Mechanisms of Inheritance 3

CONTENTS

One of the most remarkable characteristics of chromosomes is the ability to sort precisely the genetic material represented in homologous pairs of chromosomes into daughter cells and gametes, as previously discussed. This assortment is recognized through the many visible characteristics of individuals. This *phenotype*, or visible presentation of a person, is influenced by the expression of alleles at different times during development, at different efficiencies, and in different cells or tissues. Observed differences are the result of a cell's *genotype,* or molecular variation in alleles.

Mechanisms of inheritance generally refer to traits resulting from a single factor or gene, called *unifactorial inheritance*, or from the interaction of multiple factors or genes, called *multifactorial inheritance*. Because it is the simplest inheritance pattern, unifactorial inheritance is the best understood. Gregor Mendel first investigated this type of inheritance in his famous studies of garden peas in 1865. Because the underlying principles of Mendel's work became hallmarks to understanding inheritance, mechanisms of unifactorial inheritance are often called *mendelian inheritance* and the other mechanisms are referred to as *nonmendelian inheritance.*

Multifactorial inheritance is more complex because of the variation of traits within families and populations. Individual genes within a disease demonstrating multifactorial inheritance may have a dominant or recessive inheritance pattern; but when numerous nongenetic factors and genes interact to cause the disease, the mechanisms can be difficult to interpret and explain.

MENDELIAN INHERITANCE

Genes are found on autosomes and sex chromosomes, and evidence for the existence of genes prior to the molecular revolution was based on measurable changes in phenotype. These changes resulted from allelic variation. Observing variation depends on the relationship of one allele to another. The terms used to describe this relationship are dominant and recessive. If only one allele of a pair is required to manifest a phenotype, the allele is *dominant*. If both alleles must be the same for a particular phenotypic expression, the allele is *recessive*. This is described by the notation AA, Aa, aa, where "A" is dominant and "a" is recessive. The AA condition is called *homozygous dominant*, Aa is called *heterozygous*, and aa is called *homozygous recessive*.

Sex chromosomes also have alleles with dominant and recessive expression. However, this situation is different because for males all X chromosome genes are expressed from the same single chromosome. Females have two X chromosomes, but the scenario is different from that of autosomes because of lyonization.

Variation in alleles results from mutations. The effects of any mutation may influence the character and function of the protein formed. Many times the mutation will create a protein with a recessive nature, but this is not always the case. Several mechanisms through which an allele can affect a function are shown in Table 3-1. These mechanisms are independent of mode of inheritance.

Autosomal Dominant Inheritance

Mendelian inheritance is classified as autosomal dominant, autosomal recessive, and X-linked (Box 3-1). A diagram representing family relationships is called a *pedigree* and can be informative about inherited characteristics. Figure 3-1 shows conventional symbols used in pedigree construction.

The family pedigree shown in Figure 3-2 has features suggesting autosomal dominant inheritance. It can be noted

TABLE 3-1. Selected Mechanisms of Allele Action

Mechanism		Example
Loss-of-function	Gene product or activity is reduced.	Waardenburg syndrome results from mutations in *PAX*3, a DNA binding protein important in regulating embryonic development.
Gain-of-function	Gene product is increased. Gene expression occurs at the wrong place or time. Gene product has increased activity.	Charcot-Marie-Tooth disease results from the overexpression of *PMP*22 (peripheral myelin protein) caused by gene duplication.
Protein alteration	Normal protein function is disrupted.	Kennedy disease results from CAG (polyglutamine) expansion at the 5´ end of the androgen receptor. The mutant protein misfolds, aggregates, and interacts abnormally with other proteins, leading to toxic gain of function and alteration of normal function.
Dominant effects of recessive mutation	Alleles are recessive at the molecular level but show a dominant mode of inheritance.	Retinoblastoma is inherited as a recessive allele. A mutation in the second, normal allele (also known as the two-hit hypothesis) results in tumor formation.

Box 3-1. EXAMPLES OF INHERITED DISORDERS

Mendelian

Autosomal dominant
- Achondroplasia
- Marfan syndrome
- Neurofibromatosis type 1
- Brachydactyly
- Noonan syndrome

Autosomal recessive
- Albinism
- Cystic fibrosis
- Phenylketonuria
- Galactosemia
- Mucopolysaccharidoses

X-linked dominant
- Hypophosphatemic rickets
- Orofaciodigital syndrome

X-linked recessive
- Duchenne/Becker muscular dystrophies
- Hemophilia A and B
- Glucose-6-phosphate dehydrogenase deficiency
- Lesch-Nyhan syndrome

Nonmendelian

Triplet repeats
- Fragile X syndrome
- Myotonic dystrophy
- Spinocerebellar ataxia
- Friedreich ataxia
- Synpolydactyly

Genomic imprinting
- Prader-Willi syndrome
- Angelman syndrome

Mitochondrial
- LHON
- MERRF
- MELAS

that each affected person has at least one affected parent. Moreover, the normal children of an affected parent, when they in turn marry normal persons, have only normal offspring. In this particular instance, the mutant allele is dominant and the normal allele is recessive. In nearly all instances of dominant inheritance, as exemplified by the pedigree, one parent carries the detrimental allele and shows the anomaly, whereas the other parent is normal. The affected parent will pass on the defective dominant allele, on average, to 50% of the children. Normal children do not carry the harmful dominant allele, hence their offspring and further descendants are not burdened with the dominant trait.

There are numerous examples in humans of defective genes that are transmitted in a dominant pattern. *Achondroplasia*, a form of dwarfism, is inherited as an autosomal dominant trait. Achondroplasia is a *congenital disorder,* a defect present at birth. Affected individuals are small and disproportionate, with particularly short arms and legs. With an estimated frequency of 1 in 15,000 to 40,000 live births, achondroplasia is one of the more common mendelian disorders. Most infants affected by achondroplasia with two mutated alleles, representing a homozygous condition, are stillborn or die in infancy; heterozygous individuals surviving to adulthood produce fewer offspring than normal. This observation underscores an important point for many autosomal dominant disorders—two mutated alleles often have severe clinical consequences.

Characteristics of Autosomal Dominant Inheritance

Guidelines for recognizing autosomal dominant inheritance in humans may be summarized as follows:

1. The affected offspring has one affected parent, unless the gene for the abnormal effect was the result of a new mutation.
2. Unaffected persons do not transmit the trait to their children.
3. Males and females are equally likely to have or to transmit the trait to males and females.
4. The trait is expected in every generation.
5. The presence of two mutant alleles generally presents with a more severe phenotype. Detrimental dominant traits are rarely observed in the homozygous state.

Autosomal Recessive Inheritance

A gene can exist in at least two allelic forms. For the sake of simplicity, two will be considered—A and its alternative (mutant) allele, a. From these two alleles, there are three

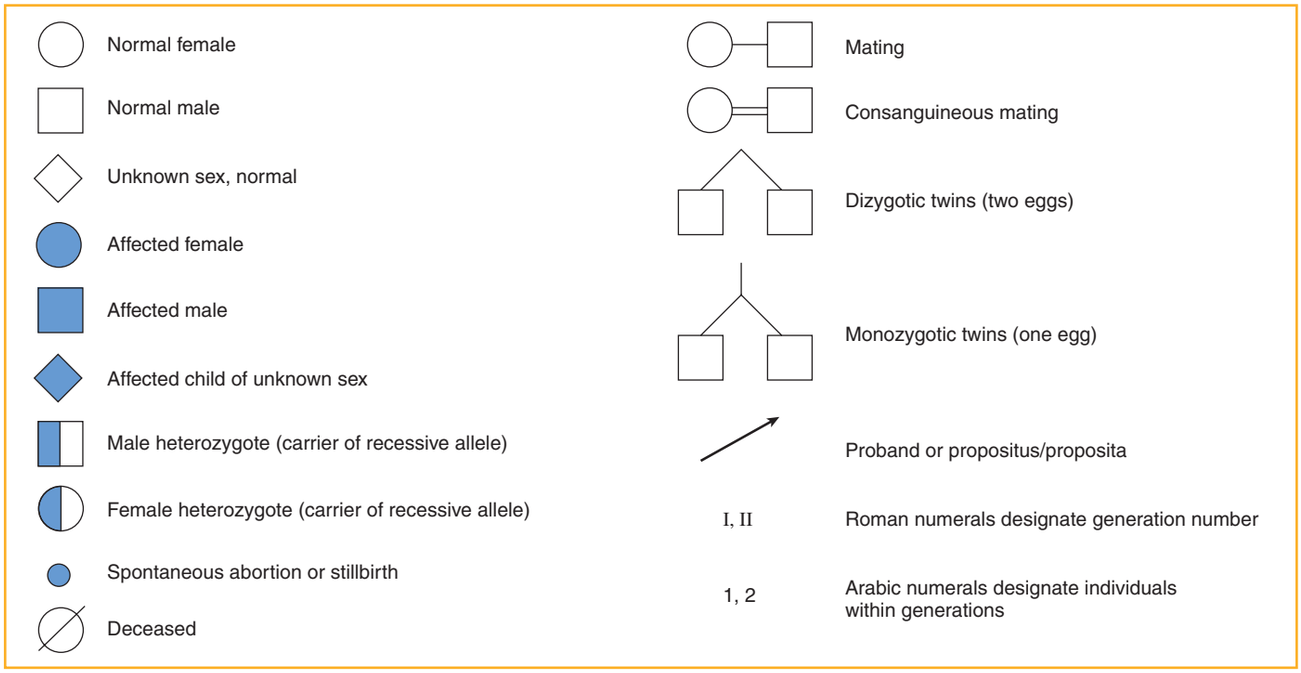


Figure 3-1. Conventional symbols used in pedigrees.

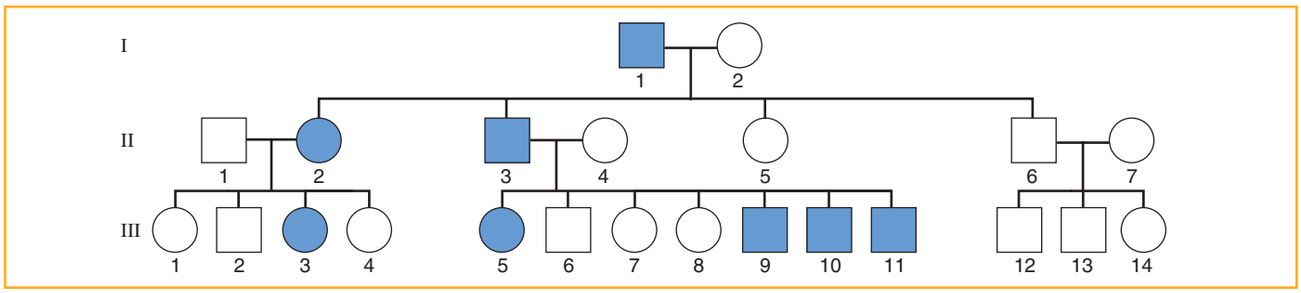


Figure 3-2. Pedigree of a family with an autosomal dominant trait.

different genotypes, AA, Aa, and aa, that can be arranged in six types of marriages. These genotypes and their offspring are listed in Table 3-2. The outcome of each type of marriage follows the mendelian principles of segregation and recombination.

In the vast majority of cases of recessive inheritance, affected persons derive from marriages of two heterozygous carriers; affected individuals receive a mutant allele from each parent and represent homozygous recessive expression. In other words, recessive disorders in family histories tend to appear only among siblings and not in their parents. This is demonstrated by the family pedigree in Figure 3-3. This pedigree shows that a normal male marries a normal woman. Apparently, both were heterozygous carriers, since one of the four children (the first child, designated II-1) exhibited the recessive trait. This son, although affected, had two normal offspring (III-1 and III-2). These two children must be carriers (Aa), having received the a allele from their father (II-1) and the A allele from their unaffected mother (II-2). The genetic constitution of the mother (II-2) cannot be ascertained; she may be either homozygous dominant (AA) or a heterozygous carrier (Aa). The marriage of first cousins (III-3 and III-4) increases the risk that both parents of IV-1 and IV-3 have received the same detrimental recessive gene through a common ancestor. In this case, the common ancestors are the parents in generation I.

It can be deduced from this pedigree that the daughter (II-6) of the first marriage was a carrier (Aa). Her two children were normal, but it is noted that her first child (III-4) married a first cousin (III-3), and from this marriage affected children (IV-1 and IV-3) were born. Accordingly, the daughter of the third generation (III-4) must have been heterozygous, and in turn, her mother (II-6) was most likely heterozygous (or else she married a heterozygous man). Similarly, the male involved in the cousin marriage (III-3) must have been heterozygous, as was his father (II-3).

TABLE 3-2. Possible Combinations of Genotypes and Phenotypes in Parents and the Possible Resulting Offspring

Mating Type		Gametes				Offspring	
		First Parent		Second Parent			
Genotype	Phenotype	50%	50%	50%	50%	Genotype	Phenotype
AA x AA	Normal x normal	A	A	A	A	100% AA	100% Normal
AA x Aa	Normal x normal	A	A	A	a	50% AA 50% Aa	100% Normal
Aa x Aa	Normal x normal	A	a	A	a	25% AA 50% Aa 25% aa	75% Normal 25% Abnormal
AA x aa	Normal x abnormal	A	A	a	a	100% Aa	100% Normal
Aa x aa	Normal x abnormal	A	a	a	a	50% Aa 50% aa	50% Normal 50% Abnormal
aa x aa	Abnormal x abnormal	a	a	a	a	100% aa	100% Abnormal

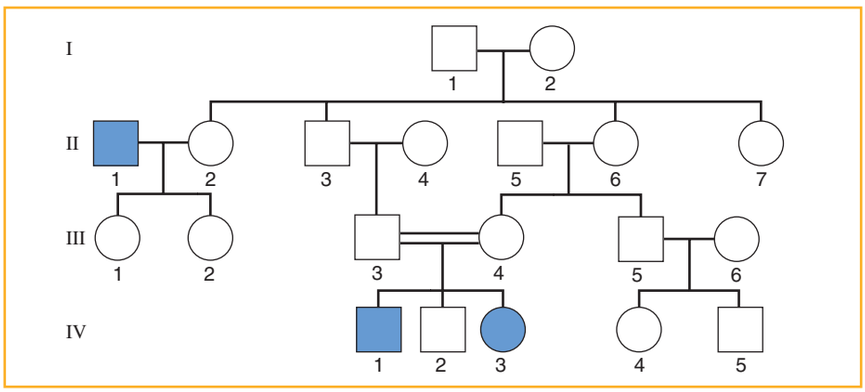


Figure 3-3. Pedigree of a family with an autosomal recessive trait.

Pedigrees of the above kind typify the inheritance of such recessively determined traits in humans as *albinism*, *cystic fibrosis*, and *phenylketonuria*. Special significance is attached to the *heterozygous carrier*—the individual who unknowingly carries the recessive allele. It is usually difficult to tell, prior to marriage, whether the individual bears a detrimental recessive allele. Thus, a recessive allele may be transmitted without any outward manifestation for several generations, continually being sheltered by the dominant normal allele. The recessive allele, however, becomes exposed when two carrier parents happen to mate, as seen in Figure 3-3. This explains cases in which a trait, absent for many generations, can suddenly appear without warning.

Often only one member in a family is afflicted with a particular disorder. In such an event, it would be an error to jump to the conclusion that the abnormality is not genetic solely because there are no other cases in the family. Without a positive family history, and sometimes the corroboration of diagnoses, the occurrence of a single afflicted individual may represent a new, sporadic mutation.

Characteristics of Autosomal Recessive Inheritance

Guidelines for recognizing autosomal recessive inheritance may be summarized as follows:

1. Most affected individuals are children of phenotypically normal parents.
2. Often more than one child in a large sibship is affected. On average, one fourth of siblings are affected.
3. Males and females are equally likely to be affected.
4. Affected persons who marry normal persons tend to have phenotypically normal children. (The probability is greater of marrying a normal homozygote than a heterozygote.)
5. When a trait is exceedingly rare, the responsible allele is most likely recessive if there is an undue proportion of marriages of close relatives among the parents of the affected offspring.

Consanguinity and Recessive Inheritance

Offspring affected with a recessive disorder tend to arise more often from consanguineous unions than from marriages

of unrelated persons (see Chapter 12). Close relatives share more of the same alleles than persons from the at-large population. If a recessive trait is extremely rare, the chance is very small that unrelated marriage partners would harbor the same defective allele. The marriage of close relatives, however, increases the risk that both partners have received the same defective allele through some common ancestor. Not all alleles are equally detrimental. Stated in another way, identical alleles may produce an extreme phenotype, whereas two different alleles of the same gene may appear mild or even normal.

With increasing rarity of a recessive allele, it becomes increasingly unlikely that unrelated parents will carry the same recessive allele. With an exceedingly rare recessive disorder, the expectation is that most affected children will come from cousin marriages. Thus, the finding that the parents of Toulouse-Lautrec, a postimpressionist artist who documented bohemian nightlife, particularly at the Moulin Rouge in Paris, were first cousins is the basis for the current view that the French painter was afflicted with *pycnodysostosis,* characterized by short stature and a narrow lower jaw. This condition is governed by a rare recessive allele unlike achondroplasia, another form of short stature that is determined by a dominant allele. Thus, it was more likely that Toulouse-Lautrec suffered a rare disorder expressed as a result of his parents' relatedness rather than a common disorder that could only be explained by a new mutation.

Codominant Expression

In some heterozygous conditions, both the dominant and recessive allele phenotypes are expressed. From a molecular viewpoint, the relationship between the normal allele and the mutant allele is best described as *codominant*. This means that, at the molecular level, neither allele masks the expression of the other. An example of codominance is sickle cell anemia. In this example, two types of hemoglobin are produced: normal type hemoglobin A and a mutant form, called hemoglobin S. Another example is the expression of both A and B antigens on the surface of red blood cells in individuals with type AB blood.

The terms dominant and recessive have little, if any, utility when both gene products affect the phenotype. Dominance and recessiveness are attributes of the trait, or phenotype, *not* of the gene. An allele is *not* intrinsically dominant or recessive—only normal or mutant.

BIOCHEMISTRY & PHYSIOLOGY

Hemoglobin

Hemoglobin is composed of *heme,* which mediates oxygen binding, and *globin,* which surrounds and protects the heme. Hemoglobin is a tetramer of globin chains (two α-chains and two β-chains in adults), each associated with a heme. There are many variants of hemoglobin. In sickle cell, the β-globin chain is a mutation and is known as hemoglobin S (HbS). A missense mutation causes valine to be placed in the protein in place of glutamic acid.

The mutation that causes HbS produces oxygenated hemoglobin that has normal solubility; however, deoxygenated hemoglobin is only about half as soluble as normal HbA. In this low-oxygen environment, HbS molecules crystallize into long fibers, causing the characteristic sickling deformation of the cell. The deformed cells, which can disrupt blood flow, are responsible for the symptoms associated with sickling crises such as pain, renal dysfunction, retinal bleeding, and aseptic necrosis of bone, and patients are at an increased risk for anemia owing to hemolysis of the sickled cells.

IMMUNOLOGY

ABO Blood Groups

There are 25 blood group systems that account for more than 250 antigens on the surface of red blood cells. The ABO blood group is one of the most important, and the antigens expressed are produced from alleles of one gene. There are three major alleles—A, B, and O—but more than 80 have been described.

The ABO gene encodes glycosyltransferases, which transfer specific sugars to a precursor protein known as the H antigen. The H antigen is a glycosphingolipid consisting of galactose, *N*-acetylglucosamine, galactose, and fructose attached to a ceramide. In the absence of sialic acid, it is a globoside rather than a ganglioside. The A allele encodes α1, 3-*N*-acetylgalactosamyl transferase, which adds *N*-acetylgalactosamine to the H antigen to form the A antigen. The B allele produces α1,3-galactosyltransferase, which transfers galactose to the H antigen, thus forming the B antigen. The O allele produces the H antigen, but it has no enzyme activity.

X-Linked Recessive Inheritance

No special characteristics of the X chromosome distinguish it from an autosome other than size and the genes found on the chromosome, but these features distinguish all chromosomes from each other. X chromosome inheritance, often called X-linked or sex-linked, is remarkable because there is only one X chromosome in males. Most of these alleles are therefore *hemizygous*, or present in only one copy, in the male because there is no corresponding homologous allele on the Y chromosome. Presence of a mutant allele on the X chromosome in a male is expressed, whereas in the female a single mutant allele may have a corresponding normal allele to mask its effects, as expected in the situation of dominance versus recessiveness.

The special features of X-linked recessive inheritance are seen in the transmission of hemophilia A (Fig. 3-4). This is a blood disorder in which a vital clotting factor (factor VIII) is lacking, causing abnormally delayed clotting. Hemophilia exists almost exclusively in males, who receive the detrimental mutant allele from their unaffected mothers. Figure 3-4 shows part of the pedigree of Queen Victoria of England. Queen Victoria (I-2) was a carrier of the mutant allele that

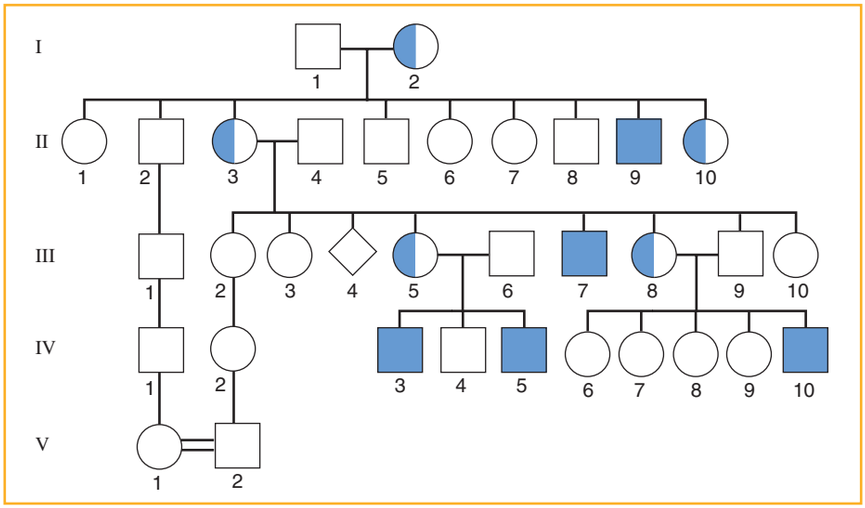


Figure 3-4. X-linked inheritance of hemophilia A among descendants of Queen Victoria (I-2) of England.

occurred either as a spontaneous mutation in her germline or was a mutation in the sperm of her father, Edward Augustus, Duke of Kent. Queen Victoria had one son (II-9) with hemophilia and two daughters (II-3 and II-10) who were carriers. The result of these children marrying into royal families in other countries spread the mutant factor VIII allele to Spain, Russia, and Germany. The children of II-3 have hemophilia in two more generations (III-7, IV-3, IV-5, and IV-10). The families of II-9 and II-10 also revealed hemophilia through two more generations (not shown). Though the grandson of III-2 married V-1, no hemophilia allele was introduced back into the family of the first son of Queen Victoria, Edward VII, and the royal family of England has remained free of hemophilia. Generation V is represented by Queen Elizabeth and Prince Philip.

For alleles on the X chromosome, each son of a carrier mother has a 50% chance of being affected by hemophilia, and each daughter has a 50% chance of being a carrier. Hemophilic females are exceedingly rare, since they can only derive from an extremely remote mating between a hemophilic man and a carrier woman. A few hemophilic women have been recorded in the medical literature; some have married and given birth to hemophilic sons.

Characteristics of X-Linked Recessive Inheritance

Guidelines for recognizing X-linked recessive inheritance may be summarized as follows:

1. Unaffected males do not transmit the disorder.
2. All the daughters of an affected male are heterozygous carriers.
3. Heterozygous women transmit the mutant allele to 50% of the sons (who are affected) and to 50% of the daughters (who are heterozygous carriers).
4. If an affected male marries a heterozygous woman, half their sons will be affected, giving the erroneous impression of male-to-male transmission.

X-Linked Inheritance and Gender

As noted, X-linked inheritance is distinguished by the presence of one chromosome in males but two in females. To explain the appearance of a condensed body in female cells, known as a *Barr body*, and to justify the possibility of twice as many X chromosome gene products in females as in males, the Lyon hypothesis was proposed. This hypothesis, which has been become well established, recognizes the Barr body in female cells as an inactivated X chromosome. Through inactivation, dosage compensation occurs in a female that generally equalizes the expression between males and females.

In general, lyonization suggests that (1) alleles found on the condensed X chromosome are inactive, (2) inactivation occurs very early in development during the blastocyst stage, and (3) inactivation occurs randomly in each blastocyst cell. Lyonization is more complicated than this simplistic presentation because some alleles are expressed only from the inactive X chromosome, other alleles escape inactivation and are expressed from both X chromosomes, and still other alleles are variably expressed. It is easiest to understand X inactivation as a random event, or that about 50% of cells have the maternal X chromosome inactivated and about 50% of cells have the paternal X chromosome inactivated; however, this situation does not always occur. It is possible to have *skewed inactivation*, whereby the X chromosome from one parent is more or less likely to become inactivated. Depending on the degree of skewing, a clinical presentation will be affected. The more extreme the skewing in favor of keeping the mutant X active, the poorer the prognosis for the individual.

The onset of X inactivation is controlled by the *XIST* gene. This gene is expressed only from the inactive X chromosome and is a key component of the X inactivation center (XIC) found at the proximal end of Xq. The cell recognizes the number of X chromosomes by the number of XICs in the cell. In the presence of two X chromosomes, *XIST* is activated and

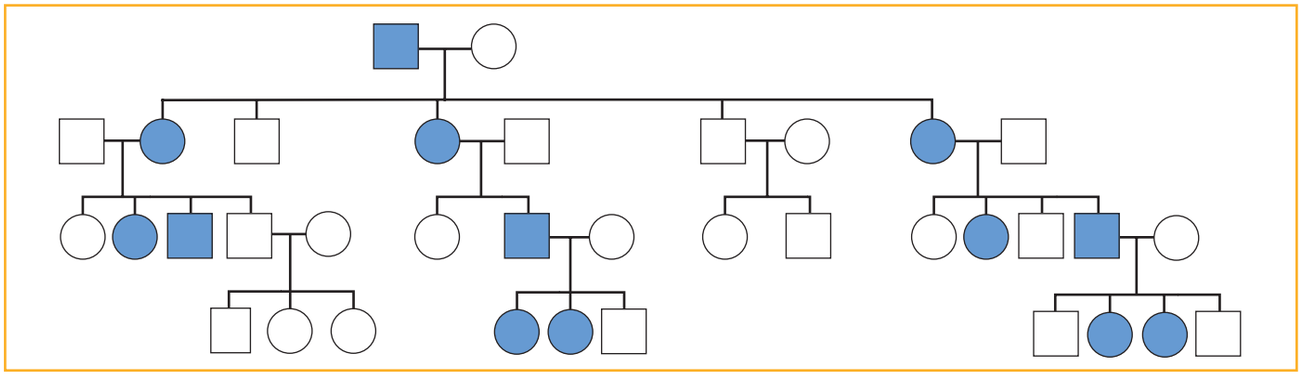


Figure 3-5. Inheritance of an X-linked dominant trait. Note that daughters always inherit the trait from an affected father whereas sons of an affected father never inherit the trait.

RNA molecules are produced that bind to regions of the X chromosome, rendering it inactive. It is not known how some genes escape the influence of the RNA molecules and remain active.

X-Linked Dominant Inheritance

Disorders resulting from X-linked dominant inheritance occur far less frequently than other forms of inheritance. As noted, X-linked recessive inheritance can occur, and males are almost always the affected gender although in very rare cases it is possible for females to acquire two mutant alleles or express milder phenotypes as carriers. With X-linked dominant inheritance, *there are no carriers;* expression of the disease occurs in both males and females, and only one mutant allele is required. As might be expected, heterozygous females may be less affected than males because of the presence of a normal, nonmutated allele. The distinguishing feature between an X-linked dominant and an autosomal disorder is that an autosomal mutation is transmitted from males and females to male and female offspring. When a mutation is located on the X chromosome and expressed in a dominant manner, females transmit the mutant allele to both male and female offspring; however, *males can only transmit it to females* (Fig. 3-5). In addition, affected females may only transmit the mutant allele to 50% of offspring; males will transmit the mutant allele to 100% of females.

Penetrance and Expressivity

Not every person with the same mutant allele necessarily manifests the disorder. When the trait in question does not appear in some individuals with the same genotype, the term *penetrance* is applied. Penetrance has a precise meaning—namely, the percentage of individuals of a specific genotype showing the expected phenotype. If the phenotype is always expressed whenever the responsible allele is present, the trait is *fully penetrant*. If the phenotype is present only in some individuals having the requisite genotype, the allele expressing the trait is *incompletely penetrant*. For a given individual, penetrance is an all-or-none phenomenon; i.e., the phenotype is present (penetrant) or not (nonpenetrant) in that one individual. In penetrant individuals, there may be marked variability in the clinical manifestations of the disorder. When more than one individual is considered, such as a population of individuals, a percentage is usually applied to the proportion of individuals likely to express a phenotype. To illustrate this point, if a trait occurs with 80% penetrance, expression is expected in 80% of individuals with the trait.

Nonpenetrance is a cul-de-sac for clinicians and genetic counselors. Figure 3-6 demonstrates a pedigree with an autosomal dominant trait in which nonpenetrance is pervasive. Individual II-2 most likely carries the disease allele, unless offspring III-2 arose from a new dominant mutation. The future offspring III-4 is at risk for the dominant disease. The calculated mathematical risk would take into consideration the empirical penetrance percentage for the trait (say, 60%) and the probability that a person from the general population (spouse II-6) would harbor the disease allele.

Expressivity is the term used to refer to the range of phenotypes expressed by a specific genotype. This is much more frequent than nonpenetrance. A good example of expressivity is seen in neurofibromatosis (NF). NF consists of two disorders, NF1 and NF2, caused by mutations in different genes. NF is an autosomal dominant disorder, and in both

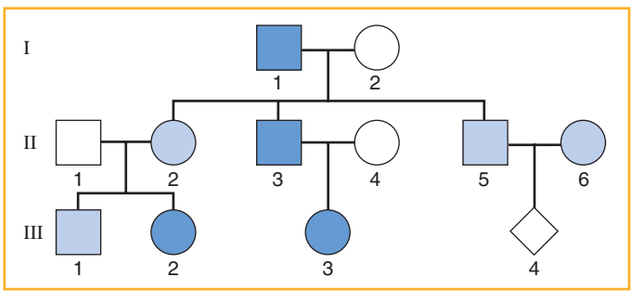


Figure 3-6. Nonpenetrance in a family with an autosomal dominant disorder. The light-colored boxes indicate individuals who do not express the phenotype for the disorder.

forms over 95% of affected individuals have café-au-lait spots. Café-au-lait spots are flat, coffee-colored macules. The expressivity of these spots, which resemble birthmarks, is variable and differs in number, shape, size, and position among individuals.

Late-Acting Genes

Proper interpretation of penetrance and expressivity may be complicated when the genes involved are expressed in the adult rather than the child. These late-acting genes include many genes involved with aging but may also include certain disease genes. *Huntington disease* is an inherited disorder characterized by uncontrollable swaying movements of the body and the progressive loss of mental function. The mutation in the gene is present at birth in all cells of the individual, but the effect of the protein is not evident until much later. The symptoms usually develop in an affected person between the ages of 30 and 45 years. Penetrance is 100%, there is no cure, and the progress of the disease is relentless, leading to a terminal state of helplessness. No therapy can significantly alter the natural progression of the disease, and there are no states of remission. Death occurs typically 12 to 15 years after the onset of the involuntary, jerky movements.

NONMENDELIAN INHERITANCE

Some clinical presentations do not fit the classical patterns of mendelian inheritance and represent examples of nontraditional or nonmendelian inheritance (see Box 3-1). These include triplet repeats, genomic imprinting, mosaicism, and mitochondrial inheritance.

Triplet Repeats

The expansion of short tandem arrays of di- and trinucleotides from a few copies to thousands of copies demonstrates a new type of mutation with the potential of having profound effects on the phenotype of offspring through an unusual mode of inheritance. First demonstrated with fragile X syndrome, the expansion of triplet repeats is found in several neurologic disorders. The expansion probably occurs as a result of faulty mismatch repair or unequal recombination in a region of instability. The proximity of the region of instability to an allele is of paramount importance. Trinucleotide repeats can be found in any region of gene anatomy: the 5′-untranslated promoter region, an exon, an intron, or the 3′ untranslated region of the gene. Interestingly, trinucleotide expansions in any of these regions can also result in disease (Table 3-3). The effects of location may result in a *loss of function*, as seen with fragile X syndrome. A *gain of function* is seen with amplification of CAG, resulting in polyglutamine tracts that cause neurotoxicity in several other neurodegenerative diseases. Finally, RNA can be detrimentally affected if the expansion occurs within a noncoding region. In myotonic dystrophy, the expanded transcript is unable to bind RNA proteins correctly for splicing and remains localized in the nucleus (see Chapter 8).

During normal replication, when the double helix separates into small, single-stranded regions, secondary structures can form with complementary and repeated sequences. These structures, represented as loops and hairpins, hinder the

TABLE 3-3. Neurologic Disease Due to Triplet Repeat Amplification

Location/Disorder	Chromosome Locus	Repeat	Normal Range (repeats)	Disease Range (repeats)
In the 5' Untranslated Region				
Fragile X-A	Xq27.3	CGG in *FMR*1 gene	6–54	50–1500
Fragile X-E	Xq28	CGG/CCG in *FMR*2 gene	6–25	200+
Within the Translated Region of the Gene				
Spinobulbar muscular atrophy (Kennedy disease)	Xq21.3	CAG in androgen receptor gene	13–30	30–62
Huntington disease	4p16.3	CAG in *HD* gene	9–37	37–121
Spinocerebellar ataxia type 1	6p24	CAG in ataxin-1 gene	25–36	43–81
Spinocerebellar ataxia type 3 (Machado-Joseph disease)	14q	CAG in undescribed gene	13–36	68–79
Dentatorubropallidoluysian atrophy (DRPLA)	12p13.31	CAG of atrophin gene	7–23	49–88
In the 3' Untranslated Region				
Myotonic dystrophy	19q13.3	CTG of cAMP-dependent muscle protein kinase	5–37	44–3000
In an Intron				
Friedreich ataxia	9q13	GAA in the first intron of the *FRDA* gene	7–20	200–900

progression of replication by DNA polymerase. An example is $(GAA)_n/(TTC)_n$ expansions that bind to each other. As a result, the polymerase may dissociate either slightly or completely. If its realignment or reassociation does not occur at the exact nucleotide where it should, *DNA has slipped*. Consequently, synthesis continues, but it may "resynthesize" a short region, resulting in amplification. This amplified region distorts the helical structure of DNA—a distortion under the surveillance of mismatch repair proteins. Ordinarily, proteins stabilize the DNA not matching the template strand into a loop that can be excised followed by repair and ligation of any correct nucleotides inserted with the DNA strand. Mismatch repair is the mechanism responsible for slippage repair. Failure of the mismatch repair mechanism to remove the extra DNA does not imply a mutation of any of the repair proteins but rather an inability to adequately repair all regions involved in slippage. This suggests that triplet repeat amplification may occur through events of large slippage that overwhelm the repair system, through unequal recombination, or both. The mechanism by which DNA avoids repair during amplification is unknown.

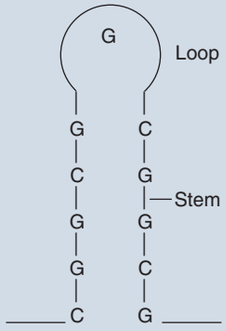
A process known as unequal crossing-over, or recombination, may further amplify duplications. In this process, there is physical exchange of genetic material between chromosomes. During meiosis, homologous chromosomes may mispair with each synapsis. Should a crossover event occur, the DNA breaks, an exchange occurs, and the DNA ends are ligated. The resulting chromatids have gained or lost genetic material if the exchange is unequal (Fig. 3-7). For amplifications, the result is a gain of triplet repeats for one chromatid.

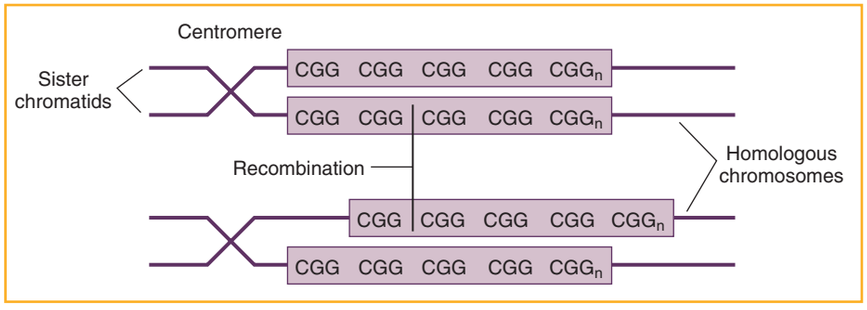
The presence of triplet repeats is not an abnormal condition. It is when the number of repeats reaches a threshold number that disease is expressed (see Table 3-3). When the number of repeats remains stable in the absence of amplification, or with limited amplification below a threshold number, a *normal* condition exists. Once amplification begins to occur, a *premutation* may exist in which some individuals, but not all, may express some symptoms. At this stage, amplification can proceed in the gametes of a premutation individual to a *full mutation* in which all individuals are

BIOCHEMISTRY

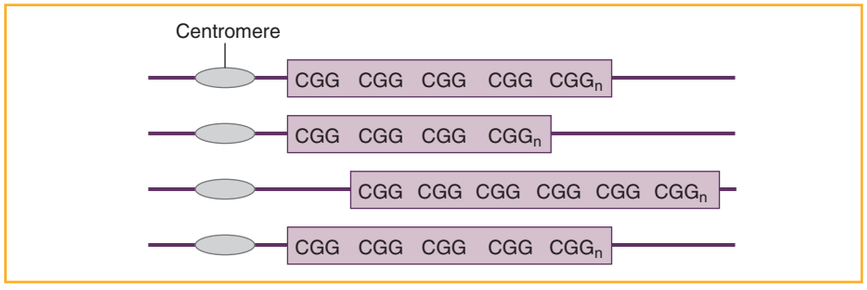
Hairpin Structure

Hairpins are fundamental structural units of DNA. They are formed in a single-stranded molecule and consist of a base-paired stem structure and a loop sequence with unpaired or mismatched nucleotides. Hairpin structures are often formed in RNA from certain sequences, and they may have consequences in DNA transcription such as causing a pause in transcription or translation that results in termination.





A



B

Figure 3-7. Unequal crossover and sister chromatid exchange. **A**, One chromatid of sister chromatids incorrectly pairs with its corresponding sister chromatid. **B**, The outcome shows one chromosome gained DNA, one lost DNA, and two remained the same.

affected. Depending on the gene affected and its chromosomal location, a triplet repeat disease may demonstrate autosomal dominant, autosomal recessive, or X-linked expression.

Unlike most X-linked or recessive disorders, the premutation phenotype presents a different clinical image than expected. Neither males nor females show any outward signs of fragile X syndrome. However, male carriers of the fragile X premutation are at a high risk for fragile X associated tremor/ataxia syndrome (FXTAS), an adult-onset neurologic disorder characterized by ataxia, intention tremor, short-term memory loss, atypical Parkinson's disease, loss of vibration and tactile sensation and reflexes, and lower limb weakness. Penetrance of this disorder increases with age. With the appearance of these features in this group of males (premutation males occur at a frequency of 1 in 813), the premutation presentation is a more common cause of tremor and ataxia in men over age 50 (1 in 3000) than are other ataxia-tremor associated disorders.

Females with premutations are also reported with FXTAS although the incidence is lower. Two additional effects seen in these females is premature ovarian failure occurring before age 40 and an increased incidence of dizygotic twins. Women with full mutations do not experience these features, just as men with full mutations have a different constellation of physical features. Approximately 22% to 28% of women in this group experience premature ovarian failure. Some studies suggest the increase in twinning may be linked more closely to premature ovarian failure than to the premutation itself.

A particularly interesting feature of triplet repeat amplification is that, in many disease presentations, the amplification is parental-specific during gametogenesis. This is the underlying cause of confusion about its mode of inheritance. For fragile X syndrome, two elements contribute to the expression of trinucleotide repeats and disease expression. First, expansions tend to occur through female meiosis I gamete formation. Second, males are more often affected than carrier females due to X chromosome inactivation. This explains why in fragile X syndrome the sons of carrier females are more affected than daughters and why offspring of carrier males do not express the disorder. The risk of mental retardation and other physical features depends on the position of an individual in a pedigree relative to a transmitting male. The daughters of normal transmitting males inherit the same regions of amplification as are present in the transmitting father.

During oogenesis in the daughter of a normal transmitting male, further amplification occurs that is inherited by sons and daughters. Because males carry only a single X chromosome, the effect is more pronounced than in females carrying two X chromosomes, one of which presumably is normal. Females are therefore obligate carriers. The reverse occurs in Huntington's disease, in which amplification occurs preferentially in meiotic transfer from the father. In either situation, a molecular explanation now exists for the observation in some neurologic disorders of an increase in disease severity through successive generations. Referred to as *genetic anticipation*, repeat amplification provided a scientific explanation to allay fears in an affected family that the disease was occurring earlier and with greater severity in successive generations because the mothers were worrying during pregnancy and beyond and somehow contributing to the disease etiology.

Genomic Imprinting

For most autosome genes, one copy is inherited from each parent and generally both copies are functionally active. There are some genes, however, whose function is dependent on the parent from whom they originated. Stated another way, allelic expression is parent-of-origin specific for some alleles. This phenomenon is known as *genomic imprinting*. Genomic imprinting differs from X chromosome inactivation in that the latter has a somewhat random nature and involves most of the chromosome. Genomic imprinting involves specific alleles on a particular chromosome.

DNA is imprinted through methylation, though the signal for initiating this process is unknown. It is a reversible form of allele inactivation. During gametogenesis, most DNA is demethylated to remove parent-specific imprints in germ cells. Remethylation then occurs on alleles specific to the sex of the parent (Fig. 3-8); some alleles are methylated specifically in the copy inherited from the father, inactivating that

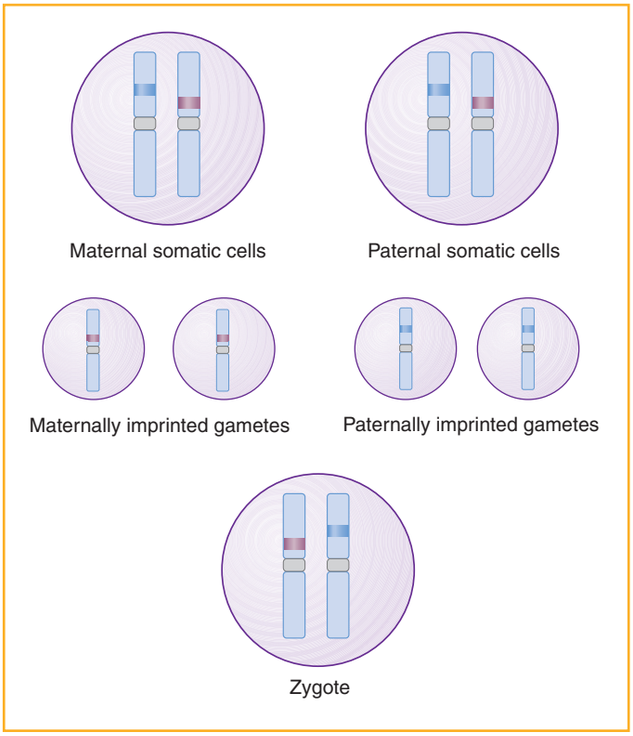


Figure 3-8. Genomic imprinting. Somatic cells have methylated alleles from a specific parent. At gamete formation, the imprint is removed and all alleles are imprinted for the sex of the parent. When gametes form a zygote, parent-specific alleles are present. Blue is a paternal imprint and pink is a maternal imprint.

copy of the gene, while others are methylated specifically in the maternally inherited copy. In females, methylation occurs prior to ovulation when oocyte development resumes. In males, imprinting in spermatogonia is less clear but probably occurs at birth when spermatogonia resume mitosis. However, it is clear that DNA methyltransferase expression in the nucleus correlates with maternal and paternal imprinting. Methylation remains throughout embryogenesis and postnatally. *The consequence of imprinting is that there is only one functional allele for these imprinted genes.* This has significant clinical implications if the functionally active allele is inactivated by mutation.

A number of clinically important genetic diseases are associated with imprinting errors. The first recognized genomic imprinting disorder was Prader-Willi syndrome. It is also the one of the most common microdeletion syndromes and involves at least 12 genes at the chromosome 15q11.2-q13 locus. At least two of these are imprinted genes depending on the parent of origin and hold special importance for Prader-Willi and Angelman syndromes: *SNRPN* and *UBE3A*, respectively. The *SNRPN* gene, producing small nuclear ribonucleoprotein N, is methylated during oogenesis but not spermatogenesis. The *UBE3A* gene, producing ubiquitin-ligase, is methylated during spermatogenesis but not oogenesis (Fig. 3-9). As a common microdeletion, or contiguous gene, syndrome, deletion of a region of the paternal chromosome 15 results in Prader-Willi syndrome because no SNRPN protein is expressed from the imprinted maternal chromosome 15 *SNRPN* allele. Likewise, deletion of the same region from the maternal chromosome 15 yields Angelman syndrome and not Prader-Willi syndrome. SNRPN protein is produced in Angelman syndrome, but UBE3A protein is not expressed from the imprinted paternal chromosome.

Prader-Willi and Angelman syndromes occur from microdeletions in 75% to 80% of cases and can be detected by FISH analysis. However, as seen in Figure 3-9, other mechanisms exist including the possibility of mutations within the individual genes. These represent the major mutation mechanisms. Gross deletion of the promoter and exon 1 of *SNRPN* has been reported; most mutations reported in the *UBE3A* gene are nonsense mutations

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DNA Methylation

DNA methylation occurs by the addition of a methyl group to cytosine. With the presence of "CpG islands," or regions of adjacent cytosines and guanines in promoter regions, methylation of these cytosines is an important aspect of gene regulation. Promoter regions that are highly methylated provide fewer readily available target sites for transcription factors to bind. Therefore, methylation is associated with down-regulation of gene expression and demethylation is associated with up-regulation of gene regulation. Methylation occurs in the presence of DNA methyltransferase, which transfers a $-CH_3$ group donated by S-adenosylmethionine. The $-CH_3$ group is added to carbon 5 of cytosine and becomes 5-methylcytosine (m^5C).

Barr bodies, the physical presentation of inactive X chromosomes, are heavily methylated. Aberrant DNA methylation can lead to disease.

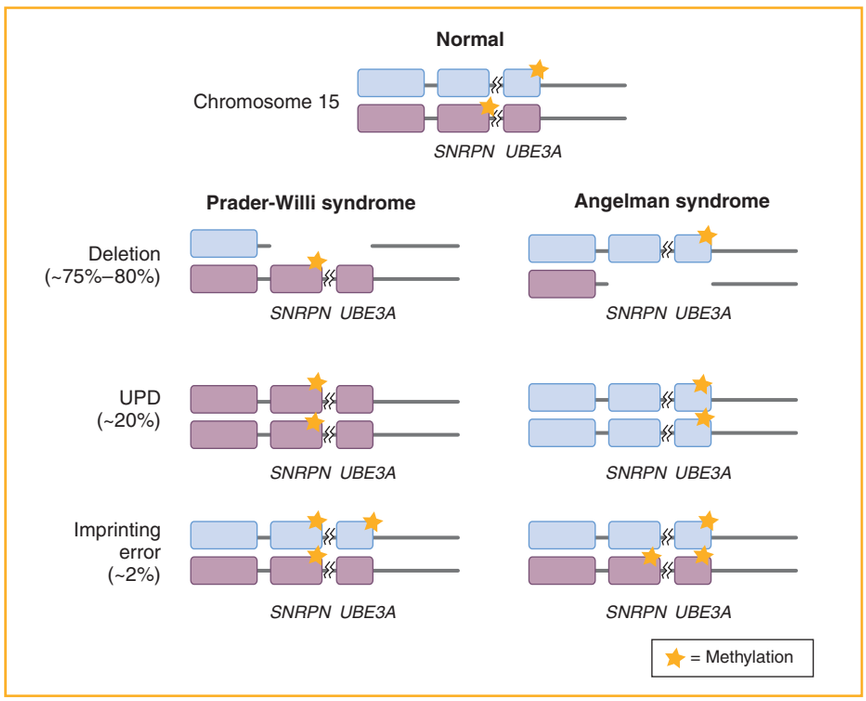

Figure 3-9. Differences between Prader-Willi and Angelman syndromes. The genes *SNRPN* and *UBE3A* are shown to demonstrate the effect of parent-specific methylation. Prader-Willi and Angelman syndromes may occur selectively from a microdeletion of chromosome 15q11.2-q13, uniparental disomy, or an imprinting error. Deletion areas contain several genes (e.g., contiguous gene sign/microdeletion). Not represented are individual gene mutations.

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Ubiquitin

Ubiquitin is a highly conserved, small protein of 76 amino acids involved in protein degradation and found in all cells. It attaches to proteins targeted for degradation by proteasomes or occasionally lysosomes.

- UBE1: ubiquitin-activating enzyme, which converts ubiquitin to a thiol ester
- UBE2: family of carrier proteins
- UBE3: protein ligase that binds ubiquitin to proteins

resulting in a nonfunctional protein. Molecular analysis with restriction enzymes can reveal changes in methylation sites. Not all chromosomes have imprinted genes. In fact, only nine chromosomes with imprinted alleles have been reported. Most of the genes that are imprinted occur in clusters and probably number only a few hundred.

Uniparental disomy (UPD) is responsible for approximately 20% of Prader-Willi and Angelman syndromes and occurs when two copies of one chromosome originated from one parent by nondisjunction. This differs from a complete hydatidiform mole, which receives an entire complement of chromosomes from one parent and is incompatible with life. When a homologous pair of chromosomes is inherited from a single parent, consequences may arise if some genes on the chromosome are imprinted and thus not expressed (see Fig. 3-9). As seen in Prader-Willi and Angelman syndromes, UPD is a factor in a significant number of cases.

Uniparental disomy occurs in Prader-Willi and Angelman syndromes when a gamete has two of the same chromosome from nondisjunction of chromosome 15. Upon fertilization, trisomy 15 occurs but fetal demise is avoided through "rescue" and loss of one of the three copies. Most of the time, normal disomy is restored. However, about a third of the time uniparental disomy occurs. Most nondisjunction occurs in maternal meiosis I. Therefore, the resulting UPD is a *heterodisomy*, or the presence of two different homologous chromosomes from a parent, rather than an *isodisomy*, or the presence of two chromosomes with identical alleles. If genomic imprinting exists on these chromosomes, genetic disease occurs. The fetus may have escaped the consequences of trisomy but not the necessity of fine regulation of gene expression.

Clinically, Prader-Willi and Angelman syndromes present quite differently. Angelman's syndrome is characterized by microcephaly, severe developmental delay and mental retardation, severe speech impairment with minimal or no use of words, ataxia, and flapping of the hands. Symptoms become apparent beginning around age 6 months and are fully evident by age 1. Because affected individuals often have a laughing, smiling facies, the term "happy puppet" was used in the past to describe them.

Prader-Willi syndrome may first be apparent in utero, where the fetus is hypotonic and displays reduced movements. This hypotonia is apparent at birth; feeding may be difficult owing to a poor sucking reflex, and nasogastric feeding may be required. Between the ages of 1 and 6 years, the child develops hyperphagia, leading to morbid obesity. Individuals have short stature. Children have cognitive learning disabilities but are generally only mildly mentally retarded. Their behaviors are distinctive and characterized by tantrums, stubbornness, manipulative behaviors, and obsessive compulsiveness, such as picking at sores. Both males and females demonstrate hypogonadism and incomplete pubertal development with a high incidence of infertility. Other features include small hands and feet, almond-shaped eyes, myopia, hypopigmentation, and a high threshold for pain. Obesity can be managed by diet and exercise to yield a more normal appearance.

Mosaicism

The presence of cells with different karyotypes in the same individual is mosaicism. It arises from a mutation occurring during early development that persists in all future daughter cells of the mutated cell. If the mutation occurs early in development, more cells as well as tissues will be affected; thus, clinical presentations are generally more pronounced the earlier a mutation occurs.

Mosaicism may either be *chromosomal mosaicism* or *germline mosaicism*. With chromosomal mosaicism, the presence of an additional chromosome or the absence of a chromosome from nondisjunction will create some trisomic or monosomic cells. Monosomic cells are likely to die, but trisomic cells may persist, yielding a clinical presentation less severe than complete trisomy in which all cells have an extra chromosome. This underscores an important concept about chromosomal mosaicism: the more cells with an extra chromosome, the more severe the clinical presentation. Mosaicism may also result from a less dramatic event than nondisjunction. A new mutation may occur on a particular chromosome in some cells that persists in some tissues but not necessarily all. If the expression of the mutated gene or region of chromosome adversely affects the cells or tissues in which it is located, a more discrete effect will occur. If germ cells are not affected by chromosomal mosaicism, gametes will be normal and offspring will be unaffected. A minority of Down syndrome cases as well as many types of cancers are examples of somatic mosaicism affecting chromosomes.

In germline mosaicism, the mutation is not in somatic cells and an individual is unaware of the mutation until an affected offspring is born. All cells of the affected offspring will carry the mutation. Parental testing will not reveal the mutation unless germ cells are tested. With one affected child, the occurrence of a de novo mutation in the child or gamete cannot be distinguished from a germline mosaicism. De novo mutations are also called *spontaneous mutations*. However, the occurrence of the same mutation or condition in more than one offspring is suggestive of a parental germline mutation (Fig. 3-10). Germline mosaicism is suspected in

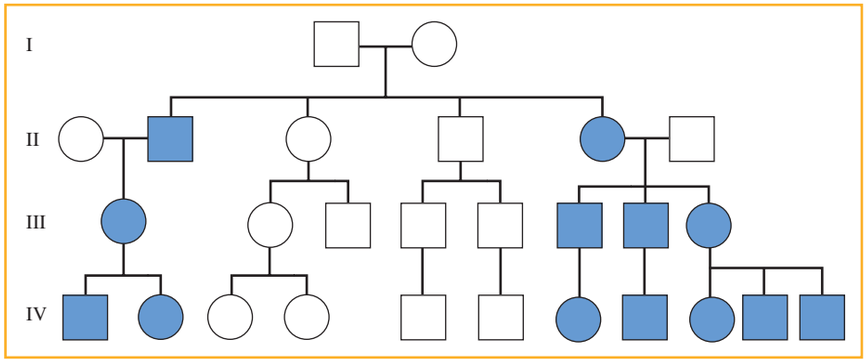

Figure 3-10. Pedigree suggesting a germline mutation in individual I-1 or I-2.

about one third of young males developing Duchenne type muscular dystrophy (see Chapter 7).

Mitochondrial Inheritance

All inheritance models, with the exception of mitochondrial inheritance, involve genes found on chromosomes in the nucleus. These genes are contributed to offspring through gametes from each parent. Mitochondria also contain DNA (mtDNA) that contributes genes to the process of cellular energy production. Mitochondria, however, are contributed to the zygote only from the maternal gamete and thus represent a maternal inheritance pattern. Females always pass mitochondrial mutations to both sons and daughters, but males never pass these mutations to their offspring (Fig. 3-11).

Human mtDNA is a circular molecule that encodes 37 gene products on 16.5 kb of DNA. There may be a few to thousands of mitochondria per cell. If all copies within a cell are the same, the cell is *homoplasmic*. In part owing to a very high sequence evolution rate, some mtDNAs may become mutated while others remain normal within the same cell. This situation in which normal and mutated mtDNAs exist in the same cell is termed *heteroplasmy*. Segregation of mtDNA during cell division is not as precise as chromosomal segregation, and daughter cells may accumulate different proportions of mutated and normal mtDNA. The random segregation of mtDNA during mitosis may yield some cells that are homoplasmic or cells with variable heteroplasmy. For this reason, many members of the same family may have different proportions of mutated mtDNAs. Unlike nuclear chromosomal allele mutations demonstrating autosomal dominant, autosomal recessive, or X-linked inheritance, *a threshold of mutated mtDNAs is generally required before a disease results*. Typically, clinical manifestations result when the proportion of mutant mtDNA within a tissue exceeds 80%. This threshold is tissue- and mutation-dependent. As a result, there is variability in symptoms, severity, and age of onset for most mitochondrial diseases. Stated another way, both penetrance and expressivity are dependent on the degree of heteroplasmy within an individual with a mitochondrial disease.

Mitochondria are extremely important in producing ATP through oxidative phosphorylation. It may then be intuitive that those tissues with the highest energy requirements might be the most highly affected by mtDNA mutations. This also suggests that those tissues with the greatest energy demands may also have a lower threshold for mtDNA mutations (i.e., a lower proportion of heteroplasmy will result in disease). Mitochondrial diseases often involve muscle, heart, and nervous tissues and present with CNS abnormalities with or without neuromuscular degeneration. Examples of mitochondrial disease are Leber's hereditary optic neuropathy

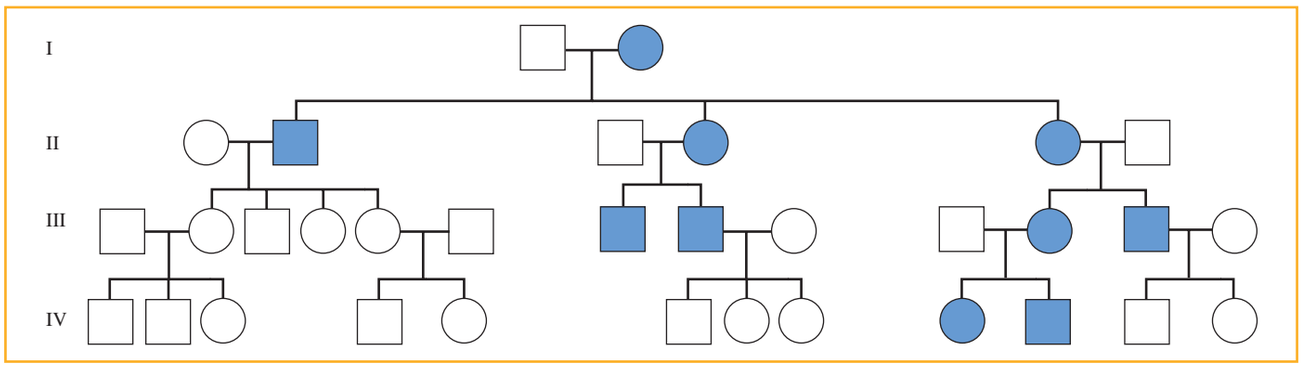

Figure 3-11. Mitochondrial inheritance. mtDNA is inherited from females only.

Box 3-2. EXAMPLES OF MULTIFACTORIAL INHERITANCE

Congenital Malformations	Adult-Onset Diseases
Cleft lip/palate	Diabetes mellitus
Congenital dislocation of the hip	Epilepsy
Congenital heart defects	Hypertension
Neural tube defects	Manic depression
Pyloric stenosis	Schizophrenia

(LHON), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), and myoclonic epilepsy and ragged red fibers (MERRF) (see Chapter 7).

It is important to point out that mitochondrial diseases have two different origins. Mutations within mtDNA lead to mitochondrial disease dependent on the degree of heteroplasmy in cells containing the mutation and exhibiting a maternal inheritance pattern. A second type of mitochondrial disease results from mutations in nuclear genes affecting the expression and function of proteins required in mitochondria. There are approximately 3000 of these proteins, and not all have been identified. The criterion for distinguishing between the two forms of mitochondrial disease is that one is maternally inherited and the other demonstrates mendelian patterns of inheritance, the latter reflecting nuclear chromosome expression. Risk to families with mitochondrial disease is different with the two modes of inheritance.

MULTIFACTORIAL INHERITANCE

Many conditions are represented by a complex interaction of several to many genes, and environmental factors may also influence their expression. Individual alleles in this complex interaction may individually demonstrate any of the mendelian or nonmendelian inheritance patterns previously discussed. However, the expression of these individual alleles is dependent on other alleles and factors. Therefore, the understanding of these types of interactions and the diseases demonstrating *multifactorial inheritance* is quite complex (Box 3-2). Several examples will be discussed briefly to demonstrate the principles of multifactorial inheritance. A more detailed discussion of diabetes will ensue to illustrate a disease with genetic and nongenetic influences that affects millions of individuals each year.

Phenotypic Distribution

Many genes influence phenotypes such as height and weight. As a result, the distribution of the many phenotypes demonstrated by multifactorial inheritance is expected to form a bell-shaped curve. For example, the normal curve of distribution of heights of fully grown males is shown in Figure 3-12. The average, or *mean*, is 68 inches, with a *standard deviation* of 2.6 inches. Standard deviation (SD) is a measure

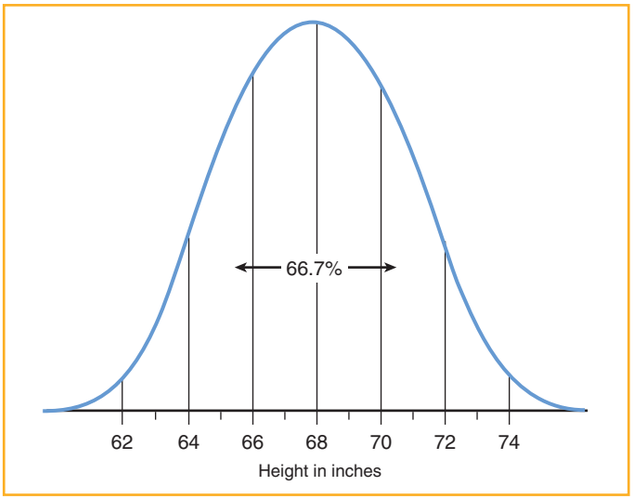

Figure 3-12. Height in adult males demonstrates a bell-shaped curve as expected for multifactorial, polygenic traits.

of the variability of a population. Briefly, if a given population is normally distributed, then approximately two thirds of the population lies within 1 SD on either side of the mean—in this case, 68 – 2.6 and 68 + 2.6, or between 65.4 and 70.6 inches. Ninety-five percent of the individuals, or 19 in 20, may be expected to fall within the limits set by 2 SD on either side of the mean. Exceptionally short people (<62.8 inches) and exceptionally tall people (>73.2 inches) occupy the extreme limits of the curve.

The bell-shaped distribution characterizes traits such as height and weight in which there is *continuous variation between one extreme and the other*. In regard to height, those at the extremes of the curve—the exceedingly short and the exceptionally tall—are not generally recognized as having a disorder. An exceptionally tall person is not judged as having a clinical condition! In certain other situations, however, those individuals at the tail of the distribution curve are potential candidates for a congenital disorder such as spina bifida. The point in the distribution curve beyond which there is a risk that a particular disorder will emerge is called the *threshold level* (Fig. 3-13). All individuals to the left of the threshold level are not likely to have the disorder and those to the right of the threshold value are predisposed to the disorder.

Liability and Risk

The term liability expresses an individual's genetic predisposition toward a disorder and also the environmental circumstances that may precipitate the disorder. As an analogy, in the case of an infectious disease, an individual's susceptibility to a virus or bacterium depends on inherent immunologic defenses, but the liability includes also the degree of exposure to the infective agent. In the absence of exposure to an infectious virus or bacterium, the genetically vulnerable person does not become ill. Likewise, in spina

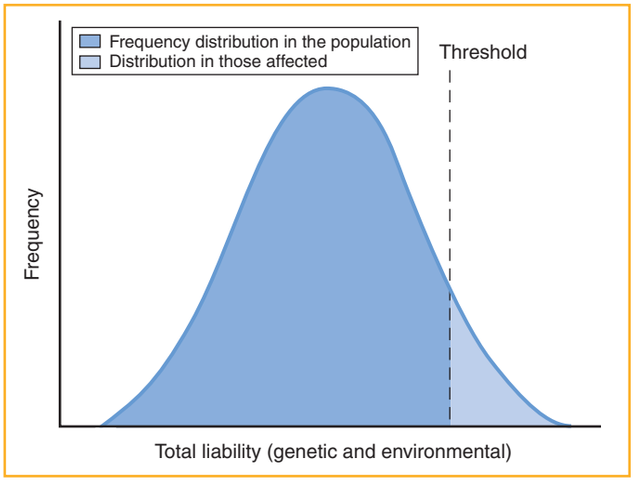

Figure 3-13. The threshold level is shown for the continuous variation of a multifactorial, polygenic trait.

bifida, a strong genetic predisposition renders the fetus susceptible or at a risk, but the intrauterine environment may turn the risk into the reality of the disorder. Environmental influences are thus superimposed on the polygenic determinants for high risk. A condition such as spina bifida or cleft palate is often referred to as a *multifactorial trait*, since it results from the interaction of both genetic factors involving multiple genes and environmental agents.

The greater the number of risk genes possessed by the parents, the greater the probability that they will have an affected child. It also follows that the larger the number of risk genes in an affected child, the higher the probability that a sib will be affected. As a general rule, the closer the relationship, the greater the number of genes that are shared. Table 3-4 shows the proportion of genes that relatives have in common. A parent and child share 50% of their genes, since the child receives half of his or her genes from a single parent.

TABLE 3-4. Family Relationships and Shared Genes

Relationship to a Given Subject	Proportion of Genes in Common (Coefficient of Relationship, *r*)
Identical twin	1
Fraternal twin	1/2
First-degree relatives	1/2
Parent-child	
Siblings	
Second-degree relatives	1/4
Grandparent-grandchild	
Uncle-nephew	
Aunt-niece	
Third-degree relatives	1/8
First cousins	

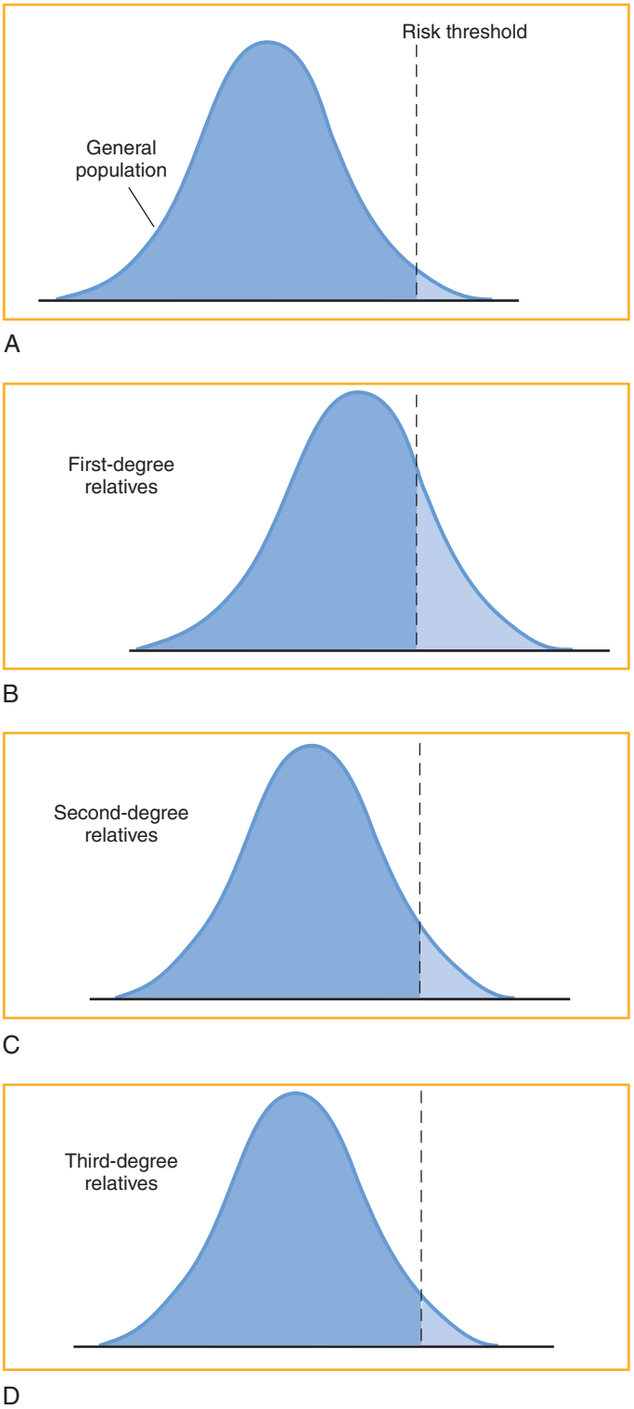

Figure 3-14. Risk factors and therefore the risk threshold for relatives increase with degree of relatedness.

Figure 3-14 illustrates the liabilities of a disorder determined by many genes, with a population incidence of 0.005, for relatives; the risk factors for relatives are respectively 1, 5, and 10 times the general incidence. On average, 50% of the genes of first-degree relatives (parents,

children, and siblings) are shared with the affected individuals. The mean of the distribution for first-degree relatives is shifted to the right. Thus, first-degree relatives have more risk genes than does the general population, and the incidence of the disorder among first-degree relatives can be expected to be higher than in the general population. The distribution of second-degree relatives is also shifted to the right, but in a direction less than that of first-degree relatives. Third-degree relatives exhibit a distribution curve that tends to approximate that of the general population. Although first cousins do not share as many genes as first-degree relatives, the risk of a polygenically determined disorder is higher when the parents are first cousins than when they are unrelated.

Risk and Severity

The risk to relatives varies directly with the severity of the condition in the proband. Individuals with the more severe cases possess a higher number of predisposing genes and accordingly tend to transmit greater numbers of risk genes. For example, for cleft lip, if the child has unilateral cleft, the risk to subsequent siblings is 2.5%. If the child has bilateral cleft lip and palate, the sibling risk rises to 6%. In the most severe cases, the individual is at the extreme tip of the tail of the curve, having inherited a vast number of predisposing genes.

Gender Differences

Both anencephaly and spina bifida occur more frequently in females than in males. Anencephaly has a male to female ratio of 1 to 2, while spina bifida approximates a male to female ratio of 3 to 4. This suggests that there are sex-specific thresholds.

Children of affected females with pyloric stenosis are more likely to be born with the pyloric stenosis than children of affected males. The threshold value for the *female who is affected* is shifted to the left, with the consequence that the affected female possesses a large quantity of predisposing genes required for the expression of the disorder. The affected female imposes a greater risk to relatives, particularly to the male child or sibling, because of the larger number of predisposing genes. The threshold level of the male is closer to the population mean than that of the female. Strange as it may seem, the less frequently affected sex, or the female, in the case of pyloric stenosis, transmits the condition more often to the more frequently affected sex, or the male in this example.

Environmental Factors

Neural tube defects are multifactorial traits, reflecting a genetic predisposition that is polygenic, with a threshold beyond which individuals are at risk of developing the malformation if environmental factors also predispose. We are largely ignorant of the predisposing environmental triggers. We do know that the dietary intake of folic acid by women tends to protect their fetuses against neural tube defects.

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Folic Acid

Folic acid is a vitamin, a water-soluble precursor to tetrahydrofolate. It plays a key role in one-carbon metabolism and the transfer of one-carbon groups. This makes it essential for purine and pyrimidine biosynthesis as well as for the metabolism of several amino acids. It is also important for the regeneration of S-adenosylmethionine, known as the "universal methyl donor."

Folate deficiency is also the most common vitamin deficiency in the United States. The classic deficiency syndrome is megaloblastic anemia. However, the group most likely to be deficient in folate is women of childbearing age, whose deficiency should be treated. Folic acid prevents neural tube defects and is recommended for all women prior to conception and throughout pregnancy in doses ranging from 0.4 to 4.0 mg per day.

Characteristics of Multifactorial Inheritance

The unique characteristics of multifactorial inheritance as they pertain to certain congenital conditions are as follows:

1. The greater the number of predisposing risk genes possessed by the parents, the greater the probability that they will have an affected child.
2. Risk to relatives declines with increasingly remote degrees of relationship.
3. Recurrence risk is higher when more than one family member is affected.
4. Risk increases with severity of the malformation.
5. Where a multifactorial condition exhibits a marked difference in incidence with sex, the less frequently affected sex has a higher risk threshold and transmits the condition more often to the more frequently affected sex.

Diabetes

Diabetes mellitus (DM) is an example of a complex disease that is not a single pathophysiologic entity but rather several distinct conditions with different genetic and environmental etiologies. Two major forms of DM have been distinguished: insulin-dependent diabetes mellitus (IDDM), or type 1, and non-insulin-dependent diabetes mellitus (NIDDM), or type 2. A difference between these types is whether endogenous insulin is available to reduce glucose and prevent ketoacidosis, as in NIDDM, or whether exogenous insulin is required, as in IDDM.

IDDM has been referred to by obsolete expressions such as "juvenile-onset diabetes," "ketosis-prone diabetes," and "brittle diabetes." NIDDM has been called "maturity-onset diabetes," "ketosis-resistant diabetes," and "stable diabetes." NIDDM is the more prevalent type, comprising 80% of the cases. IDDM is predominantly a disease of whites or populations with an appreciable white genetic admixture. In the United States, the prevalence of IDDM is about 1 in 400 by age 20. The mean age of onset is approximately 12 years.

BIOCHEMISTRY

Insulin

Insulin is produced by the β-cells of the pancreatic islets of Langerhans, which are found predominantly in the tail of the pancreas. Insulin is translated as preproinsulin and cleaved to proinsulin in the endoplasmic reticulum. During Golgi packaging, proteases cleave the proinsulin protein, yielding C peptide and two other peptides that become linked by disulfide bonds. This latter structure is mature insulin. C peptide has no function but is a useful marker for insulin secretion, since these should be present in a 1:1 ratio. Because the liver removes most insulin, measurements of C peptide reflect insulin measurements.

Insulin secretion is initiated when glucose binds to GLUT2 glucose transporter receptors on the surface of β-cells and the glucose is transported into the cell, thereby stimulating glycolysis. The increase in ATP or ATP/ADP inhibits the ATP-sensitive membrane K^+ channels, causing depolarization and leading to the activation of voltage-gated Ca^{++} membrane channels. Calcium influx leads to exocytosis and release of insulin from secretory granules into the blood.

In addition to this primary pathway, the phospholipase C and adenyl cyclase pathways can also modulate insulin secretion. For example, glucagon stimulates insulin via the adenylyl cyclase pathway by elevating cAMP levels and activating protein kinase A. Somatostatin, however, inhibits insulin release by inhibiting adenylyl cyclase.

PHARMACOLOGY

Insulin Therapy

First-line therapy for type 2 diabetes (NIDDM) are "insulin sensitizers" such as the thiazolidinediones and metformin. Insulin is used when this first approach fails to completely resolve the situation. Exogenous insulin, used for type 1 diabetes mellitus (IDDM) and NIDDM, can be administered intravenously or intramuscularly. For long-term treatment, subcutaneous injection is the predominant method of administration.

Several aspects of subcutaneous injection of insulin differ from its physiologic secretion. The kinetics of the injected form of insulin does not parallel the normal response to nutrients. Insulin from injection also diffuses into the peripheral circulation instead of being released into the portal circulation. Preparations are classified by duration of action: short, intermediate, or long-acting.

- Short: lasts 4 to 10 hours (insulin lispro/insulin aspart, regular)
- Intermediate: lasts 10 to 20 hours (insulin)
- Long-acting: lasts 20 to 24 hours (insulin glargine)

The two broad categories of DM are separable on the basis of several observations, such as mean age of onset, the association with certain genes within the major histocompatibility complex (MHC), the presence of circulating islet-cell antibodies, and the predisposition of β-cells to destruction by certain viruses and chemicals. Evidence supports the view that early-onset IDDM is a genetic autoimmune disease in which insulin-producing β-cells of the pancreas are ultimately and irreversibly self-destroyed by autoreactive T lymphocytes. NIDDM and IDDM are genetically distinct, inasmuch as NIDDM is not known to be associated with any particular HLA haplotype.

Family Studies

NIDDM tends to be familial—i.e., it "runs in families." Most studies show that at least one third the offspring of NIDDM parents will exhibit diabetes or abnormalities in glucose intolerance in late life. Specifically, the prevalence of NIDDM among children of NIDDM parents is 38%, compared with only 11% among normal controls. *In sharp contrast*, familial aggregation of IDDM is *uncommon*. The usual finding in family studies is that 2% to 3% of the parents and 7% of the siblings of a proband with IDDM have diabetes (Table 3-5). Stated another way, the likelihood that a parent with IDDM will have a child with IDDM is only 2% to 3%. If one child has IDDM, the average risk that a second child will have IDDM is only 7%.

Children of a diabetic father have a greater liability to IDDM than children of a diabetic mother. By the age of 20, 6.1% of the offspring of diabetic fathers had diabetes, whereas only 1.3% of the offspring of diabetic mothers had the disease. Hence, IDDM is transmitted less frequently to the

TABLE 3-5. Lifetime Risk of IDDM in First-degree Relatives*

Relative	Risk (%)
Parent	2.2 ± 0.6
Children	5.6 ± 2.8
Siblings	6.9 ± 1.3
HLA nonidentical sib	1.2
HLA haploidentical sib	4.9
HLA identical sib	15.9
Identical twin	30–40
General population	0.3

Data from Harrison LC. Risk assessment, prediction and prevention of type 1 diabetes. *Pediatr Diabetes.* 2001;2(2):71–82.
*When diagnosed in the proband before age 20 years.

ANATOMY

Pancreas

The pancreas is a retroperitoneal organ except for the tail, which projects into the splenorenal ligament. It is an exocrine gland and produces digestive enzymes. It is also an endocrine gland and produces insulin and glucagon. The main pancreatic duct joins the bile duct, which runs through the head of the pancreas, to form the hepatopancreatic ampulla that enters the duodenum.

offspring of diabetic mothers than to those of diabetic fathers. The mechanism responsible for the preferential transmission is not clear.

In essence, the low incidence of hereditary transmission of IDDM suggests the intervention of one or more critical environmental insults. One hypothesis suggests that IDDM requires two hits, analogous to the two hits required in the development of some cancers. The first hit is an infection, and the second hit is the selection of self-reactive T cells, which is influenced genetically through the MHC. The incisive questions are: What are the nongenetic (environmental) factors that trigger IDDM, and how do they interact with the genetic factors?

Monozygotic Twin Studies

To elucidate the role of genetic and environmental factors in the etiology of diabetes, pairs of identical (monozygotic) twins have been studied. Theoretically, if diabetes is influenced strongly by inherited factors and one identical twin manifests the disease, the other would be expected to display the disease. The extent of genetic involvement is estimated from the degree of *concordance* (both twins developing diabetes) as opposed to *discordance* (only one twin developing diabetes).

In a study of 100 pairs of identical twins for NIDDM, it was found that when one twin of a pair developed diabetes after age 50, the other twin developed the disease within several years in 90% of cases. Thus, older (i.e., > 50 years) identical twins are usually concordant for NIDDM. The very high concordance rate for late-onset NIDDM is impressive in that the diabetic condition arises at a time when twins usually live apart and ostensibly share fewer environmental factors than during early childhood. The twin studies support the hypothesis that NIDDM is determined primarily by genetic factors.

On the other hand, when one twin developed the disease before age 40, the other twin developed the disease in only half the cases. Accordingly, younger (i.e., <40 years) identical twins are 50% discordant for IDDM—i.e., if one has IDDM, the other does not and shows no signs of becoming so in half the cases. These findings demonstrate that genetic factors are predominant in NIDDM, and additional factors, presumably environmental, are required to trigger IDDM.

HLA Studies

Studies in several laboratories have revealed a strong association between IDDM and HLA antigens at the DR locus of the MHC. The major antigens conferring enhanced risk to IDDM are DR3 and DR4. Indeed, 95% of white patients with IDDM express either DR3 or DR4, or both. Individuals who express both DR3 and DR4 antigens are at the highest risk, whereas DR2 and DR5 expression is uncommon in IDDM. The DR3 and DR4 alleles are *not in themselves* diabetogenic but, rather, are *markers* for the true susceptibility allele in the HLA region.

The DQ locus consists of two tightly linked genes: DQA1 and DQB1. These encode α- and β-chains. Both loci are highly polymorphic. There are 8 and 15 major allelic variations in DQA1 and DQB1, respectively. Alleles at both loci demonstrate susceptibility to IDDM. Certain DQ alleles that are usually inherited in conjunction with DR3 and DR4 are recognized as prime susceptibility alleles. In white patients, DR3 and DR4 are almost universally associated with the DQB1*0302 and DQB1*0201 antigens.

It is clear that both HLA-DQA1 and HLA-DQB1 alleles are important in establishing a susceptibility to diabetes. DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 haplotypes, representing closely linked markers that are inherited together, confer the highest risk for IDDM. In combination, their effect is even stronger than that observed for individuals homozygous for DQA1*0501-DQB1*0201 or DQA1*0301-DQB1*0302, suggesting that heterodimers formed from gene products in *trans* conformation (i.e., DQA1*0501 and DQB1*0302) may be particularly diabetogenic. Other DQ haplotypes conferring a high risk for IDDM include DQA1*0301-DQB1*0201 among blacks, DQA1*0301-DQB1*0303 in the Japanese, and DQA1*0301-DQB1*0401 in the Chinese. The DQA1*0102-DQB1*0602

ANATOMY & EMBRYOLOGY

Twins and Fetal Membranes

Monozygotic (MZ) twins are identical twins that originate from one zygote, a process that usually begins during the blastomere stage. Dizygotic (DZ) twins are fraternal twins that originate from two zygotes.

The type of placenta depends on when twinning occurs. Most MZ twins have monochorionic-diamniotic placentas (65% to 70%). If twinning occurs later (9 to 12 days after fertilization), then monochorionic-monoamniotic placentation may occur, but this is rare (1%). In this latter case, twin-to-twin transfusion syndrome can occur. If twinning occurs after day 12, separation is incomplete and conjoined twins are the result.

DZ twins have dichorionic-diamniotic placentas, most of which are separate (60%). If implantation sites are close, placentas may fuse (40%). Since DZ twins occur more frequently than MZ twins, the most prevalent placentation is dichorionic-diamniotic.

IMMUNOLOGY

Human Leukocyte Antigens

Human leukocyte antigens (HLAs) are alloantigens important for maintaining tolerance, and they serve as antigen-presenting receptors for T lymphocytes. HLA genes are clustered on chromosome 6p. Class I proteins such as HLA-A, HLA-B, and HLA-C are each independent allele products. Class II proteins such as HLA-D (DP, DQ, DR) are formed from admixing maternal and paternal allele products. Each person has one haplotype from each parent.

haplotype is protective and is associated with a reduced risk for IDDM in most populations.

Autoimmunity

IDDM is an autoimmune disease. Sera from newly diagnosed IDDM patients contain an antibody that reacts with the β-cells in the islets of Langerhans taken from normal, nondiabetic individuals. IDDM represents the culmination of a slow process of immune destruction of insulin-producing β-cells (Fig. 3-15) and is also classified as an HLA-associated autoimmune disease.

What triggers the production of antibodies against the pancreatic β-cells? A promising hypothesis is that the antibody is the remnant of an immune response to components of the islet cells that were altered or damaged by viruses. An intriguing association suggests a viral triggering event from the observation that 20% of all children with congenital rubella—primarily those who are DR3-positive or DR4-positive—become diabetic later in life. This form of diabetes may be a consequence of the widespread effects of congenital rubella on the immune system.

Whatever triggering event may be operative, it is clear that destruction of insulin-producing cells is a slowly developing process, not an acute one. There is definitive evidence that T lymphocytes are the major determinants of this process. Essentially then, the current popular theory of the pathogenesis of IDDM encompasses β-cell damage by a foreign viral antigen, activation of the immune system, and the subsequent induction of autoimmunity directed against the β-cells.

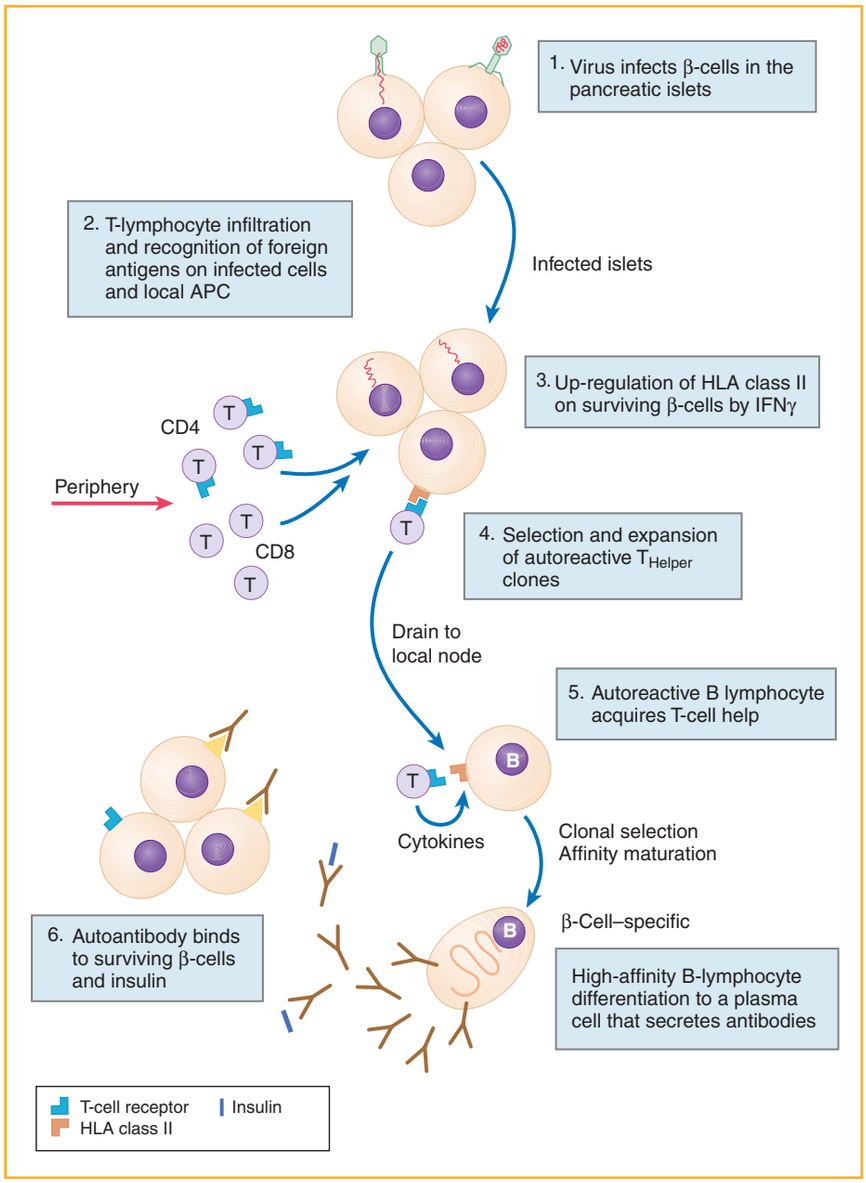

Figure 3-15. Process depicting destruction of insulin-producing β-cells in a hypothetical model of viral-induced islet cell autoimmunity. Infection of the pancreatic islet by a virus (e.g., coxsackie B4 or cytomegalovirus) may lead to a robust intra-islet T lymphocyte-mediated response. As a result of T lymphocyte infiltration, local inflammation, and/or IFN secretion, induction of HLA class II expression on the β cell is enhanced, leading to the selection of T lymphocyte clones. Through mimicry, reactivation of these T lymphocyte clones occurs when antigen-presenting, auto-reactive B lymphocytes capture and present specific β-cell antigens released from the damaged islet. The specific B/T lymphocyte interaction provides co-stimulation and avoids anergic deactivation of auto-reactive B cells. As these clones survive and expand, islet-specific auto-antibodies accumulate in the circulating immunoglobulin pool. This view is supported by studies of high-risk subjects showing that antibodies to candidate auto-antigens may exist long before disease develops. The presence of islet immunity, however, does not necessarily imply loss of β-cell function. (Courtesy of Dr. Ronald Garner, Mercer University School of Medicine.)

IMMUNOLOGY

Autoimmunity

Autoimmunity is loss of self-tolerance in humoral or cellular immune function. Helper T cells (T_H) are the key regulators of immune responses to proteins and are MHC restricted. Major factors contributing to autoimmunity are genetic susceptibility and environmental triggers. Autoimmune diseases may be systemic, as seen in systemic lupus erythematosus, or organ specific, as demonstrated by IDDM.

Several studies have identified susceptibility genes for diabetes. As noted, IDDM is associated with the HLA region of chromosome 6. For NIDDM, which is the most prevalent form of diabetes, several susceptibility genes have been identified in different groups including Mexican Americans, an isolated Swedish population living in Bosnia, Pima Indians in the southwest United States, and Utah families of European descent. Each of these studies identified different genes specific to that population. These data suggest that different combinations of susceptibility genes have different effects within populations and increase the incidence of disease within individuals and populations.

Molecular Mimicry

There is evidence that a defect in the expression of HLA-directed class II molecules may establish the conditions for autoimmune disease. Class II molecules, which enable T cells to perceive antigen, are normally expressed on antigen-presenting cells that interact with helper T cells—namely, dendritic cells, macrophages, and B cells. The usual inability of nonlymphoid cells, such as pancreatic cells, to express class II surface markers apparently serves as protection against autoimmunity, preventing nonlymphoid cells from presenting their own proteins as antigens. If pancreatic cells were to express class II molecules inadvertently, they could cause an autoimmune response via T cells.

What triggers the expression of class II antigens in the pancreatic cells? A promising hypothesis is that the production of class II molecules is the consequence of an immune response to pancreatic cells, specifically to islet β-cells, that have been altered or damaged by viruses. A viral infection insult activates, in some manner vaguely understood, the pancreatic cells to express class II molecules (see Fig. 3-15). A plausible scenario is that a viral protein shares appreciable amino acid sequences with a pancreatic islet protein—an instance of molecular mimicry.

When the pancreatic cells are abnormally triggered to express class II molecules, they can then present their antigens to helper T cells, just like macrophages. Stated another way, the pancreatic cell protein receptor alongside the class II molecule forms a functional unit capable of interacting with helper T cells. The outcome is a large-scale activation of T cells and a cascade of effects that include the production of circulating antibodies by plasma cells specifically directed against the surface receptors on the pancreatic B cells and other components.

Viruses may be only one of many triggering agents of IDDM. Other environmental insults such as drugs and toxic chemicals might similarly damage β-cells and give rise to diabetes. In experimental animals, drugs such as alloxan and streptozotocin can induce diabetes by destroying β-cells. In 1975, a rodent poison known as Vacor, which has a molecular structure resembling that of streptozotocin, was introduced in the United States. It was accidentally ingested by a number of people, several of whom developed acute diabetes with clear evidence of β-cell destruction. Not all of these people developed diabetes, indicating that the environmental insult interacts with a complex genetic background, which can be protective.

IMMUNOLOGY

Lymphocytes

Lymphocytes are responsible for antigen recognition. B lymphocytes—antibody-producing cells—make up 10% to 15% of circulating lymphocytes. Antigen recognition is accomplished by antibodies.

T lymphocytes recognize antigens on antigen-presenting cells and make up 70% to 80% of circulating lymphocytes. Most T cells are distinguished by the presence of CD4 or CD8 glycoproteins on their surface that determine function. CD8+ molecules, expressed on most cells, bind class I histocompatibility molecules. CD4+ molecules bind class II histocompatibility molecules and are present on antigen-presenting cells such as B cells, macrophages, and dendritic cells. CD8+ T lymphocytes are cytotoxic killer cells, while other lymphocytes produce interferons, tumor necrosis factor, and interleukins. CD4+ T lymphocytes, also known as T helper cells, produce cytokines and are important in cell-mediated and antigen-mediated immunity.

NIDDM

As stated earlier, NIDDM has a greater genetic component than does IDDM in that concordance for IDDM among monozygotic twins approaches 100%. Yet environmental factors also play a role; ironically, environmental factors are better known in IDDM than in NIDDM.

NIDDM most often occurs in individuals who are over age 40 and overweight. Obesity facilitates expression of the genetic predisposition to NIDDM. The changes in lifestyle that result in both obesity and NIDDM are vividly exemplified by the urbanization of the Pima Native Americans of Arizona. The exceptionally high prevalence of NIDDM among the Pima (affecting 50% of the adult population) reflects a modern change in dietary pattern from low caloric intake, in which both obesity and diabetes were rare, to caloric abundance, in which both clinical conditions are common.

The susceptibility gene among the Pima Indians is calpain-10, a protease that regulates the function of other proteins. It is composed of 15 exons and undergoes differential splicing

to form at least 8 different proteins expressed in a tissue-specific manner. Calpain-10 is found only in pancreatic islet cells. A specific A-to-G mutation in an intron 3, referred to as UCSNP-43 (for University of Chicago single nucleotide polymorphism 43), increases the risk for diabetes. Two other mutations, UCSNP-19 in intron 6 and UCSNP-63 in intron 13, also affect risk. Two mutated UCSNP-43 alleles and two different alleles at the other two sites are associated with the greatest risk for developing diabetes. The presence of two different DNA sequences at three sites in the same gene allows for eight different combinations of sequences. It is hypothesized that these alterations affect expression in different tissues: the UCSNP-43 alleles alter calpain-10 expression in the pancreas and the other alleles affect expression in muscle or fat cells.

Pima Indians with two UCSNP-43 mutations but without diabetes produced 53% less calpain-10 mRNA in muscle. These same individuals have a lower metabolism and increased insulin resistance suggestive of mild diabetes, characteristics that also increase obesity. Calpain-10 itself does not cause diabetes, but it does interact with other factors such as diet and exercise to cause diabetes. These mutations have also been found in other populations and when present increase the risk for diabetes.

Restriction endonuclease analyses of the insulin gene and an adjacent large, "hypervariable" region proximal (5′) to the gene itself have revealed an array of mutational events, but thus far it has been difficult to associate most known nucleotide changes with specific physiologic mechanisms. It can be asserted that the risk for transmission of NIDDM is greater than that for IDDM because of the need of an environmental stress or insult to the B cells. For first-degree relatives, the risk is 10% to 15%; the risk of impaired glucose tolerance, which is the usual precursor of NIDDM, is 20% to 30%. A good case can be made for periodic screening of first-degree relatives with oral glucose tolerance tests: those with impaired tolerance should be advised to maintain ideal body weight. In a minority, but significant percentage, of families, NIDDM occurs without the precondition of obesity. In those families, NIDDM is probably caused by a different mechanism.

Maturity-onset Diabetes of the Young

A small subset, representing about 2% to 5% of individuals with diabetes, have maturity-onset diabetes of the young (MODY). As the oxymoronic name suggests, this form of disease resembles "normal" NIDDM but can be present in young adulthood, usually occurring before age 25 as opposed to after age 40. MODY is transmitted as an autosomal dominant disease with high penetrance; 50% of the offspring of an affected parent exhibit at the least impaired glucose tolerance, which usually progresses to frank, but often mild, diabetes. The symptoms of MODY are quite variable, reflecting its genetic heterogeneity.

MODY, characterized by defects in pancreatic β-cell function, is caused by mutations in at least six genes representing six MODY types (Table 3-6). Five of these are transcription factors, and mutations in all six genes are loss-of-function mutations. Seventy-five percent of cases of MODY are caused by transcription factor mutations. The most common form, MODY3, representing 69% of cases, is caused by mutations in a transcription factor (*TCF*1) gene that regulates expression of several liver genes, including the hepatic nuclear factor–1α (HNF-1α) protein. The second most common presentation is MODY2, caused by mutations in the glucokinase (*GCK*) gene. For these individuals, glucose levels may be elevated to twice normal, whereas patients with mutations in HNF-1α may have glucose levels increased up to five times normal (Fig. 3-16).

Gestational Diabetes

Finally, diabetes may also develop during pregnancy from an unknown cause. Gestational diabetes occurs in approximately 4% of all pregnancies and usually resolves after pregnancy. Insulin resistance is thought to occur as a result of hormone levels during pregnancy. Symptoms generally occur in the second half of pregnancy and are characterized by fatigue resulting from a lack of glucose in tissues.

If untreated, maternal hyperglycemia is harmful to the developing fetus. Since insulin does not cross the placenta and glucose does, the fetal pancreas responds by increasing insulin secretion. Extra glucose is stored and is responsible for the large size of newborns, a condition known as *macrosomia*.

TABLE 3-6. Comparison of MODY Types

MODY Type	Gene	Protein	Protein Function*	Mutation Effect	Prevalence
1	*HNF4α*	Hepatocyte nuclear factor–4α	Transcription factor		
2	*GCK*	Glucokinase	Phosphorylates glucose		Common
3	*TCF1*	Hepatic nuclear factor–1α	Transcription factor		Most common
4	*IPF1*	Insulin promoter factor–1	Transcription factor	Loss of function	
5	*TCF2*	Hepatic nuclear factor–1β	Transcription factor		
6	*NEUROD1*	Neurogenic differentiation factor–1	Transcription factor		

*Each of these transcription factors is involved in the regulation of the insulin gene through a complex process affecting the gene directly or through regulation of each other. Thus, mutations decrease transcription, leading to increased blood glucose. Ultimately, complete β-cell failure occurs.

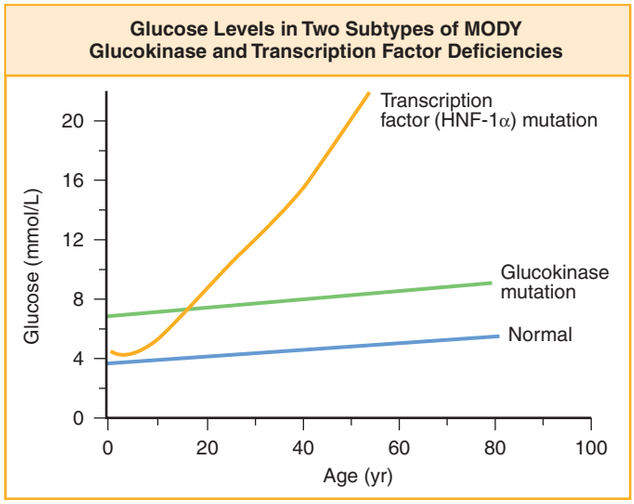

Figure 3-16. MODY3 (HNF-1α) and MODY2 (glucokinase) cause more than 80% of maturity-onset diabetes of the young. Glucose is elevated in both types but may be dramatically increased in MODY3. (Redrawn with permission of The American Diabetes Association from Pearson ER, Velho G, Clark P, et al. β-Cell genes and diabetes: quantitative and qualitative difference in the pathophysiology of hepatic nuclear factor-1α and glucokinase mutations. *Diabetes* 2001;50(1):S101–S107.)

Newborns subsequently suffer from hypoglycemia because of elevated insulin and have an increased risk of perinatal mortality and morbidity. This must be corrected to prevent mental retardation and other signs of failure to thrive. These infants are at an increased risk for breathing problems. They also have an increased risk of later developing obesity and NIDDM. Similarly, up to 50% of the mothers of these infants will develop NIDDM. In addition, the risk of a mother experiencing gestational diabetes in future pregnancies is 67%. Clearly, there are many aspects to diabetes that result from a complex interaction between genetic factors and nongenetic factors.