but occasionally Hirschsprung's disease, imperforate anus, or

tracheoesophageal atresia. Other birth defects include congenital cataracts (3%), syndactyly, 11 pairs of ribs, and urinary tract anomalies (2–3%) (20). Anomalies can be detected by prenatal ultrasound, particularly the heart defects and duodenal atresia. If either of these types of anomalies is seen, prenatal testing for Down syndrome should be considered.

Postnatal medical problems, in addition to developmental delay and mental retardation, include poor feeding and hypotonia in infancy, obesity later in life, visual impairment (myopia in 70%), recurrent otitis media and hearing loss, hypothyroidism (20%), atlantoaxial or atlanto-occipital instability (5–10%), and childhood leukemia (1%) (20).

Trisomy 18

Trisomy 18 (Edward syndrome) is a much less common autosomal trisomy, seen in approximately 1 in 7000 liveborn infants (21). It has been estimated that at least two-thirds of trisomy 18 conceptuses are lost by spontaneous abortion. Trisomy 18 is caused by the presence of three copies of the number 18 chromosome, again arising by nondisjunction in meiosis I or meiosis II.

The prognosis for trisomy 18 is extremely poor and the majority of infants die within the first 3 months of life. There are, however, a few individuals who have survived to their teens and twenties. The associated mental retardation is severe to profound. In general, the birth defects seen in trisomy 18 are more frequent and much more severe than that in trisomy 21 (Fig. 8). Most infants have significant intrauterine growth retardation, which may be identified by prenatal ultrasound. Approximately 85% of infants have a congenital heart defect, including septal defects, multivalvular dysplasia, and complex anomalies. Other anomalies are dysmorphic facial features, short sternum, joint contractures, overlapping digits, rocker bottom feet, renal abnormalities, and occasionally gastrointestinal tract anomalies and neural tube defects.

Trisomy 13

Trisomy 13 (Patau syndrome) is the third autosomal trisomy, which can be seen in liveborn infants. It is less common than trisomy 18 and occurs in about 1 in 12,000 live births (19). As in trisomy 18, the prognosis for trisomy 13 is very poor, with most infants dying within the first 3 months. Depending on the severity of the associated birth defects, longer-term survival is possible. Infants with trisomy 13 tend to be better grown than those with trisomy 18 (Fig. 9). However, they are more likely to have microcephaly, which can be seen by prenatal ultrasound. Congenital heart defects are present in 80% to 85%, again often of a complex nature. Those with less significant heart defects may survive longer. Holoprosencephaly, cleft lip and palate, renal anomalies, and omphalocele are frequent abnormalities in trisomy 13, which may be detected by prenatal ultrasound.

Sex Chromosome Abnormalities

Sex chromosome abnormalities as a group are very common. Approximately 1 in 500 individuals has a sex chromosome abnormality, although it has been suggested that the true incidence may be higher. Some cases may never be ascertained due to the relatively mild signs and symptoms associated with most of the common sex chromosome problems. Many patients

Figure 9 Newborn infant with trisomy 13, showing microphthalmia, bilateral cleft lip and palate, and postaxial polydactyly of the hands.

are not diagnosed until adulthood when they may undergo evaluation for infertility or other reproductive problems.

Turner Syndrome

Turner syndrome is one of the most familiar sex chromosome abnormalities. It is caused by the presence of only one X chromosome (45,X) in phenotypic females. This classic Turner karyotype is seen in 50% of cases; other cases may be mosaic (45,X/46,XX), or may be caused by an abnormal X chromosome, such as an isochromosome or ring X (1). There may be portions of a Y chromosome present or mosaicism for a 46,XY cell line in some cases. FISH testing for SRY is recommended for detection of Y chromosome material. Turner syndrome is seen in about 1 in 2500 newborn girls. However, it is well known that the incidence is much higher in early pregnancy, with more than 98% of 45,X embryos and fetuses being lost by spontaneous miscarriage. This would suggest that the incidence among conceptuses could be as high as 1.5%, an extraordinarily high frequency (5).

Features of Turner syndrome detectable by ultrasound include cystic hygroma, lymphedema, or hydrops fetalis; when these abnormalities are seen, the prognosis for survival of the pregnancy is poor (22). Smaller cystic hygromas may resolve during pregnancy, leaving a webbed neck or excess nuchal skin. Other anomalies seen in Turner syndrome include

congenital heart defects, frequently coarctation of the aorta, and renal anomalies, often horseshoe kidney. Lymphedema may be seen at birth, often involving the dorsum of the hands and feet, as well as a shield-shaped chest and wide-spaced nipples. For surviving girls with Turner syndrome, the prognosis for good health and a normal lifespan is excellent. Intelligence is normal, although there may be some specific learning disabilities. However, due to the “streak” ovaries, puberty and the onset of menses will not usually occur without hormone therapy and the average adult height without growth hormone treatment is 57 inches. The long-term outcome with estrogen/progesterone replacement and growth hormone therapy is very good. A number of successful pregnancies in women with Turner syndrome have been reported, with the use of ovum donation and in vitro fertilization techniques (23).

Klinefelter Syndrome

Klinefelter syndrome is caused by the presence of an extra X chromosome in a phenotypic male (47,XXY) and occurs in approximately 1 in 500 to 1 in 1000 males. If Klinefelter syndrome is detected prenatally, it is usually by chance, when an amniocentesis is being done for other reasons. There are no expected fetal anomalies in Klinefelter syndrome and most cases are not diagnosed in infancy. The diagnosis may be suspected in a boy with tall thin body habitus and mild learning disabilities. Testicular size is usually normal in prepubertal boys. However, boys with Klinefelter syndrome fail to go through puberty normally and eventually have small, firm testes and relatively hypoplastic external genitalia. They are usually sterile, and the diagnosis is sometimes made in an adult infertility or urology clinic.

47, XXX Syndrome

This chromosome abnormality is thought to occur in 1 in 1000 women, although the true incidence may be higher. Many cases are probably never ascertained because of the mild nature of the associated signs and symptoms. There is no recognizable phenotype, except perhaps tall stature and mild learning problems. Fertility is normal and the risk of sex chromosome aneuploidy in the offspring of an affected woman appears to be low.

47, XYY Syndrome

This chromosome abnormality is also fairly common, occurring in about 1 in 800 men. Again, there is no definite phenotype except taller than expected stature, slightly long facies and long digits. Most individuals have normal intelligence, but there have been reports of personality disorders and antisocial behavior. The incidence may be underestimated due to the nonspecific phenotype. This condition is most often diagnosed serendipitously.

Structural Alterations

In addition to aneuploidy, in which there is loss or gain of an entire chromosome, rearrangements, deletions, or duplications of portions of chromosomes can occur. These structural alterations can result in either balanced or unbalanced chromosome abnormalities. In unbalanced abnormalities, there is missing and/or additional chromosome material, which often causes multiple birth defects and developmental problems. Balanced chromosome abnormalities involve rearrangements of

the chromosomes without loss or gain of chromosome material and are usually found in normal individuals.

One frequent cause of structural alteration is a translocation. There are two basic types of translocations. A reciprocal translocation occurs when two chromosomes break and the pieces exchange places (Fig. 10). The resulting abnormal chromosomes are called “derivative chromosomes.” If the reciprocal translocation is balanced, the individual is usually

Figure 10 Reciprocal translocation between chromosomes 3 and 21. The break points on the long arm of each chromosome are indicated by the wavy line. Source: From Ref. 6.

normal. Translocations such as these are found in approximately 1 in 500 normal individuals in the general population and are usually never detected. However, if a parent carries a balanced translocation and passes only one of his or her derivative chromosomes to an offspring, along with the other normal chromosomes, the child will have an unbalanced chromosome complement and will likely have multiple problems as a result (Fig. 11). Inheritance of a derivative chromosome in this situation causes a partial duplication (partial trisomy) of one chromosome and a partial deletion (partial monosomy) of another chromosome.

In Robertsonian translocations, the satellites of two acrocentric chromosomes are lost and the q arms of the chromosomes fuse at the centromeres, resulting in a single chromosome (Fig. 12). Thus, a carrier of a Robertsonian translocation has a total of only 45 chromosomes, but has a balanced chromosome complement and is a normal individual. The loss of the satellites poses no problems since these regions of chromosome material encode repetitive sequences that are not essential. A Robertsonian translocation in a parent can result in an unbalanced chromosome abnormality in his or her offspring. If the zygote inherits the translocation chromosome along with either one of the parents’ corresponding normal chromosomes, the child will have trisomy for that chromosome. If the zygote receives the translocation chromosome alone, a normal carrier would be produced. If the zygote does not receive the translocation chromosome, and thus has one of the single involved chromosomes, a monosomy would be produced, which would cause a miscarriage. As previously discussed, most autosomal trisomies are lethal. The only unbalanced Robertsonian translocations seen in liveborn babies with trisomies are in Down syndrome and trisomy 13. Other trisomies for acrocentric autosomes, such as trisomy 14, trisomy 15, or trisomy 22 are lethal abnormalities and

Figure 11 Meiotic segregation in a reciprocal translocation involving chromosomes 3 and 21. Source: From Ref. 6.

would cause miscarriages. The most frequent Robertsonian translocation encountered in the general population is a 13;14 translocation, followed in frequency by a 14;21 translocation (22). The former would place a carrier at high risk for having a child with trisomy 13, while the latter would result in a high risk for trisomy 21, and both would be expected to cause a higher risk for recurrent pregnancy loss due to lethal trisomies and monosomies.

Another type of structural abnormality is a deletion, in the absence of a translocation. Deletions can be terminal, that is, at either end of the chromosome, or interstitial, within the short or long arm of the chromosome. As can be imagined, there is an almost infinite number of possible deletions that can occur as a result of accidents during meiosis. However, some specific deletions are seen more frequently. One such relatively common terminal deletion is 5p- or cri du chat syndrome. Cri du chat syndrome is associated with microcephaly and mental retardation in all cases, congenital heart defects in about 30%, and craniofacial dysmorphic features, including a round face, epicanthal folds, hypertelorism, and dysplastic ears (19). Newborns with cri du chat syndrome have a mewing or cat-like cry, for which the syndrome was named. Another fairly frequent terminal deletion is 4p- or Wolf–Hirschhorn

syndrome, a disorder associated with mental retardation, growth deficiency, and distinctive craniofacial dysmorphic features. Patients with Wolf–Hirschhorn have microcephaly, a prominent glabellar region, hypertelorism, strabismus, cleft lip and/or palate, and micrognathia (19). They may have associated cardiac, renal, and genital anomalies. These types of deletions are usually visible on routine karyotype analysis due to their large size. However, deletions can obviously vary widely in size; some are too small to be seen on routine studies and will require FISH analysis or chromosome microarray analysis to be detected.

There are an increasing number of recognizable microdeletion syndromes (Table 2). For example, Prader–Willi and Angelman syndromes are microdeletion syndromes, both involving a deletion at 15q11q13. Prader–Willi syndrome is a complex disorder associated with hypotonia and failure to thrive in early infancy, followed by rapid weight gain after about 2 years of age and severe obesity, mental retardation, hypogonadism, and craniofacial dysmorphic features. By contrast, the Angelman syndrome phenotype is very different, even though both syndromes are associated with the same microdeletion. Angelman syndrome, also called the “happy puppet” syndrome, presents with severe mental retardation,

Figure 12 Meiotic segregation in Robertsonian translocation involving chromosomes 14 and 21. Source: From Ref. 6.

Table 2 Microdeletion Syndromes		
Syndrome	Clinical features	Chromosome deletion
Alagille	Cholestasis, pulmonary atresia, vertebral anomalies, dysmorphic facial features	20p11.23-p12.2
Angelman	Mental retardation, seizures, ataxic gait	15q11.2q13
DiGeorge/velocardiofacial	Characteristic facies, cleft palate, heart defect, thymic hypoplasia	22q11.2
Langer–Giedion	Characteristic facies, sparse hair, exostoses, mental retardation	8q24
Miller–Dieker	Lissencephaly, characteristic facies	17p13.3
Prader–Willi	Mental retardation, obesity, short stature, hypotonia, hypogonadism	15q11.2q13
Smith–Magenis	Dysmorphic facies, sleep disturbance, mental retardation, self-destructive behavior	17p11.2
Williams	Mental retardation, supravalvular aortic stenosis, characteristic facies	7q11.23
Retinoblastoma	Eye tumor	13q14
Wilms tumor/aniridia	Mental retardation, aniridia, Wilms tumor, genital defects	11p13

Figure 13 Two unrelated boys with Williams syndrome. Note epicanthal folds, upturned nose, and wide mouth with full lips. Source: From Ref. 25.

seizures, ataxia, and inappropriate laughter. It is now known that genomic imprinting is responsible for the phenotypic differences between these two syndromes. Genomic imprinting implies that maternally and paternally derived genetic information at the same locus is not identical and interchangeable, at least for some portions of the genome. In Prader-Willi, the deletion always occurs on the paternally derived number 15 chromosome, resulting in lack of genetic influence from the father in this region. About 70% to 75% of Prader-Willi patients have such a deletion, while the remainder have no visible abnormalities; virtually all of these remaining patients have maternal disomy, meaning that they have two copies of the mother's chromosome 15 and neither of the father's, again resulting in lack of paternal genetic influence in this region (1). The disomy can be either isodisomy, in which there are two identical copies of one of the maternal 15 chromosomes, or heterodisomy in which the two copies represent one of each of the maternal number 15 chromosomes. The mirror image situation exists in Angelman syndrome; if a deletion is found, it is always on the maternally derived number 15, and if there is no deletion, there is paternal disomy for 15, both cases resulting in loss of maternal genetic influence in this region of 15 (1). Thus, genomic imprinting of the genes in this area at 15q11q13 is taking place, since lack of influence from either parent can result in two very different syndromes.

Many other microdeletion syndromes have been identified (24). Many of these conditions were well recognized by clinical geneticists for years before the microdeletion could be detected. With the improvement of cytogenetic technology, smaller fragments of missing chromosome material can be identified using FISH. Studies in this area have been aided by the use of chromosome microarray analysis. Microdeletion syndromes have also been referred to as contiguous gene syndromes because of the loss of multiple adjacent genes. It is understandable that the phenotypes of these patients may vary depending on the size of the deletion and the number and importance of the deleted genes. One good example of this is Williams syndrome, which is readily diagnosed by FISH (Fig. 4). Williams syndrome is a well-recognized multiple

anomaly syndrome characterized by moderate mental retardation, craniofacial dysmorphic features, hypercalcemia, and cardiovascular abnormalities. Facial features include epicanthal folds with periorbital fullness, stellate iris pattern, long philtrum, and wide mouth with prominent lips (Fig. 13). The cardiovascular abnormalities typically involve supravalvular aortic stenosis, but other blood vessels, such as pulmonary and renal arteries, may be affected. Most cases are sporadic, but there is autosomal dominant inheritance in some families. There is a separate, clearly autosomal dominant disorder of isolated supravalvular aortic stenosis, which is not associated with the other features of Williams syndrome. It has been discovered that the supravalvular aortic stenosis and other vascular abnormalities in both of these disorders are due to abnormalities in the gene for elastin, which is located on chromosome 7 at 7q11.23 (26). In the case of Williams syndrome, one copy of the elastin gene is deleted, along with other genes in the surrounding regions. Williams syndrome patients without deletion of the elastin gene do not have arterial disease. Thus, variable expression of contiguous gene or microdeletion syndromes can be directly related to the extent of the deleted genes, but all patients share an overlapping area of involvement.

In addition to deletions, duplications of chromosome material can occur in the absence of a translocation, and again there are an increasing number of newly recognized microduplication syndromes detectable with the use of chromosome microarray analysis. Duplications can be interstitial, within the body of the chromosome, or terminal, at the end of the chromosome. Duplication of a portion of chromosome material results in partial trisomy for that region. As with deletions, there are an almost endless number of possible duplications that could result from errors in meiosis during gamete formation. Some of the common duplications have been found in regions associated with deletions of the same region, including the 22q11.2 microduplication syndrome at the same locus as the DiGeorge/velocardiofacial syndrome deletion (Fig. 14) and the 7q11.23 microduplication syndrome at the same locus as the Williams syndrome deletion.

Figure 14 Array comparative genomic hybridization for 22q11.2 microdeletion and microduplication syndromes. (A) A 3Mb loss of chromosomal material in a patient with the 22q11.2 microdeletion syndrome (DiGeorge/velocardiofacial syndrome) is shown on the left and its reciprocal gain in a patient with the 22q11.2 microduplication syndrome is shown on the right. (B) Deletion confirmed by metaphase fluorescence in situ hybridization (FISH) analysis, with a single red 22q11.2 signal and two green control signals. (C) Duplication confirmed by interphase FISH showing three red signals and two control green signals. *Source:* From Ref. 7.

Another type of structural rearrangement is a ring chromosome. A ring occurs when the terminal p and q arms are deleted and the “sticky ends” anneal to each other. This can occur with any chromosome, perhaps most frequently with

the X chromosome. The seriousness of the effects is related to the extent of the deletions produced.

Inversions refer to breakage of a chromosome at two places with secondary inversion and reinsertion of the

Figure 15 Structural abnormalities of chromosomes (see text for details).
Source: From Ref. 6.

material back into the same chromosome. If this occurs within either the p arm or the q arm, it is said to be a paracentric inversion, since the centromere is not affected. If it occurs on either side of the centromere, it is a pericentric inversion (Fig. 15). Most individuals with inversions are

normal. Sometimes, an inversion results in a deletion or duplication of chromosome material, leading to phenotypic or developmental abnormalities. If a parent has an inversion, his or her offspring could have an unbalanced chromosome constitution if proper pairing does not occur during meiosis and abnormal gametes are formed. If an inversion is present, a loop forms during chromosome pairing in meiosis I. Recombination, or crossing over, which normally occurs in meiosis I, can take place in the loop as well, potentially leading to unbalanced gametes. However, normal carriers of paracentric and pericentric inversions have different risks of producing abnormal offspring. If the inversion is paracentric, the unbalanced gametes produced are likely to be acentric or dicentric, with a very low chance of resulting in a viable liveborn child with a chromosome abnormality. If the inversion is pericentric, the centromere is not affected by recombination and abnormal gametes with duplication and deletion of material distal to the inversion can be produced, potentially leading to viable offspring with chromosome anomalies. The larger the pericentric inversion, the more likely it is that abnormal offspring can be produced, since the duplicated or deleted segments are smaller. The overall risk that a pericentric inversion carrier will have karyotypically abnormal children is estimated to be between 1% and 10% (16).

Finally, isochromosomes result from loss of either the p arm or the q arm of a metacentric or submetacentric chromosome, with duplication of the remaining arm, thus causing trisomy for the p or q arm of a pair of chromosomes and monosomy for the opposite arm. This is seen most commonly in some cases of Turner syndrome, when the X chromosome is involved. Isochromosomes for the autosomes would be expected to cause very severe and likely lethal abnormalities in most cases.

Chromosome Abnormalities in Spontaneous Abortions

Chromosomes examined from products of conception from pregnancy losses at various stages of gestation show an exceptionally high rate of chromosomal abnormalities. In fact, as many as 10% to 15% of early embryos have a chromosome abnormality, usually leading to a spontaneous pregnancy loss (27). Approximately 50% to 60% of spontaneous abortions are caused by a chromosome anomaly in the embryo or fetus (28). At the time of live birth, approximately 1 in 150 newborns is chromosomally abnormal. Thus, as stated by John Opitz, "it seems established that most of, 'humanity' dies before, rather than after birth, and that perhaps only a third survive from the earliest beginnings till [sic] birth..." (29). The most frequent abnormalities seen are autosomal trisomies (52%), especially trisomy 16 (15%), trisomy 13, 18, and 21. 45,X accounts for 18% of abnormalities and triploidy is found in 17% (30). There is a steady loss of these abnormal pregnancies throughout the first trimester. By 20 weeks, the incidence has fallen to 27 in 1000 or 2.7%, and by full term, the incidence is 6 in 1000 or 0.6% (28).

In the vast majority of cases, the occurrence of a chromosomally abnormal pregnancy with spontaneous abortion does not increase the risk of recurrence in a future pregnancy. A general rule of thumb is that products of conception should be evaluated by karyotype analysis on the third pregnancy loss. Parental blood chromosomes should also be analyzed at this point, looking for a balanced translocation in

Table 3 Common Single-Gene Disorders	
Disease	Incidence
<i>Autosomal dominant</i>	
Adenomatous polyposis coli	1 in 6000
Adult polycystic kidney disease	1 in 1000
Familial hypercholesterolemia	1 in 500
Hereditary nonpolyposis colorectal cancer	up to 1 in 200
Huntington disease	1 in 20,000
Marfan syndrome	1 in 10,000 to 1 in 20,000
Myotonic dystrophy	1 in 7000 to 1 in 20,000
Neurofibromatosis type I	1 in 3000 to 1 in 5000
Osteogenesis imperfecta	1 in 10,000 to 1 in 15,000
<i>Autosomal recessive</i>	
A1-Antitrypsin deficiency	1 in 2500 to 1 in 10,000 Caucasians
Cystic fibrosis	1 in 2000 Caucasians
Hemochromatosis	1 in 5000
Phenylketonuria	1 in 10,000 to 1 in 20,000
Sickle cell disease	1 in 400 African-Americans
Tay-Sachs disease	1 in 3000 Ashkenazi Jews
Thalassemia	1 in 50 to 1 in 100 Asians and in Mediterranean populations
<i>X-linked</i>	
Duchenne muscular dystrophy	1 in 3500 males
Fragile X syndrome	1 in 1200 males; 1 in 2500 females
Hemophilia A	1 in 10,000 males
Color blindness	8% of males
Glucose 6-phosphate dehydrogenase deficiency	10–15% of African-American males

one of the partners. A translocation is found in approximately 5% to 10% of couples with recurrent miscarriages (31).

MENDELIAN INHERITANCE

In 1865, an Austrian monk named Gregor Mendel published his now-famous treatise on the inheritance of characteristics in garden peas, leading eventually to the modern study of genes and inheritance, including human genetic disorders. However, Mendel’s original work was largely ignored during his lifetime. In fact, his contemporary, Charles Darwin, apparently never knew of Mendel’s research and hypotheses. Mendel’s work was “rediscovered” at the beginning of the 20th century. In 1902, Archibald Garrod reported the first known human Mendelian disorder, alkaptonuria. Over the last century, thousands of human single-gene traits and disorders have been described (Table 3). These conditions were catalogued over many years by Victor McKusick in his *Mendelian Inheritance in Man* and now in an online version OMIM (Online Mendelian Inheritance in Man) (3).

Since humans have two copies of each chromosome, we also have two copies of each gene. Alternative forms of the same gene are called “alleles.” Each chromosome contains hundreds of different genes. At the time of conception, we inherit one of each pair of genes from our mother and the other of each pair from our father. Thus, we receive an equal amount of genetic information from each parent.

There are four basic inheritance patterns of human single-gene disorders. These include autosomal dominant and

Figure 16 Autosomal dominant inheritance. Both males and females are affected and there is male-to-male transmission of the disorder. Source: From Ref. 25.

autosomal recessive inheritance, in which the mutant gene is on an autosome, and X-linked dominant and X-linked recessive inheritance, in which the mutant gene is on the X chromosome. Individuals can be heterozygous for a given gene, meaning that they have two different forms, or alleles, of the gene at the same locus on each of the two different chromosomes. Individuals can also be homozygous for a gene, in which the two alleles are identical. Males are said to be hemizygous with respect to genes on the X chromosome, since they have only one copy of the X.

In autosomal dominant genetic disorders, only one copy of a mutant allele is necessary for expression of the disease (Fig. 16). Thus, heterozygotes will be affected. The risk of passing on the mutation to an offspring is 50% for each pregnancy. Vertical transmission of the disorder is seen on pedigree analysis, and the condition can often be traced back many generations. However, there are certain caveats to keep in mind when evaluating autosomal dominant disorders. First, there is a high degree of variability of expression of dominant conditions. Even within the same family, some individuals may be severely affected while others may have very mild and medically insignificant features. Thus, thorough review of medical histories and physical examinations on multiple family members are often parts of a genetic evaluation. Second, there may be reduced penetrance of a dominant disorder. Thus, a heterozygote may not show any manifestations of the mutant gene after a complete medical evaluation. Finally, many autosomal dominant disorders have a high new spontaneous mutation rate and a dominant condition may

Figure 17 Autosomal recessive inheritance. Note that both parents must be carriers and the affected offspring may be male or female. Source: From Ref. 25.

appear de novo in an isolated individual in a pedigree. However, once present, it can be passed on to subsequent generations. In general, abnormal genes in autosomal dominant disorders likely encode structural proteins, such as collagen, which is abnormal in osteogenesis imperfecta, or fibrillin, which is mutant in Marfan syndrome.

In autosomal recessive conditions, two copies of a mutant allele are necessary for expression, and only homozygotes will be affected (Fig. 17). Heterozygotes for recessive genes are usually normal and do not have any manifestations of the disease, but they are carriers. Autosomal recessive disorders manifest only if both parents are carriers of the same mutant gene and the offspring receives one mutant allele from each parent. Individuals with autosomal recessive disorders are often compound heterozygotes, meaning that they have two different mutant alleles of the same gene, resulting in expression of the disorder. Recurrence risk for a recessive

disorder is 25% for each pregnancy. There is a 25% chance of having a normal noncarrier child and a 50% chance of having a carrier child. Pedigree analysis usually does not reveal other affected family members outside of the nuclear family group, unless there is consanguinity. The incidence of an autosomal recessive disorder in a population is related to the frequency of heterozygotes. The carrier rate for some conditions is high, while it is very low for others. Recessive conditions have a higher incidence in isolated or inbred populations. Recessive disorders show little variation in expression among affected members within a pedigree, although there may be inter-familial variation due to different mutant alleles. In general, recessive disorders more likely involve genes that encode enzymes or molecules important in cellular functions. Examples include inborn errors of metabolism caused by enzyme deficiencies, hemoglobinopathies, and defects in cellular membrane transport mechanisms, as in cystic fibrosis.

Figure 18 X-linked recessive inheritance. Males can be affected in multiple generations and they inherit the trait from their mothers. Source: From Ref. 25.

X-linked recessive conditions are caused by mutations in genes on the X chromosome. Since males are hemizygous for X-linked genes, they will express such mutations, while females with a mutant recessive gene on one X chromosome will be carriers with a normal phenotype (Fig. 18). There are

exceptions to these rules. For example, if the X-inactivation process results in a larger proportion of active X chromosomes carrying the mutant allele, a female may show expression or partial expression of the disorder. In X-linked dominant conditions, only one copy of the mutant allele is needed for full

Figure 19 X-linked dominant inheritance. Males and females are affected, but male-to-male transmission does not occur.

expression of the disorder (Fig. 19). In this situation, males and females are both affected, but males will show much more severe manifestations than females, or the mutation may even be lethal in males.

There are fewer Y-linked genes known at this time. The most recent update on OMIM lists 59 genes identified on the Y chromosome, including the SRY gene, which is involved in male sex determination (3). Y-linked, or holandric,

conditions, such as maleness itself, are passed exclusively from father to son.

MULTIFACTORIAL AND POLYGENIC INHERITANCE

In multifactorial inheritance, it is postulated that genetic influences from both parents, in combination with environmental factors, lead to a specific birth defect or disorder. In polygenic inheritance, multiple genetic influences in

Table 4 Multifactorial Disorders

Disorder	Incidence
<i>Congenital malformations</i>	
Cleft lip with/without cleft palate	1 in 500 to 1 in 1000
Cleft palate	1 in 1000
Club foot	1 in 1000
Congenital heart defects	1 in 200 to 1 in 500
Neural tube defects	1 in 500 to 1 in 1000
Pyloric stenosis	1 in 300
<i>Adult diseases</i>	
Alcoholism	1 in 10 to 1 in 20
Alzheimer's disease	1 in 10
Bipolar affective disorder	1 in 100 to 1 in 200
Cancer	1 in 3
Diabetes (types I and II)	1 in 10
Coronary artery disease	1 in 3 to 1 in 5
Schizophrenia	1 in 100

combination act together to produce a disorder. Examples of birth defects with multifactorial inheritance include isolated congenital heart defects, cleft lip with or without cleft palate, cleft palate, neural tube defects, pyloric stenosis, and congenital dislocation of the hips (Table 4). In multifactorial disorders, there is no evidence to suggest simple Mendelian inheritance. For example, the defect does not necessarily appear in sequential generations of a family, as would an autosomal dominant condition due to a single-gene mutation. However, there may be clustering of the defect in more than one member of a pedigree. The appearance of multifactorial disorders has been explained by a threshold model in which certain genes present in an individual, along with prenatal environmental factors, accumulate toward a threshold. If an individual has enough of these genes and factors, a threshold will be crossed and the defect will appear (32).

Multifactorial disorders often show an increased incidence in one sex or the other. Pyloric stenosis is much more common in males, while dislocation of the hips is more common in females. These sex differences have been explained by a shift in the threshold depending upon the sex.

Once a couple has had a child with a multifactorial condition, the risk of having a second child with the same disorder is increased over the background population risk. Recurrence risk figures can be estimated for a given defect based on population studies. For example, the incidence of cleft lip with or without cleft palate is 1 in 700 to 1 in 1000 newborns. This risk increases to approximately 4% or 1 in 25 after a family has had one child with cleft lip, representing a 40-fold increased risk. However, the risk is still relatively small, with a 96% chance of having a normal child.

The exact environmental factors contributing to multifactorial conditions remain unknown in most cases. Regional factors must play a role, since it is well known that some areas of the world have a markedly increased risk for certain defects. For example, the incidence of neural tube defects is approximately 1 in 1000 in the general population, but in parts of Great Britain, especially Ireland and Wales, the rate is as high as 7 to 9 in 1000 births (1). Genetic influences must also play a role, since immigrants from these areas to other parts of the world still have a somewhat higher rate of neural tube defects (1). However, other

Table 5 Diseases Caused by Mitochondrial DNA Mutations

Kearns–Sayre disease, due to large mtDNA deletion
Leber hereditary optic neuropathy, due to G3460A, G11778A, or T14484C mutation in complex I subunits
Leigh syndrome (subacute necrotizing encephalopathy), due to T8993G or C mutation in complex V in 10% of cases
Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes, due to A3243G or T3271C mutations in Leucine tRNA
Mitochondrial myopathy, due to mutations in various mt-tRNA genes
Myoclonic epilepsy and ragged-red fiber disease, due to A8344G in Lysine tRNA
Neurogenic muscle weakness, ataxia, and retinitis pigmentosa, due to T8993G or C mutation in complex V

environmental variables such as diet must also be important. It has been shown that folic acid consumption in the diet of pregnant women can significantly reduce the recurrence risk of neural tube defects in subsequent pregnancies. The current recommendation by the Centers for Disease Control states that all women of childbearing age who are capable of becoming pregnant should consume 400 mg of folic acid per day (33). All women who have had a previous child with a neural tube should take 4 mg of folic acid per day, beginning 2 to 3 months prior to conception and continuing for the first 3 months of gestation (34). Flour and other grain products are now supplemented with folic acid in the United States to ensure proper intake for all women, since it is difficult to obtain more than 200 mg of folic acid a day from a typical American diet.

MITOCHONDRIAL INHERITANCE

There are an increasing number of recognized human genetic disorders caused by mutations in the mitochondrial genome (Table 5) (35). Within the cytoplasm of each cell are several hundred mitochondria, which are involved in energy production for the cell. Each mitochondrion has multiple copies of its own circular DNA (mtDNA), which is 16.5 Mb in size, encoding 37 genes for tRNA, rRNA, and some of the enzymes of oxidative phosphorylation. Because mtDNA is cytoplasmic in location, inheritance is exclusively maternal. Sperm contain very few mitochondria and, therefore, both males and females inherit essentially all of their mtDNA from their mothers. There is variable expression of mitochondrial disorders due to heteroplasmy; this term refers to the variability of the proportion of mutant mtDNA within each cell. The proportion of abnormal mtDNA at the somatic level may change over time due to replicative segregation. This can account for the apparent degenerative nature of these disorders noted clinically, which corresponds to an increase in the percentage of abnormal mitochondria (36,37). A woman who has a mitochondrial DNA disorder will pass it on to all of her offspring. However, because of the highly variable expression of these conditions, not all offspring would be expected to be affected to the same degree. Offspring of men with mitochondrial DNA disorders cannot inherit the condition because all mitochondria originate from the mother. It is important to remember that there are also many disorders associated with mitochondrial dysfunction that are inherited in a Mendelian fashion, since most

proteins active within the mitochondria are encoded by nuclear DNA genes.

COMMON METABOLIC DISORDERS

Inborn errors of metabolism are due to enzyme deficiencies that cause abnormal elevations of intermediary compounds of biochemical pathways in tissues and body fluids, with resultant medical problems. Most have an autosomal recessive inheritance pattern, but a few are X-linked recessive disorders. There are several hundred known inborn errors of metabolism, most of which are very rare (38). However, they are collectively common and affect about 1 in 1000 newborns.

One of the most common metabolic disorders is the autosomal recessive aminoacidopathy phenylketonuria (PKU), which has an incidence of 1 in 10,000 to 1 in 15,000 newborns (38). It is caused by a deficiency of the enzyme phenylalanine hydroxylase, which normally converts phenylalanine to tyrosine. Patients with PKU have elevated blood levels of phenylalanine and excretion of phenylketones in the urine. If left untreated, affected individuals will develop microcephaly and profound mental retardation due to the toxic effects of the elevated compounds. However, if diagnosed within the first few weeks of life, with institution of a low phenylalanine diet, mental retardation can be prevented. PKU is the first genetic disorder for which there has been generalized population screening. Since the 1960s, all newborns in the United States have been tested for the disorder. This has nearly eliminated PKU as a cause for mental retardation. However, a new problem has emerged; women who were allowed to stop the dietary restriction of phenylalanine after age 6 years are now of childbearing age. The elevated blood level of phenylalanine in a pregnant PKU woman has deleterious effects on the developing fetus, with increased risk for spontaneous pregnancy loss, congenital heart defects, severe microcephaly, and mental retardation. Therefore, the PKU diet must be reinstituted prior to conception and continued throughout pregnancy. New guidelines state that all people with PKU should remain on the diet for life.

The United States and most developed countries of the world perform newborn screening for many inborn errors of metabolism, endocrine disorders including hypothyroidism and congenital adrenal hyperplasia, cystic fibrosis, hemoglobinopathies, and hearing loss. All states now have significantly expanded newborn metabolic disease screening, largely due to the development of tandem mass spectrometry methods to detect many amino acid disorders, organic acid disorders, and disorders of fatty acid oxidation. For most of these disorders, it has been shown that early diagnosis in the newborn period can significantly reduce morbidity and mortality. As mentioned above for PKU, institution of dietary restriction of phenylalanine before 1 month of age can prevent mental retardation. For other inborn errors of metabolism, such as fatty acid oxidation defects, maple syrup urine disease and galactosemia, illness, mental retardation, and even death can be prevented by early detection and treatment.

POPULATION SCREENING FOR GENETIC DISEASE

Screening tests are a routine part of medical care, with the goal of early identification and treatment of specific and common diseases. For genetic diseases, population screening involves

not only affected individuals, but also additional family members. Screening for genetic disease has been defined as the "search in a population for persons possessing certain genotypes that (i) are already associated with disease or predisposition to disease or (ii) may lead to disease in their descendants" (39). As outlined in the previous section, newborn screening for metabolic diseases exemplifies the first type of screening, which will result in proper identification of infants with these disorders and institution of treatment. Other examples include presymptomatic genetic testing for Huntington disease, breast cancer, or colon cancer.

The second type of screening includes testing of targeted ethnic or racial populations to identify carriers of genetic disease. Examples are cystic fibrosis in Caucasians, Tay-Sachs disease in Ashkenazi Jews, and hemoglobinopathies in African-Americans (10). On a smaller scale, genetic screening can involve karyotype analysis on family members at risk for having a chromosome translocation. Carrier testing is also available for women who are at risk to be carriers for X-linked disorders such as Duchenne muscular dystrophy or Fragile X syndrome. This type of population screening will not affect the health of the carrier, but will have a significant impact on reproductive choices for the family. It is essential that appropriate genetic counseling accompany the information given regarding carrier status.

GENETIC COUNSELING

Genetic counseling is an important part of the practice of medical genetics. The field of genetics is unique in that a diagnosis of a genetic disorder in an individual has an impact on his or her entire family. Multiple family members and their present or future offspring may be at risk for the genetic disorder as well. Genetic counseling involves the explanation of the manifestations of the disorder, the natural history and treatment, the inheritance pattern, the risks of recurrence, and the methods of prenatal and postnatal diagnosis. The genetic counselor also helps the family make the best possible adjustment to the disorder and the risks of recurrence. This often involves referral to a support organization through which the family can make contact with other affected individuals. The vast majority of genetic counselors and clinical geneticists seek to provide nondirective counseling, whereby information about the disorder and the recurrence risk is given in an unbiased and neutral way, and the family makes decisions about reproductive options in accordance with their own beliefs and values.

Reasons for referral for genetic counseling include a family history of a known or suspected single-gene disorder, chromosomal disorder, or multifactorial condition, carrier status for a genetic disease, consanguinity, advanced maternal age during pregnancy, abnormal screening tests during pregnancy, repeated pregnancy loss or infertility, or teratogen exposure during pregnancy.

In the prenatal clinic setting, the genetic counseling session often precedes any diagnostic testing. During the session, the family history is reviewed in detail, as well as the reasons for referral, the information obtainable by the prenatal testing, and the risks of the procedure. If fetal abnormalities are detected, then additional counseling sessions may be required to explain the findings to the family and to discuss options for

Figure 20 General structure of a typical human gene. Source: From Ref. 40.

pregnancy management. If special care or surgery will be needed for the baby immediately after birth, the genetic counselor and the obstetrician may help arrange meetings with the appropriate surgeons or pediatric specialists so that the family is optimally prepared. For cases that are likely to be lethal, it is best to introduce the idea of a postmortem examination before the birth of the baby. In the event of death, a complete autopsy is recommended to search for additional anomalies and to confirm the diagnosis. This helps ensure that accurate genetic counseling, especially with respect to recurrence risk, can be provided in subsequent pregnancies.

MOLECULAR GENETICS

Genes are the basic units of inheritance. As mentioned previously, there are several thousand single-gene diseases with a known molecular genetic cause, representing only a fraction of the estimated total 20,000 to 25,000 genes in the human genome. Over the past 5 to 10 years, there has been a dramatic increase in the number of identified, cloned, and sequenced disease genes. With this new knowledge has come increased availability and demand for both prenatal and postnatal molecular genetic testing. Molecular genetic testing is done for diagnostic purposes to confirm the presence of a disorder, as well as to identify conditions presymptomatically.

Genes are composed of deoxyribonucleic acid (DNA) and are contained on the chromosomes. Each strand of DNA has a specific sequence of four nucleotides, each containing a different base, adenine, thymine, cytosine, or guanine. Adenine pairs with thymine and cytosine pairs with guanine as two complementary strands of DNA are wound together to form a double helix. Genes have a common basic structure (Fig. 20). First, there are upstream sequences that regulate transcription, known as promoters and enhancers. Then, there is a transcription initiation site, followed by a series of alternating exons and introns. The DNA sequence serves as a template from which messenger RNA (mRNA) is made; this process is known as transcription. As transcription proceeds, a primary mRNA is made from the DNA sequence of the gene, which includes the introns. The intron sequences are then spliced out and the exons are linked together to form the mature mRNA molecule. Thus, the exons are the only portions of the gene that specify the final protein product. The mature mRNA molecule is used to make the protein product by the process of translation. Groups of three nucleotides, called codons, code for specific amino acids. Transfer RNA (tRNA) and ribosomal RNA (rRNA) interact with the mRNA to assemble the amino acids into a polypeptide chain to form the final protein molecule.

Errors in the sequence of nucleic acids of the DNA produce mutations in genes and often lead to genetic diseases. There are a variety of different kinds of mutations that can occur in genes. Some result in genetic disorders, while others may have no detrimental effect. The most common type of mutations include missense (single amino acid change), nonsense (insertion of stop codon with premature termination of translation), frameshift (insertion or deletion of nucleotides that alters downstream codons), splice site (incorrect splicing of introns), and promoter mutations (decreased transcription of mRNA). An expansion mutation occurs when there is insertion of many extra copies of a trinucleotide repeat; examples of disorders with this type of mutation are fragile X syndrome, Huntington disease, and myotonic dystrophy.

Diagnosis is possible for many genetic disorders using molecular genetic techniques. Genomic DNA can be obtained from peripheral blood leukocytes, solid tissues, or cultured cells. Testing can be accomplished by either direct mutation analysis or by linkage analysis (Fig. 21). In linkage analysis, information about nondisease producing variation, or polymorphisms, such as restriction fragment-length polymorphisms, variable number of tandem repeats, or microsatellite repeat polymorphisms, can trace inheritance of a chromosome containing a disease-producing gene through a family. This method of testing has disadvantages. First, multiple family members are needed to establish the phase of linkage. Second, some families may not be informative for some linked markers. Third, recombination between the marker and the disease gene may occur, causing inaccuracies in the test results.

In direct DNA testing, the disease gene must have been isolated and disease-producing mutations are identified. Specific mutations can then be sought by a variety of methods. Segments of DNA from the gene of interest is amplified by (PCR) polymerase chain reaction and then sequenced, or deletions or rearrangements of the gene can be identified by Southern blotting. Direct testing is advantageous, since it is likely to be more accurate than linkage analysis, and a single individual can be tested without requiring family information. For example, molecular genetic testing for sickle cell disease is highly accurate, since all affected individuals have the same missense mutation at position six of the beta-globin chain, which results in a substitution of valine for glutamic acid. This point mutation results in a loss of one MstII restriction enzyme site in the mutant gene, making direct DNA diagnosis very straightforward. For other genetic disorders characterized by a number of common mutations, such as cystic fibrosis, allele-specific oligonucleotide probes (ASOs) of 18 to 20 nucleotides in length complementary to known normal and

Figure 21 Linkage of the gene for neurofibromatosis 1 to a marker locus. The mother (I-1) is affected with this dominant disease and is heterozygous at the NF1 locus (Dd), as well as at two other loci, 1 and 2, on chromosome 17. She carries the A and B alleles on the same chromosome as the mutant NF1 allele (D). The unaffected father is homozygous normal (dd) at the NF1 locus, as well as at the two marker loci (AA and BB). All three affected offspring have inherited the A allele at marker 1, whereas the three unaffected offspring have inherited the a allele. Thus, all six offspring are nonrecombinant for NF1 and marker locus 1. However, individuals II-1, II-3, and II-5 are recombinant for NF1 and marker locus 2, indicating that meiotic crossing over has occurred between these two loci. Source: From Ref. 40.

mutant DNA sequences can be devised. ASOs are then hybridized to patient DNA samples to identify homozygotes or heterozygotes for specific mutations. Thus, a panel of ASOs for the most common mutations seen in a disorder can be used to rapidly screen patient DNA samples. For disorders in which there are no common mutations or when common mutations have been ruled out, the entire gene can be sequenced to look for rare or new disease-causing mutations. In the future, identification of the molecular basis for genetic defects or syndromes may be achieved by exome sequencing, in which all of the exons in the genome are sequenced to identify mutations in candidate genes, or by whole genome sequencing. Up-to-date information about genetic disorders for which molecular genetic testing is clinically available can be found at the GeneTests website (<http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests>).

TREATMENT OF GENETIC DISEASES

With the isolation of disease genes and an increasingly better understanding of the molecular genetic bases for many disorders, it has become feasible to consider treatments and potentially cures for genetic disease (41).

Gene therapy involves insertion of normal copies of genes into individuals who have genetic diseases. This can potentially be accomplished by either somatic cell or germ cell gene therapy. Most work thus far has focused on somatic cell gene therapy. There are two ways to approach somatic cell gene therapy. Ex vivo gene therapy involves removing a patient's cells from the body, inserting the normal gene copy into the cells, and then returning the cells to the body. In vivo gene therapy, cells are treated while inside the patient's body. For successful gene therapy, the cell requiring treatment must be easily accessible and relatively long-lived. Some of the earliest human gene therapy trials were performed for severe combined immune deficiency due to adenosine deaminase deficiency, using bone marrow stem cells. Other cells under consideration for therapy have included lymphocytes, hepatocytes, muscle cells, and respiratory epithelial cells. More

recently, gene therapy for Leber's congenital amaurosis has been accomplished by replacement of the defective gene locally to the retina of the eye. Types of genetic diseases that are amenable to somatic cell gene therapy are primarily autosomal recessive or X-linked disorders that result in almost total lack of normal protein. Reconstitution of even 5% to 10% of normal protein levels appears to be sufficient to treat these diseases. Dominant disorders that are caused by heterozygosity for mutant and normal genes (dominant-negative mutations) are not likely to be treatable by gene replacement; methods to block production of the mutant protein will be required.

In conditions associated with an enzyme deficiency, methods have been developed to provide systemic delivery of the missing enzyme to the patient. Enzyme replacement therapy for lysosomal storage diseases, such as Gaucher disease, Fabry disease, Hurler syndrome, and Pompe disease, has been very successful. Enzyme replacement therapy for other enzyme deficiency disorders is under development. Another approach has been to decrease the substrate for the deficient enzyme by giving a drug that inhibits an upstream enzyme in the pathway, thus decreasing accumulation of the toxic compound. This type of drug treatment has been approved for Gaucher disease and is being developed for other conditions.

With the elucidation of the molecular genetic pathways involved in certain genetic disorders, it has been possible to devise new uses for FDA-approved drugs. Examples of this include the experimental use of farnesyltransferase inhibitors in progeria, a premature aging syndrome, and an angiotensin II type 1 receptor antagonist for treatment of the dilated aortic root in Marfan syndrome. Pharmacologic chaperones are also being developed to help stabilize mutant proteins and enhance residual activity.

Finally, methods for manipulating the expression or splicing of mutant genes are being investigated. Drugs that induce read-through of a stop codon during translation of a mutant gene can result in increased expression of full-length protein. This approach has been tried experimentally with cystic fibrosis and Duchenne muscular dystrophy. In other studies, antisense oligonucleotides have been used to induce skipping

during mRNA splicing of exons that contain mutations; this approach will produce a smaller protein, but one that might retain some residual function. This method of therapy has been investigated for Duchenne muscular dystrophy. It is anticipated that more such opportunities will become available in the future for targeted therapy of the disease manifestations of genetic disorders.

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