

Chapter 10 GENETIC DISEASE

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CHROMOSOME ABNORMALITIES

Genomic abnormalities that are large enough to be detected by the examination of chromosomes by microscopy are usually referred to as chromosome abnormalities. Numerical chromosome abnormalities include polyploidy and aneuploidy. Polyploidy means an abnormal multiple of the number of chromosomes; diploidy is normal, triploidy and tetraploidy are abnormal and, therefore, polyploid. Ployploidy is almost always lethal *in utero*, resulting in spontaneous abortion. Aneuploidy refers to the presence of an extra copy of a chromosome, trisomy, or the absence of a copy of a chromosome, monosomy. Aneuploidy frequently results in spontaneous abortion but live birth and even a near-normal life span are possible. The aneuploid conditions most frequently encountered clinically are listed in Table 10.1

The structural abnormalities involving one chromosome include deletions, inversions, duplications, ring chromosomes, and isochromosomes. A ring chromosome arises when the end of each arm of a chromosome has been deleted and the arms have joined at the sites of the deletions. Isochromosomes are the result of abnormal centromere division in which there is duplication of one arm of the chromosome and deletion of the other.

Insertions and translocations are structural abnormalities that involve two, and sometimes more, chromosomes. An insertion occurs when part of one chromosome is inserted into another, nonhomologous chromosome. A reciprocal translocation consists of the exchange of chromosomal material between two nonhomologous chromosomes so that

two translocation chromosomes are formed. A Robertsonian translocation consists of the fusion of two acrocentric chromosomes at the centromere with loss of the short arms of both so that a single translocation chromosome is formed. Acrocentric chromosomes have their centromeres located far from the center of the chromosome; the short arms are very short, consisting of stalks with satellites at the end. The acrocentric chromosomes are 13, 14, and 15 (group D) and 21 and 22 (group G).

Chromosome analysis

There are two methods currently in use for chromosome analysis, G-banding and fluorescence *in situ* hybridization. G-banding refers to Giemsa staining of chromosomes in their condensed state. The cells that are used for this analysis are peripheral blood lymphocytes, which form the overwhelming majority of the mitotically active cells in peripheral blood. An anticoagulated blood specimen is added to tissue culture medium and incubated in the presence of phytohemagglutinin. The phytohemagglutinin agglutinates the red cells from the specimen and stimulates the lymphocytes to divide. A microtubule inhibitor, such as colchicine, is also added to the medium to arrest the mitotic cells in metaphase. Alternatively, the mitotic cells can be arrested in prometaphase. The cells are harvested, lysed with hypotonic buffers, fixed, and mounted on cover slips. Following gentle trypsinization, the chromosomes are stained with Giemsa. The Giemsa staining results in a banding pattern that is characteristic for each chromosome (Figure 10.1). Metaphase chromosomes produce approximately 350 to 550 bands per haploid set, prometaphase chromosomes produce approximately 850 bands, and mechanically stretched metaphase chromosomes produce upwards of 1400 bands (Claussen *et al.* 1994). The bands are caused by alternating portions of the chromosome that are darkly stained by Giemsa (G-bands) and lightly stained by Giemsa (R-bands). There is believed to be an underlying structural explanation for the banding pattern. The usual explanation is that G-bands contain DNA that is rich in AT base pairs and R-bands contain DNA

Table 10.1
Most Frequent Abnormalities of Chromosome Number

Abnormality	Chromosome	Disorder
trisomy	21	Down syndrome
	X XXY	Klinefelter syndrome
	Y XYY	XYY syndrome
monosomy	X XO	Turner syndrome

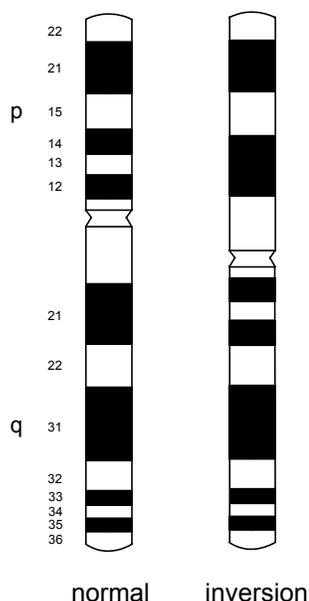


Figure 10.1 The G-banding pattern for chromosome 7 at a resolution of 450 bands per haploid set. A normal chromosome 7 is shown on the left. The band numbering schemes for the short (p) and long (q) arms are indicated. A chromosome 7 with a pericentric inversion is shown on the right.

that is rich in GC base pairs. More recently, it has been suggested that the R-bands are regions of the chromosome that are stretched during routine chromosome preparation and that G-bands are unstretched, and therefore DNA-rich, regions of the chromosome; the banding pattern would then arise from “a fixed hierarchy of the stretchability of chromosomes” (Hliscs *et al.* 1997).

The individual chromosomes are identified by size, position of the centromere, and the G-banding pattern. The chromosomes are then arranged into a karyotype wherein the chromosome pairs are set out in numerical sequence. The sex chromosomes may be paired separately or grouped with the chromosome pairs of similar size: the X chromosome with chromosomes 6 to 12 (group C) and the Y chromosome with chromosomes 21 and 22 (group G). Abnormalities of chromosome number are apparent by a simple count. If there is an extra chromosome, it is identified by the presence of a threesome rather than a pair of one of the chromosomes. The XO monosomy is identified by the presence of a single sex chromosome, an X chromosome. Structural chromosome abnormalities are detected as chromosomes which do not show any of the normal G-banding patterns for a chromosome of their size and centromere location. If the structural

abnormality involves one chromosome, the karyotype will show one abnormal chromosome and one unpaired chromosome. By comparing the G-banding pattern of the abnormal chromosome to the normal pattern of the involved chromosome, the nature of the abnormality can be characterized. For example, Figure 10.1 shows the G-banding pattern in the case of an inversion of chromosome 7. It is clear that the aberrant portion of the abnormal chromosome is between band 15 of the short arm and band 22 of the long arm. It is also clear that the abnormal portion has a G-banding pattern that corresponds to an inversion of the normal portion. If the structural abnormality involves two chromosomes, the karyotype will show two abnormal chromosomes and either two unpaired chromosomes or a missing chromosome pair. Here also the abnormality can be characterized by comparison with the normal G-banding pattern of the involved chromosomes.

In fluorescence *in situ* hybridization, chromosome identification is accomplished by using single-strand nucleotide probes that hybridize to chromosome-specific DNA sequences. The probes are labeled with a fluorochrome (a fluorescent chemical). Because the fluorescence signal does not require that the chromosomes be condensed in order to be seen, this method can be applied to interphase nuclei of nondividing cells. It can also be used as an alternative technique for the analysis of metaphase chromosome preparations.

The probes used for fluorescence *in situ* hybridization are of three types: chromosome-specific painting probes, centromeric repeat probes, and locus-specific probes. Chromosome-specific painting probes hybridize to many sites along the length of a single chromosome, thereby “painting” the chromosome. To paint each of the 22 autosomes and each of the sex chromosomes uniquely so that a complete karyotype can be analyzed, 24 unique colors are needed. This could be accomplished by using 24 different fluorochromes but that number exceeds the number of fluorochromes available. Instead, by labeling each probe with a more than one fluorochrome in a unique combination, 24 distinct signals can be generated with many fewer fluorochromes—only 5 are needed. Karyotype analysis using this approach is called multiplex fluorescence *in situ* hybridization or spectral karyotyping (SKY). For a number of full-color examples of the application of this approach, the reader is directed to the article by Speicher *et al.* (1996). The advantages of

Table 10.2
Microdeletion syndromes

Chromosome	Disorder
7	Williams syndrome
15,maternal	Angelman syndrome
15,paternal	Prader-Willi syndrome
22	CATCH-22 syndrome

multiplex fluorescence *in situ* hybridization over G-banding include improved accuracy in the detection and characterization of translocations and an appreciable shortening of the time required for performance of an chromosome analysis. Disadvantages of the method include an inability to detect paracentric inversions (those not involving the centromere) or insertions involving a single chromosome arm and decreased accuracy in the detection of small duplications and small deletions (La Beau 1996), including those giving rise to the microdeletion syndromes (Table 10.2).

Centromeric repeat probes hybridize to chromosome-specific, tandemly repeated DNA sequences present at the centromeres. Because the DNA sequences are repeated, a number of copies of the probe bind to the respective centromere, resulting in an intense fluorescence signal. For this reason, centromeric repeat probes are especially useful in the enumeration of specific chromosomes. For instance, trisomy 21 can be efficiently evaluated in cultured lymphocytes using a centromeric repeat probe for chromosome 21. If trisomy is present, three chromosomes will fluoresce in metaphase cells and three fluorescent domains will be present in interphase nuclei. A number of specific chromosomes can be enumerated if multiple probes, each labeled with a different fluorochrome, are used. This technique is called multicolor fluorescence *in situ* hybridization.

Another use for multicolor fluorescence *in situ* hybridization is in the evaluation of chromosome microdeletions. Microdeletions are not detected by metaphase G-banding and are only sometimes seen using high-resolution prometaphase G-banding. They are readily identified using multicolor fluorescence *in situ* hybridization. For such investigations two probes are used. One probe is either a centromeric repeat probe for the chromosome of interest or a locus-specific probe for an uninvolved site on the chromosome. The other probe is locus-specific for DNA at the site of the microdeletion. In a

metaphase chromosome preparation, the first probe will hybridize to both copies of the chromosome of interest. If the suspected microdeletion is present, the second probe will hybridize to only one of the two homologous chromosomes. Similarly, if interphase nuclei are studied, two fluorescent domains will mark the hybridization of the first probe but the second probe will show only a single fluorescent domain.

DNA MUTATION

As used in this chapter, DNA mutation refers to an alteration in DNA that is too small to be detected by microscopy. The enormous variation in DNA sequences found among humans (and other species) arises from the accumulation of mutations over time. Much of the variation is found only sporadically in the population. Sometimes, however, a particular gene variant may be present with a frequency that exceeds that attributable to *de novo* mutational production of the variant. Such gene variants are called alleles and the genetic locus is said to show polymorphism.

Mutations are caused by nucleotide substitution, deletion or insertion of one or a few nucleotides, deletion, fusion, duplication or insertion of large nucleotide sequences, and expansion of trinucleotide repeat sequences (Table 10.3; Reddy and Housman 1997). Single nucleotide substitutions are the most frequent cause of mutation. When they involve coding DNA, they are also further categorized according to the effect of the mutation on the transcription of the DNA. Silent mutations, which usually involve the third base in a codon, do not result in a change of the amino acid specified by the involved codon. Missense mutations do cause a change in the amino acid specification. Nonsense mutations convert amino acid-specifying codons into stop codons, resulting in truncation of the protein product. Stop codon mutations have the opposite effect, converting a stop codon into one specifying an amino acid, thereby resulting in an elongated protein product.

Mutations usually cause disease by reducing the synthesis of a normal gene product or by replacing the synthesis of the normal gene product with a modified product that is not fully functional (Ravine and Cooper 1997). Reduced synthesis of a normal product results from mutations that cause a defect in promoter function, a disruption of gene structure

Table 10.3
Trinucleotide repeat syndromes

Repeat	Location in gene	Gene product	Disorder
CAG	open reading frame	huntingtin ataxin-1,2,3,6 atrophin androgen receptor	Huntington's disease spinocerebellar ataxia type 1, 2, 3, 6 respectively dentatorubral-pallidoluysian atrophy spinobulbar muscular atrophy
CTG	3' flanking region	myotonin	myotonic dystrophy
GAA	intron	frataxin	Friedreich's ataxia
CGG	5' flanking region	FMR-1	fragile X syndrome

(such as mutations leading to frameshifts), or a decrease in RNA transcription. Mutations that give rise to RNA that is not processed or translated properly will also reduce the synthesis of normal product. The severity of the disease caused by a mutation varies depending upon whether the mutation results in a reduction in gene product synthesis or the production of a modified product, upon the degree of dysfunction or the modified product, and upon the gene dosage (Table 10.4). In addition, there is considerable between-family and between-individual variability in the severity of disease caused by the same mutation (Wolf 1997).

Molecular diagnostic studies

A mutation produces an abnormal segment of DNA. If transcription of the DNA is possible, the messenger RNA (mRNA) transcribed will be abnormal as will the protein that is translated. The laboratory diagnosis of a genetic disease caused by a mutation is accomplished by demonstration of the abnormal DNA, of the abnormal mRNA, or of the abnormal protein product. Subsequent sections of this chapter (Genetic disorders of metabolism, Genetic disorders of plasma proteins, and Genetic disorders of blood cells) describe ways in which some genetic diseases can be diagnosed by evaluation of protein products. The techniques used to evaluate mRNA and DNA for evidence of a mutation are referred to as molecular diagnostic studies.

The first step for many molecular diagnostic studies is amplification of the nucleic acid target sequences by use of the polymerase chain reaction (PCR). This step, which generates a large amount of target DNA, is included as a means of achieving or improving the detectability of the analytic signal

(Eisenstein 1990, Ferrari *et al.* 1996). PCR uses two oligonucleotide primers, one that binds at or near the 3' end of the target sequence on the sense strand of the DNA and one that binds at or near the 3' end of the target sequence on the antisense strand of the DNA. A reaction mixture containing the DNA and an excess of the primers is heated to a temperature at which the double-stranded DNA dissociates into single-stranded DNA. Upon cooling, the primers hybridize to their complementary sequences on the single DNA strands. Also present in the reaction mixture is a thermostable DNA polymerase (e.g., the *Taq* polymerase). Starting from the ends of the bound primers, the polymerase catalyzes the synthesis of new DNA strands using the original DNA strands as the templates. The mixture is then heated and cooled again followed by a second round of primer-directed DNA synthesis. This cycle is repeated numerous times with the amount of target DNA doubling with each cycle. After a few cycles, the major synthetic product is a segment of DNA that contains only the target sequence, that is, the DNA between and including the primer binding sites.

mRNA sequences can also be amplified using a variant of PCR called reverse transcriptase-PCR (RT-PCR). In this technique, DNA that is complementary to the mRNA in a sample is synthesized using a reverse transcriptase. The mRNA-DNA product is then used as the nucleic acid source for the first round of the PCR. The primer for the antisense target sequence hybridizes to the single-stranded DNA leading to the synthesis of double-stranded DNA. Subsequent cycles of the reaction amplify this DNA.

PCR-based diagnostic techniques. Besides amplifying DNA for subsequent methodologic steps,

Table 10.4
Severity of Genetic Disease

Gene Product	Genotype	
	heterozygous	homo/hemizygous
functional	normal	normal
hypofunctional	mild to moderate	mild to severe
nonfunctional	mild to moderate	severe
not produced	mild to moderate	severe

there are a number of ways in which the PCR can itself function as a diagnostic technique (Ferrari *et al.* 1996, Nollau *et al.* 1997). A multibase deletion, insertion, or expansion of trinucleotide (triplet) repeat sequences can be detected by electrophoretic analysis of the length of the DNA products. Using primers that bracket the target region of DNA, the presence of a deletion will result in the major product of the PCR having a reduced length compared to the major product of normal DNA. Similarly, the presence of an insertion will lead to the major product of the PCR being a DNA segment with a length greater than that of the major product of normal DNA. When performed using DNA from an individual homozygous for the mutation, the PCR will generate products of abnormal length. If an individual is normal, products of normal length will be generated and if an individual is heterozygous for the mutation, products of both normal and abnormal length will be generated.

Small deletions and nucleotide substitutions can be detected by using primers that bind at the site of the mutation. Under certain defined reaction conditions, primers will not hybridize with their DNA binding site if there is a base mismatch within the primer and they will not support DNA synthesis if there is a base mismatch at their 3' residue. If the primer is specific for the normal DNA sequence, the PCR will generate a product from normal DNA but not from DNA with a mutation in the primer binding site. If the primer is specific for the mutant sequence, normal DNA will not generate a product but the mutant DNA will. The analysis is conducted by performing PCR twice, once with each kind of primer. DNA from an individual who is normal generates products only with the primer for normal DNA, DNA from an individual who is homozygous for the mutation generates products only with the primer for mutant DNA, and DNA from an individual who is heterozygous for the mutation generates products with both primers.

Another way to detect a nucleotide substitution is to use a primer that introduces an allele-specific restriction enzyme cleavage site in the amplified DNA. The primer, which binds close to the site of the mutation, has a single base mismatch which, in combination with the normal base at the mutation site, produces a defined restriction enzyme cleavage site in the newly synthesized DNA. A cleavage site is not formed from DNA containing a mutation because the mutant base does not generate DNA with the proper sequence. The appropriate restriction enzyme is added to the reaction mixture following the first round of DNA amplification and normal DNA thereby eliminated. Then, subsequent PCR cycles will produce DNA products only if the individual is hetero- or homozygous for the mutation. Alternatively, the restriction enzyme digestion step can be deferred until the PCR is complete and the DNA products can be evaluated by Southern blot hybridization (as discussed below).

Allele-specific oligonucleotide hybridization. Nucleotide substitutions can be demonstrated directly in PCR-amplified DNA by utilizing labeled oligonucleotide probes that bind at the site of the mutation. By adjusting the hybridization conditions so that only perfectly matched duplexes are stable, signal generation occurs only if the allele match is exact. If the probe is complementary to the normal sequence, a signal will be generated by normal DNA. Signal generation will occur with mutant DNA if the probe is complementary to the mutant sequence. By testing DNA with both types of probe, the genotype of the individual can be ascertained. DNA from an individual who is normal will generate a signal with the probe for normal DNA but not with the probe for mutant DNA. DNA from an individual who is homozygous for a mutation will generate a signal with the probe for the mutant DNA but not with the probe for normal DNA. DNA from an individual who is heterozygous will give a signal with both probes.

Southern blot hybridization. Southern blot hybridization is based on the analysis of the lengths of DNA fragments derived by the digestion of whole DNA with a restriction endonuclease. The DNA fragments are separated according to length by electrophoresis in agarose gel, and transferred to a nitrocellulose filter by blotting. The location, and hence the size, of the DNA fragment of interest is indicated by hybridization of the fragment with a radiolabeled oligonucleotide probe complementary to

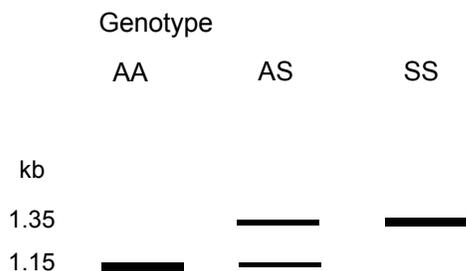


Figure 10.2 Schematic depiction of the molecular diagnosis of the sickle hemoglobin mutation using Southern blot analysis. The restriction endonuclease is *MstII* and the labeled oligonucleotide probe is complementary to a portion of the 5' flanking DNA of the β -globin gene.

some defined portion of the fragment. The length of a DNA fragment will be altered if a deletion, insertion, or expansion of trinucleotide (triplet) repeat sequences is present within the fragment. If the alteration in fragment length is larger than 50 to 100 base pairs, it can be detected as a change from normal in the electrophoretic location of the fragment.

A change in the length of a DNA fragment can also be caused by a nucleotide substitution that creates or destroys a cleavage site for a restriction endonuclease. For instance, the mutation that produces sickle hemoglobin (6 Glu \rightarrow Val) fortuitously alters the sequence at a site for the restriction enzyme *MstII*. Following incubation with *MstII* and using a probe to the 5' flanking DNA of the β -globin gene, normal DNA generates a 1.15-kb fragment due to cleavage at codon 6. In contrast, sickle DNA generates a 1.35-kb fragment as the next *MstII* restriction site is 200 base pairs downstream from codon 6 (Figure 10.2). Unfortunately, most nucleotide substitutions do not alter a restriction endonuclease cleavage site. Southern blot hybridization can still be used as a diagnostic approach for such mutations by performing the analysis on DNA that has been amplified by PCR using a primer that introduces an allele-specific restriction enzyme cleavage site (as discussed above).

Southern blot hybridization is also frequently used to diagnose genetic disease when the gene responsible for the disease has not been identified or when the site and character of the mutation causing the disease is unknown even though the gene involved has been identified. In these circumstances it is often possible to identify a polymorphic DNA sequence that, while not itself being the site of the

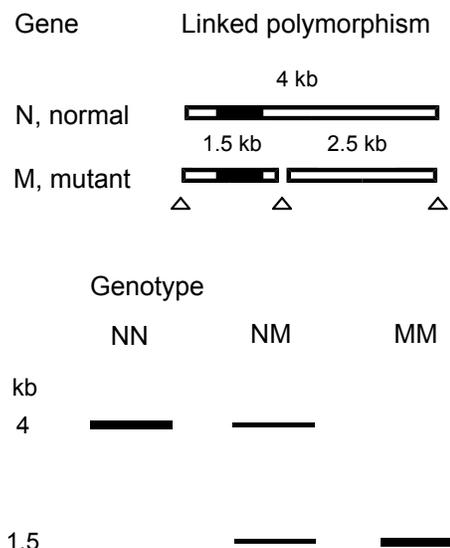


Figure 10.3 Schematic depiction of the molecular diagnosis of a genetic disease using an RFLP. The polymorphism linked to the mutant gene has a novel endonuclease restriction cleavage site. Southern blot hybridization shows a shorter than normal DNA fragment in individuals with the mutant gene. Triangles, restriction endonuclease cleavage sites; black box, probe site.

mutation, is in close linkage with the mutation. Hence the polymorphism and the mutation are, for the most part, co-inherited and the presence of the polymorphism serves as a marker of the mutation. Polymorphisms that arise from nucleotide variability at the site of a potential restriction endonuclease cleavage site, called restriction fragment length polymorphisms (RFLPs), are particularly useful. For example, a mutation may be linked to DNA that has a polymorphism characterized by the presence of a novel endonuclease restriction cleavage site (as in Figure 10.3). Following digestion with the appropriate endonuclease restriction and Southern blot hybridization with a probe to DNA near the novel cleavage site, the polymorphism will show a shorter than normal DNA fragment. Conversely, if the mutation is linked to a polymorphism that lacks a restriction endonuclease cleavage site that is usually present, Southern blot hybridization will reveal a DNA fragment that is longer than normal. In either case, the presence of the polymorphism as demonstrated by Southern blot hybridization can be used as a diagnostic marker for the linked mutation. However, because genetic linkage is never absolute, the diagnostic performance of polymorphisms is not as good as that of direct molecular studies of the mutation. Additionally, polymorphisms can only be

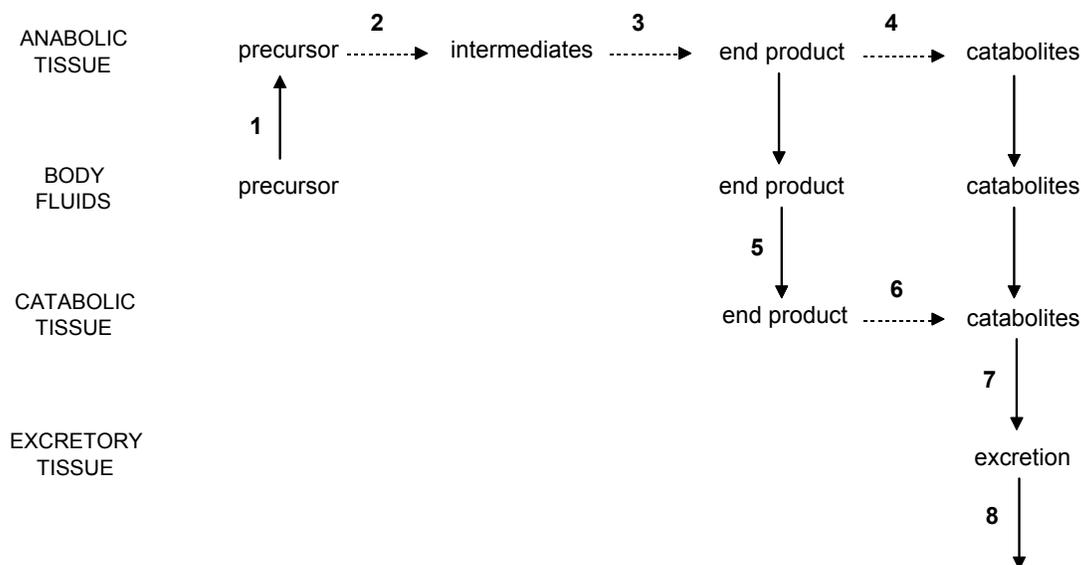


Figure 10.4 A model of metabolic fluxes. Numbers mark potential sites of genetic defects.

identified by studying a number of related individuals both with and without the disease. Conducting family studies is at best difficult and is sometimes impossible (Korf 1996).

Northern blot hybridization. Genetic abnormalities that result in abnormally sized mRNA can be demonstrated in a manner similar to that of the Southern blot technique with the exception that enzymatic digestion is not required. mRNA is extracted from a tissue in which the gene is expressed. It is then separated according to length by gel electrophoresis and transferred to a nitrocellulose filter by blotting. Labeled DNA probes hybridize to the sequence of interest marking the location of the mRNA.

Under carefully standardized and controlled conditions, the intensity of the hybridized label is proportional to the level of expression of the gene so this technique can be used to quantify transcriptional activity. This can be helpful in the evaluation of diseases in which the mutation resides in the regulatory DNA rather than the coding DNA.

Genetic disorders of metabolism

The genetic disorders of metabolism arise from abnormalities of membrane transport proteins and of the enzymes that catalyze the anabolic and catabolic reactions of intermediate metabolism. The general model of metabolic fluxes illustrated in Figure 10.4 reveals the numerous locations at which such a genetic defect can appear. There may be impaired transport of a precursor substance into the anabolic

tissue (site 1 in the Figure), of end product into its catabolic tissue (site 5), or of catabolites through the excretory pathway (sites 7 and 8). Disordered metabolic transformations can occur in the anabolic tissue (sites 2, 3, and 4) or in the catabolic tissue (site 6).

As a rule, the presence of a metabolic defect results in the decreased appearance of substance in the metabolic pathway distal to the defect and the accumulation of substances proximal to the defect, especially of the substance immediately proximal. Table 10.5 gives a scheme for substance accumulation based upon the site and mechanism of the metabolic defect. Note that when there is no reflux of accumulated substance out of the cell, accumulation is strictly intracellular and can only be evaluated by examination of involved tissues. When reflux does occur, substance accumulates in the body fluids as well as intracellularly. Substance accumulation can then be detected and quantified by the measurement of the substance in readily obtained and analyzed specimens such as plasma and urine.

When there is a defect in a biosynthetic pathway (Figure 10.4, sites 2 and 3), the synthesis of alternative end products may be increased due to shunting of accumulated precursor or intermediate substances into alternative metabolic pathways.

The pattern of end product deficiency, intermediate product accumulation, and alternative end product appearance is often highly diagnostic for the underlying defect. Congenital adrenal hyperplasia, serves as an interesting example (Miller 1997). The

Table 10.5
Substance Accumulation in Genetic Disorders of Metabolism

Defect	Substance	Accumulation		Example
		Intracellular	Systemic	
<i>Anabolic tissue</i>				
1. ↓ uptake	precursor		x	familial hypercholesterolemia
2. ↓ metabolism	precursor	x	x	galactosemia
3. ↓ metabolism	intermediates			
	with reflux	x	x	alkaptonuria
	without reflux	x		glycogen storage disease
4. ↓ metabolism	end products			
	with reflux	x	x	mucopolysaccharidosis
	without reflux	x		lysosomal storage disease
<i>Catabolic tissue</i>				
5. ↓ uptake	end products		x	dysbetalipoproteinemia
6. ↓ metabolism	end product	x	x	Gilbert's hereditary jaundice
7. ↓ input into	catabolites	x	x	Dubin-Johnson hereditary jaundice
	excretory path			
<i>Excretory tissue</i>				
8. ↓ excretion	catabolites	x	x	renal tubular acidosis

most common defect causing this disorder is a deficiency of 21-hydroxylase, an adrenal enzyme that is involved in the biosynthesis of cortisol and aldosterone. The production of cortisol would be impaired as a result of such a defect were it not for the fact that the plasma concentrations of cortisol are homeostatically regulated. By maintaining a high plasma concentration of corticotropin, and thereby producing a generalized increase in adrenal biosynthetic function, the body is able to overcome mild to moderate forms of 21-hydroxylase deficiency. Severe forms of 21-hydroxylase deficiency, on the other hand, cannot be overcome; they present in the newborn period with life-threatening salt wasting due to aldosterone deficiency.

The combination of 21-hydroxylase deficiency coupled with the corticotropin-driven hyperfunction of adrenal biosynthesis results in increased plasma concentrations of many intermediate substances in the steroid biosynthetic pathway. The most pronounced elevations are in 17-hydroxyprogesterone, the substrate for 21-hydroxylase in the pathway to cortisol. In moderate and severe forms of 21-hydroxylase deficiency, the plasma concentration of 17-hydroxyprogesterone is markedly increased in the basal state. In mild forms, the basal plasma concentrations of 17-hydroxyprogesterone are less distinctly

elevated and the diagnosis necessitates the use of a corticotropin stimulation test (Azziz *et al.* 1994).

There are also increased plasma concentrations of the alternative end products, androstenedione and testosterone, that arise from the peripheral biotransformation of 17-hydroxyprogesterone. It is the increased concentrations of these androgens that cause the clinical findings of virilization in newborn females with severe forms of the deficiency and oligomenorrhea and hirsutism in females with mild and moderate forms of the deficiency.

Genetic disorders of plasma proteins

The plasma proteins serve various specialized functions in the extracellular fluids. A genetic disorder involving one of these proteins usually presents as an impairment in the function served by the protein. The coagulation proteins provide excellent examples of the derangement of function that results from genetic disorders of a plasma protein. If there is a functional deficiency of a procoagulant protein, such as factor VIII, the patient will suffer from a bleeding diathesis. If the patient has a functional deficiency of an anticoagulant protein, such as antithrombin III, he or she will have a clotting tendency. The usual diagnostic approach to a suspected plasma protein deficiency consists of the

laboratory demonstration of the deficiency. If the deficiency is due to the production of a hypofunctional or nonfunctional protein, the mass concentration of the protein may be normal, so the deficiency must be demonstrated using a functional assay. If the deficiency results from decreased protein synthesis, decreased protein secretion, or an increased plasma clearance rate of the protein, both the functional activity of the protein and its mass concentration will be decreased. In these conditions, the deficiency can be demonstrated using either a functional assay or a mass assay. Functional protein assays measure the specialized function of the protein. Mass assays for the plasma proteins are for the most part based on immunologic methods. Additionally, semiquantitative measurement of the mass concentration of two plasma proteins, α_1 -antitrypsin and IgG, is possible using protein electrophoresis. The α_1 and γ fractions of plasma consist almost entirely of α_1 -antitrypsin and IgG, respectively, so the intensity of each band when visualized with a protein stain is proportional to the mass concentration of the respective protein.

The foregoing approach cannot be used in the evaluation of all plasma protein deficiencies, however. For instance, in the case of α_1 -antitrypsin deficiency, measurement of plasma α_1 -antitrypsin concentration, whether by functional or mass assay, can be diagnostically unreliable because α_1 -antitrypsin is an acute phase reactant. Its plasma concentration can increase several-fold over baseline values in response to infections and inflammatory conditions. Hence, even patients with severe α_1 -antitrypsin deficiency can have low normal protein concentrations during an acute phase reaction.

A more reliable approach for the diagnosis of α_1 -antitrypsin deficiency is to characterize the phenotype of the patient's α_1 -antitrypsin using a phenotypic marker that distinguishes the proteins produced by the major alleles for the protein. This approach is referred to as protein phenotyping. The marker employed in the protein phenotyping of α_1 -antitrypsin is the isoelectric point of the protein as revealed by isoelectric focusing on acrylamide gels (Jeppsson and Franzén 1982).

The most common alleles for α_1 -antitrypsin are the M alleles. The M allele products have normal elastase inhibitory function and are present in normal concentration in the plasma. The common allelic variants that cause α_1 -antitrypsin deficiency are the S

allele and the Z allele (Norman *et al.* 1997). The S allele product (264 Glu \rightarrow Val) is functional and is synthesized in normal quantities but undergoes accelerated intracellular degradation so that subnormal amounts are secreted. Homozygous individuals have plasma α_1 -antitrypsin concentrations that are about half normal. The Z allele product (342 Glu \rightarrow Lys) is somewhat hypofunctional. It is synthesized in normal quantities but tends to polymerize in the endoplasmic reticulum so that only small amounts are secreted. Individuals homozygous for the Z allele have plasma α_1 -antitrypsin concentrations that are 10 to 15 percent of normal.

The amino acid substitutions of the S and Z alleles result in α_1 -antitrypsins with isoelectric points that are different from the normal M allele product and from one another. Examination of the band pattern of a sample following isoelectric focusing reveals the α_1 -antitrypsin phenotypes present in the plasma and thereby allows the genotype of the patient to be inferred (Figure 10.5).

When there are numerous allelic variants for a protein, it is generally not possible to find a practical phenotypic marker that can distinguish all of the variant forms of the protein. It is usually considered adequate that the phenotype succeed in identifying the most common variants.

Genetic disorders of blood cells

The proteins involved in the genetic disorders of blood cells encompass enzymes of cellular metabolism, structural proteins of the cytoskeleton and the membrane, and specialized proteins of cell function. Disorders that serve as examples from the red cell lineage are, respectively, glucose-6-phosphate dehydrogenase (G6PD) deficiency, hereditary spherocytosis, and sickle cell anemia. Many of the genetic disorders of blood cells can be diagnosed with molecular diagnostic studies but, at the present, these studies are used mostly for prenatal diagnosis (discussed later in this chapter). Postnatal diagnosis, which is usually pursued in childhood, depends, for the most part, upon laboratory approaches appropriate to the involved protein. For instance, disorders of the enzymes of cellular metabolism are evaluated using *in vitro* assays of blood cell enzyme activity. Genetic disorders of structural proteins may be diagnosed by the characteristic morphologic abnormalities that result from the deficient or dysfunctional protein product. Alternatively, *in vitro* functional studies may reveal function deficiencies



Figure 10.5 Schematic depiction of the isoelectric focusing band patterns for the major variants of α_1 -antitrypsin. Each allelic product has two major and three minor forms that differ in their isoelectric points. The different forms arise from heterogeneity in the post-translational processing of the protein. Only the major form bands are shown.

that attend the disorder of a structural protein. The impairment in glycosylphosphatidylinositol (GPI) anchoring that occurs in paroxysmal nocturnal hemoglobinuria, for example, leads to a partial deficiency of the membrane-bound proteins that modulate membrane lysis by complement. Consequently, the red cells have a heightened sensitivity to complement that can be demonstrated very reliably by a laboratory study of complement-mediated hemolysis, the Ham test.

The specialized proteins of blood cell function include red cell hemoglobin, the membrane adhesion proteins and granule proteins of phagocytes and platelets, and the immunoglobulins of lymphocytes.

The genetic disorders of hemoglobin consist of the hemoglobinopathies and the thalassemias. The hemoglobinopathies are disorders with variant hemoglobin products. They are diagnosed by protein phenotyping using hemoglobin electrophoresis. The thalassemias are disorders with reduced synthesis of β hemoglobin chains (β -thalassemia) or α hemoglobin chains (α -thalassemias). Their diagnosis depends, in part, upon the demonstration of a reduced production of hemoglobin chain products. In β -thalassemia there is a decrease in the intracellular concentration of hemoglobin A ($\alpha_2\beta_2$) but a normal concentration of hemoglobin A₂ ($\alpha_2\delta_2$) and hemoglobin F ($\alpha_2\gamma_2$). This is shown by semiquantitative hemoglobin electrophoresis. In α -thalassemia the intracellular concentrations of hemoglobin A, F, and A₂ are all decreased. This alone is not diagnostic of α -thalassemia, however, as a number of acquired disorders, most notably iron deficiency, share this finding. The presence of hemoglobin H

(β_4), which forms in red cells deficient in α hemoglobin chains, is highly suggestive of α -thalassemia. It is demonstrated by supravital staining.

Deficiencies of blood cell membrane adhesion proteins are evaluated by *in vitro* adhesion studies. The assessment of granule protein deficiencies is guided by the function of the protein believed to be deficient. Disorders of granule proteins that mediate adhesion processes are evaluated using *in vitro* adhesion studies; disorders of granule enzymes are evaluated using *in vitro* assays of enzyme activity. Deficiencies of immunoglobulins are assessed semiquantitatively by protein electrophoresis and quantitatively by mass assay using immunologic methods.

SCREENING FOR GENETIC DISEASE

Screening studies are performed to detect serious, treatable disorders before they are clinically manifest. Early detection allows for the early institution of therapy, thereby minimizing any serious or irreversible consequences that may arise during the preclinical stage of the disorder.

Typically, screening studies for genetic disease are designed as dichotomous diagnostic tests. They have a critical value which separates screen-positive results from screen-negative results. Individuals who are found to be screen-positive undergo further diagnostic evaluation. To be useful for screening, a laboratory study must have a diagnostic performance that satisfies two criteria. First, the screen-positive study values must have likelihood ratios that exceed the threshold likelihood ratio for followup,

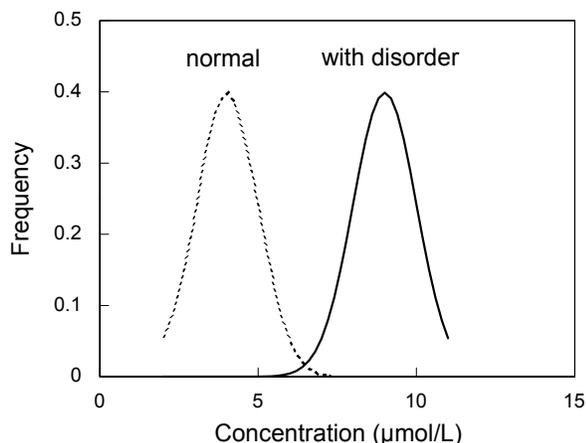


Figure 10.6 Reference frequency distributions for a hypothetical candidate screening study.

threshold likelihood ratio for followup =

$$\frac{(1 - \text{prevalence}) P[\text{rejection}]}{\text{prevalence} (1 - P[\text{rejection}])}$$

where *prevalence* is the frequency of the genetic disease in the clinical population and $P[\text{rejection}]$ is the level of diagnostic probability at which the disease is so unlikely that it can be excluded. The second criterion is that the sensitivity of the study at the stipulated critical value must be large enough to justify the expense and inconvenience of the screening program.

As an example, consider a candidate screening study with the diagnostic performance illustrated in Figure 10.6. If a sensitivity of 0.90 is considered necessary for the screening program, the maximum critical value that can be used is $3.72 \mu\text{mol/L}$ as this is the result value at which the sensitivity just equals 0.90. If the prevalence of the genetic disorder is 1 in 200,000 and if the threshold probability for rejection $P[\text{rejection}]$ is 0.02, the threshold likelihood ratio for followup is 4082. Likelihood ratios of this magnitude are only achieved at study concentrations greater than $4.16 \mu\text{mol/L}$. As this value is larger than the maximum critical value permitted by the program sensitivity criterion, the performance of the candidate study falls short of that needed. If, however, there is a subpopulation that is at risk for the genetic disorder, the increased prevalence in that group could still justify the limited use of the study. For a subpopulation in which the prevalence of the disorder is 1 in 10,000, for instance, the threshold likelihood ratio for followup is 204. If the diagnostic performance of the study is the same in the

subpopulation as in the general population, likelihood ratios as large as 204 are found at concentrations greater than $3.56 \mu\text{mol/L}$. Here the threshold likelihood criterion can be achieved while at the same time satisfying the program sensitivity criterion. Therefore, the candidate study could be used to screen for the genetic disorder among members of the subpopulation.

Use of the threshold likelihood for followup as a criterion of study performance has as an implicit assumption that facilities are available for the diagnostic followup of screen-positive newborns. If followup is difficult or unavailable, the diagnostic performance of the screening study has to be good enough that it can serve as a confirming study. In that case, screen-positive study results must have likelihood ratios that exceed the threshold likelihood ratio for acceptance,

threshold likelihood ratio for acceptance =

$$\frac{(1 - \text{prevalence}) P[\text{acceptance}]}{\text{prevalence} (1 - P[\text{acceptance}])}$$

where $P[\text{acceptance}]$ is the level of probability at which the disease is so likely that it is deemed confirmed.

Newborn screening

There are a number of serious genetic disorders of metabolism that can be significantly ameliorated if treatment is begun soon after birth (Treacy *et al.* 1995). All of these disorders are potential candidates for inclusion in a newborn screening program. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is one such disorder. The aldosterone deficiency seen in severe forms of the disease can be treated by replacement therapy with a mineralocorticoid and the cortisol deficiency can be rectified by glucocorticoid replacement therapy. Cortisol therapy also leads to normalization of the pituitary-adrenal homeostatic axis, thereby reducing the hypersecretion of androgens. Besides being treatable, severe congenital adrenal hyperplasia due to 21-hydroxylase deficiency occurs with a fairly high incidence—1 in 10 to 15,000 births—therefore making it an excellent disorder to consider screening for in the newborn period. As discussed earlier in this chapter, there is a pronounced elevation in the plasma concentration of 17-hydroxyprogesterone in the severe forms of 21-hydroxylase deficiency. The concentration is almost always more than 20 ng/ml while the concentration in healthy newborns is less

than 2 ng/ml if measured 24 hours or more after birth. The concentration of 17-hydroxyprogesterone is higher in the immediate newborn period (less than 24 hours), in premature infants, and in ill term infants, although, even in these newborns, the concentration is rarely as high as 20 ng/ml (Miller 1997). Using 20 ng/ml as the critical value yields very large likelihood ratios for screen-positive study results. The likelihood ratios exceed the threshold likelihood ratio for followup as calculated using the prevalence of severe 21-hydroxylase deficiency mentioned above and any reasonable value for P[rejection]. Additionally, a critical value of 20 ng/ml yields a high sensitivity for the detection of severe 21-hydroxylase deficiency. Thus, the use of 17-hydroxyprogesterone as a screening study for severe 21-hydroxylase deficiency is justified. The study is included in the set of newborn screening studies performed in a number of states in the United States.

Screening for adult-onset genetic disease

Diagnostic studies are available for a number of genetic disorders that present in adulthood (Ravine and Cooper 1997). Currently, none of the studies is used to screen the general population or even at-risk subpopulations; the studies are used exclusively in the diagnostic evaluation of individuals determined to be at risk on the basis of family history. There are two major reasons for this. The first reason is that, for some of the disorders, there are no effective measures for the prevention of the onset of clinical disease or effective therapy for the amelioration of the disease. The second reason is that most of the disorders show substantial inter-family variability in the phenotypic expression of the disorder even when the genotypic forms are identical. Consequently, it would not be possible to distinguish screen-positive individuals in whom the disorder would be serious from those in whom the clinical effects would be slight.

REPRODUCTION AND GENETIC DISEASE

There are two primary medical goals for human reproduction as far as genetic disease is concerned. First, couples contemplating having a child must be aware of the risks of producing a child with a genetic disorder. Second, affected offspring must be identified while *in utero* so that the parents can make a decision about bringing the pregnancy to term.

Table 10.6
Probability of parenting a child expressing a genetic disorder (-, normal gene; m, mutant gene)

Mutant Gene Expression	Genotype of Parents		Probability
Dominant	- -	- -	mutation rate
	- -	- m	0.5
	- m	- m	0.25 homozygous 0.5 heterozygous
Recessive	- -	- -	zero
	- -	- m	mutation rate
	- m	- m	0.25
	m m	- m	0.5
	m m	m m	1
X-linked	♂ -	♀ - -	♂ mutation rate ♀ zero
	♂ -	♀ - m	♂ 0.5 ♀ mutation rate
	♂ m	♀ - -	♂ mutation rate ♀ mutation rate
	♂ m	♀ - m	♂ 0.5 ♀ 0.5

Risk assessment and carrier detection

The risk of having a child with a particular genetic disorder depends upon the form of expression of the disease, i.e. whether it is dominant or recessive, upon the genotype of the parents, and upon the rate at which mutations causing the disease arise *de novo* (Table 10.6). The probabilities depend upon a knowledge of the genotypes of the parents. While the parental genotypes for a dominant disease are made manifest by the presence or absence of the disease, the genotypes for a recessive disease are not. A parent who does not have a recessive disorder may be homozygous for the normal gene or he or she may be heterozygous for the disease-producing gene. Individuals who are heterozygous for a recessive disorder are often referred to as carriers and the laboratory evaluation of the heterozygous genotype is called carrier detection. At present, there are no screening programs for carrier detection in the general population. Evaluation of carrier status is limited to individuals who have a family history for the disease or who are members of subpopulations that have a high carrier prevalence. One such at-risk subpopulation is the Ashkenazi Jews. Approximately 3 in every 100 members of this subpopulation are carriers for Tay-Sachs disease. Therefore, in the absence of a positive family history, the probability

that an Ashkenazi parent is a carrier is 0.03. The probability that both parents are carriers is 0.0009 and the probability of an affected child is 0.25 times 0.0009, which equals 0.000225.

If a parent has a positive family history, the probability of the individual being a carrier can be calculated according to Mendelian and Bayesian formulas. Say that a mother has a brother with hemophilia, an X-linked disorder. That means that her mother is an obligate carrier and that the Mendelian probability that she is a carrier is 0.5. An obligate carrier is an individual who can be deduced to be heterozygous from the family history. Another example of an obligate carrier is the daughter of a man with hemophilia. If the couple already has disease-free children, this additional information about the likelihood of carrier status can be incorporated into the calculation of the probability of being a carrier. If there are two disease-free boys, the probability is 0.2. Using Bayes' formula,

$$\text{probability of being a carrier} = \frac{0.5 \times 0.25}{0.5 \times 0.25 + (1 - 0.5) \times 1} = 0.2$$

where 0.5 is the prior probability of the mother being a carrier, 0.25 is the probability of having two disease-free sons if the mother is a carrier, and 1 is the probability of having two disease-free sons if the mother is homozygous for the normal gene. The probability estimate can be refined further by measuring the plasma concentration of factor VIII in the mother. Factor VIII is the deficient coagulation factor in hemophilia. Carriers of hemophilia secrete half as much factor VIII into the circulation so that their plasma factor VIII concentrations are, on average, only half normal. The frequency distributions for factor VIII derived from the data reported by Duncan *et al.* (1984) are shown in Figure 10.7. In this figure, the separation between the two diagnostic classes has been improved by correcting for the effect of von Willebrand factor concentration upon the plasma factor VIII concentration (Noe 1996). Likelihood ratios for carrier status can be calculated from the data depicted in the figure. For instance, at a corrected factor VIII concentration of 0.9 U/ml, the likelihood ratio for being a carrier is approximately 0.08. Using Bayes' formula,

$$\text{probability of being a carrier} = \frac{0.2 \times 0.08}{0.2 \times 0.08 + (1 - 0.2)} = 0.02$$

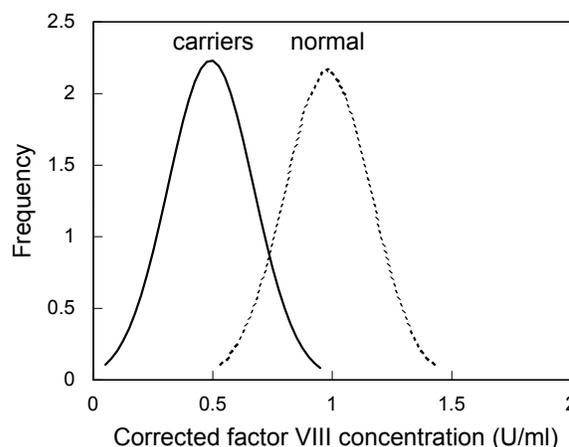


Figure 10.7 Reference frequency distributions for corrected plasma factor VIII concentration. The distributions are derived from normal distribution models of the data reported by Duncan *et al.* (1984).

This means that the probability that the mother will have an affected son is 0.01.

The utility of a phenotypic marker as a tool for carrier identification is assessed by determining how well it performs in classifying parents according to their correct phenotype. As discussed in Chapter 3, the index of classification accuracy that is most often used is the area under the ROC curve. The area under the ROC curve for corrected plasma factor VIII concentration as a marker of being a carrier of hemophilia is 0.97. This is quite high remembering that an ideal study has an area of 1. Still, it does not mean that the study can provide the desired degree of diagnostic certainty in any specific case. Consider, for example, a mother who wants to know that the probability of her son having hemophilia will be less than 0.001. If her prior probability of being a carrier is 0.5 (say her brother has hemophilia and she has no children yet), she can be assured of the stipulated low risk of bearing an affected son only if her corrected plasma factor VIII concentration is 1.15 U/ml or more. At lower factor VIII concentrations her probability of being a carrier is greater than 0.002 so the probability of an affected son is more than 0.001. If she has a normal genotype, the probability she will have a corrected factor VIII concentration greater than 1.15 U/ml is only 0.17. So the chances that the factor VIII measurement will provide her the confidence to bear a son is quite low.

Instead of inferring the genotype using a phenotypic marker, most of which are subject to diagnostic uncertainty, the genotype can be evaluated directly

by molecular diagnostic studies. When a disease is always caused by the same mutation, this is a very accurate way to ascertain carrier status. Sickle cell anemia is an example.

When more than one mutation can cause a genetic disease, carrier detection by molecular methods is usually somewhat less sensitive because it is usually only practical to detect the most prevalent mutations. In the case of Tay-Sachs disease, for instance, the three mutations that cause 98 percent of the disease among Ashkenazi Jews are detected in routine testing. That means that the sensitivity of the study is, at best, 0.98 in this subpopulation. The sensitivity is lower in the general population where there is a greater variety in the mutations that cause the disease.

There are a large number of mutations that cause hemophilia and no one mutation is responsible for more than a small fraction of the cases. As a consequence, molecular diagnostic evaluation of carrier status in hemophilia is very involved and is usually only performed at larger medical centers. If there are a number of family members with the disease, gene linkage studies can be conducted with the hope of identifying a DNA sequence polymorphism that is linked reliably to the disease-producing mutation. If family studies are unrewarding, or if there are not enough evaluable family members with the disease, the DNA of the suspected carrier can be screened for abnormalities. Deletions and nucleotide substitutions that disrupt the site of a restriction enzyme can be identified by Southern blot analysis (Peake 1995). Deletions are demonstrated by probing restriction enzyme-treated DNA with the complete factor VIII cDNA. Nucleotide substitutions are found by analysis of the fragment patterns of the exons, intron/exon borders, and 5' and 3' non-coding regions of the factor VIII gene following incubation with each of many different restriction enzymes. Mutations that do not disrupt restriction enzyme sites can be screened for using mismatch detection techniques (Ferrari *et al.* 1996, Nollau *et al.* 1997). Such studies do not reveal a defect in approximately half of the cases in which the mutation results in no gene product. Most of these cases are caused by an inversion of the distal portion of the X chromosome that leads to disruption of the factor VIII gene. The location of the inversion is always the same so it can be easily detected. Using a probe for the DNA locus responsible for the inversion, Southern blot analysis of the DNA fragments generated by the restriction

enzyme *BclI* reveals fragment polymorphisms that are characteristic for the inversion (Peake 1995).

Prenatal detection of genetic disease

Prenatal laboratory screening for genetic disease is limited to studies that can be carried out on specimens that can be collected with very low risk to the mother and fetus. By far the safest specimen to obtain is maternal blood.

In cases in which the fetus has an increased probability of having a genetic disease, the physician and parents may agree to pursue more definitive laboratory studies. As most genetic diseases can only be diagnosed with high reliability using laboratory studies performed on cells, this amounts to a decision to obtain and study fetal cells. This necessitates invasive specimen collection techniques that entail some risk to the mother or fetus.

Maternal blood. The placental trophoblasts synthesize various hormones and nonhormonal proteins. All of these substances are able to diffuse into the maternal circulation (Chard 1991). The concentrations of the placental products in the maternal plasma reflect the rate of synthesis of the respective products in the placenta. Thus, if a genetic disease alters the rate of placental synthesis of a product, the concentration of the product will be altered in the maternal plasma. In this way, placental products can be used as diagnostic markers for some genetic diseases. In trisomy 21, for example, the placental production of intact human chorionic gonadotrophin and its free beta subunit are increased (Newby *et al.* 1997) resulting in increased concentrations of these products in the maternal plasma.

The placenta also permits the ready exchange of lipophilic substances between the fetal circulation and the maternal circulation (Chard 1991). Thus, any lipophilic substance that accumulates in the fetal plasma as a result of a genetic disorder will also distribute into the maternal plasma and may reach concentrations that allow it to be used as a diagnostic marker. In addition, certain substances are transported from the fetal plasma into the maternal plasma by the placenta. A transported substance can serve as a diagnostic marker if the rate of transport of the substance is altered by a genetic disease. The placental transport of α -fetoprotein appears to be diminished in trisomy 21 (Newby *et al.* 1997) leading to decreased α -fetoprotein concentrations in the maternal plasma. There is substantial overlap of the marker reference frequency distributions in

normal pregnancies and in pregnancies in which the fetus has trisomy 21. Consequently, neither human chorionic gonadotrophin nor α -fetoprotein alone is highly discriminatory for trisomy 21. However, combination testing with the two markers is a fairly reliable way to detect pregnancies that are at high enough risk of trisomy 21 to justify the use of an invasive technique of prenatal testing.

Fetal cells. Amniotic fluid and placenta are sources of fetal cells that can be sampled with relative ease and safety. Amniotic cells are obtained by amniocentesis. Chromosome analysis, biochemical studies, and molecular diagnostic studies can be performed directly on the collected cells but, more commonly, the studies are carried out on cells obtained by tissue culture of the mitotically active cells in the specimen.

Placental cells are obtained by chorionic villus sampling. This technique involves suction biopsy of a small amount of chorion frondosum, the progenitor of the placenta. The yield of fetal DNA with this procedure is often large enough to permit molecular diagnostic studies to be performed directly on the fetal cells contained in the specimen. Chromosome analysis and biochemical studies are performed on cells cultured from the mesenchymal core of the villi. If necessary, molecular diagnostic studies can be conducted on cultured cells rather than on directly on the collected cells.

Chromosome analyses can also be conducted on fetal lymphocytes cultured from fetal blood obtained by percutaneous umbilical cord blood sampling. The risk of pregnancy loss with this procedure is greater than for amniocentesis or chorionic villus sampling.

An extremely low risk source of fetal cells is maternal blood. Starting late in the first trimester, trophoblasts and fetal blood cells can be found in the maternal circulation. The cells are present in very low concentration but they can be concentrated using various cell sorting techniques (Martin *et al.* 1998). Unfortunately, cell sorting does not produce a homogenous fetal cell sample; maternal cells always constitute a large fraction of the cells in the sorted sample. Consequently, the material cannot be used to diagnose recessive and X-linked genetic disorders because molecular diagnostic studies cannot distinguish the mutant DNA of the mother from that of the fetus. Dominant genetic disorders can be diagnosed if the father carries the gene, but not if the mother does. Post-sorting isolation of individual fetal cells

greatly extends the usefulness of this approach. It permits the performance of single cell interphase chromosome analysis by fluorescence *in situ* hybridization and single cell DNA analysis by high-sensitivity molecular diagnostic techniques.

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