LANGE

GEORGE H. SACK

USMLE ROAD MAP

GENETICS

- HIGH-YIELD FACTS
- ILLUSTRATIONS
- CLINICAL PROBLEMS
- CLINICAL CORRELATIONS

The Best Route to Step 1 Success

LANGE

USMLE ROAD MAP

GENETICS

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To the honor and memory of my parents, Sophia and George Sack

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PREFACE

The principles of genetics are relatively simple. However, the complexity of the 3 billion nucleotides in the human genome means that these simple principles must be applied to a remarkably variable information base. This book emphasizes the structure, organization, and physiologic consequences of genetic variations in humans. Sequencing the human genome has identified an unanticipated range of variations; newer techniques likely will find many more. Thus, how the basic principles will be translated from this variant base of DNA through cellular metabolism and physiology cannot currently be predicted. The high frequencies of single nucleotide polymorphisms, copy number variations, inversions, deletions, amplifications, and epigenetic changes already discovered have no precedents—-fully integrating their consequences likely will be complicated. All of this means that applying genetics to human and medical biology will remain a challenge.

In the past, medical genetics often has been viewed as an obscure collection of observations about rare anomalies. Now, the striking variations found in sequence data mean that any aspect of medicine will require awareness of fundamental biologic differences, eg, in disease pathogenesis, natural history, reactions to the environment and drugs, and neoplasia. Large amounts of sequence information will soon become available for individual patients; how we use this will be related to our understanding of basic mechanisms and their interactions. No longer will genetics be limited to quaint, arcane rarities; it will have become part of the medical mainstream. I invite readers to embark on a fascinating journey.

Consistent with the plan of the Road Map series, this book emphasizes fundamental principles. No attempt has been made to be encyclopedic. Instead, specific disorders are presented as examples of these principles. Some of these disorders (eg, Down syndrome, phenylketonuria, sickle cell disease, neurofibromatosis, G6PD deficiency) appear in multiple contexts, emphasizing their relative frequency. Although these are quite illustrative, many others could have been chosen and the basic notions can be applied broadly.

ACKNOWLEDGMENTS

Teachers have given me important personal examples of many of the principles presented here. They have included Margaret Abbott, Sam Asper, Mac Harvey, Debbie Meyers, Dan Nathans, Ham Smith and Phil Tumulty. I am particularly grateful to current faculty colleagues for their encouragement with this project: Charles Cummings, Jerry Hart, and Dan Lane have been particularly important in different ways. Support for the time devoted to this book has come from the generosity of Nell and George Berry, Nathan Cohen, F. Michael Day, Shirley and Bill Griffin, Ruth and George Harms, and Steve Lazinsky. I remain grateful for support of these and other friends including Anne and Michael Connelly, Marylynn and John Roberts, and, especially, Elizabeth.

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CHAPTER 1
PRINCIPLES

I. Proteins

A. Proteins are polymers of **amino acids** linked by **peptide bonds** (Figure 1–1).

B. Amino acid sequences reflect the sequence of **nucleotides** in the responsible gene.

C. The three-dimensional protein structure reflects complex interactions among amino acid **side chains** (Figure 1–2).

D. Proteins may function alone or in complexes with identical (**homopolymer**) or different (**heteropolymer**) partners (see Figure 1–2).

E. Changing a single amino acid can modify the structure, function or stability of a protein, depending on the location and the specific change; alternatively, it may have no effect.

SICKLE CELL DISEASE

CLINICAL CORRELATION

Changing a single amino acid from valine to glutamic acid at the sixth position in the β chain destabilizes the entire protein in low oxygen environments, distorting the shape of red cells (sickling) and leading to their destruction (see Figure 1–2; see Chapter 4 for further discussion).

F. Amino acids themselves can be modified by adding (or removing) phosphate, methyl (or other alkyl) groups, sugars, or lipids.

G. The function and structure of proteins is the basis for evolutionary selection.

II. Nucleic Acids

A. DNA

1. DNA is a very long helical polymer composed of two strands of nucleotides individually linked by **phosphodiester bonds** and cross-linked by **hydrogen bonds** (Figure 1–3).

2. The nucleotides**,** known by their initials (A for adenine, C for cytosine, G for guanine, T for thymine) are paired across the helix (**A with T; G with C**). This strict **base pair (bp) complementarity** means that the nucleotide sequence on one strand determines the complementary sequence of the other (pairing) strand (Figure 1–4).

3. Any given DNA (or RNA) strand has **polarity** from the 5′ end to the 3′ end of the sugar; the two strands in a double helix have *opposite* polarity.

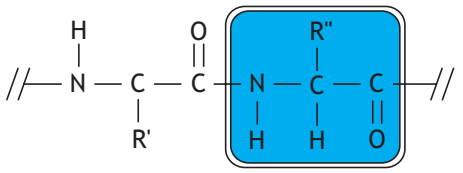


Figure 1–1. Two amino acids linked by a peptide bond. This is the basic unit of all proteins. The substituents (R) can vary from a proton in glycine, to imidazole (tryptophan), to a carboxylic acid (eg, glutamic acid).

4. The DNA in the nucleus of a single human cell contains ~3×10^9 bp whose prototypic sequence is known. (A kilobase [kb] = 1000 [10^3] bp; a megabase [mb] = 10^6 bp.)
5. Each **chromosome** contains one continuous DNA molecule.
6. During cell division (mitosis, discussed further in Chapter 2) each DNA strand serves as a template for the enzymatic synthesis of a complementary strand yielding two full-length double-stranded polymers (**replication**) (Figure 1–5).

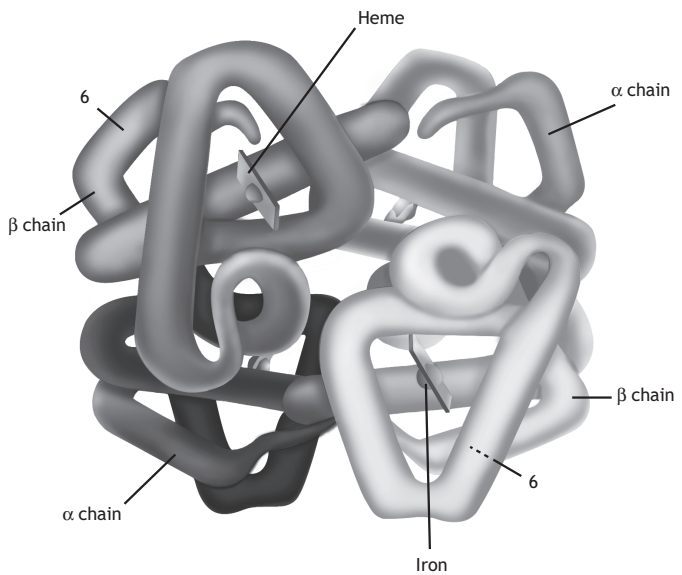


Figure 1–2. Model of the three-dimensional structure of globin. Note that it is a heteropolymeric tetramer with two α chains and two β chains, each of which contains a heme group and iron atom. The sickle cell mutation occurs in the β chains, as indicated.

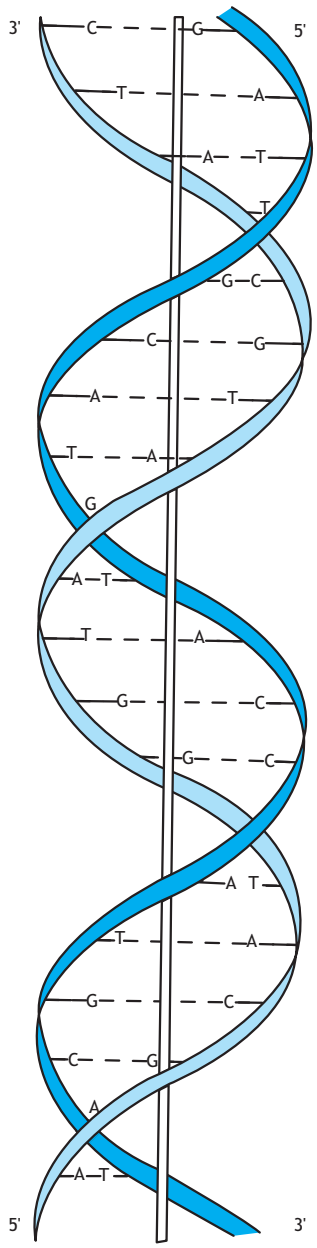


Figure 1–3. Model of DNA showing stacks of base pairs joined by phosphodiester bonds. Note that the strands have opposite polarity (5′→3′).

7. Changing any nucleotide in the template strand causes a corresponding, complementary, change of the pairing nucleotide on the newly synthesized strand, thus propagating the change.
8. The sequence of nucleotides in DNA determines
   a. The amino acid sequences of individual proteins

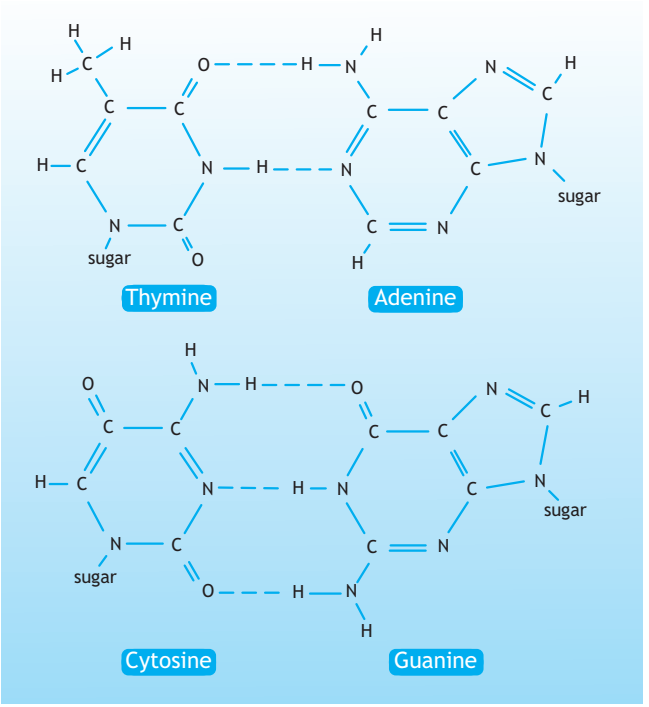


Figure 1–4. Pairing of bases in DNA. The hydrogen bonds hold the complementary strands together.

- **b.** The physical limits of a gene
- **c.** Signals controlling gene expression
- **d.** Signals controlling replication
- **e.** Regions to assist DNA packing in the nucleus and the organization of chromosomes (see Chapter 2)

9. Successive groups of 3 nucleotides within a gene (read from 5′ to 3′ on a given strand) direct incorporation of specific amino acids into a protein (the **triplet code;** see Table 1–1).

- **a.** Most proteins contain combinations of all 20 amino acids.
- **b.** Some amino acids have more than one triplet code (or **codon**); this is called **degeneracy.**
- **c.** Some triplets indicate the end of a protein (**termination;** see Table 1–1).

10. Within the DNA of a human cell, only ~5% of the sequence is evolutionarily conserved and only ~1.5% **represents codons.** The functions of the remainder are not known but a large fraction is represented as RNA that likely helps mediate control of gene expression in development and differentiation.

11. A **gene** contains all information needed to synthesize a protein, including signals showing where the gene begins and ends and how it is controlled (Figure 1–6A).

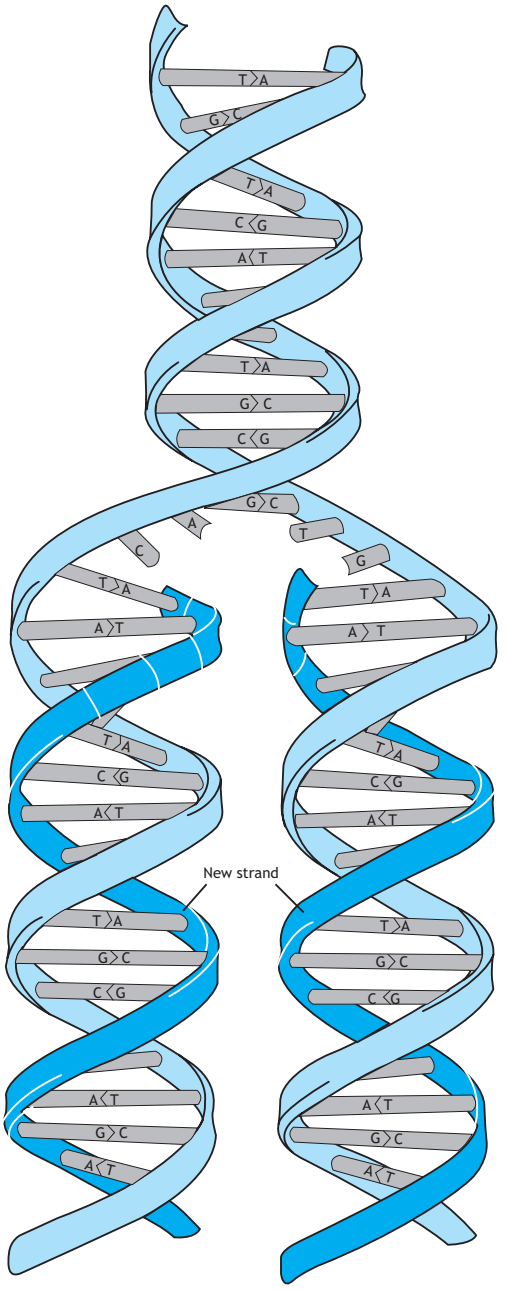


Figure 1–5. DNA replication leads to formation of two strands using complementary nucleotides. (Reproduced with permission from Gelehrter TD, Collins FS. *Principles of Medical Genetics,* LWW, 1990.)

Table 1–1. Three-letter (triplet) codons.

First Position (5′ end)	Second Position U	C	A	G	Third Position (3′ end)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term	Term	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	GluN	Arg	A
	Leu	Pro	GluN	Arg	G
A	Ileu	Thr	AspN	Ser	U
	Ileu	Thr	AspN	Ser	C
	Ileu	Thr	Lys	Arg	A
	Meth	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

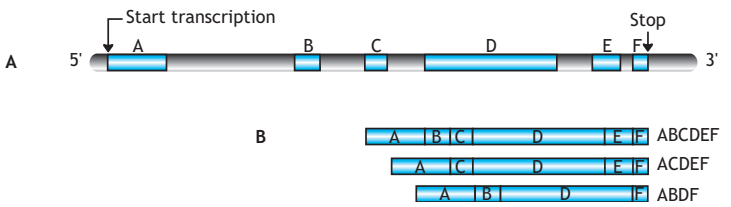


Figure 1–6. ***A.*** Gene model showing exons, introns, and essential signals. ***B.*** Splicing of the primary transcript joins exons to form mature mRNA. Differential splicing patterns can lead to mRNA molecules sharing only part of their sequence (and, thus, encoding very different proteins), as shown.

12. Within a gene, contiguous groups of nucleotides called **exons**, containing the codons and control information, are separated by regions called **introns**.
13. Enzymes in the nucleus use the sequence of one strand of the gene's DNA as a template to make a complementary single strand of RNA (**transcription**).

B. RNA and Messenger RNA

1. In RNA, **U pairs with A** (in place of T, as in DNA).
2. The primary transcript is modified by removing introns and joining exons by **splicing**.
   - **a.** Splicing leads to an uninterrupted codon sequence.
   - **b.** Different exons of a single gene may be spliced together. Known as **differential splicing,** this process yields multiple coding sequences sharing some common regions (Figure 1–6B).
3. After splicing and additional modifications, the mature messenger RNA (**mRNA**) enters the cytoplasm.
4. DNA complementary to mRNA (**cDNA**) can be synthesized in vitro for diagnostic and basic studies.
5. mRNA is translated into protein on the ribosome by linking amino acids corresponding to codons.
6. The growing polymer folds into a mature protein (which may then be modified by adding sugars, lipids, etc).
7. Newly synthesized proteins are transported either to specific sites within the cell or out of the cell for use elsewhere.

C. Other RNA Molecules

1. Some RNA molecules do not encode proteins.
2. **Short (micro) RNA** molecules (~22 nucleotides) have multiple roles.
   - **a.** By **binding** (**hybridizing**) to mRNA, a micro RNA molecule can cause degradation of the message; this is called **RNA inhibition** (**RNAi**).
   - **b.** Short RNA molecules are synthesized by the cell, but RNAi also can work with RNA molecules introduced *into* a cell. Hence, RNAi can mediate *both* endogenous and exogenous control of gene expression (see also Chapter 12).
   - **c.** Micro RNA molecules appear to be essential for control of cell differentiation and growth.
3. RNA encoded by *Xist* gene helps mediate **X-chromosome inactivation** (see Chapter 2).
4. RNA molecules are central to ribosome structure and function.
5. RNA and protein complexes are important in splicing and in maintaining telomeres (ends) of chromosomes (see Chapter 2).

III. Tools of Molecular Genetics

A. Constituents of gene expression and control—DNA, RNA, proteins, enzymes, and others—can be isolated or synthesized de novo.

B. **Sequencing** of DNA, RNA, and proteins can be automated.

C. The DNA sequence can identify genes and suggest their function(s).

D. The length of most DNA molecules complicates their study, but **restriction enzymes** can cut DNA at specific nucleotide sequences wherever they appear in the DNA to produce smaller fragments (Figure 1–7).

E. **Electrophoresis** separates DNA fragments according to length and permits their transfer to a support called a Southern blot (Figure 1–8).

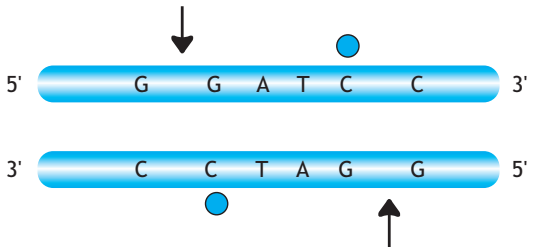


Figure 1–7. The restriction enzyme *BamH1* cuts double-stranded DNA at a specific nucleotide sequence producing discrete fragments of the long polymer. The dots show positions where methylation (such as might occur in imprinting; see text) can block recognition of this sequence by the enzyme and thus prevent cleavage.

F. **Hybridization** (also called **annealing**) is the formation of double-stranded DNA (or RNA, or DNA and RNA) by matching complementary sequences.
1. **Hybridization accuracy** is related to the **media** (temperature, ionic strength, etc) and the **sequence length.**
2. A stretch of 20 or more nucleotides usually identifies a unique complementary sequence in the genome.

TECHNICAL ILLUSTRATION

Because any nucleotide has a 1 in 4 chance of having a complement, the likelihood that a stretch of 20 consecutive nucleotides will have a precise complement is, on average, $1/4 \times 1/4 \times 1/4... = (1/4)^{20} \cong 1/1.1 \times 10^{12}$. This usually assures a single match.

G. An **oligonucleotide** is a short length of DNA or RNA.

H. Oligonucleotides (often also called **probes**) are useful for hybridization.
1. If the probe is labeled with ^{32}P, the site(s) of its hybridization on a Southern blot can be revealed by exposing the blot to film (**autoradiography**) (see Figure 1–8).
2. Alternatively, the probe can be labeled with a **fluorescent tag.**
3. cDNAs also are useful as probes.

I. **Multiple probes** can be arranged on a solid matrix (**microarray**) so that expression or variation of thousands of genes can be determined in a **single hybridization** (Figure 1–9).

J. A **DNA fragment** can be inserted into a self-replicating bacterial **plasmid** to become a **recombinant DNA** molecule and propagated in bacteria as a **molecular clone** (Figure 1–10).

K. The **polymerase chain reaction** (**PCR**) exploits hybridization, complementarity, and DNA enzymes (Figure 1–11).

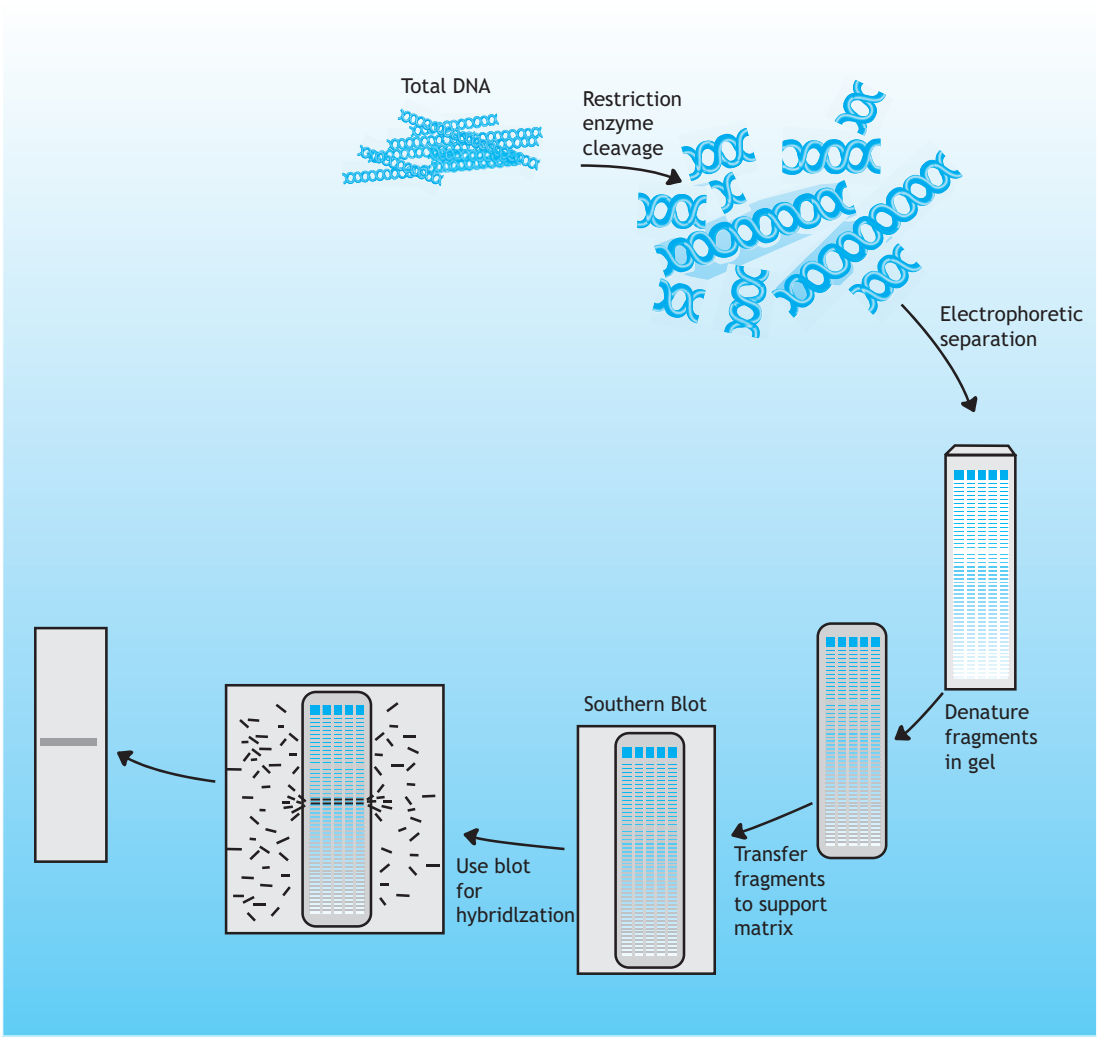


Figure 1–8. DNA fragments (usually produced by restriction enzyme cleavage) are separated by electrophoresis and then transferred to a solid support (Southern blot). A labeled probe can be hybridized to the DNA on the blot and then be detected by exposure to x-ray film (autoradiography).

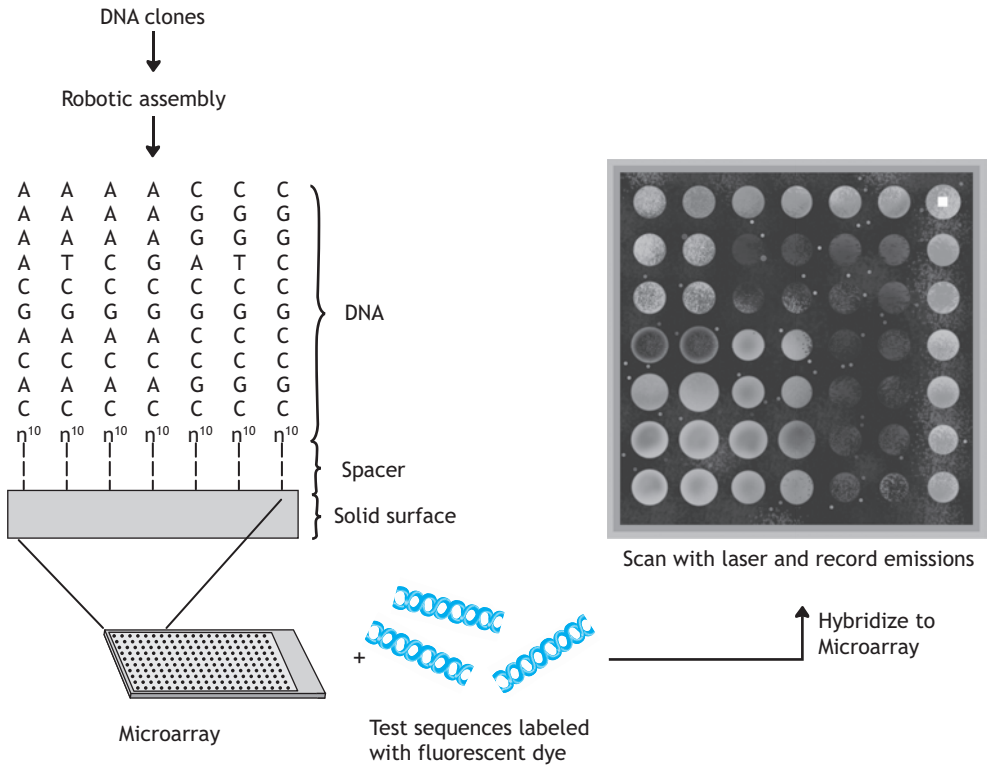


Figure 1–9. A microarray contains multiple (often thousands) of oligonucleotides of unique sequences. Hybridization can quantify fluorescence intensity to detect sequence variants as well as the presence or absence of sequences in the applied specimen.

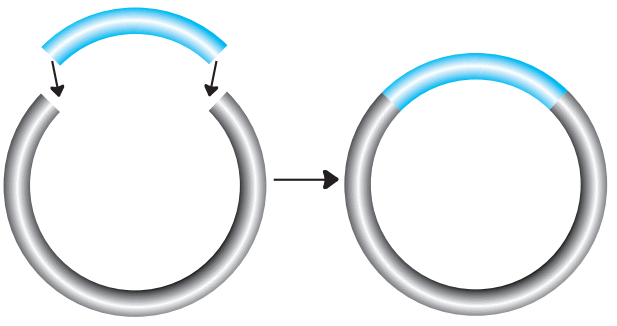


Figure 1–10. A DNA fragment can be inserted into a plasmid vector to obtain a molecular clone that can be propagated in bacteria. In theory, *any* sequence can be cloned based on this approach.

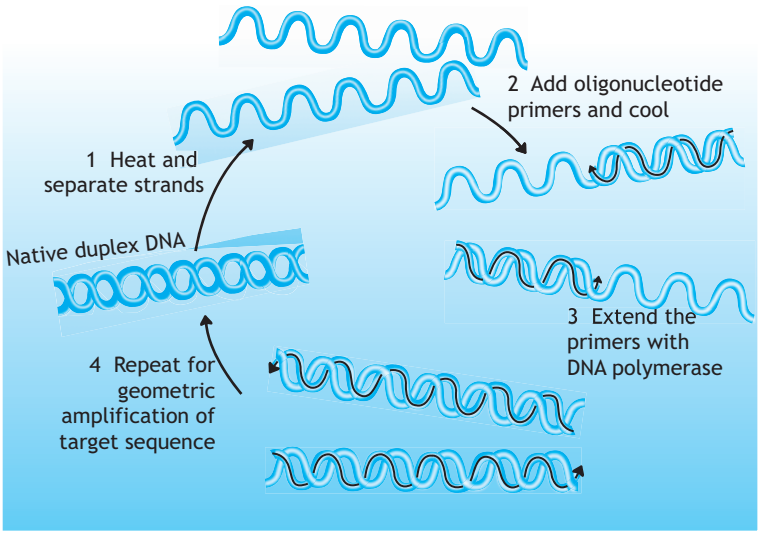


Figure 1–11. Basic steps in the polymerase chain reaction (PCR). *1*. PCR begins by separating the original two complementary strands. 2. Short, single-stranded "primers" are hybridized to single-stranded templates. *3*. The primers are then extended enzymatically to the full length of their templates to produce double strands. *4*. Individual double strands are separated by heating (melting), and the process is repeated after cooling to reaction temperature. PCR geometrically amplifies the starting sequence (in theory, it can begin with only a single DNA molecule) and can be quantified.

IV. Variations

A. The integrity of DNA, RNA, and proteins depends on **cellular enzymes.**

1. The enzymes of DNA replication have proofreading functions to help maintain fidelity in mitosis and meiosis, but they are not perfect.
2. **Environmental damage** (sunlight, radiation, drugs, chemicals, toxins, etc) also must be detected and reversed by repair enzyme systems.
3. Transcription and translation also are subject to error.
4. Errors help explain polymorphisms and mutations.

XERODERMA PIGMENTOSUM (OMIM 287—)

CLINICAL CORRELATION

- *Xeroderma pigmentosum encompasses a group of disorders characterized by poor maintenance of DNA integrity.*
- *Affected individuals often have extreme sensitivity to sunlight and* ***accumulate DNA damage,*** *resulting in frequent skin cancers.*
- *Molecular defects underlying different forms of xeroderma pigmentosum include genes essential for maintaining DNA integrity.*

B. **Polymorphisms** occur throughout DNA.

1. When two corresponding DNA sequences differ, they can be considered **alleles**.
2. By current estimate, ~4 Mb of variation exists per haploid genome.
3. Alleles are considered polymorphic when the most common allele has a frequency of < 99 in 100.
4. **Single nucleotide polymorphisms** (**SNPs,** pronounced "snips") occur in ~1 in 500 nucleotides.
5. **Deletions or insertions** of single or multiple nucleotides (called **indels**) also occur frequently.
6. **Variations in the number of tandemly repeated sequences** (**VNTRs**) are seen at about half the frequency of indels. The original sequence may be short—usually 1–4 nucleotides, giving **short tandem repeats** (**STRs**)—or long.
7. **Copy number variations** (**CNVs**) are frequent.
   a. CNVs range from single gene (or gene fragment) lengths (10–50 kb) to large regions containing many genes (> 100 kb).
   b. CNVs can be detected with automated sequencing and SNP studies.
   c. More than 1500 regions with CNVs have already been identified.
8. Because individuals have two copies of all autosomal chromosomal regions (sometimes more in the presence of CNVs) they can have two alleles of each corresponding region.
   a. If the alleles are identical, the individual is said to be **homozygous** at that site (or locus).
   b. If the alleles differ, the individual is **heterozygous**.
9. Any two random genomes contain millions of polymorphisms.
10. Polymorphisms can lead to
    a. No clinically detectable consequences
    b. A major difference in a gene or protein (eg, sickle cell disease, see Figure 1–2)
    c. Differences in the quantity or half-life of a gene product
    d. Relatively minor changes in the biology of an individual that may become cumulatively consequential in common diseases (see Chapter 10)
    e. Observable differences without likely medical consequence

C. A set of sequence variation(s) over a long stretch of DNA that is usually transmitted intact across generations is called a **haplotype.**

1. Identifying and localizing haplotypes along and among chromosomes delineates a **HapMap,** a long-range, sequence-based, set of easily measured markers.
2. The HapMap and related marker systems are basic to gene mapping and linkage analysis (see Chapter 2).
3. These marker systems also are used in genome-wide association studies for common diseases (see Chapter 10).

D. **Mutations** are changes in DNA sequence with biologic consequences.

1. A **point mutation** is the exchange of one DNA nucleotide for another. Exchanging one purine for another (eg, A for G) or one pyrimidine for another (eg, C for T) is called a **transition;** the alternative is a **transversion.**
2. Because of **codon degeneracy** (recall Table 1–1) some nucleotide changes do not change the encoded protein.
3. A nucleotide change causing substitution of one amino acid for another is a **missense mutation** (eg, sickle cell disease; see earlier discussion and Chapter 4).

4. A change leading to a termination codon (see Table 1–1) is a **nonsense mutation**.
5. Changes in noncoding regions can affect transcription, translation, or splicing or may be silent.
6. Short (even single nucleotide) or long indels are relatively common.
   a. Because of the triplet code, **adding or subtracting 3 nucleotides** (or multiples of 3) in a coding region **preserves the reading frame.**
   b. **Changing other numbers** of nucleotides **alters the reading frame** and affects all downstream codons and their corresponding amino acids (Figure 1–12).
7. Changes in splicing due to any of the preceding mechanisms generally change the gene product.

E. Transposition, the movement of a DNA sequence to a new site on the same or a different chromosome, can completely alter gene structure, expression, or both.
1. **Insertion** is the introduction of a sequence into a new location.
2. **Fusion** places together DNA sequence(s) that are not normally adjacent.
3. These events are frequent in cancer cells (see Chapter 9).

F. Amplification is local repetition of a (usually) short DNA sequence leading to an extended repeat.
1. This process underlies **triplet repeat disorders** (see Chapters 3 and 5).
2. It can generate minor differences in repeat lengths that can be valuable haplotype markers (eg, VNTRs, STRs, $[AT]_n$).

G. Changes in gene number are of two types.
1. **Duplications** (often large) can include one or more genes, thus increasing gene dosage. CNVs, discussed earlier, are an example.

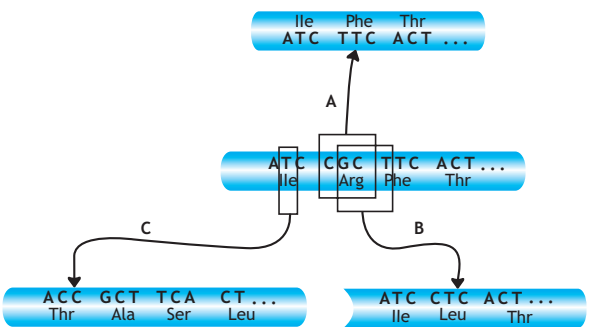


Figure 1–12. The original DNA sequence is shown in the center with its encoded amino acids noted. Deleting 3 contiguous nucleotides *within* the reading frame (***A***) simply removes a single encoded amino acid. Deleting 3 contiguous nucleotides outside the reading frame (***B***) inserts (in this example) a novel amino acid in place of the original two but reestablishes the correct reading frame. Deleting a single nucleotide (***C***) puts the reading frame completely out of alignment and directs polymerization of an incorrect chain of amino acids that continues until a termination codon is encountered. Consider the effect(s) of inserting nucleotide(s).

2. **Deletions** remove DNA and its encoded genes from a chromosome.
3. When an individual is heterozygous for a gene, loss of one of the two alleles can be detected as **loss of heterozygosity** (see Chapter 9).

H. **Imprinting** occurs when genes have different expression depending on their parent of origin.

1. Imprinting is **reversible** and mediated, at least in part, by modifying chromatin or DNA in discrete areas (eg, adding or removing a methyl [$-CH_3$] group to or from the C in the –CG– pair).
2. Expression of the imprinted allele is suppressed.
3. Imprinting permits transmission of information during cell division without a change in the DNA sequence itself; this process is now defined as **epigenetics.**
4. Imprinting is important in **controlling gene expression** during development and in common diseases (particularly those of later onset; see Chapter 10).

V. Pedigree Analysis

A. A **pedigree** is a graphic interpretation of family or kindred relationships, providing a simple graphic record for communicating genetic data (Figure 1–13).

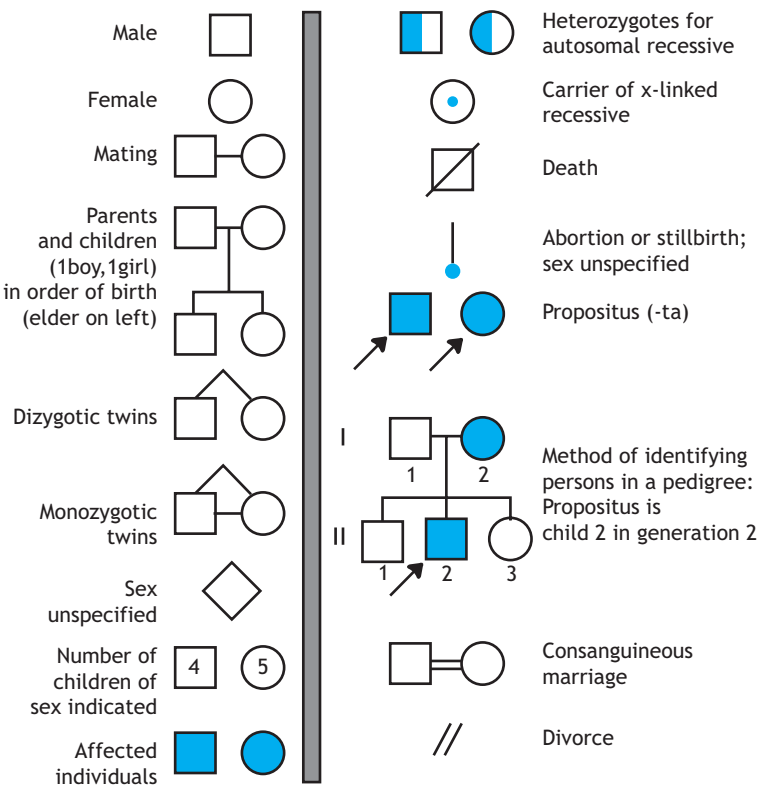


Figure 1–13. Conventional pedigree symbols. Other symbols are illustrated in later chapters.

1. Each generation is recorded on a new line with the oldest member entered on the left.
2. The symbol for the male in a mating is entered on the left.
3. Individuals are easily identified numerically.

B. Compiling a pedigree may be a complicated task.
1. All individuals may not be available for study.
2. All medical information may not be complete.
3. Individuals may refuse to participate.
4. The trait of interest may not present in the same way in each individual (see Chapters 3–5).

C. Pedigree information is valuable.
1. It is essential for linkage studies (see Chapter 2).
2. It often can show the mode of inheritance of a trait.
3. It may identify individuals at risk.
4. It is the basis for much genetic counseling.

VI. Genetic Testing

A. Preimplantation, prenatal, neonatal, symptomatic, or diagnostic evaluation is possible.
1. Testing can confirm or establish a diagnosis.
2. Having the correct diagnosis may clarify prognosis and suggest interventions.
3. Asymptomatic individuals can be offered testing based on their pedigree and genetic counseling.

B. Study methods depend on age, findings, or both**.**
1. Clinical and general techniques are the place to begin.
   - **a.** The history and physical examination are basic to classification.
   - **b.** Pedigree analysis can suggest individual (high or low) risk and also may be the basis for linkage analysis.
   - **c.** Radiologic studies (eg, computed tomography, magnetic resonance imaging, positron emission tomography, and ultrasound) can confirm diagnoses and identify affected organs.
   - **d.** Routine laboratory tests may suggest follow-up studies.
2. **DNA electrophoresis** may localize the underlying changes.
   - **a.** Altered DNA **restriction fragment length** can incriminate a DNA region.
   - **b.** A **single-strand conformation polymorphism** (**SSCP,** revealed by altered **electrophoretic mobility** of single-stranded DNA fragments containing nucleotide changes) may indicate the need for sequencing.
3. Sequencing is being simplified and automated.
   - **a.** **Short-range study** can be directed toward *known* mutations (or changes) in a DNA region implicated by clinical findings.
   - **b.** **Large-scale analysis** of an entire gene or region may be needed when a mutation is suspected but *known* mutations have not been found.
   - **c.** **Microarrays** can simplify sequencing by identifying changes either regionally or across complete genomes (see Chapter 2).
4. **Imprinting patterns** may be revealed by altered restriction enzyme susceptibility, electrophoretic migration patterns, or antibody reactivity (Figure 1–14).
5. Enzymes and other proteins may be studied directly.
   - **a.** The amount or catalytic activity of an enzyme can assess its synthesis, degradation, or integrity.

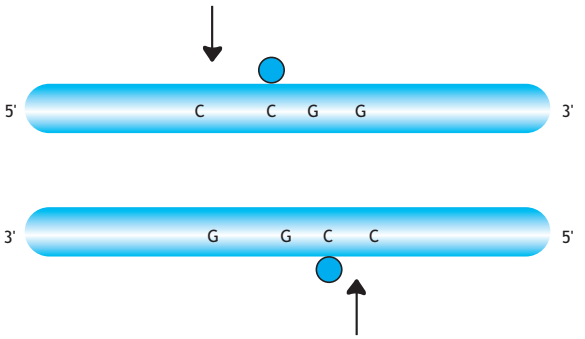


Figure 1–14. When methyl groups are present on the DNA sequence, as shown, it can still be cleaved by restriction enzyme *MspI* but not by *HpaII,* thus identifying one form of imprinting.

b. Physical changes in proteins, including size and charge, are clues to variations.
c. **Two-dimensional electrophoresis** can compare multiple proteins at once, although the patterns may be complex.
d. Proteomics techniques, including **mass spectrometry,** can improve detection.

6. Changes in substrate or metabolite levels may identify the aberrant pathway.
   a. High (phenylalanine in PKU) or low (thyroid hormone in hypothyroidism) levels can provide or suggest a diagnosis.
   b. Detecting a novel metabolite can aid diagnosis of a metabolic disorder.
7. Chromosome studies may precede molecular analysis (see also Chapter 2).
   a. Complete **karyotype analysis** can reveal number and gross structural changes; this often is done using arrays of SNPs.
   b. **FISH** (see Chapter 2) uses hybridization with multiple chromosome-specific probes to achieve chromosome painting and is a faster alternative to manual karyotypes.
   c. FISH also can use selected probe(s) to determine gene number or presence.
8. **Linkage analysis** (see Chapter 2) may be useful when pedigree data are available.
   a. The goal is to identify **polymorphic markers** that associate with the trait.
   b. **HapMap markers** are often helpful for identifying chromosome regions that may be related to the gene or trait.
9. Methods must balance risk (population frequency), diagnostic accuracy (false-positive vs false-negative results), cost, and treatment options.
10. Many new tests are being developed.

C. **Prenatal testing** has many uses.
1. Indications include
   a. A previously affected child or an affected individual in the kindred
   b. A history suggesting a chromosome abnormality

   c. Advanced maternal age
   d. Screening for specific risk
2. Benefits of prenatal testing include
   a. Assisting informed parental decisions
   b. Possible prenatal treatment
   c. Anticipating obstetric complications
   d. Arranging for specific neonatal care
3. Tissue sources vary with the time and clinical indications for the study (Figure 1–15).

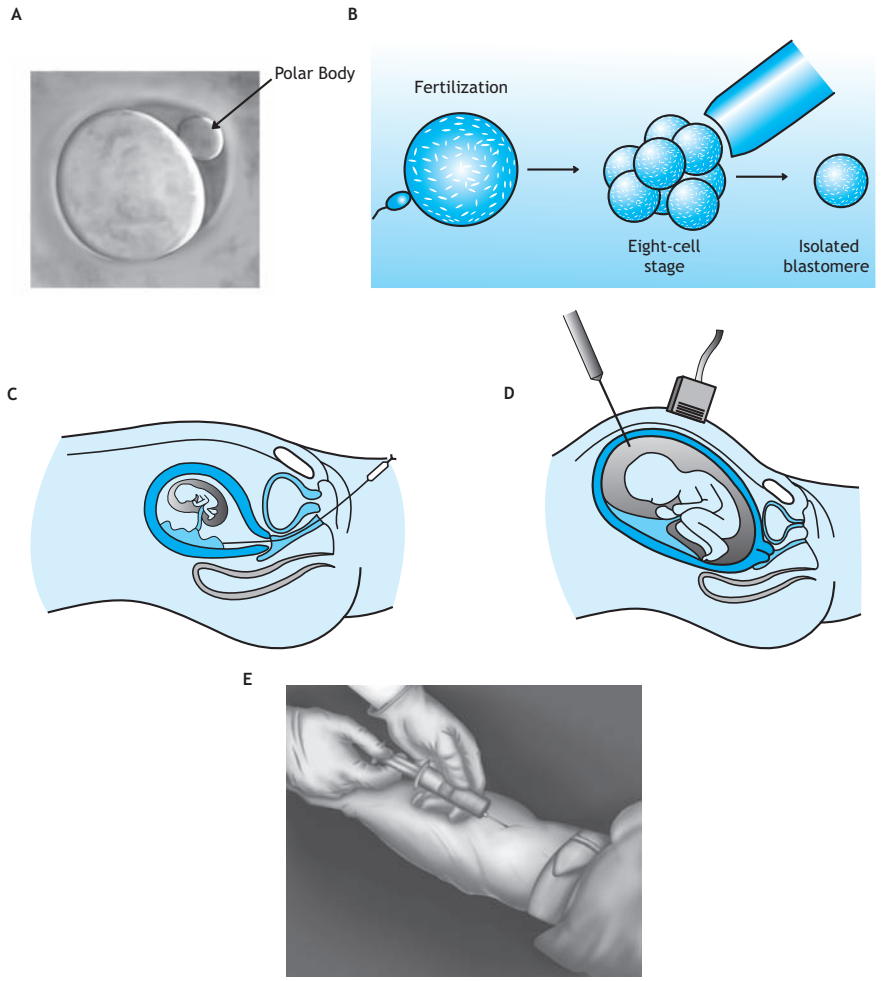


Figure 1–15. Sources of cells for prenatal diagnosis. ***A.*** Polar body. ***B.*** Single blastomere (from in vitro fertilization). ***C.*** Chorionic villus biopsy. ***D.*** Amniocentesis. ***E.*** Maternal circulation.

a. Preimplantation study can be combined with in vitro fertilization (IVF; see Chapter 2).
(1) **Polar body DNA** analysis detects ***maternal*** **genes**.
(2) DNA from a single **blastomere** (usually from the 8-cell stage) can evaluate all genes.

b. Prenatal studies use several sources.
(1) **Chorionic villus sampling** (**CVS**) at 8–10 weeks permits chromosome and DNA studies.
(2) **Amniocentesis** at 16–20 weeks permits chromosome, DNA, and some metabolic studies.
(3) Maternal serum levels of **human chorionic gonadotropin** (**β-HCG**) and **pregnancy-associated plasma protein A** (**PAPP-A**) are used in first-trimester testing; **alpha-fetoprotein (AFP)**, unconjugated **estriol** (**uE_3**), and **inhibin A** are more helpful in the second trimester**.**
(4) **Fetal cells or DNA** in maternal circulation can be detected by **PCR.**

DOWN SYNDROME

CLINICAL CORRELATION

- *Down syndrome is an example of a **trisomy,** a genetic disorder characterized by the presence of three copies of an individual chromosome, in this case chromosome 21.*

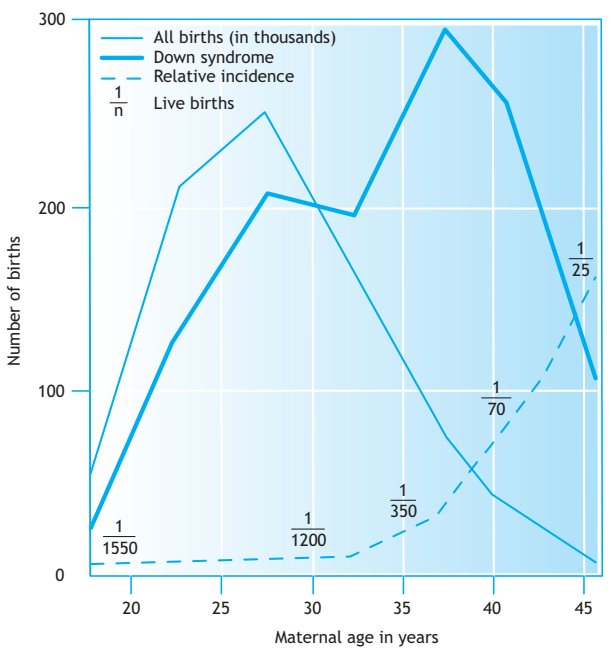


Figure 1–16. Incidence of Down syndrome with maternal age. Note the prominent increase after age 35.

- *The most common current indication for prenatal diagnosis is **advanced maternal age** (Figure 1–16).*
- *The **maternal serum tests** used depend on the stage of gestation; at 11–13 weeks, PAPP-A and β-HCG are helpful (Figure 1–17).*
- *These can be combined with measurement of fetal nuchal thickness by ultrasound (Figure 1–18) to achieve ~85% detection with a 5% false-positive rate.*
- *AFP, uE_3, and inhibin A may be studied in the second trimester; when combined with the results of the first-trimester studies the detection rate is ~95% with 5% false positivity.*
- ***Chromosome studies** are definitive (see Chapter 2); karyotyping, SNP testing, or chromosomal painting (fluorescence in situ hybridization [FISH]) can be performed either on cells from preimplantation sampling or on cells obtained by CVS or amniocentesis (see Figure 1–15).*

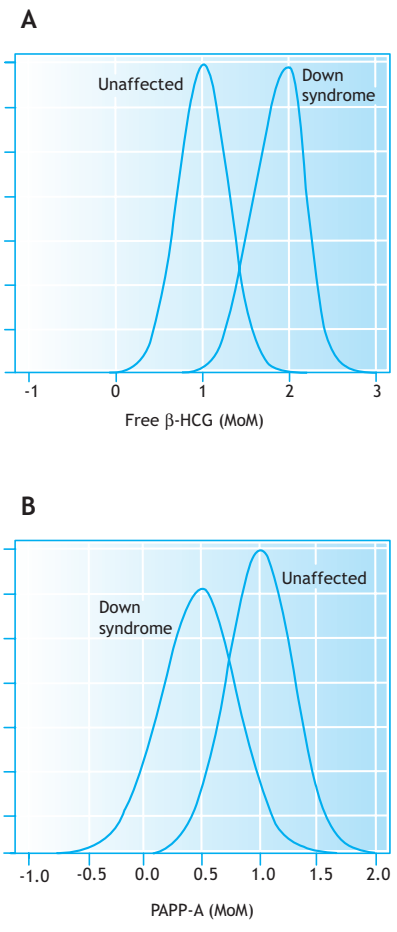


Figure 1–17. Maternal serum levels of β-human chorionic gonadotropin (β-HCG) (**A**) and pregnancy-associated plasma protein A (PAPP-A) (**B**) in the first trimester in unaffected pregnancies and Down syndrome, expressed as multiples of the median (MoM).

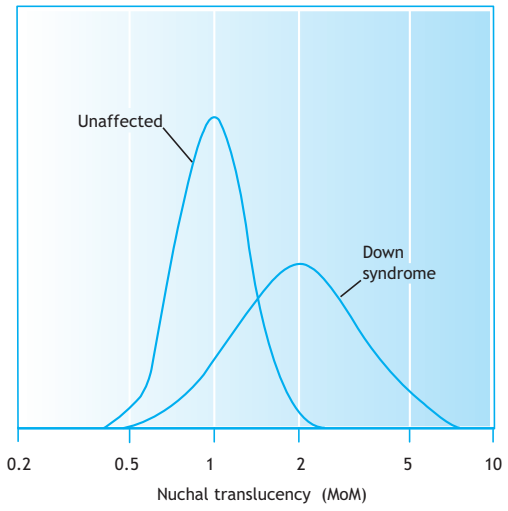


Figure 1–18. First trimester fetal nuchal translucency (multiples of the median, MoM) in Down syndrome and unaffected pregnancies.

NEURAL TUBE DEFECTS

- *Screening for these defects (eg, spina bifida) involves analysis of **maternal serum AFP levels** (Figure 1–19).*
- *If an elevated level is found, further studies (eg, ultrasound or imaging) can help confirm the diagnosis.*

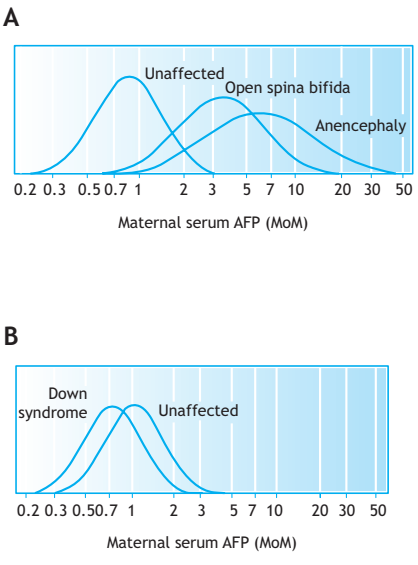


Figure 1–19. Maternal serum alpha-fetoprotein (AFP) levels during gestation expressed as multiples of the median (MoM). In neural tube defects (***A***), levels are usually elevated; and in Down syndrome (***B***), they are lower.

D. **Neonatal screening** is now performed for many conditions.
1. Both blood and urine specimens are used.
2. **Early detection** of treatable conditions such as **phenylketonuria** (**PKU**) and **hypothyroidism** is the goal.

PHENYLKETONURIA (OMIM 261600)

- *The Guthrie test uses blood from a newborn infant (usually obtained by a heel stick) to detect elevated phenylalanine levels.*
- *Infants so identified must then be studied further to clarify the diagnosis and arrange treatment (see also Chapter 4).*

CLINICAL PROBLEMS

Down syndrome occurs more frequently in pregnancies of older mothers, and screening is usually able to identify affected fetuses. These pregnancies often are subsequently terminated.

1. What accounts for the continued widespread birth of affected individuals?

A. The threshold for screening data is set high.

B. CVS samples often are contaminated with maternal cells.

C. Pregnancies are more common in younger women.

D. Ultrasound is unreliable when a fetus is moving rapidly.

E. Karyotype results often are delayed.

A laboratory worker is frustrated by the solid, dark pattern seen when a radioactive probe (10 nucleotides long) is hybridized to a Southern blot of DNA from cultured cells.

2. The most likely reason for this pattern is that

A. The temperature of the hybridization mix is too low, resulting in aberrant signals.

B. The salt concentration of the mix needs to be increased.

C. The cell culture is contaminated with mycoplasma.

D. A longer probe is needed to increase specificity.

E. The blot contains incompletely digested DNA and hence the complementary sites are not adequately separated.

A medical student is using electrophoresis to analyze a urinary protein that has been detected with a specific antibody. The student has taken 50 specimens from healthy classmates and is surprised to find that 11 of them show a distinctly different migration distance (two-dimensional gels show a pK_a change).

3. The student suspects that

A. The antibody is not as specific as was thought.

B. Earlier attempts at making gels led to distorted size estimates.
C. The newly discovered mutation may be associated with early-onset baldness.
D. The protein may have variable glycosylation.
E. The protein is polymorphic.

ANSWERS

1. The answer is C. Although the incidence of Down syndrome clearly rises with maternal age, the number of pregnancies is much higher in younger than in older mothers, and some younger mothers are not screened aggressively for the disorder. The screening thresholds are based on large population studies to both maximize detection and minimize unnecessary testing; lowering them would greatly increase the need for studies, with attendant costs and anxiety (choice A). Contamination of CVS with maternal cells is infrequent (choice B). Ultrasound is occasionally uninterpretable (due to fetal position or movement), but an experienced operator is usually successful. The blood tests also provide another source of information (choice D). Delays in obtaining results of karyotyping are not a problem when rapid methods such as FISH are used (choice E).

2. The answer is D. A probe of only 10 nucleotides (even if it does not contain an obviously repetitive sequence) will have over 1000 matches in the genome. (Recall that $[1/4]^{10}$ means that the sequence will occur ~$1/10^6$, and with ~10^9 bp in the genome the hybridization sites will be unresolvable.) Raising the temperature of the hybridization mix may improve the signal but eliminating competition from ~1000 sites is unlikely (choice A). Increasing the salt concentration also can increase stringency but is not likely to eliminate the overwhelming number of competitive sites (choice B). Although the cell culture might be contaminated, unless the probe is related to the contaminating sequence(s) this will not be the problem (choice C). Incompletely digested DNA on the blot can lead to aberrant hybridization, but using a short probe is likely to give a broad smear of signals (choice E).

3. The answer is E. The data suggest a simple polymorphism of high frequency, and the two-dimensional pK_a gel findings hint that a single amino acid may be changed. Many polymorphisms have been found in this way. The novel migration pattern was described as "distinct," implying that the antibody has good specificity but that some feature of the antigenic protein had changed (choice A). Gel preparation improves with practice and the student's finding was consistent, eliminating choice B. No data are given that would connect the change to hair growth, and the sample is too small to draw conclusions (choice C). With variable glycosylation of the underlying protein the migration might not be discrete (choice D).

CHAPTER 2
CHROMOSOMES AND CHROMOSOMAL DISORDERS

I. Chromosome Biology

A. A chromosome is a large macromolecular complex comprising a single molecule of DNA and multiple proteins.

B. **Histones** are an important family of chromosomal proteins.

1. Histones and other proteins organize DNA into compact arrays, and aid in **transcription** and **replication**.

2. Histones can be modified (eg, by adding methyl, acetyl, or phosphate groups) to add another level of control, as in **imprinting** (see Chapter 1).

C. There are **46 chromosomes** in the nucleus of a human somatic cell: 22 pairs of autosomes (numbered 1–22) and either two X-chromosomes (for females) or one X- and one Y-chromosome (for males).

D. All **nuclear chromosomes** have several common structures (Figure 2–1).

1. The **primary constriction** is a visible, narrow region containing the centromere.

2. The **centromere** contains proteins of the **kinetochore,** which is the site of sister chromatid attachment, and also microtubule binding sites for chromosome movement during cell division (see section IV, later).

3. Chromosome arms (two), usually a **short arm** (identified by the letter p, as in *petit*) and a **long arm** (q), are separated by the centromere.

4. The **telomere** is the chromosome end, containing repetitive DNA sequences.

E. The **mitochondrial chromosome** differs; it is a circular DNA molecule containing 16,569 base pairs (bp) (see Chapter 6).

II. Chromosome Analysis

A. Dyes bind differently to distinct regions on chromosomes, providing microscopically visible landmarks called **bands**.

B. Bands are numbered, providing reference locations along each chromosome.

C. A **karyotype** shows all chromosomes, usually with bands (Figure 2–2).

D. Assembling a karyotype by hand is slow and has largely been supplanted by molecular approaches.

E. Hybridization to single nucleotide polymorphisms (SNPs) arranged on a **microarray** can rapidly reveal changes throughout all chromosomes or in specific regions (recall Chapter 1).

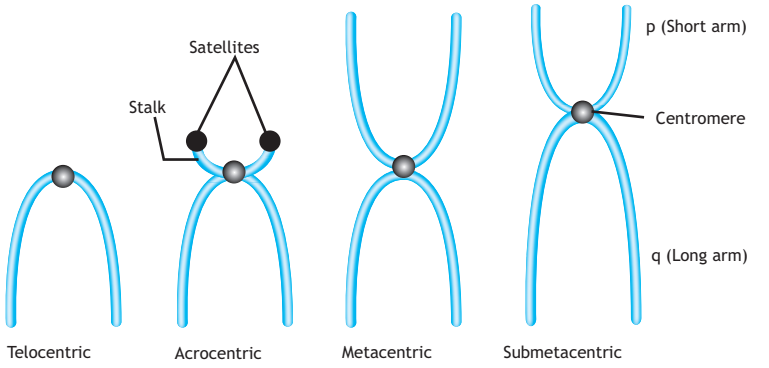


Figure 2–1. Common features of chromosome structure.

F. Fluorescence in situ hybridization (**FISH**) uses a DNA probe labeled with a fluorescent dye (see Chapter 1).

1. **Fluorescence microscopy** can detect the probe hybridized to intact chromosomes (Figure 2–3).
2. Multiple probes (often with different emission wavelengths) can be used together.

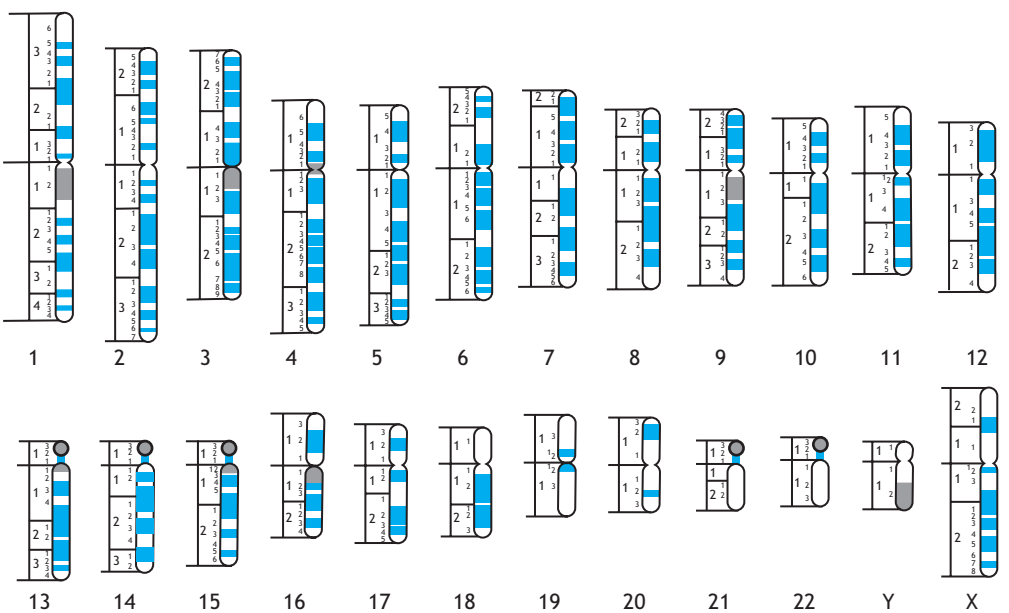


Figure 2–2. Human karyotype showing G-(Giemsa-staining) band patterns and regional numbering.

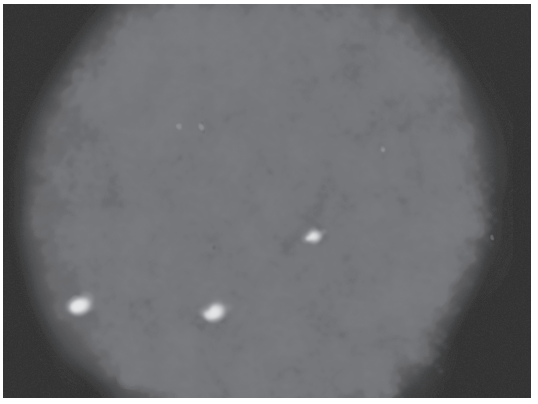


Figure 2–3. Fluorescence in situ hybridization (FISH) pattern using a probe for a single copy gene on chromosome 21. Note three signals, corresponding to three copies (trisomy) of chromosome 21. *(TriGen assay, courtesy of Vysis Abbott Group.)*

G. **Chromosome painting** uses a set of probes, each representing a unique sequence along a *single* chromosome and labeled with the *same* dye.
   1. Such a set of probes can hybridize with and identify a single pair of chromosomes.
   2. Multiple sets of probes can identify constituents (and many change[s]) of an entire karyotype.

III. Mitosis

A. Mitosis is the process by which chromosomes are replicated and distributed to daughter cells in somatic cell division.

B. Mitosis is part of the cell cycle, defined by events in the nucleus (Figure 2–4).

C. The cell spends most of the time in **G1 phase.**

D. When cell division begins, the cell enters **S phase,** during which DNA and chromosomal replication occur.

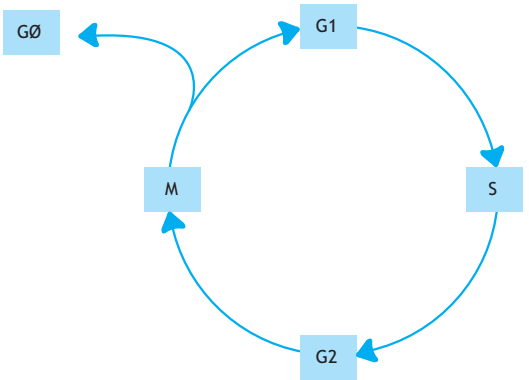


Figure 2–4. The cell cycle. Chromosome replication occurs during S phase.

1. Chromosomes first become visibly distinguishable during **prophase** (Figure 2–5).
2. At **metaphase,** the chromosomes are aligned along the equatorial plane.

TECHNICAL ILLUSTRATION

Chromosomes are most readily identified during metaphase, and the drug **colchicine** *can be used to arrest cells at this point in the cycle, permitting* **karyotype analysis.**

E. A delay (**G2 phase**) occurs prior to separation of newly replicated chromosomes, represented by two **chromatids**, joined at the centromere (see Figure 2–5).

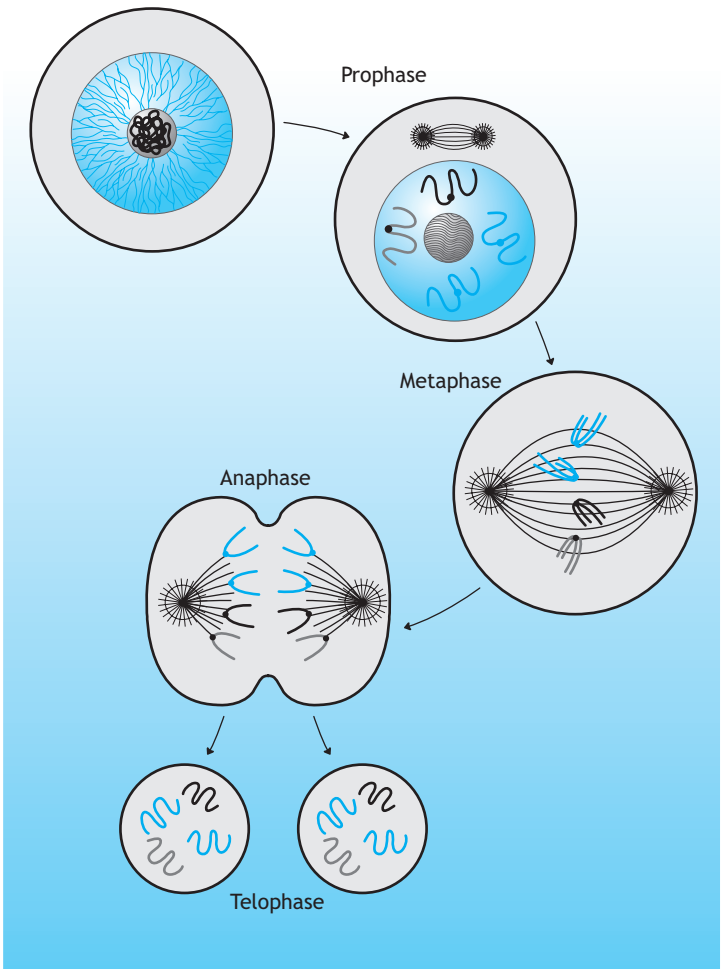


Figure 2–5. Events of mitosis, showing only four chromosomes for clarity.

F. During **M phrase,** chromatids are drawn apart (**anaphase**) along microtubules (attached to kinetochores) to form the nuclei of daughter cells (**telophase**).

G. The cytoplasm then divides (**cytokinesis**), forming two identical daughter cells.

IV. Meiosis

A. Meiosis occurs in the formation of **germ cells** and has two functions.

1. The chromosome number is halved from 46 in the somatic cell (**diploid** or 2n) to 23 in the germ cell (**haploid** or n), resulting in a cell that contains one chromosome from each pair of autosomes and either an X or a Y.

2. Physical DNA exchange occurs between homologous chromosome pairs (**crossing-over** or **recombination**).

B. Meiosis comprises several distinct events (Figure 2–6).

1. Meiosis I begins as each individual chromosome of a homologous pair is replicated to form two sister chromatids that are held together along their length by proteins.

a. The homologous maternal and paternal chromosomes (two chromatids each) align with one another through specific pairing, called **synapsis,** to form a bivalent in a synaptonemal complex (**zygotene** stage).

b. During synapsis there is physical exchange of DNA segments between sister chromatids of the paired parental chromosomes (recombination or crossing-over).

c. Due to **crossing-over** (completed in **pachytene** stage), each chromatid becomes a mosaic of regions derived from the two parental chromosomes (ie, it is a "recombinant").

(1) This is the basis for establishing linkage (see section IV, later).

(2) *At least one* and often multiple crossover events per chromosome occur in meiosis.

d. The proteins holding the paired chromatids (now mosaics) together are released (recall that they are still joined at their respective centromeres) and the pairs of chromatids are pulled apart in **anaphase I.**

(1) The pairs of chromatids are separated without regard to their parent of origin; thus, even had crossing-over *not* occurred there would still be mixing of the progeny of the original *parental* chromosomes. This separation of chromatid pairs is called **disjunction.**

(2) Failure to separate pairs of sister chromatids is called **nondisjunction** and is an important cause of chromosomal disorders.

DOWN SYNDROME

- *Down syndrome is usually a result of nondisjunction leading to three copies (***trisomy***) of* **chromosome 21** *in the maternal germ line.*
- *The rising incidence with increasing maternal age (recall Figure 1–16) likely is due to the fact that older oocytes have been inactive for many years.*

e. At the end of **telophase** there are two cells, each with **23 *pairs*** of sister chromatids.

(1) A diploid *amount* of DNA is still present.

(2) Chromatid pairs represent copies of *either* the maternal *or* the paternal chromosome (except for the small regions exchanged in crossing-over).

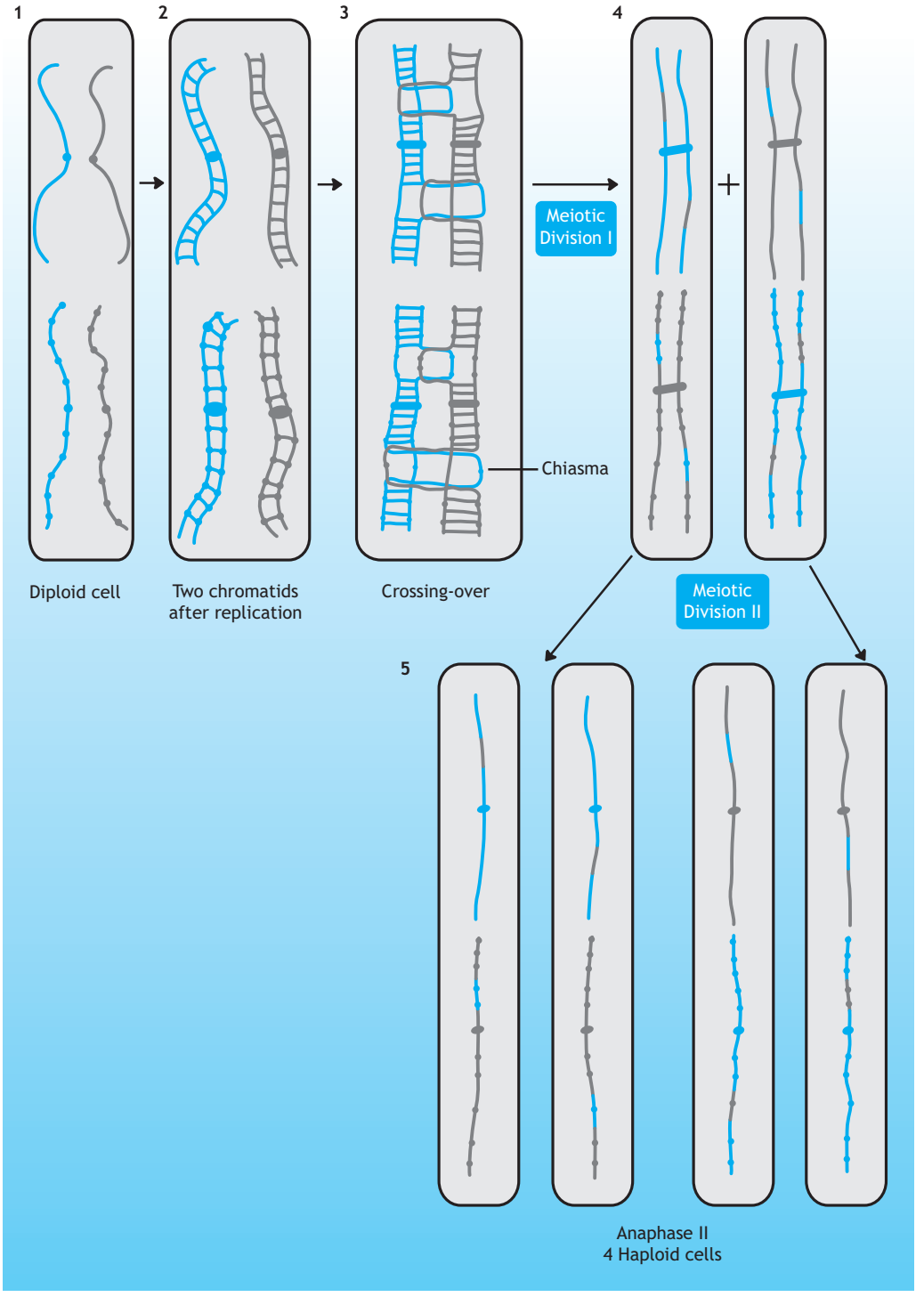


Figure 2–6. Events of meiosis, showing only two autosomal chromosome pairs for clarity. Recall that for females meiosis II is delayed until ovulation.

2. **Meiosis II** begins *without* further replication as the proteins holding the centromeres together come apart. Sister chromatids are drawn apart, resulting in four haploid germ cells, each containing 23 chromosomes (and a haploid amount of DNA).
   - **a.** The 22 pairs of autosomes can pair throughout their length.
   - **b.** The **X- and Y- chromosomes** have only short regions of homology, including centromeres and telomeres. There is thus essentially no recombination between X and Y, but their centromeres (and their attachment proteins) assure appropriate meiotic movement.
   - **c.** In males, one type of germ cell contains 22 autosomes and an X-chromosome; the other contains 22 autosomes and a Y-chromosome.
   - **d.** In females, *each* germ cell contains 22 autosomes and an X-chromosome.

C. In males, **sperm formation** begins with the spermatogonium, which undergoes meiosis and loss of cytoplasm to yield mature sperm (Figure 2–7).

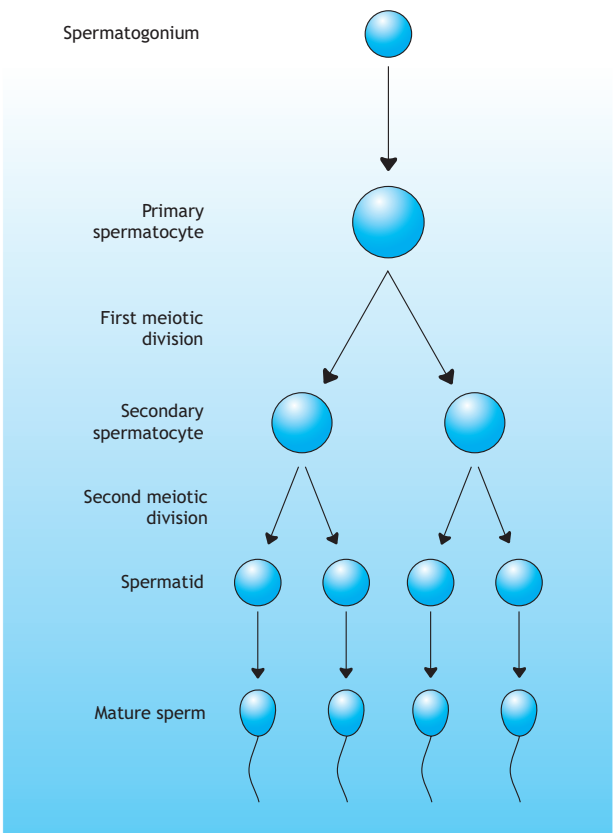


Figure 2–7. Sperm formation events (a continuous process after puberty in the adult male).

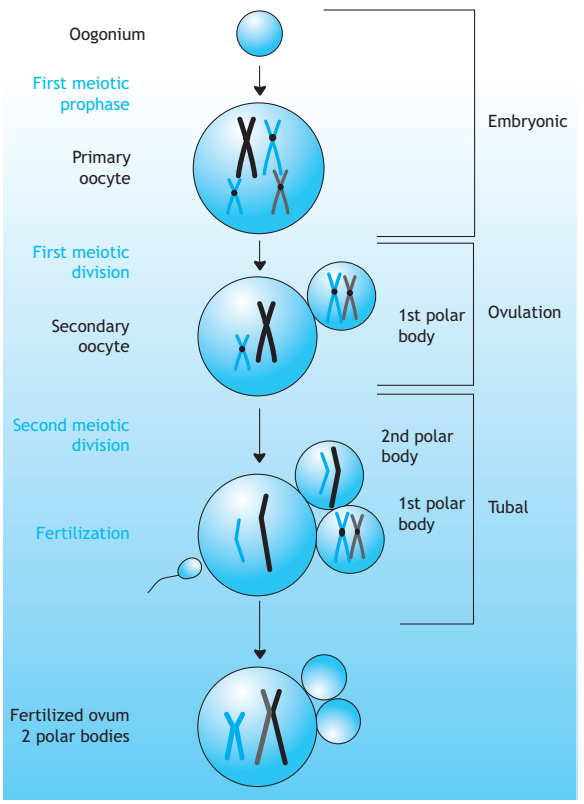


Figure 2–8. Oocyte formation, including processes occurring before and after ovulation. Note that the second meiotic division occurs late in the maturation process. There is striking asymmetry with respect to the cytoplasm.

D. In females, the process of **oocyte formation** is more complex (Figure 2–8).

1. During embryonic development, the oogonium becomes a primary oocyte that enters meiosis but stops at the first meiotic prophase where it remains until ovulation.
2. At ovulation, the maturing oocyte is stimulated to complete meiosis I asymmetrically, and a secondary oocyte and a tiny **first polar body** are formed.
3. Meiosis II in the secondary oocyte follows ovulation, usually in the fallopian tube.
4. Meiosis II is completed *after* fertilization and results in formation of a **second polar body,** containing a haploid chromosome set (identical to the maternal set remaining in the oocyte) and a small amount of cytoplasm.
5. The fertilized oocyte is **diploid** (ie, 2n; it has 46 chromosomes) with a haploid set of chromosomes from each parent; development continues by means of mitosis.

E. Meiosis thus embodies **two** *different* processes contributing to separation of maternally and paternally derived information.

1. **Separation** of sister chromatid pairs during anaphase I is random with respect to parent of origin.
2. **Crossing-over** mixes fragments of maternal and paternal chromosomes.

F. **In vitro fertilization** (**IVF**) encompasses several techniques for assisted reproduction.
   1. Oocytes are harvested from the ovary, where their number usually has been increased by prior hormone treatment (Figure 2–9).
      a. These oocytes will have completed meiosis I.
      b. Exposing the oocytes to sperm leads to meiosis II and polar body formation.
      c. The fertilized cell(s) then proceed(s) through several cycles of mitosis. A single cell can be removed (eg, at the 8-cell stage) for preimplantation genetic study (see Chapter 1).
   2. The developing embryo (with or without genetic study) is then implanted into the uterus to continue development.

V. Linkage

A. Linkage refers to the likelihood that one trait or haplotype marker will be transmitted with another through meiosis.
   1. The likelihood of mixing maternal and paternal chromosomal regions by crossing-over in meiosis is related to the **physical distance** between them on the chromosome.
      a. Markers located physically close to one another on a chromosome are more likely to be transferred together between generations than those located far apart (recall the definition of haplotype in Chapter 1).
      b. Haplotypes are distinguished by **allelic variation(s),** as described in Chapter 1.

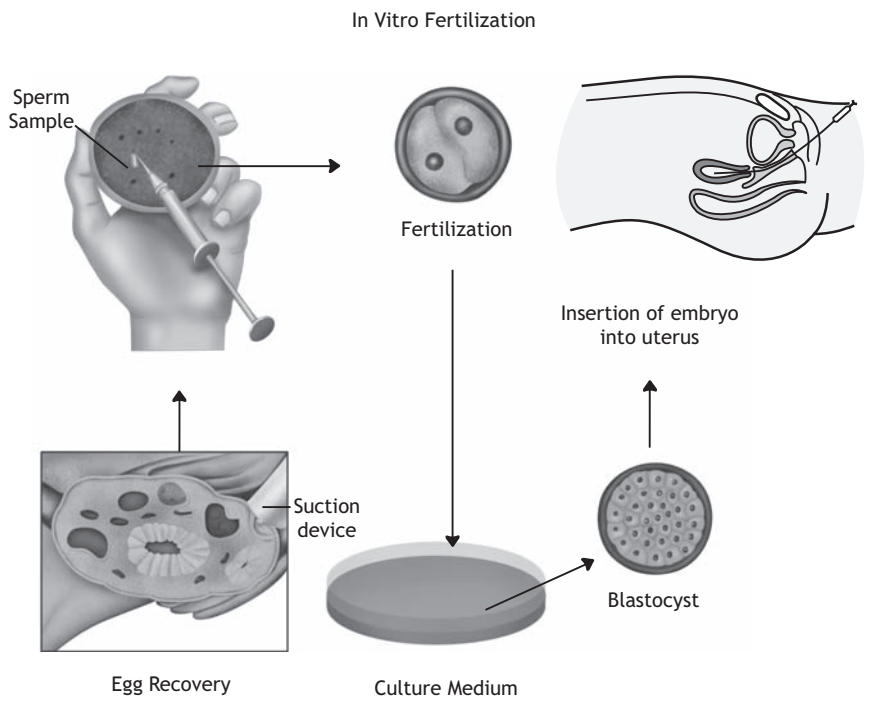


Figure 2–9. Basic scheme for in vitro fertilization (IVF) involving oocyte harvesting, sperm exposure, and early embryo growth followed by uterine implantation.

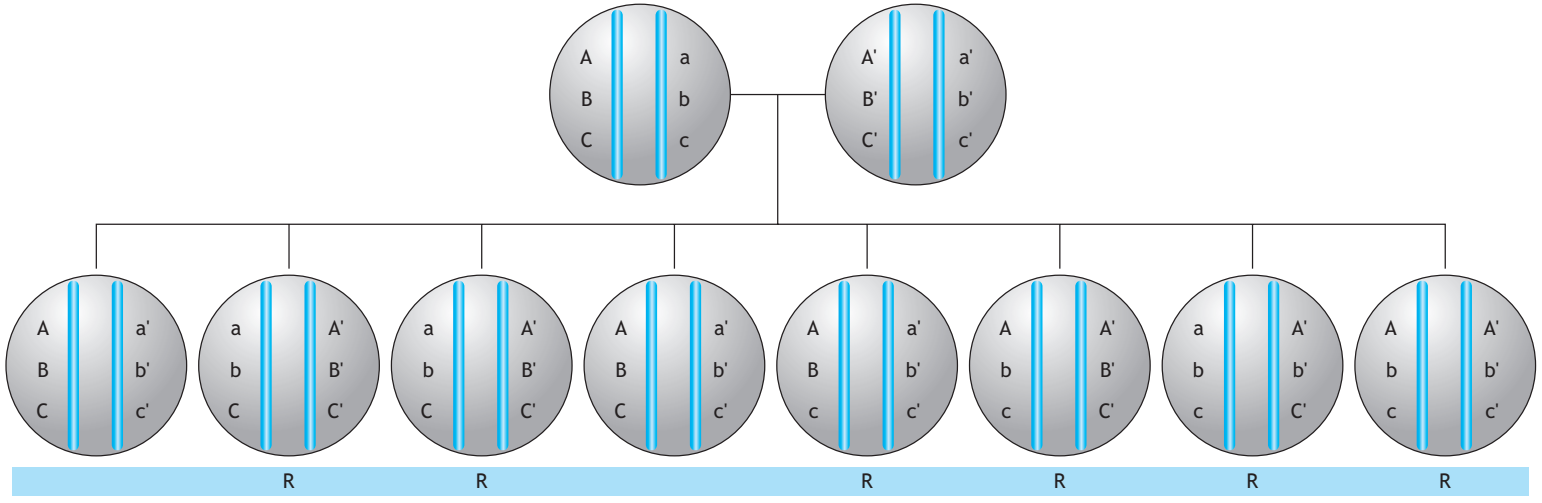


Figure 2–10. Haplotype transmission in a sibship of eight. Several of the siblings are recombinant for certain markers as shown. Data such as these are the basis for linkage analysis.

2. Haplotypes in offspring can be compared with those in parents to identify DNA regions (and the gene[s] within them) that have been transferred together (Figure 2–10).
3. Haplotypes of parents and offspring can be compared with the **movement** (**segregation**) of an inherited trait in the kindred.
   a. All markers on the *same* chromosome are said to be **syntenic,** but not all syntenic markers will be closely linked, particularly those far apart.
   b. Because at least one crossover event occurs between each pair of autosomes during meiosis, the maximum frequency for transmitting any two syntenic markers together is 50%.
   c. Linkage thus refers to both the ***order* of markers** and the **physical *distance*** between them and must ultimately be related to the responsible DNA sequence(s).

B. Linkage can be expressed mathematically as the **likelihood ratio** based on the **recombination fraction** noted as θ.
1. θ is the ratio of the likelihood that a given marker pattern would appear in the kindred if linkage and a given recombination fraction were present to the likelihood that the same pattern would be seen if the markers were not linked at all.
2. The ratio is expressed as $\log_{10}$ and referred to as the **LOD score** (for *l*og of the *od*ds).
3. The higher the LOD score the more likely it is that there is *actual* linkage between the markers rather than a chance association.
4. Conventionally, an LOD score of 3 or greater (ie, a likelihood ratio of 1000:1) is considered good evidence for linkage.

C. When a trait is seen in multiple family members, establishing linkage to a specific marker (or set of markers) identifies the DNA region where the responsible gene must reside and often is the first step in identifying the gene itself (Table 2–1).

D. The HapMap provides useful markers for linkage analysis.

E. Both evolutionary and forensic studies rely on analysis of DNA markers.

VI. Chromosomal Disorders

A. Most changes in structure, replication, and movement of chromosomes have prominent consequences.

Table 2–1. LOD scores for three chromosome 4 markers and Huntington disease (OMIM 143100).

Test and Marker	Recombination Fraction (θ)					
	0.00	**0.05**	**0.10**	**0.20**	**0.30**	**0.40**
HD vs G8	6.72	5.96	5.16	3.46	1.71	0.33
HD vs MNS	−∞	−3.22	−1.70	−0.43	−0.01	0.07
HD vs GC	−∞	−2.27	−1.20	−0.32	0.00	0.07

HD, Huntington disease.
Data from Gusella JF, Wexler NS, Conneally PM, et al. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 1983;306:234–238.

1. More than 50% of **early pregnancy losses** are due to chromosome abnormalities.
2. Most affected pregnancies are lost spontaneously in the first trimester.

B. Changes in chromosome number are important.

1. Departure from the diploid number of 46 chromosomes in somatic cells (**aneuploidy**) always is consequential.
2. **Trisomies** account for ~50% of all chromosome-related pregnancy losses, but most affected fetuses are lost spontaneously as pregnancy progresses; thus few individuals with somatic trisomy are seen as live births (Figure 2–11).

DOWN SYNDROME

CLINICAL CORRELATION

- *Although Down syndrome is the most frequently encountered human trisomy, liveborn affected individuals are only a fraction of those conceived (Figure 2–11). The* average *incidence is ~1 in 660 live births.*
- *Affected individuals are now reaching older ages, and attention to their care as adults is important.*
- *Characteristic features of Down syndrome are shown in Table 2–2 and Figure 2–12.*
- **Triplication** *of all or part of chromosome 21 is the common finding.*
  –Most individuals with Down syndrome have complete trisomy 21.
  –Triplication of the segment 21q22→telomere can explain most, but not all, of the findings seen in these individuals.
- **Translocations** *also are seen.*
  –The most common is 14–21, in which entire chromosomes are joined.
  –An individual with a translocation has a normal chromosome number (46) but also a chromosome with abnormal structure that can be passed in a kindred, leading to fetal loss, "balanced" carriers, and affected individuals (see later discussion).
- *Rarely, individuals are chromosomally* **mosaic,** *that is, not all of their cells are trisomic. Clinical features vary in severity, depending on the type(s) and number(s) of cells involved.*

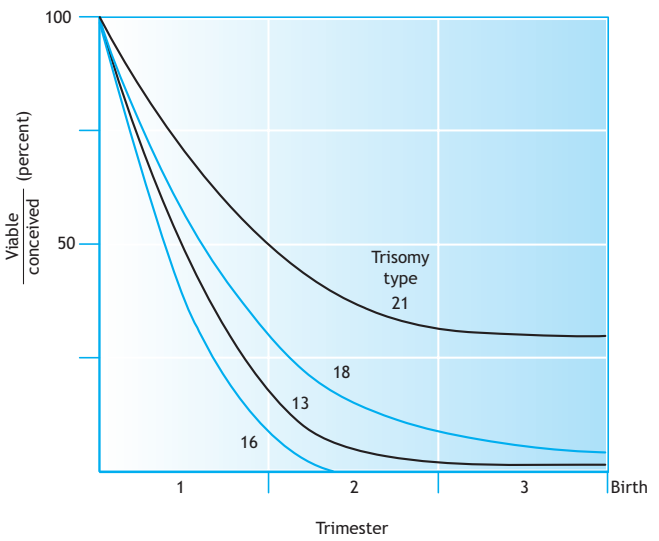


Figure 2–11. Most trisomy conceptions are lost early in pregnancy. There is no survival of trisomy 16 conceptions, although they are prominent early.

Table 2–2. Features of Down syndrome.

Short stature
Flat facial profile
Hypotonia, lax joints, transverse (simian) palmar crease
Congenital cardiac defects, usually due to incomplete septation (~40% of affected individuals)
Moderate to severe mental retardation
Loss of cognitive skills, with brain changes similar to those of Alzheimer disease, as patients age

- *Maternal* **gonadal mosaicism** *may explain some recurrences. Because the trisomic cells are confined to the gonad, they may be passed to progeny although the parent is clinically unaffected.*
- *Although the incidence of trisomy 21 Down syndrome rises with maternal age (historically, the most frequent indication for prenatal studies, recall Figure 1–16), trisomy 21 conceptions can occur at any maternal age, making noninvasive screening with serum markers and ultrasound valuable in all pregnancies.*
- *Although older fathers also have been implicated as the source of the "extra" chromosome, > 80% of trisomy 21 conceptions result from nondisjunction in meiosis I prophase in the oocyte.*

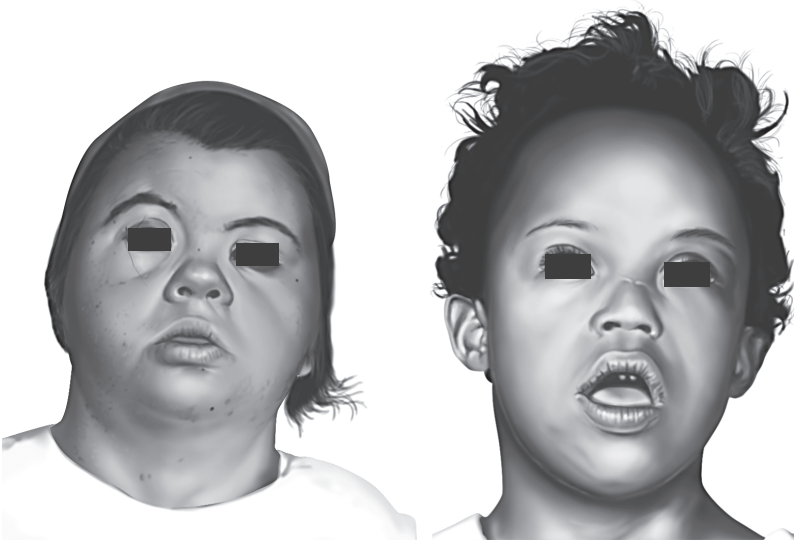


Figure 2–12. Characteristic facial features in Down syndrome.

C. Changes in sex chromosomes have predictable effects.

1. The **X-chromosome** has unique biology.
2. Females have two X-chromosomes and, thus, two copies of all X-linked genes.
3. The two X-chromosomes in somatic cells of all females appear physically different; one is said to be inactivated and is largely **transcriptionally silent.**
4. **Inactivation** of one X-chromosome occurs randomly in *all* female somatic cells soon after fertilization.
5. The inactive state is associated with transcription of the ***Xist*** gene on the X-chromosome (Figure 2–13).
   a. The *Xist* gene product is an **RNA** that spreads the inactivation signal throughout the chromosome.

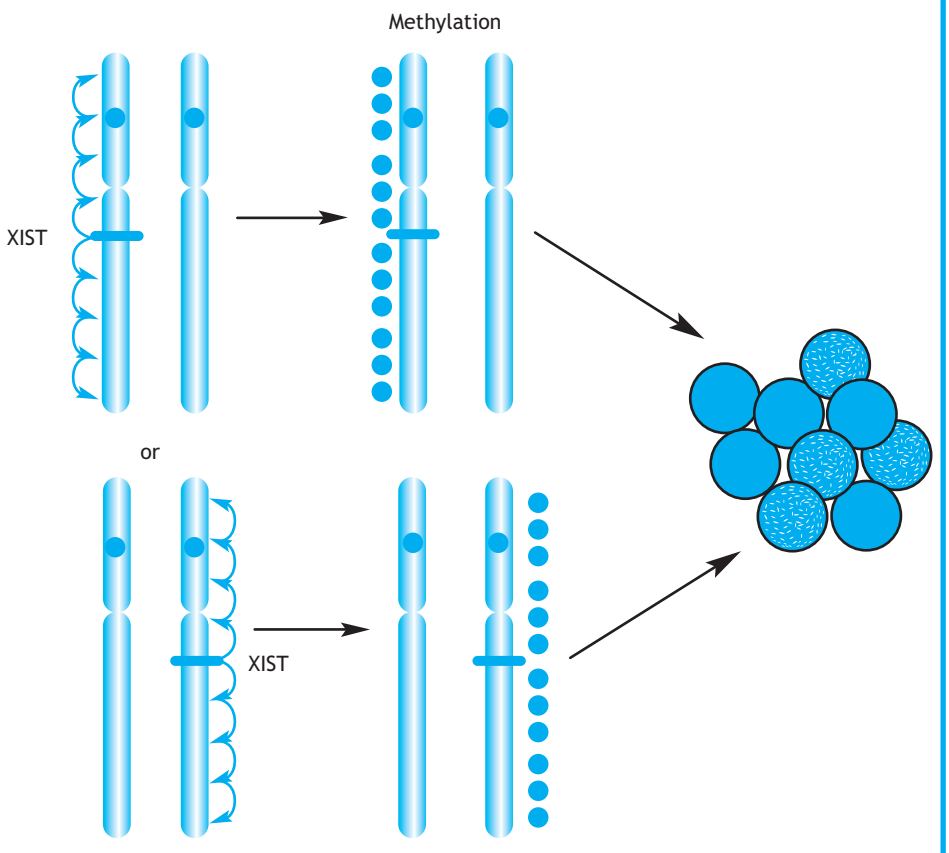


Figure 2–13. X-chromosome inactivation is a complex process. RNA transcribed from the *Xist* gene on the X-chromosome interacts with multiple sites on the chromosome, as shown, leading to the addition of methyl groups (dark circles). This process is initially random but, once established, is maintained through subsequent cycles of mitosis.

b. Inactivation is associated with **methylation of cytosine nucleotides** on X-chromosomal DNA.

6. Because X-inactivation is random early in development, the pattern in mature somatic cells also is random and **women are mosaics** for expression of **most X-linked genes.** This was first proposed by Mary Lyon (the "Lyon hypothesis"), and the inactive X-chromosome is sometimes said to be *Lyonized.*

TURNER SYNDROME

CLINICAL CORRELATION

- **XO females** *(Turner syndrome) have* **45 chromosomes.**
- *A female habitus, relatively short stature, and primary amenorrhea are common features (Figure 2–14).*
- *Many of these individuals are mosaic for XO* **and** *XX cells.*
- *Although one might suspect that the information from only a single X-chromosome should be adequate for female development (after all, X-inactivation largely eliminates transcription from one X in every somatic cell), some contribution from two X-chromosomes must be needed.*
- *Normal response to exogenous estrogens can assist adolescent development, and most affected individuals have good adjustment in life.*

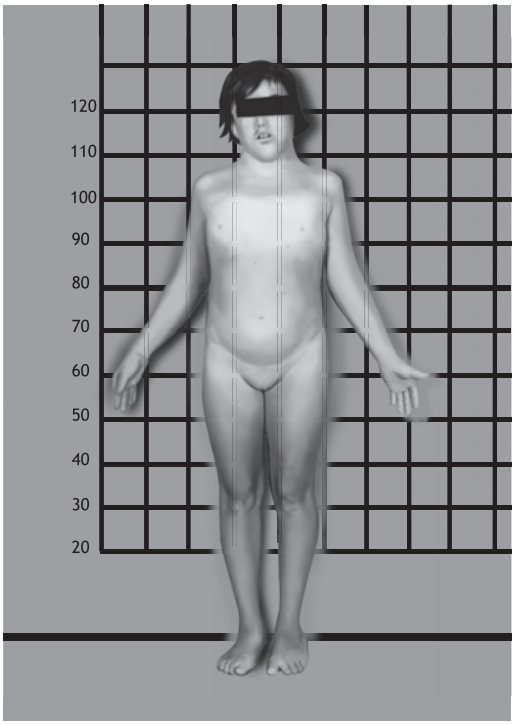


Figure 2–14. Characteristic physical features in Turner syndrome (XO).

KLINEFELTER SYNDROME

- **XXY males** *(Klinefelter syndrome) have* **47 chromosomes** *and are relatively frequent (1 in 600–1000) (Figure 2–15).*
- *Although these males have a prominent pubertal growth spurt with increased adult height, they are hypogonadal and infertile.*
- *Treatment with exogenous androgens can help somatic and psychologic development.*
- *These patients have an increased frequency of breast cancer in adulthood (otherwise rare in males).*

D. Changes in chromosome structure have both phenotypic and meiotic consequences.

1. **Errors in replication or separation** of intact chromosomes as well as in their complex structure(s) can lead to visible and submicroscopic changes.
2. **Altered structure(s)** can have serious consequences for cell division and gene expression.

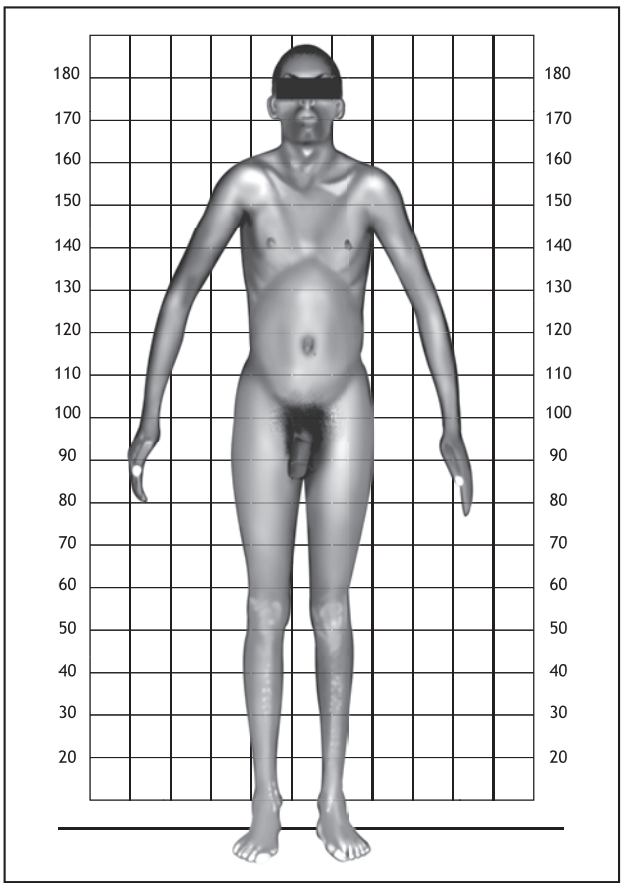


Figure 2–15. Characteristic physical features in Klinefelter syndrome (XXY).

3. Such changes are frequent in (and sometimes diagnostic of) certain malignancies (see Chapter 9).
4. **Deletions** are due to loss of DNA.
   - **a.** The extent varies from a single nucleotide through a segment to loss of an entire chromosome.
   - **b.** **Karyotype analysis, FISH,** and **sequencing** can each define the region in many cases.
   - **c.** A cell containing a deletion will be **haploid** for coding information from the region involved.
   - **d.** A microscopically visible deletion generally involves a large DNA region, often encompassing multiple genes.
     - ***(1)*** A so-called **"contiguous gene defect"** can be complex due to the many genes affected.
     - ***(2)*** Deletions of X-chromosome sequences in males cause loss of *all* information from the region and have been particularly helpful for localizing X-linked genes.

DUCHENNE MUSCULAR DYSTROPHY (OMIM 310200)

CLINICAL CORRELATION

- *This* **X-linked trait** *is the most common form of early onset muscular dystrophy in males.*
- *Karyotypes of rare affected individuals revealed overlapping deletions in the Xp21 region, which was then shown to contain the dystrophin gene (Figure 2–16).*

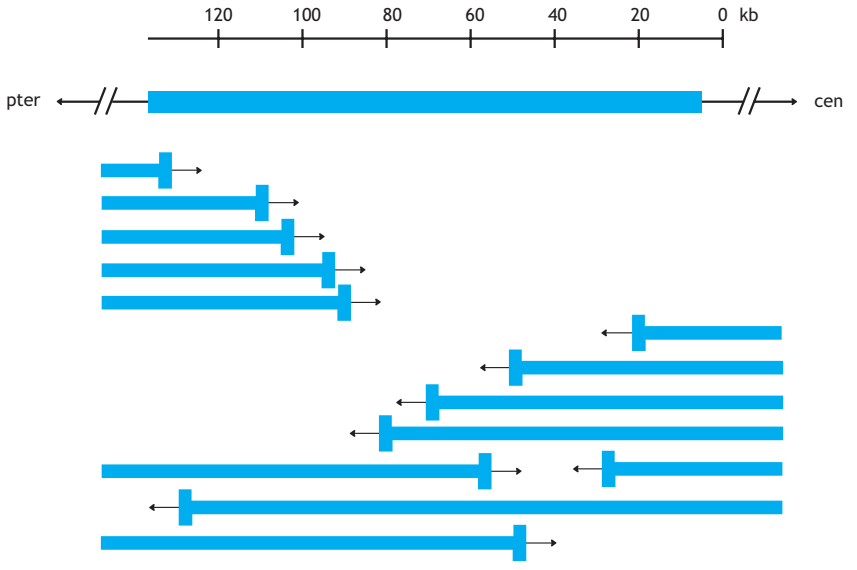


Figure 2–16. Overlapping deletions on the short arm of the X-chromosome (Xp21) helped localize the gene (dystrophin) for Duchenne muscular dystrophy, which is quite large. These deletions were visible by microscopy and implicated a common region by their overlap.

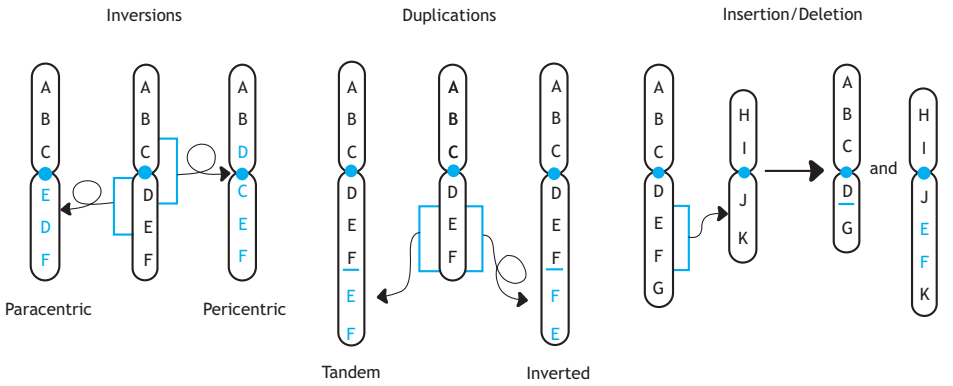


Figure 2–17. Chromosome changes involving inversions, duplications, deletions, and insertions.

5. **Inversions**, **duplications,** and **insertions** all reflect changes in **chromosome organization** (Figure 2–17).
   - **a.** Changes can alter gene control or interrupt a coding sequence(s).
   - **b.** **Copy number variations** (**CNVs;** see Chapter 1) of single genes can be difficult to detect.
   - **c.** Insertions can move material between chromosomes.
   - **d.** Some rare insertions are caused by mobile genetic elements.
   - **e.** These changes can disrupt meiotic pairing.
6. **Translocations** are due to simple or complex movement of fragments or complete chromosomes.
   - **a.** **Isochromosomes** join short or long arms of a chromosome at the centromere (Figure 2–18A).
     - ***(1)*** Duplication of such a large region usually interferes with development.
     - ***(2)*** These changes are readily recognized by **mirror image banding** or **FISH patterns.**
   - **b.** **Reciprocal translocations** usually exchange entire regions (Figure 2–18B).
   - **c.** **Robertsonian translocations** join long arms of **acrocentric chromosomes** at the centromere, but usually no coding information is lost because the short arms of acrocentric chromosomes contain only repeated **ribosomal RNA genes** (Figure 2–18C).
   - **d.** **Translocation chromosomes** are usually easily identified.
   - **e.** Clinically unaffected individuals can be found in a balanced situation (but they carry the translocation).
   - **f.** Complications develop with **meiotic pairing.**

TRANSLOCATION DOWN SYNDROME

CLINICAL CORRELATION

- *This relatively infrequent situation usually reflects a* **Robertsonian translocation** *between* **chromosomes 14 and 21**.
- *Affected individuals usually cannot be distinguished clinically from those with trisomy, but their pedigree may be complex, as shown in Figure 2–19.*

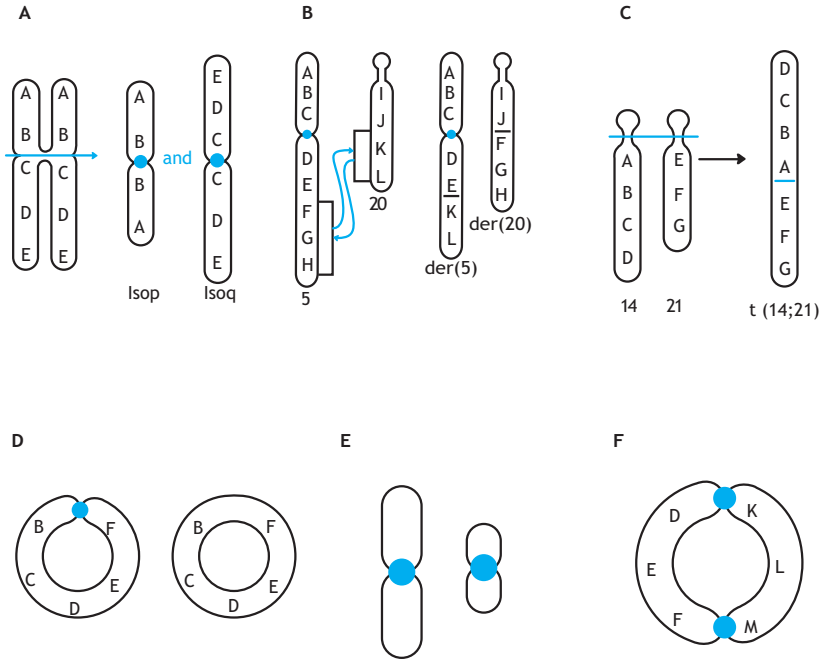


Figure 2–18. Variant chromosome structures. ***A.*** Isochromosome. ***B.*** Reciprocal translocation. ***C.*** Robertsonian translocation. ***D.*** Rings. ***E.*** Markers. ***F.*** Dicentric chromosome.

g. Translocations cause ~4% of spontaneous first trimester fetal loss.
h. One or both parents carries(y) a translocation in ~6% of couples with **recurrent spontaneous abortion;** chromosomes 13, 14, 15, 21, 22 are most frequently involved.
i. Many men carrying a translocation are sterile, with only a 2–5% chance of transmitting the change.
j. A woman with a translocation has a 10–20% chance of transmitting it.

7. Examining the **karyotype** of cells from a spontaneously aborted fetus can assist infertility counseling.
8. Other structural changes are less frequent.
   a. **Ring chromosomes** result from joining two ends of a broken chromosome (Figure 2–18D).
      (1) Rings lacking a centromere will be lost.
      (2) Rings containing a centromere usually are unstable in mitosis, often breaking and separating unevenly leading to mosaic cell progeny.
      (3) **FISH** can be helpful in identifying the DNA sequences in rings.
   b. **Marker chromosomes** are usually short, with a centromere and minimal adjacent sequence(s) (Figure 2–18E).
      (1) They may be difficult to identify due to limited sequence data.

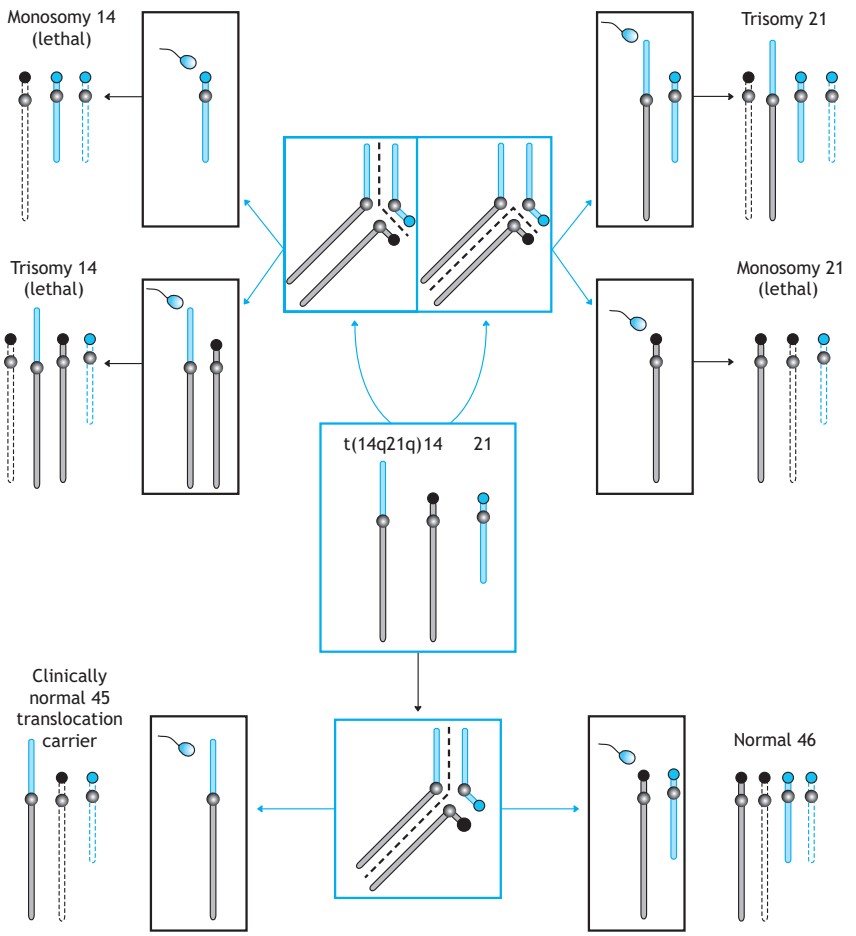


Figure 2–19. Chromosome 14–21 translocation can lead to Down syndrome but also has important consequences for other fertilization events, as shown. Illustrated is a typical Robertsonian translocation (see text). Note that an individual with Down syndrome due to this translocation will have 46 chromosomes but will be trisomic (ie, will have three copies) for the sequences on chromosome 21.

(2) When they are encountered in prenatal studies, interpretation can be difficult.

(3) Rare examples contain functional (usually excess) gene(s), leading to developmental problems.

c. **Dicentric chromosomes** contain two centromeres and adjacent fragments of the same or different chromosome(s) (Figure 2–18F)

(1) If both centromeres are functional, the structure will break in mitosis and usually will be lost.

(2) If one centromere is inactive, the dicentric chromosome can be stable through mitosis.

CLINICAL PROBLEMS

A CVS sample is sent for routine study to a prenatal diagnostic laboratory. Routine analysis using a FISH probe for chromosome 16 reveals three prominent signals.

1. What conclusion can be drawn from this finding?

A. The stringency of the hybridization needs to be increased.

B. This trisomy likely will abort spontaneously.

C. A dicentric chromosome is present.

D. Chromosome painting is needed.

E. The finding of three signals indicates fetal viability.

A 31-year-old man has a unilateral breast lump. A biopsy is performed, and the pathology report, surprisingly, shows an early-stage carcinoma. The man's family history reveals no history of breast cancer.

2. What would the physician be most likely to advise?

A. Hormone studies

B. Karyotype

C. Prophylactic mastectomy

D. *BRCA1* and *BRCA2* gene studies for the patient's younger sister

E. IQ test

In the course of a routine examination, a 29-year-old woman tells the physician that she and her husband have been unsuccessful at conceiving a baby. Her health has been excellent, and she will soon be a partner in her law firm. She had an older brother who died "of some sort of heart disease" at 6 months of age but no other siblings. Her parents are apparently healthy; her mother is 56 years old, and her father is 62. She wonders what might be causing her conception problems.

3. The physician would most likely advise that

A. The chance that the woman has undiscovered congenital heart disease is ~10%.

B. The woman's husband should have a sperm count and motility assay performed.

C. A thorough gynecologic history is needed.

D. The couple should undergo IVF.

E. The couple should continue their attempts to conceive.

The woman returns a year later, feeling well but more concerned about infertility. Since her last visit, she had two delayed menstrual periods 4 months apart, but her cycle is once again normal.

4. The physician would most likely advise that

A. The couple should undergo IVF.

B. The couple should consider artificial insemination.

C. The woman should have chromosome studies performed.

D. The woman should consider 3-month cycling with hormones.

E. The likely cause is failure of implantation.

The woman is not eager to have chromosome studies performed. She returns 4 months later, accompanied by her mother, who recalls a personal history of menstrual irregularities and miscarriages, in addition to the death of her infant son (the patient's brother). The mother adds that he was born and died while she was working for the Peace Corps in Africa.

5. The physician would most likely to conclude that

A. An infectious condition, acquired during the mother's Peace Corps service, has caused health problems in her offspring.

B. The patient's hormone levels should be measured throughout her cycle.

C. Chromosome studies are unlikely to be helpful, but linkage analysis may provide useful data.

D. The patient's chromosome number should be determined.

E. The patient history does not reflect a familial problem.

Two months later, the patient returns. A community laboratory reports that she is 46 XX.

6. Which of the following actions is the physician most likely to advise?

A. A trial of hormone cycling

B. Karyotyping

C. IVF

D. Ultrasound upon confirmation of pregnancy

E. Adoption

A review of 482 CVS samples analyzed by a prenatal laboratory over a 5-year period shows two cases of trisomy 16, four cases of XO, one case of trisomy 21, and three ring chromosomes of unknown origin.

7. What can one conclude from these findings?

A. Careful study of the quality of drinking water in the community is warranted.

B. The data are not unusual for pregnancies monitored at 10–12 weeks.

C. The data should be compared with those from another county to resolve concerns about their quality.

D. The data cannot be analyzed properly without information about the age of the mothers.

E. Determining the DNA sequences in the rings may clarify implications of the data.

ANSWERS

1. The answer is B. Trisomy 16 pregnancies usually abort early. Increasing the stringency of the hybridization (choice A) will likely cause all of the signals to disappear together. A dicentric chromosome would be unlikely to have two hybridization sites for the probe and, if it did, they would appear very close together (possibly not even distin-

guishable (choice C). Chromosome painting (choice D) will add little needed information because FISH has already identified the chromosomes. Finding three signals implies a high risk of fetal loss (choice E, recall Figure 2–11).

2. The answer is B. The rarity of breast cancer in men makes the possibility of Klinefelter syndrome (XXY) likely. Hormone studies (choice A) will not add to the diagnosis (although they may become useful later in his care). The patient's other breast should be evaluated carefully, although mammography may not be useful at this stage and it is premature to suggest prophylactic mastectomy (choice C). Unless suggested by the pedigree, studies of the patient's sister (choice D) are not indicated. An IQ test (choice E) is not indicated unless concerns arise about his mental status.

3. The answer is C. The physician needs to know more about the patient's cycles and their regularity. Has she ever been pregnant? What are her medications? A 10% risk of congenital heart disease (choice A) is quite high given the available data (see also Chapter 10). The physician would be justifiably curious about the woman's older brother, but she may be able to provide few details. Choices B and D are premature until more is known. Choice E is not helpful.

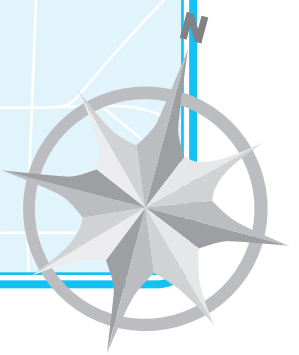
4. The answer is C. The patient may be having spontaneous early fetal loss and, recalling the story about her brother, the physician would most likely suspect a chromosome change. IVF (choice A) is premature and would be more valuable when her chromosome status is known. Artificial insemination (choice B) would not help if she has a chromosome abnormality. Her cycles have returned to normal, so exogenous cycling (choice D) is not indicated. She may have had successful implantation that was followed by fetal loss (choice E).

5. The answer is D. Chromosome studies are the appropriate next step to follow up the physician's suspicion in question 4. An earlier infectious problem did not apparently affect the patient's own development (choice A). Because most of her cycles have been normal, measuring hormone levels is not likely to be a problem (choice B). There are no clinical or laboratory markers to use for linkage analysis (choice C). The concern about her older brother justifies considering familial problems (choice E).

6. The answer is B. The laboratory has reported only the chromosome *number*. Based on the fact that the patient is healthy and intelligent, she is unlikely to have a chromosome deletion. The physician would most likely suspect a translocation, which would explain the occurrence of what might have been Down syndrome in the patient's older brother. A karyotype would confirm this suspicion. Hormone cycling (choice A) remains inappropriate. IVF does not directly address the suspected problem, which is fetal viability, not fertilization itself (choice C). Ultrasound is now standard care and thus not specifically indicated for her (choice D). Adoption is always an option but still does not address her personal situation (choice E).

7. The answer is B. Finding three trisomies in these early samples is not unexpected. Most likely they led to spontaneous fetal loss. Environmental influences (choice A) are unlikely causes of these events, which appear at frequencies similar to those reported elsewhere, and comparison with other counties is not likely to be revealing (choice C). There was only one example of trisomy 21 and two of trisomy 16; both are in an expected range for the broader population making the age(s) of the mothers (choice D) unhelpful. The rings are difficult to assess (choice E; see text), but efforts should be made find out the outcomes of these pregnancies. Prenatal studies at early dates show the highest frequencies of changes; most of these conceptions are lost later.

CHAPTER 3
AUTOSOMAL DOMINANT INHERITANCE



I. General Principles

A. Autosomal dominant (AD) conditions are detectable in the **heterozygote** (an individual with a **single mutant allele** of an autosomal pair).

B. There is **50% chance of transmitting the mutant allele** to each germ cell and, thus, a 50% chance for an affected individual to pass on the mutation at each conception.

C. The mutant allele is usually **detectable in *all* individuals** carrying it.

D. AD conditions often show a **vertical pedigree** pattern (Figure 3–1).

E. **Sexes are involved equally** although manifestations may differ between them.

F. Individuals may present with characteristics of an AD condition but without a family history; they may have ***new* mutations** that can then be transmitted in an AD pattern.

G. New mutations for some AD conditions appear more frequently in children of older fathers.

H. Several important terms are used in describing AD conditions.

1. **Phenotype** is the set of clinical (or laboratory) features observed.
2. **Genotype** is the underlying mutation.
3. AD conditions often show **pleiotropy**—multiple, overtly unconnected, biologic and clinical changes.
   - **a.** Clinical findings may involve multiple organs and systems.
   - **b.** Understanding the molecular nature of the mutation and its biologic consequences often clarifies relationship(s) between the otherwise disparate manifestations.
4. **Penetrance** refers to the detectability of the gene's effect, an all-or-none notion.
5. **Variable expressivity** of a penetrant trait with often unpredictable severity or prominence of clinical features is common and sometimes confusing.
6. Because multiple features often characterize a condition throughout the kindred, they are collectively referred to as a *syndrome* (from the Greek word meaning "running together").

I. Many AD mutations lead to structural (less frequently, enzymatic) abnormalities because one allele remains intact and at least *some* of the normal gene product can be synthesized.

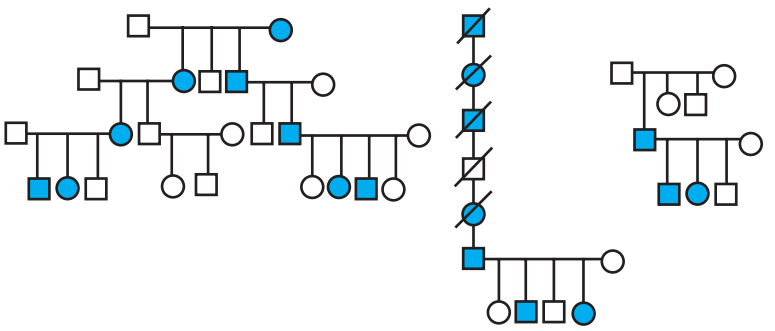


Figure 3–1. Pedigrees for autosomal dominant inheritance. Males and females are affected equally, and multiple generations are involved. Note that even partial ascertainment (not all siblings identified or some generational data missing) can still establish the basic transmission pattern.

J. AD inheritance of some **tumor syndromes** (see Chapter 9) has helped identify responsible genes and mechanisms.

NEUROFIBROMATOSIS

- *Neurofibromatosis type 1 (NF1), also called* von Recklinghausen neurofibromatosis *(VRNF) or* disease *(OMIM 162200), is the most common form of neurofibromatosis.*
- ***Café au lait spots*** *(Figure 3–2A)—easily distinguished, lightly pigmented macules resulting from melanocyte proliferation—may occur on any skin surface, and the presence of ≥ 6 is characteristic of VRNF (Figure 3–2C). Axillary freckling also is noted.*
- ***Tumors*** *(called neurofibromas or schwannomas, reflecting their frequent origin in Schwann cells) develop throughout life (Figure 3–2B).*
  –They are usually benign, causing symptoms simply due to size or location, but may become malignant.
  –They also appear as Lisch nodules, visible on ciliary nerves.
- *VRNF can result from many different mutations of the large* neurofibromin *gene on 17q.*
  –No consistent relation between mutation and phenotype exists, although more severe features have been noted in individuals with large deletions.
  –The same *mutation is present in affected members of the* same *kindred but their phenotypes usually differ (due to pleiotropy and variable expressivity).*
- *Neurofibromin, a GTPase-activating protein, helps inactivate the* ras *oncogene. Hence, its mutations are associated with proliferative effects of overactive* ras.

K. **Triplet repeat disorders** often show AD inheritance.

1. **Triplet repeat amplification** underlies nearly 30 neurologic disorders, including AD conditions such as myotonic dystrophy, Huntington disease (OMIM 143100), and Machado-Joseph disease (OMIM 109150), as well as fragile X syndrome (OMIM 309500; see Chapter 5).
2. Repeats may occur in translated regions, 5′ or 3′ untranslated regions, and within introns; their location is specific to each clinically identified disorder and the molecular consequences of their expansion(s) may differ.

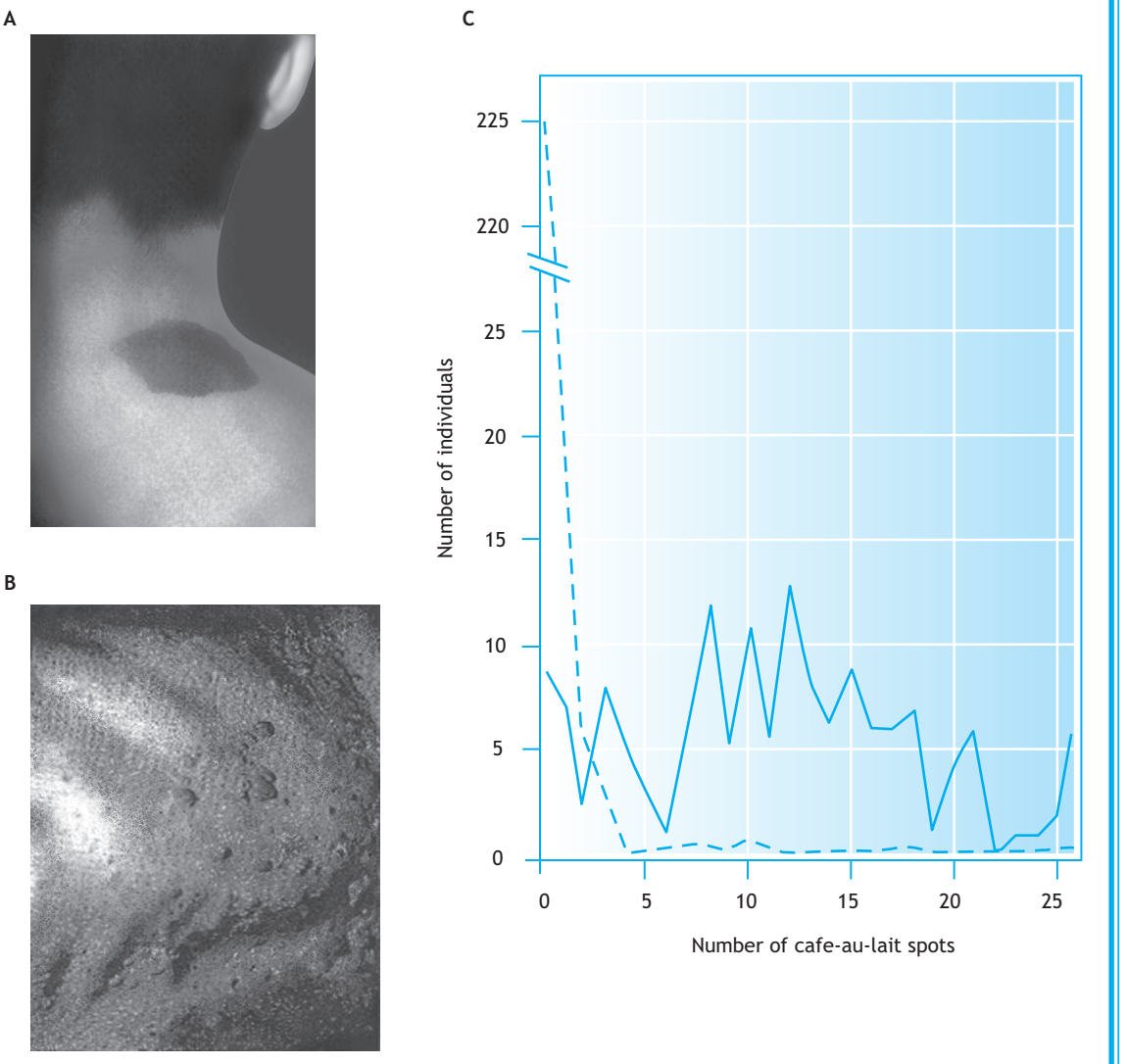


Figure 3–2. Clinical features of neurofibromatosis. ***A.*** Café-au-lait spot. ***B.*** Multiple small neurofibromas on the skin. ***C.*** Data showing that single café-au-lait spots (dashed line) are relatively frequent in the population while higher numbers (solid line) make the diagnosis of VRNF progressively more likely. (Adapted from Crowe et al. Springfield, IL, Charles C Thomas, 1956.)

MYOTONIC DYSTROPHY (DM1, OMIM 160900)

CLINICAL CORRELATION

- *Myotonic dystrophy is the most common form of adult muscular dystrophy.*
- *Characteristic features are progressive, distal muscle weakness and myotonia; cataracts, electrocardiographic changes, and frontal balding develop later. Progression of the findings may be slow.*
- *Phenotypic changes generally occur earlier, and often more severely, in later generations (a phenomenon historically termed "anticipation"; this can now be explained by the underlying molecular mechanism, and the term itself is generally no longer appropriate).*
- *The 3′ untranslated end of the responsible gene (dystrophia myotonica protein kinase on 19q) contains a CTG trinucleotide repeat; the codons for the protein itself are normal (Figure 3–3A).*

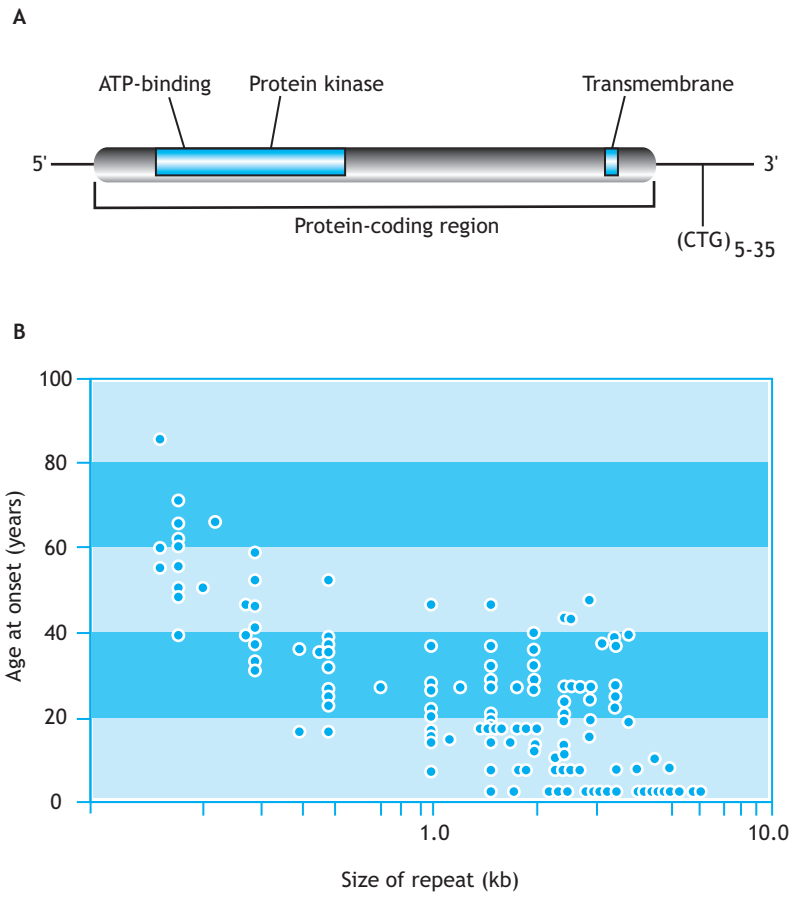


Figure 3–3. The trinucleotide repeat at the 3′ untranslated end of the gene for myotonic dystrophy (***A***) is prone to expansion with increasingly severe clinical consequences. The age of onset of problems (and clinical detection) falls as the repeat number rises (***B***).

–Healthy individuals have 5–30 tandem CTG repeats; 50–80 repeats are seen in mildly affected individuals, and > 2000 repeats are associated with a severe, often congenital, phenotype (this is the molecular basis for anticipation; Figure 3–3B).
–High repeat numbers are not transmitted by males; severe congenital phenotypes are encountered only in offspring of affected mothers.
–Long CTG triplet repeats appear unstable and prone to further amplification.
–The CTG repeat is transcribed into RNA (where it becomes CUG); the function(s) of CUG-binding proteins may be distorted by being sequestered by the repeats.

L. AD inheritance is useful for gene mapping by linkage analysis (see Chapter 2).

II. Recurrence Risks

A. The 50% transmission pattern is consistent, and an individual with a ***new*** **mutation** has the ***same*** **50% transmission risk.**

B. The phenotypic **severity** of triplet repeat disorders often is related to the **length of the repeat,** and molecular analysis may aid diagnosis and counseling.

C. Both pleiotropy and variable expressivity complicate predictions for the clinical status of offspring and siblings.

D. **Homozygotes** for an AD mutation may never be encountered because of **lethality;** alternatively, they may show an **exaggerated phenotype**.

CLINICAL PROBLEMS

Parents have brought their second child, a 1-year-old boy, for evaluation. He has short stature, and they have been told he has achondroplasia (OMIM 100800). No one in their family is short. The boy is pleasant and active, and testing confirms the diagnosis.

1. The physician would most likely advise the parents that

A. Their recurrence risk is 50% and prenatal diagnosis may be possible.

B. The reason the condition has not been seen earlier in their family is that most affected children die in infancy.

C. The recurrence risk for the boy is 25%, depending on his partner.

D. The recurrence risk for the boy is 50% regardless of his partner.

E. The boy's sibling has a risk of 25% of having a child with achondroplasia.

A 40-year-old woman seeks advice from her physician. Her 42-year-old brother has begun to show symptoms of Huntington disease (HD; OMIM 143100), and the disease has affected other members of her family, including her mother. The woman feels well but recently became pregnant for the first time. She hopes to continue the pregnancy but is concerned about having an affected child.

2. The physician would most likely advise her that

A. The child's risk of HD is 50%.

B. The child's risk of HD is ~0.

C. Her risk of HD is low because she is 40 and unaffected.

D. DNA studies may clarify her status.

E. The fetus should undergo DNA studies.

A 28-year-old man has been told he has a heart murmur. He is very active, 5′11″, and has mild myopia that he tolerates well. His father died from an aortic aneurysm and dissection and was said to have Marfan syndrome (OMIM 154700). He was 6′9″ tall with dislocated lenses and poor vision.

3. Which of the following statements is most likely to be true?

A. Given the severity of changes in the father, the man's murmur is likely functional.

B. An echocardiogram may provide helpful information.

C. An examination by an optometrist is warranted.

D. The absence of joint dislocations indicates that the likelihood of Marfan syndrome is low.

E. The man's serum cholesterol level needs to be followed closely.

The man is advised to have an echocardiogram but does not follow up on the physician's advice. At his next visit, he reports that he still feels well. Further examination shows that he has mild scoliosis, of which he was unaware.

4. The most appropriate next step is to recommend

A. A back brace

B. Physical therapy

C. Back exercises

D. An echocardiogram

E. A stress test

ANSWERS

1. The answer is D. Achondroplasia frequently presents as a new mutation but then has AD transmission. The boy should be intelligent, and he and his parents might benefit from referral to the group Little People of America. Choice A is unlikely because the parents are unaffected and this is an AD trait. Neonatal lethality (choice B) is not common in achondroplasia. As an individual with an AD condition, the recurrence risk is 50%, not 25% (choice C). An unaffected sibling has a very rare chance of having an affected child (the same as the rate of a new mutation); thus choice E is incorrect.

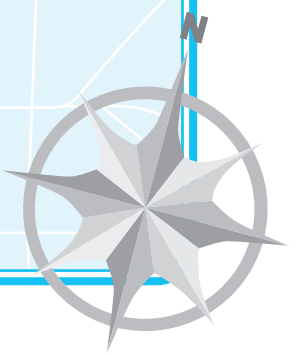
2. The answer is D. The woman is clearly at risk for Huntington disease, and her own status must be clarified prior to counseling. The risk to the fetus could be 0 or 50% (choices A and B), depending on the mother's status; however, in genetic counseling, it is essential to establish the diagnosis of the presenting individual (often called the consultand) first. Although most individuals with HD would have shown problems by age 40, she remains at 50% genetic risk (recall "penetrance"); thus choice C is incorrect.

Data reporting age of onset are derived from populations with many individual exceptions. Choice E is inappropriate; it is too early to place a fetus at risk for genetic studies when the mother's HD status is unknown. Because of her age, however, fetal screening for neural tube defects and Down syndrome is important.

3. The answer is B. An echocardiogram is a good way to clarify the man's cardiac status. The term *functional* in choice A is vague at best and, in the context of a 50% genetic risk, it is premature. An ophthalmologist, not an optometrist (choice C), should make the evaluation. It is already known that the patient has myopia, and corneal details may be missed otherwise. Joint laxity, although important for many individuals with Marfan syndrome (choice D), is variable and difficult to quantify. Although cholesterol (choice E) is important, it is a relatively low risk factor in a young man and certainly does not need to be followed "closely."

4. The answer is D. Finding scoliosis may explain why the man is not tall like his father and, combined with a "heart murmur" and mild myopia, increases the suspicion for Marfan syndrome. The simplest next step is to determine the status of his heart valves and ascending aorta with an echocardiogram. This is an example of the importance of recognizing both pleiotropy and variable expressivity as characteristics of AD conditions. The scoliosis is mild and likely stable, so bracing (choice A), physical therapy (choice B), and exercise (choice C) are not indicated. A stress test (choice E) is not likely to be very informative in a young man; far more relevant data will come from an echocardiogram.

CHAPTER 4
AUTOSOMAL RECESSIVE INHERITANCE



I. General Principles

A. Autosomal recessive (AR) patterns occur when the affected individual has a **mutation** of the copy of the gene **contributed by *each* parent.**

B. **Heterozygous** parents are generally unaffected and often are called **carriers**.

C. Affected individuals who have the ***same* mutation** in each copy of the affected gene are known as **homozygotes**; those with a ***different* mutation** in each copy of the gene are **compound heterozygotes** (unless noted otherwise, the former designation often is used for both, but the distinction can be important, as discussed later).

D. **Each heterozygous parent** has a **50%** chance of **contributing a mutant allele,** and so their chance of having a **homozygous child** is **25%** ($\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$) with *each* conception.

E. AR conditions may show a **horizontal pedigree** pattern (Figure 4–1).

F. Because carriers often are unaware of their status, finding a homozygote often is unprecedented in a kindred.

G. Two thirds of clinically unaffected siblings of an affected individual are likely to be heterozygotes.

TECHNICAL ILLUSTRATION

- *Consider a sibship of 4 where the genotype likelihoods are as follows: homozygous normal, ¼; heterozygous from mother, ¼; heterozygous from father, ¼; and homozygous affected, ¼.*
- *The first three categories are clinically unaffected, and two of them will be carriers (⅔).*

H. In most populations, the likelihood that any two individuals selected at random will be carriers is low, explaining the relative rarity of AR homozygotes.

I. **Consanguinity** increases the likelihood of carrier mating (see Figure 4–1).

1. Consanguineous matings concentrate whatever carrier status may have been present in the founders of the population (**founder effect**).

2. Founder effects also occur in communities isolated by geography or religion, leading to the relatively frequent appearance of homozygotes for otherwise rare conditions in these populations (eg, Old Order Amish, islanders, Parsis, some Jews).

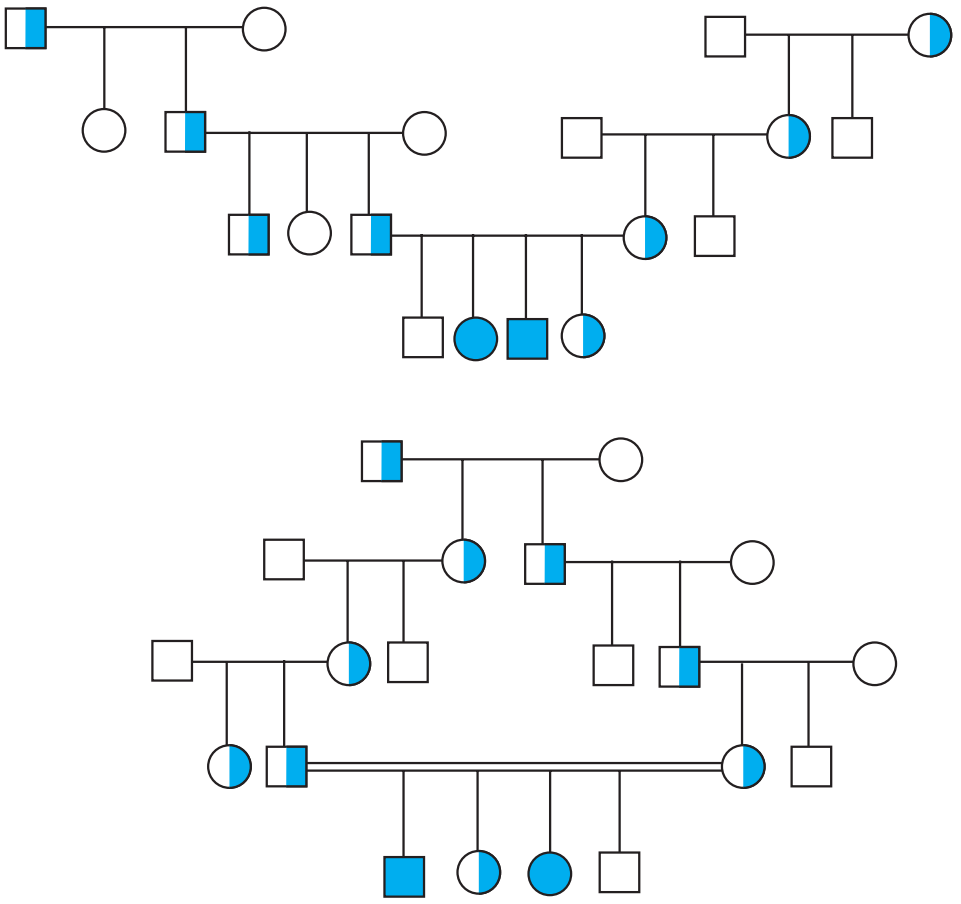


Figure 4–1. Autosomal recessive pedigrees showing what has been called a horizontal pattern. Carrier status also is shown. Note that consanguinity (lower kindred) may increase carrier frequency and the likelihood of homozygosity.

SICKLE CELL DISEASE (OMIM 603903)

- *One of the most widely studied AR conditions, sickle cell disease is characterized by the development of chronic hemolytic anemia associated with recurrent acute, painful crises due to ischemic tissue damage (see also Chapter 1).*
- *A **point mutation** in the sixth codon of the β-globin gene substitutes glutamic acid for valine, altering the structure of hemoglobin in low oxygen environments (recall Figure 1–2) and distorting the red cell shape (sickling) causing clumping, trapping, and destruction (Figure 4–2).* (Technical question: *Which nucleotide was changed? See Table 1–1.)*

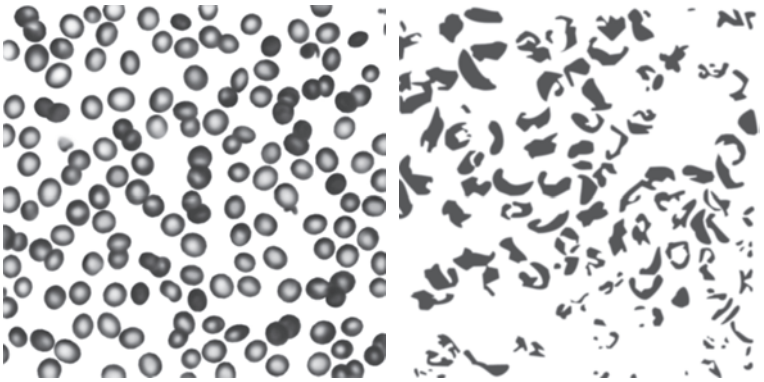


Figure 4–2. Comparison of normal erythrocytes (*left*) and those containing sickled hemoglobin (*right*). The latter cells cannot spontaneously assume their normal biconcave shape and thus undergo entrapment and hemolysis.

- *Despite having the* identical *underlying mutation, many homozygotes differ in their clinical course.*
  –Some of this variation is due to different levels of erythrocytes containing fetal *hemoglobin (HbF), an embryonic predecessor of β-globin.*
  –Erythrocytes containing HbF ("F cells") are relatively resistant to sickling and thus reduce the likelihood of a crisis by diluting the HbS cells.
  –Control of the HbF level is not *linked to the globin genes (responsible loci have been found on both 6q22 and Xp22).*
  –This is an example of controlling the consequence(s) of one mutation by an unlinked genetic change, a phenomenon called ***epistasis,*** *which has become an important consideration in common disorders (see Chapter 10).*
- *Other influences on clinical manifestations of sickle cell disease must exist but have not yet been identified.*
- *The* ***carrier frequency*** *is ~***8% *in African Americans** and even higher (up to 25%) among populations of some African and Mediterranean areas.*
- *The geographic distribution of the mutation is consistent with the relative resistance of heterozygotes to* ***falciparum malaria*** *and, hence, their having a survival advantage in endemic regions.*
- *Diagnosis can be based on testing either the erythrocyte or the gene but* every *mutation is* the same *and, thus, affected individuals are* true *homozygotes. Although often considered the prototype for AR conditions, this molecular consistency may be more the exception than the rule.*
- *The prognosis for homozygotes has improved due to improvements in acute care during crises.*
  –Transfusions can reduce anemia, dilute the sickle cells, and reduce crisis frequency and severity. Drug treatment (eg, hydroxyurea) can increase the number of F cells.
  –Techniques to replace the mutant gene are being studied; bone marrow transplantation is an option (see also Chapter 12).

J. All offspring of a homozygote must at least be **carriers**.

K. If a homozygote mates within a restricted community (with a high carrier frequency) a pattern called **pseudodominance** may occur (Figure 4–3).

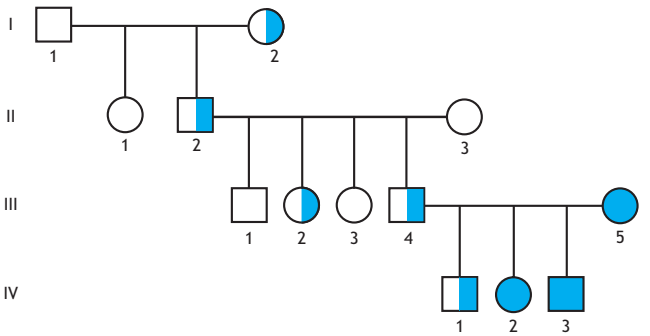


Figure 4–3. Pedigree showing pseudodominance because of a high frequency of asymptomatic heterozygotes. This raises the risk of homozygous conceptions to 50% for III-5. (Why?)

L. **Metabolic abnormalities** are common in individuals with AR disorders.

1. Recognition of this association led Archibald Garrod to coin the phrase "inborn errors of metabolism."
2. The absence *any* normal gene function may be lethal for homozygotes.
3. Carriers, being generally unaffected, can transmit the mutation widely.

PHENYLKETONURIA (OMIM 261600)

- *Phenylketonuria (PKU) is an inborn error of metabolism and an important genetic cause of* ***mental retardation.***
- *Deficiency of phenylalanine hydroxylase (PAH) leads to high blood phenylalanine levels, the proximal cause of nerve damage (Figure 4–4).*
- Many *mutations have been found in the* PAH *gene (similar to cystic fibrosis).*
- *The diagnosis is usually made in newborns by the Guthrie test for elevated blood phenylalanine levels, one of the most effective and widely used screening tests (Figure 4–5; see also Chapter 1).*
- *Children with PKU are treated by reducing* ***dietary phenylalanine,*** *thus lowering its blood level and minimizing nerve damage. Phenylalanine is an essential amino acid and so the diet requires careful attention and is quite limited.*
- *Dietary restriction in adults is being evaluated.*
- *Dietary care for mothers with* ***PKU during pregnancy*** *is complex because the fetus is at least a heterozygote and may be susceptible to damage from high phenylalanine levels even while requiring the amino acid for growth.*

M. Effective treatment of individuals with numerous AR conditions (many of which were formerly lethal in the young) has led to a growing population of older homozygotes whose clinical status and potential complications are unprecedented and challenging.

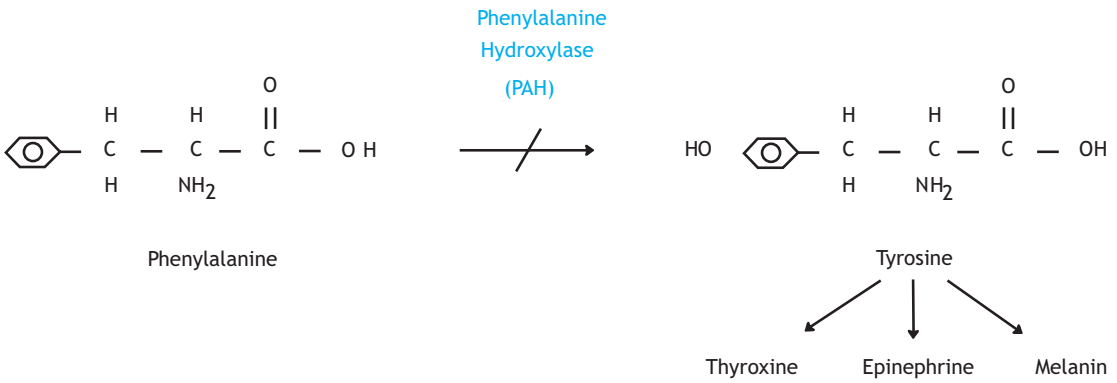


Figure 4–4. Metabolic consequences of phenylalanine hydroxylase deficiency in phenylketonuria include increased phenylalanine *upstream* of the defect and reduced tyrosine and its metabolites *downstream* of the defect.

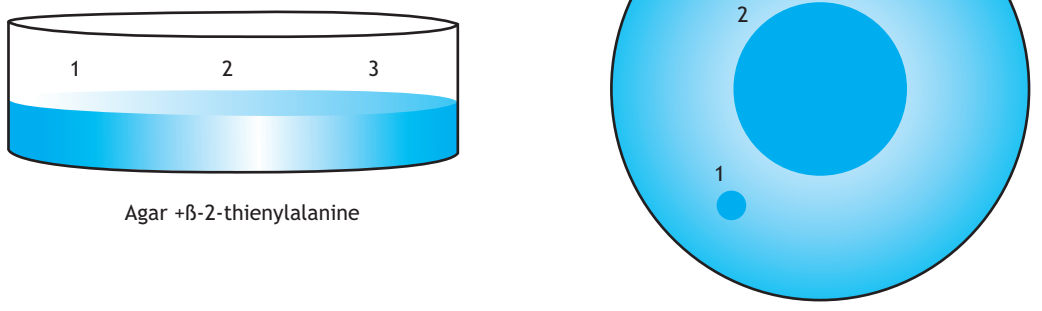


Figure 4–5. The Guthrie test. A spot of blood on filter paper establishes the potential for competition between phenylalanine in the blood and β_2-thienylalanine in the agar. High levels of phenylalanine permit bacterial growth (sample 2). Note that this finding is not diagnostic of phenylketonuria; it was designed to screen for high levels of phenylalanine.

CYSTIC FIBROSIS (OMIM 219700)

CLINICAL CORRELATION

- *Cystic fibrosis (CF) is the most frequently encountered AR condition in northern European and Caucasian populations.*
- *Defective membrane transport via chloride channels leads to gastrointestinal and pulmonary complications.*
- *The responsible gene is the* cystic fibrosis transmembrane regulator *(*CFTR*) on chromosome 7, and the most frequent mutation is loss of the entire codon for phenylalanine at position 508 (ΔF508).*
  –Unlike sickle cell disease (where all affected individuals have the same *mutation), CF has a wide range of underlying mutations and* ***compound heterozygotes*** *are common.*
  –The range of mutations at least partially underlies the broad spectrum of clinical presentations.
  –Precise molecular diagnosis may be difficult due to the multiple mutations, complicating, for example, prenatal studies (Figure 4–6).
- *The simplest assay is the sweat chloride test in skin secretions.*
- *Aggressive care of infants and adolescents has improved survival and quality of life.*
  –The diagnosis now is recognized in some older adults with chronic pulmonary disease.
  –Pulmonary care has prevented (or delayed) much chronic lung disease.
  –Infertility often is a problem in males (due to obstruction and resorption of the vas deferens during gestation).
- *The prominence of* CFTR *in the respiratory epithelium has suggested a site for experimental introduction of the exogenous normal CF gene (see Chapter 12).*

Figure 4–6. The CFTR gene contains a remarkable spectrum of mutations and polymorphisms. (Adapted with permission from Scriver CR, et al. *The Metabolic and Molecular Basis of Inherited Disease.* McGraw-Hill, 2000.)

II. Implications of the Carrier State

A. **Homozygotes for AR conditions** are generally **rare;** unless one works in a referral center or with an isolated population few will be encountered in most medical practices.

B. By contrast, **carriers** for AR conditions can be remarkably **frequent;** their frequency can be estimated by using the **Hardy-Weinberg formulation.**

TECHNICAL ILLUSTRATION

- *In a population without external constraints (also said to be at equilibrium) consider a gene with two alleles, p and q, such that the frequency of allele $p = 1 - q$.*
- *In a population where the frequency of homozygotes (two copies of allele $p = p^2$) is 1 in 10^4, $p = 0.01$ and $q\ (1 - p) = 0.99$.*
- *Based on the quadratic equation ($p^2 + 2pq + q^2 = 1$) the frequency of homozygous normals is $q^2 = .9801$. The frequency of carriers is $2pq = 198/10{,}000 \cong 2\%$.*
- *Thus, 1 in 50 individuals is a carrier for this allele even though the frequency of homozygotes is only 1 in 10,000!*

C. Several thousand AR conditions have been identified implying a wide distribution of carriers; many likely overlap.

D. Estimates of the extent of heterozygosity in normal individuals suggest at least 5–10 changes in each person (and likely far more).

E. Consequence(s) of the simultaneous presence of many heterozygous changes in multiple genes cannot be predicted currently.

1. For example, the epistatic effect of changes in the control of HbF expression in sickle cell disease involves at least two unlinked modifiers.

2. Wide variation due to a range of mutations and heterozygosity at multiple loci provides at least some explanation for different phenotypes but also complicates diagnostic studies and prognostication.

3. Such underlying variation(s) likely affect presentation and treatment of common disorders (eg, diabetes, hypertension, atherosclerosis, see Chapter 10).

CLINICAL PROBLEMS

A 24-year-old African-American woman has sickle cell disease (OMIM 603903) but has managed well, with only one crisis in the past 6 years. Both transfusion and hydroxyurea treatment have been suggested, but she has chosen not do try them yet. Her fiancée is from Nigeria and is unaffected. She is concerned about the risk of having an affected child if she becomes pregnant.

1. Which of the following statements is most likely to be true?

A. Because the woman has only mild disease, this couple can expect their child to be mildly affected.

B. Any child of this couple has a 25% chance of being a carrier.

C. The fiancée's carrier status should be determined.

D. Although her symptoms are now mild, the woman is likely to experience more problems later in life.

E. Any child of this couple has a 25% chance of being affected.

A graduate student has been studying DNA changes in an AR disease. He is surprised to find that he is a heterozygote for a mutation in the gene that he has identified in affected individuals. His wife is apparently healthy, and they have been thinking about starting a family.

2. Which of the following statements is most likely to be true?

A. The rarity of this disease makes it unlikely that his wife is a carrier.

B. If his wife does not have the mutation that he carries, their chance of having an affected child is very low.

C. His wife should be tested for all of the mutations that he has identified.

D. Any child of this couple has a 25% chance of being a carrier.

E. Because this is an AR disease, prenatal diagnosis is possible.

3. Being identified as a carrier of the ΔF508 mutation for cystic fibrosis (OMIM 261600) means that

A. An individual is likely to have Asian ancestry.

B. Clinical manifestations of CF will not occur.

C. A spouse should be studied for changes in the CF gene.

D. A sweat chloride test will be normal.

E. An individual has a 25% chance of having a child who is a carrier.

ANSWERS

1. The answer is C. The carrier status of the woman's fiancée is critical for realistic counseling. As noted in the text, individual manifestations may differ, likely reflecting modifier genes, making prognosis difficult (choice A). Having an affected mother means that any child must receive one mutant gene from her; hence any child's chance of being a carrier is at least 50% (choice B). The mother's mild involvement does not establish a later prognosis (choice D). The couple's chance of having a homozygous affected child depends on the father's genotype and could vary from 25% to ~0 (choice E).

2. The answer is C. It is important to note that the student does not yet know the spectrum and frequency of mutations in the AR disease he is studying. Although the disease is rare, his wife certainly could be a carrier (choice A, recall the Hardy-Weinberg relationship). This might be even more likely if they came from the same ethnic group. Not having identified the full spectrum of expected mutations makes it impossible to be certain that his wife is not a carrier (choice B). Even if his wife is not a carrier, he has

a 50% chance of passing his mutant gene copy to any child who will then be a carrier (choice D). Although prenatal diagnosis (choice E) may be possible, it cannot confidently be offered unless his wife's status is assured (recall the situation in CF). Artificial insemination by a donor is an option.

3. The answer is C. Excluding common mutations does *not* guarantee that a rare mutation will not be present in one's spouse. In addition, being identified as a "carrier for ΔF508" does not indicate that compound heterozygosity for it and a rare, alternative, mutation might not be present, and thus the spouse should be tested. The ΔF508 mutation, the most common, is more frequent in northern Europeans (see text), not Asians (choice A). Compound heterozygotes for CF mutations may have unpredictable manifestations or age of onset (choice B). The sweat chloride test will be normal *only* if the individual is not a compound heterozygote (choice D). Any child of such an individual has a 50% chance of inheriting the gene with the ΔF508 mutation (choice E).

CHAPTER 5
X-LINKED INHERITANCE

I. General Principles

A. Transmission of genes on the X-chromosome follows a pattern that has been called **diagonal** (Figure 5–1).

1. ***All* males** with the mutation will be **affected.**
2. Male-to-male transmission is impossible because a father must give his Y-chromosome to any son.
3. ***All* daughters** of an affected man must be **carriers** (indicated by the dot within the circle as shown).
4. Gene mapping and linkage studies often can be effective for X-linked conditions despite incomplete pedigrees (carrier status frequently can be inferred from patterns in other family members).

B. The **frequency of the phenotype** in males is the *same* as the **frequency of the mutant allele**.

C. The complement of genes on the X-chromosome has been quite stable in evolution, because there is little opportunity for meiotic exchange with the Y-chromosome.

HEMOPHILIA A (XQ28, OMIM 306700)

- *In patients with hemophilia, prominent soft tissue and joint bleeding can occur after minimal trauma due to defective blood coagulation.*
- *Mutation(s) in the gene for coagulation factor VIII can vary in position and extent. Some produce partially functional factor VIII, associated with less severe bleeding symptoms.*
- *Infusions of recombinant factor VIII protein can be prophylactic and therapeutic (see Chapter 12).*

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PD; XQ28, OMIM 305900)

- *G6PD, the first enzyme in the pentose phosphate pathway, is essential for protecting cells from oxidative damage.*
- *Reduced G6PD function makes erythrocytes susceptible to destruction (hemolysis) in the presence of oxidative stress. In general, older erythrocytes are more susceptible to hemolysis.*

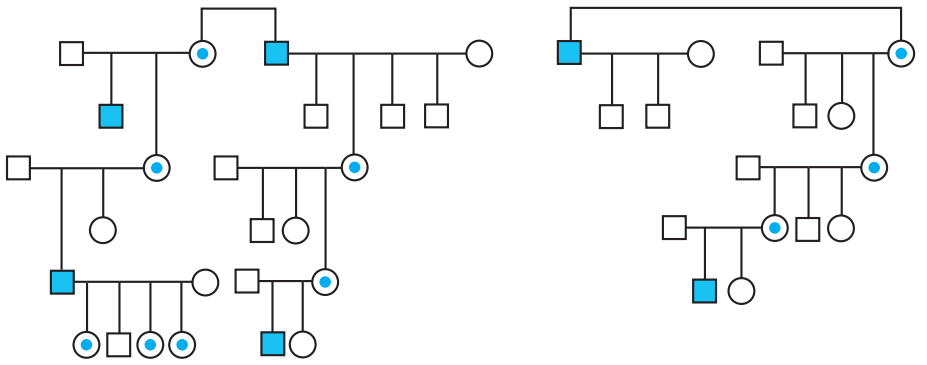


Figure 5–1. Pedigrees of X-linked traits showing diagonal transmission and female carriers. Note how several generations may appear unaffected if carrier females are not detected.

- *Severity of the hemolysis (and the resulting anemia) varies with the mutation as well as with the cause and severity of the oxidative exposure(s). Over 300 mutations are known with worldwide distribution.*
- *Affected males are susceptible to hemolysis after taking drugs that cause oxidative stress; Table 5–1 lists several examples of drugs to which these men are sensitive.*
- *G6PD deficiency is an example of gene–environment interaction and pharmacogenetics (see Chapter 11).*

Table 5–1. Drugs and chemicals causing hemolysis in G6PD-deficient individuals.

Acetanilide	Niridazole
Doxorubicin	Nitrofurantoin
Furazolidone	Phenazopyridine
Methylene blue	Primaquine
Nalidixic acid	Sulfamethoxazole

G6PD, glucose-6-phosphate dehydrogenase.
Reprinted with permission from Beutler E. Current concepts: glucose-6-phosphate dehydrogenase deficiency. *New Engl J Med* 1991;324:169.

II. The Female Carrier

A. Because of **random X-chromosome inactivation (Lyonization;** see Chapter 2) expression of the mutation in females can vary from prominent (uncommon) to absent depending on the gene involved and the assay used.

B. Symptomatic individuals often are referred to as **manifesting heterozygotes.**

C. Detecting carrier status is important for genetic counseling.

FRAGILE X MENTAL RETARDATION (Xq27, OMIM 309550)

CLINICAL CORRELATION

- *Fragile X mental retardation syndrome is a significant cause of mental retardation in males (average IQ ~40).*
- *The X-chromosome of affected individuals can undergo breakage at Xq27 in cell culture (hence the term "fragile").*
  –The gene FMR1 *contains a triplet repeat of a CCG trinucleotide in the 5' untranslated region that undergoes expansion (see also Chapter 3).*
  –No gene product is made in the presence of the expansion.
  –A threshold exists for expansion (see Figure 5–2).

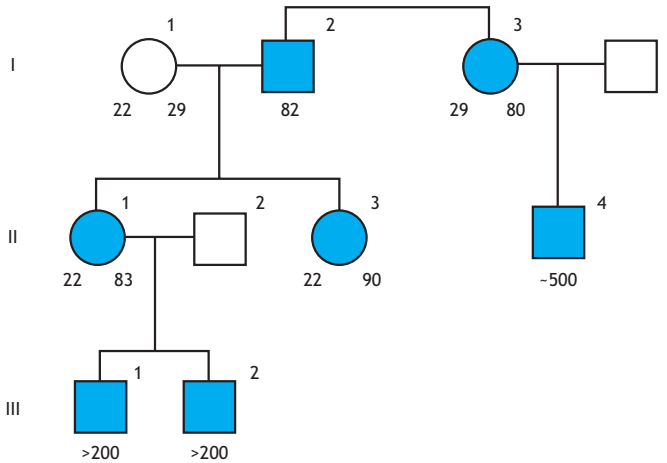


Figure 5–2. Transmission and expansion of the fragile X trinucleotide repeat. The numbers of triplet repeats are shown beneath the symbols. Note that the transmitting male (I-2) and his sister (I-3) have similar numbers of repeats in the premutation range but no symptoms. Sons of females with repeat numbers in the premutation range (II-4, III-1,2) received greatly increased numbers of repeats and showed clinical features of fragile X syndrome but there was no increase in repeat number in II-1,3, who received the premutation from their father. (Adapted from Caskey CT, et al. Triplet repeat mutations in human disease. *Science* 1992;256:784. Reproduced with permission from AAAS.)

- *Individuals with 50–200 repeats are said to have a premutation (susceptible to further expansion).*
  –Males with a premutation are clinically normal but transmit the change to all *of their daughters who have the* same *repeat numbers and are generally asymptomatic.*
  –Male and *female children of* females *with a premutation are at risk for receiving greatly increased numbers of repeats (250–4000, the full mutation), and males are fully symptomatic.*
- *Treatment is unavailable but family members may be tested to determine their repeat status for genetic counseling.*

III. X-Linked Dominant Inheritance

A. *Both* males and females are affected and able to transmit the trait (Figure 5–3).

B. **Females** transmit the trait to **50% of *both* sons and daughters.**

C. **Males** transmit to **all daughters** and ***no* sons.**

HYPOPHOSPHATEMIC RICKETS (XP22.2, OMIM 307800)

- *This is the most common form of rickets seen in the United States today. Patients are usually short with bowed legs and osteomalacia.*
- ***Defective renal phosphate transport** leads to phosphate wasting and low blood phosphate levels. Administering both phosphate and vitamin D often is effective for treatment.*

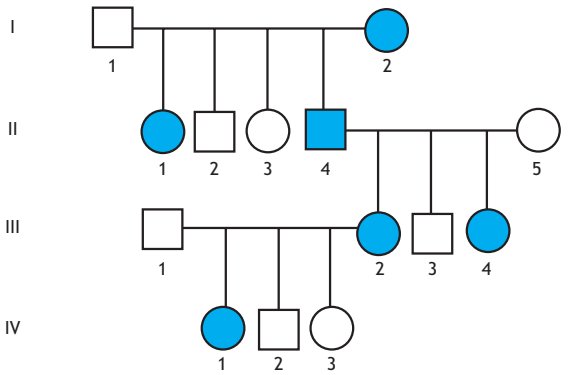


Figure 5–3. Pedigree showing X-linked dominant inheritance.

CLINICAL PROBLEMS

A healthy 41-year-old man learns that his retarded brother, who died at age 37, had fragile X mental retardation syndrome. The man and his wife, age 36 years, have a healthy daughter (age 10) and would like to have another child. They seek the advice of their physician.

1. The physician would most likely advise this couple that

A. Because the husband does not demonstrate any signs of fragile X syndrome, he is unlikely to pass on the trait to his offspring.

B. Any son would have a 50% chance of manifesting the fragile X mental retardation phenotype.

C. Their daughter is at minimal risk given the husband's status.

D. Their daughter might be affected and should be tested for the fragile X phenotype.

E. The husband should be tested for the fragile X phenotype.

A 37-year-old woman comes to the health clinic for the first time. She considers herself healthy, but the physician who examines her finds multiple bruises. The patient recalls that these have come and gone for years and does not appear to be concerned about them. Her family history includes a deceased great uncle (on her mother's side) who was thought to have hemophilia. Her own brother (aged 42) is healthy. The woman has two healthy daughters and has considered another pregnancy.

2. Which of the following statements is most likely to be true?

A. The woman's mother should undergo coagulation studies.

B. The woman's nieces may be carriers of hemophilia.

C. The woman should undergo coagulation studies.

D. The woman's asymptomatic daughters are unlikely to benefit from coagulation studies.

E. The woman should receive factor VIII replacement therapy.

A 17-year-old girl was diagnosed at age 14 with Turner syndrome and has responded well to exogenous hormone treatment. She is doing well in school but recently has had trouble with sports. She tells the physician that running leads to rapid fatigue, and recently she fell. A maternal great uncle died in his late 20s after spending almost a decade in a wheelchair, but the rest of her family history is unrevealing.

3. What is the most likely cause of the patient's difficulties with physical activities?

A. Poor coordination; she should be advised to spend time each day practicing the activities that cause difficulty.

B. Hormone treatment; weight reduction through dietary control should be advised.

C. Poor shoe wear from hallux valgus; orthotics may be beneficial.

D. Primary muscle disorder; the patient's muscle enzyme levels should be checked.

E. Unstable hips due to short stature; strengthening exercises should be prescribed.

ANSWERS

1. The answer is E. The husband may have inherited a premutation that would not be large enough to produce symptoms (choice A). Because of X-chromosome transmission patterns, there is no risk for any son the couple might conceive (choice B). However, the husband could have passed the premutation to his daughter, who, although appearing unaffected, would be a premutation carrier and at risk for having a repeat expansion and an affected son (choices C and D). Given the wife's age, prenatal screening would be recommended under any circumstances.

2. The answer is C. The woman may be a manifesting heterozygote for hemophilia. As discussed in the text, female carriers can have a broad range of presentations for X-linked conditions. If the woman's brother were affected, he likely would have shown symptoms at an earlier age. Coagulation studies in her asymptomatic mother (choice A) would add no information. Nieces (daughters of her asymptomatic brother) are unlikely to be carriers because males show the trait if it is present (choice B). If the woman is a carrier, her daughters *each* have a 50% risk of being carriers and, hence, would be candidates for coagulation screening (choice D). If the woman becomes pregnant, screening appropriate for her age should be performed, and possibly factor VIII gene studies for the fetus, depending on her own status; there is no indication for replacement factor VIII treatment (choice E).

3. The answer is D. With an affected male relative on her mother's side (providing passage through an asymptomatic carrier), this girl may be developing Becker muscular dystrophy (a less severe form than Duchenne and consistent with the later age of onset). Other X-linked neurologic and muscular disorders also might be present in this unusual situation (although recall that many individuals with Turner syndrome are XO/XX mosaics; see Chapter 2). Testing should show features of myopathy. Most teens with Turner syndrome adapt to physical activities well with reasonable joint integrity and coordination (choices A and E) and little likelihood of developing severe hallux valgus (choice C). Weight gain should not develop from carefully monitored hormone replacement (choice B).

CHAPTER 6
MITOCHONDRIAL DYSFUNCTION

I. General Principles

A. The **mitochondrial chromosome** is a double-stranded, circular DNA (16,569 bp) encoding 22 transfer RNAs (tRNAs), 2 ribosomal RNAs, and 13 proteins essential for **oxidative phosphorylation** (Figure 6–1).

B. Each **mitochondrion** (of the hundreds in any cell) contains *at least* one copy of the DNA.

1. When all mitochondrial DNAs in the same the cell are the same, the cell is said to be **homoplasmic**; when they differ the cell is **heteroplasmic**.

2. The **distribution** of mitochondrial DNA(s) may vary among cells and may change with aging.

C. Mitochondria in the egg outnumber those in sperm by 1000-fold and sperm mitochondria likely are destroyed in the egg cytoplasm. Thus, traits referable to mitochondrial DNA are always **transmitted from the mother,** giving a characteristic pedigree structure, sometimes called **cytoplasmic inheritance** (Figure 6–2).

1. Either sex can be affected.

2. Males cannot (or very rarely) transmit the trait.

II. Mitochondrial Physiology

A. Defective mitochondrial function often affects the energy supply of the cell, and thus **nerves and muscles** often show problems first because of their high energy requirements (Table 6–1).

B. **Mutations** in mitochondrial DNA develop up to 10-fold faster than those in nuclear DNA, likely due to local accumulation of reactive oxygen species during oxidative phosphorylation.

C. Integrity of oxidative phosphorylation declines with aging in somatic cells, presumably due to accumulated mutations in mitochondrial DNA (eg, a 5-kilobase [kb] deletion often accumulates in hearts with aging but rarely is seen before age 40).

D. ***Most*** **mitochondrial proteins** are encoded by ***nuclear*** **genes**.

1. Mutations affecting mitochondria can thus arise in *two* genomes.

2. The site of mutations usually can be distinguished by pedigree pattern(s).

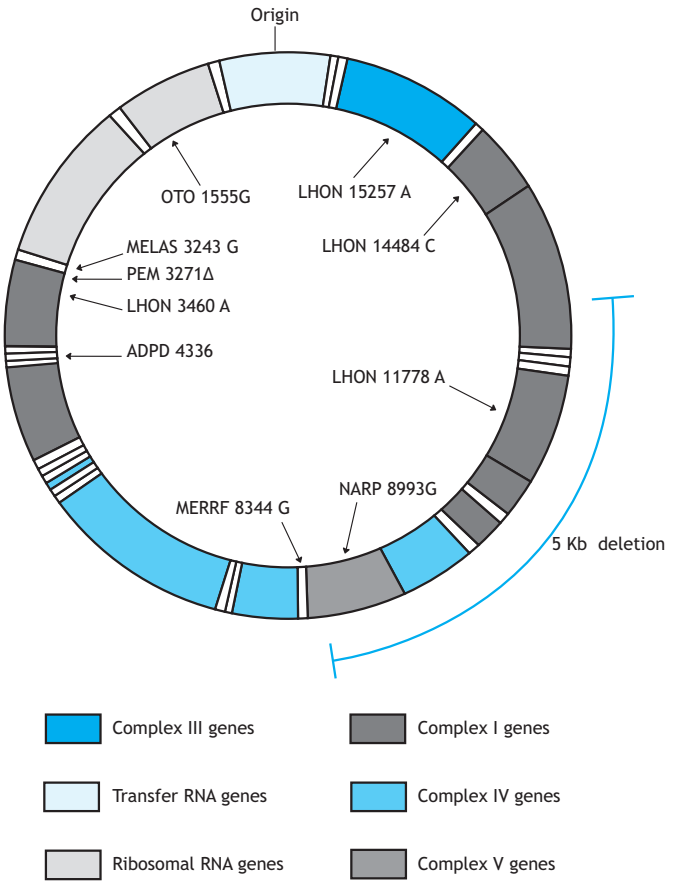


Figure 6–1. Mitochondrial DNA map showing gene locations and mutations identified for specific phenotypes. The 5-kb deletion associated with ocular myopathy is also shown. (Adapted from Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1992;256:628. Reproduced with permission from AAAS.)

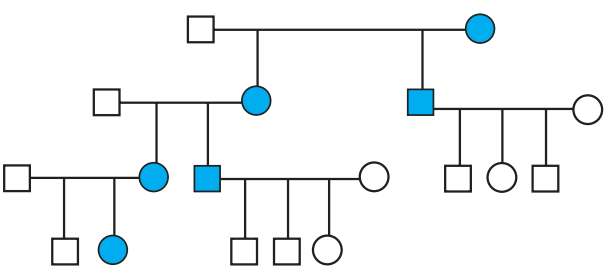


Figure 6–2. Pedigree showing that transmission of a trait encoded on mitochondrial DNA occurs only through females.

Table 6–1. Disorders with defective mitochondrial function and mutations.

Disorder	Mutation(s)	OMIM
LHON	~18	535000
Leigh syndrome NARP syndrome	Multiple (also X- linked and autosomal) T→G 8993	516060 551500
Aminoglycoside ototoxicity	A→G 1555 G→A 7444	580000
tRNA Mutations		
MELAS syndrome, also diabetes mellitus type 2 and hearing loss	Leu tRNA A→G 3243 T→C 3271	540000
MERRF syndrome	Lys tRNA A→G 8344	545000
Structural DNA Changes		
Kearns-Sayre syndrome Ocular myopathies Inherited cardiomyopathies	Deletions, duplications, rearrangements	530000

LHON, Leber hereditary optic neuropathy; MELAS, myopathy, encephalopathy, lactic acidosis, and stroke; MERRF, myoclonic epilepsy and ragged red fibers; NARP, neurogenic muscle weakness, ataxia, and retinitis pigmentosa; OMIM, Online Mendelian Inheritance in Man number.

LEBER HEREDITARY OPTIC NEUROPATHY (LHON, OMIM 535000)

- *LHON usually presents as optic nerve disease in young adults; however, peripheral neuropathies and cardiac conduction changes also occur.*
- *Inheritance is through females but family studies show more affected males than females.*
- *Multiple mitochondrial DNA mutations have been described. More than one may be found in an individual.*

CLINICAL PROBLEMS

A 76-year-old woman has been feeling "wobbly" for several months and wonders if she has had a "mini-stroke." She states that her brother had "muscular dystrophy" and died many years ago, at age 45. Her parents died in an accident when both children were young. Her three children, now in their 50s, are concerned about her health but not about their own. Physical examination shows an unsteady gait, weakness in both legs, and poor reflexes.

1. Based on the history and physical findings, the patient most likely

A. Has a late-onset recessive disorder without risk to her children

B. Should immediately begin treatment for hypertension

C. Should undergo a muscle biopsy with mitochondrial DNA analysis

D. Should undergo stress testing

E. Should be tested for mitochondrial DNA changes in peripheral leukocytes

A 20-year-old male college student visits the health center seeking information and advice because his 27-year-old sister was recently diagnosed with "Leber eye disease." He has never heard of this disease.

2. The physician's most likely response would be that

A. This problem is commonly diagnosed in women in their 20s.

B. Because he is only a bit younger than his sister and is asymptomatic, his risk for the disease is low.

C. His sister will need to undergo laser photocoagulation to correct the defect.

D. His sister's children have a 50% risk of developing this disease.

E. His sister should have mitochondrial DNA studies to confirm the diagnosis.

A physician is called by the nurse at a summer camp who is concerned because a 10-year-old boy is having difficulty walking. The nurse has been unable to reach the boy's parents and wonders if the boy's problems might be caused by myotonic or Duchenne muscular dystrophy. The boy states that he "isn't much of an athlete," but that his parents and both of his maternal uncles enjoy participating in recreational sports.

3. The physician would most likely advise that

A. The child's age and absence of affected individuals in earlier generations makes myotonic dystrophy an unlikely diagnosis.

B. The mother's age may be an important factor in this case.

C. The child should be referred to an ophthalmologist for vision testing.

D. The absence of affected males makes Duchenne muscular dystrophy an unlikely diagnosis.

E. The child should be advised to avoid contact sports.

ANSWERS

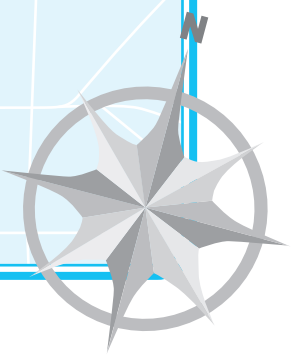
1. The answer is C. Microscopic examination of muscle integrity (and mitochondrial DNA) may help determine the patient's problem, because her findings do not suggest a mini-stroke. Different levels of heteroplasmy for a mitochondrial mutation could explain the situation and provide important counseling information for family members. This presentation of a late-onset autosomal recessive condition would be unusual and considering this as the answer would eliminate consideration of the risks to her children

(choice A). Hypertension and vascular disease (choices B and D) were not suggested by her history. Because any mutation has likely been acquired and may be limited to muscle, studying leukocyte mitochondrial DNA (choice E) may be misleading.

2. The answer is E. LHON is a rare condition, the symptoms of which are often misinterpreted. The definitive study is mitochondrial DNA analysis for the affected sister. If mutations are present, the patient's brother and children also should be examined. The disorder is not sex limited (choice A), and transmission to her children could exceed 50% (choice D). Such conditions are variable in presentation, so her brother's asymptomatic status should not exclude the diagnosis for him (choice B). Laser treatment (choice C) is not helpful for this neurologic problem.

3. The answer is C. From the available information it is difficult to distinguish the possibilities, but finding vision problems would make a mitochondrial mutation more likely. Myotonic dystrophy has autosomal dominant transmission (see Chapter 3), and family members in earlier generations are active, thus ruling out choice A. Maternal age (choice B) is not related to muscular dystrophy. Having two asymptomatic maternal uncles reduces (but does not totally eliminate) the likelihood of Duchenne muscular dystrophy (choice D). Choice E should only be considered once the patient's clinical status is established and may not be helpful.

CHAPTER 7
CONGENITAL CHANGES



I. Spectrum of Changes

A. *Congenital* means present at birth.
- **1.** Approximately 1 in 50 newborns has a recognizable physical variation, ranging from life-threatening to trivial.
- **2.** Some changes may not be discovered until later in life, despite having been present earlier.

B. *Any* organ system may show congenital changes.

C. Congenital changes raise several concerns.
- **1.** What is the extent of the changes?
- **2.** What can be done for the individual?
- **3.** What is the recurrence risk?

II. Approach

A. Congenital changes can be complex but are approached most easily through responses to several questions.

B. Is there a **family history** of a related problem?
- **1.** Because of **pleiotropy** (recall Chapter 3), recognizing at least *some* manifestations of a syndrome in a parent can clarify the diagnosis for a child.
- **2.** **Triplet repeat disorders** may be more prominent in children of an affected parent (see Chapters 3 and 5).
- **3.** The **pedigree** may identify potential carriers of an X-linked disorder, but if no affected males have been born recently the mother's carrier status may be unknown (see Chapter 5).

C. Were any **maternal problems** (illnesses, medication reactions, etc) noted during pregnancy or labor?
- **1.** The list of **teratogenic drugs** is long, frequently updated, and available online (Table 7–1 lists several examples). Some individuals may be particularly sensitive to certain drugs (see Chapter 11).
- **2.** Early **trauma or radiation** may have been forgotten.
- **3.** Recreational **drug use or alcohol abuse** is important; the fetal alcohol syndrome is usually recognizable (Table 7–2).
- **4.** **Rubella** and other **infectious problems** remain important causes of congenital problems in unprotected populations.

Table 7–1. Drugs and other exposures associated with congenital heart and vascular disease.

Drugs	Maternal Disorders
Alcohol	Connective tissue disease
Amphetamines	Diabetes mellitus
Carbamazepine	Phenylketonuria
Lithium	Rubella
Phenytoin	Thyroid disease
Retinoic acid	
Thalidomide	
Trimethadione	
Valproic acid	

D. Is there any history of **nutritional deprivation** or abnormality?

1. As discussed in Chapter 4, phenylalanine levels should be monitored in children of mothers with **phenylketonuria (PKU)**.

2. Malnutrition or vitamin deficiency may not have been noticed in the mother but may harm the developing fetus.

E. Can the observed changes be related to a **developmental stage?**

1. A specific finding may identify a critical period in fetal development; for example, a cause of cleft palate must have acted *before* palatal shelf closure in the fourth fetal month.

2. By contrast, scoliosis and microcephaly can be associated with change(s) occurring through much of fetal life.

F. What is the **spectrum of organ involvement?**

1. If a single organ (eg, skin) shows a change, is it limited to one area? For example, is a single dermatome affected?

Table 7–2. Characteristics of the fetal alcohol syndrome.

General Features	Physical Findings
Severity may be dose related	Microcephaly
Early pregnancy loss	Midfacial hypoplasia
Growth deficiency (pre- and postnatal)	Flat nasal bridge
Psychomotor retardation (common cause of mental retardation)	Epicanthal folds
	Microphthalmia
Coordination problems and hyperactivity	Upturned nose
	Joint contractures
	Congenital heart disease

2. If more than one organ or system is involved, can the changes be related pathophysiologically? (See B,1, earlier.)

G. Do **laboratory studies** add information?
1. Echocardiograms can clarify heart defect(s).
2. Hematologic changes may be associated with several syndromes.
3. Blood or urine metabolite levels may reveal a metabolic anomaly.
4. Chromosome studies may identify abnormalities.

H. Are the findings consistent with a **syndrome?** If so
1. The inheritance and recurrence pattern(s) can be predicted.
2. Later changes may be anticipated.
3. Specific treatment may be available.

CLUBFOOT

- *One of the most frequent malformations (0.6–6 per 1000) visible at birth, clubfoot involves a spectrum of changes that are usually apparent by inspection of the feet, ankles, and lower legs.*
- *Most instances are related to intrauterine pressure or positioning; clubfoot also may be associated with inherited syndromes (Table 7–3), chromosomal disorders, and drug exposures.*
- *Orthopedic intervention usually is effective.*

Table 7–3. Syndromes with clubfeet.

Drug induced	Aminopterin Methotrexate
Chromosomal	Trisomies 13 and 18 Deletions (4p, 9p, 13q, 18q) Duplications (3q, 9p, 10q)
Mendelian	Cerebrohepatorenal (OMIM 214100) Diastrophic dwarfism (OMIM 22600) Ehlers-Danlos (OMIM 130000) Larsen (OMIM 245600) Multiple pyerygium (OMIM 265000) Oral-facial-digital (OMIM 311200) Trismus-pseudocamptodactyly (OMIM 158300)

OMIM, Online Mendelian Inheritance in Man number.

Table 7–4. Drugs and environmental factors associated with CHD.

Drugs	Maternal Environment
Amphetamines	Diabetes
Diphenylhydantoin	Infections (see text)
Lithium	Phenylketonuria (PKU)
Maternal consumption of alcohol	Radiation
Retinoic acid	Thyroid disease
Trimethadione	
Thalidomide	
Valproic acid	
Warfarin	

CONGENITAL HEART DISEASE

- *Approximately 1% of liveborn US infants are diagnosed with congenital heart disease (CHD) each year; in a few affected individuals, the disease is not identified until later in life.*
- *Maternal* **rubella** *is an example of a cause of CHD (septal defects and patent ductus arteriosus) that does not involve genetic considerations and which has largely been eliminated through maternal screening and immunization.*
- **Drugs** *are recognized causes of CHD (Table 7–4); thalidomide also causes limb shortening (phocomelia).*
- *Care of mothers with* **PKU** *is a challenge (see Chapters 4 and 12). Maternal* **diabetes mellitus** *(preexisting type 1 or 2 and gestational) is common.*
- **Chromosome abnormalities** *are associated with CHD (Table 7–5).*
  –Many can be detected on a **fetal karyotype** *(see Chapters 1 and 2) as well as by studies of affected newborns.*
  –Screening for **Down syndrome** *is described in Chapters 1 and 2.*
- **Mendelian syndromes** *can include CHD (Table 7–6).*
  –A familial pattern aids both diagnosis and prognosis.
  –The molecular bases for many of these syndromes have been determined, permitting prenatal diagnosis and counseling.
- *Despite considering the specific possibilities noted above, an underlying diagnosis often cannot be established.*

Table 7–5. Chromosomal syndromes associated with CHD.

Trisomies—13, 18, 21
Deletions—4p-, 9p-, 4q-, 11q-, 13q-, 18q-, 22q11.2
Duplications—3q, 10q, 15q
X-chromosome changes—XO (Turner syndrome), XXXY, XXXXX
Triploidy

Table 7–6. Mendelian syndromes associated with CHD.

Syndrome	OMIM
Alagille	118450
Beckwith-Wiedemann	130650
Carpenter	201000
Cornelia de Lange	122470
DiGeorge	188400
Ellis-van Creveld	225500
Fanconi	227650
Holt-Oram	142900
Ivemark	208530
Myotonic dystrophy	160900
Noonan	163950
Rubenstein-Taybi	180849
Smith-Lemli-Opitz	270400
Thrombocytopenia-absent radius	274000
Velocardiofacial	192430
Weil-Marchesani	227600
Williams	194050
Zellweger	214100

- *The prominence of CHD in the absence of identifiable causes has led to compilations of* **empiric risk figures** *(Table 7–7).*
  –Such data often are the basis for genetic counseling, but they also imply contribution(s) of multiple (currently unidentified) genes to common conditions (see Chapter 10).
  –Further research on CHD likely will identify subgroups with risks considerably different from those in Table 7–7.
- *Treatment of CHD often is possible, increasing the value of recurrence risk prediction(s) and genetic counseling for affected individuals and subsequent pregnancies.*

Table 7–7. Empiric risk figures for CHD.

Condition	Suggested Risk (%) One Affected Sibling	Suggested Risk (%) One Affected Parent
Ventricular septal defect	3	4
Patent ductus arteriosus	3	4
Atrial septal defect	2.5	2.5
Tetralogy of Fallot	2.5	4
Pulmonic stenosis	2	3.5
Coarctation of the aorta	2	2
Aortic stenosis	2	4

CLINICAL PROBLEMS

A 9-year-old girl is brought to the health clinic by her parents because of a pigmented spot on her cheek that has been present since birth. No one in the family has anything like it, and they have been told that it is called a "stork bite." The girl is active and apparently healthy. Examination shows seven smaller, similar spots elsewhere with no particular distribution.

1. Which of the following actions is the most appropriate next step?

A. Laser treatment to remove the spots

B. No treatment until after adolescence, because more spots may develop.

C. Genetic tests to identify the likelihood of transmitting the condition.

D. Ophthalmologic examination to identify other manifestations of the disease

E. Psychiatric counseling to help with body image issues

A 27-year-old man sees the physician for a routine checkup. His brother has just been told that he has a heart murmur. Both brothers have always been healthy and active, and their parents are well. There is no history of heart disease in the family for several generations.

2. Which of the following statements is most likely to be true?

A. The brother should have an echocardiogram study.

B. The patient's chance of having a murmur is only about 3%.

C. The patient has a 50% chance of having a murmur.

D. The patient should have an echocardiogram study.

E. The brother has a 50% risk of having a child with a murmur.

A physician evaluating health problems among residents of remote Andean villages has discovered six children in one small village who have clubfoot deformities. They appear well otherwise.

3. The most likely next step would be to

A. Order studies of the source of the water supply

B. Put in a request for podiatry services

C. Evaluate the prevalence of gait problems in the village and try to identify a dominant trait

D. Discuss obstetric practices with the local midwife

E. Request a visit by a nutritionist

ANSWERS

1. The answer is D. The discovery of the additional café-au-lait spots is inconsistent with a "stork bite" (a common vascular lesion usually found on the forehead). Finding multiple pigmented spots suggests that the girl may have neurofibromatosis (OMIM 162200; see Chapter 3). This can be confirmed by having an ophthalmologist look for Lisch nodules. It is not unusual for tumors to appear later. The physician's careful examination has thus changed the child's diagnosis as well as the prognosis and recurrence risk prediction. Removing individual spots (choice A) is rarely indicated and is not always simple. There is no specific treatment for neurofibromatosis at any age (choice B) and no way to anticipate specific later developments. Genetic testing (choice C) may be complex (recall that the gene is very large with multiple known mutations) and would not change the simple recurrence risk of 50% for an autosomal dominant (AD) trait. Psychiatric counseling (choice E) is rarely indicated, and these children generally adapt well to the changes.

2. The answer is A. This often is the presenting picture for isolated CHD, and the first step is to define the lesion in the affected person (ie, the patient's brother). No statements can be made about the patient's status (choices B, C, and D). Because no details are known about the brother's diagnosis, suggesting a high transmission risk of 50% (choice E, which could be the case for an AD trait), is inappropriate (recall Table 7–7).

3. The answer is D. As noted in the text, clubfoot deformities are common and most frequently are associated with intrauterine developmental and obstetric problems. It would not be surprising for an isolated community to have recurrences. Exogenous causes such as contaminated water (choice A), likely to be rare in the mountains, or nutritional deprivation (choice E) are unlikely. Rather than podiatry (choice B), orthopedic services are needed. Given the high frequency in a single generation and no evidence for transmission (which would be expected for at least some of the cases if an AD pattern were present) a search for an AD trait will likely be futile (choice C).

CHAPTER 8
GENETICS AND IMMUNE FUNCTION

I. Self versus Nonself

A. Distinctions between self and nonself are mediated by cellular and protein components of the immune system.

B. All of these components are subject to genetic variation.

II. Major Histocompatibility Complex (MHC)

A. General Concepts

1. Proteins of the MHC determine much of the molecular individuality of human cell surfaces.

2. As the name implies, many details of the MHC have been elucidated by tissue transplantation studies (see later discussion).

3. Genes of the MHC are clustered on **chromosome 6** (Figure 8–1).

a. **Three groups** (or **classes**) are recognized.

b. There is enormous polymorphism within the MHC but very little recombination, and thus this region contains many useful **genetic markers** (recall Chapters 1 and 2).

c. Many of the encoded proteins have been defined by their **reactivity to antibodies,** hence the proteins *themselves* are often referred to as **antigens,** and their presence on leukocytes has led to the term **human leukocyte antigen(s) (HLA).**

4. Based on structural and sequence similarities, genes of class I and II antigens, the T-cell receptor, and immunoglobulins often are grouped as the **immunoglobulin gene superfamily** (see later discussion).

B. Class I

1. **Class I antigens** are found on the surface of ***all*** **nucleated cells** and comprise two proteins, a large (44 kDa) molecule encoded by a class I gene and **β_2-microglobulin** (small [~12 kDa], invariant, and encoded on chromosome 15) (Figure 8–2).

2. The α_1 and α_2 **domains** form a **binding site for short polypeptides;** these regions have the most variation.

3. There is usually a short (~9 amino acid) polypeptide in the binding site on the cell surface derived from intracellular antigen processing.

4. There are 15 class I genes, but only three types—**A, B, and C**—are considered here (and the C type is not as variable).

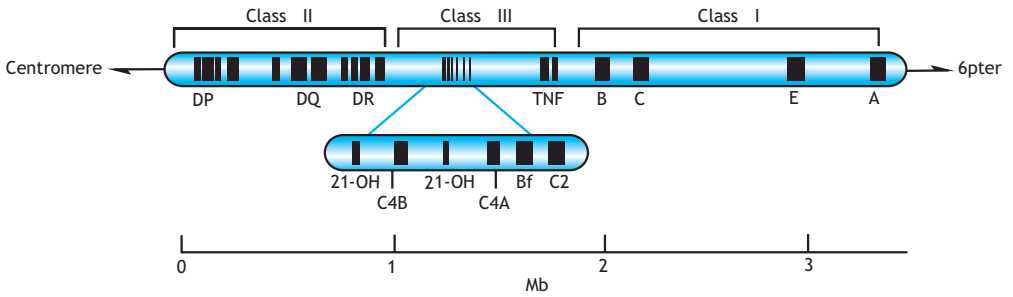


Figure 8–1. Genetic map of MHC cluster on chromosome 6 showing the three gene classes. As noted in the text, the class III gene region contains other genes, including those for 21-hydroxylase (21-OH), complement factors (C2, C4A, C4B), properdin factor B (Bf), and tumor necrosis factor (TNF) -α and -β.

C. Class II

1. **Class II antigens** are found on **T cells,** activated **B cells,** and **macrophages.**
2. Instead of containing β_2-microglobulin, these antigens are heterodimers with **α** (30–32 kDa) and **β** (27–29 kDa) **protein chains** (see Figure 8–2).
3. Most of the polymorphisms in these proteins reside in the α_1 **and** β_1 **regions.**
4. Although most class II antigens on the cell surface are bound to small polypeptides, this does not appear to be essential; however, having a bound polypeptide appears to increase stability.
5. There are ~23 class II genes, organized into **three clusters (DP, DQ,** and **DR)** called **isotypes** (see Figure 8–1).
   a. Most class II antigens contain α and β chains of the *same* isotype.
   b. Class II **DR antigens** have only β chain polymorphisms (and, hence, an individual can have only two types of DR antigens, one from each parent).
   c. **DP and DQ antigens** can be of four types by mixing the chains in *cis* (maternal α/maternal β, paternal α/paternal β) or *trans* (maternal α/paternal β or paternal α/maternal β).

D. Expression of Classes I and II

1. Genes of the class I and II families are **codominant,** meaning that an individual can express two alleles of class I and class II DR genes (one from each copy of parental chromosome 6).
2. Combined with the possible four types of class II DP and DQ antigens, there is a theoretical possibility of having > 10^7 combinations, but many of these have not been found.
3. The **high degree of polymorphism** within MHC genes and the **low level of recombination** have important genetic (and immunologic) consequences.
   a. A parent and child share one class I haplotype.
   b. There is a 1 in 4 chance that any two siblings will have inherited the *same* class I haplotype (and are thus said to be "HLA identical").
   c. HLA haplotypes are central to the biology of **tissue transplantation.**

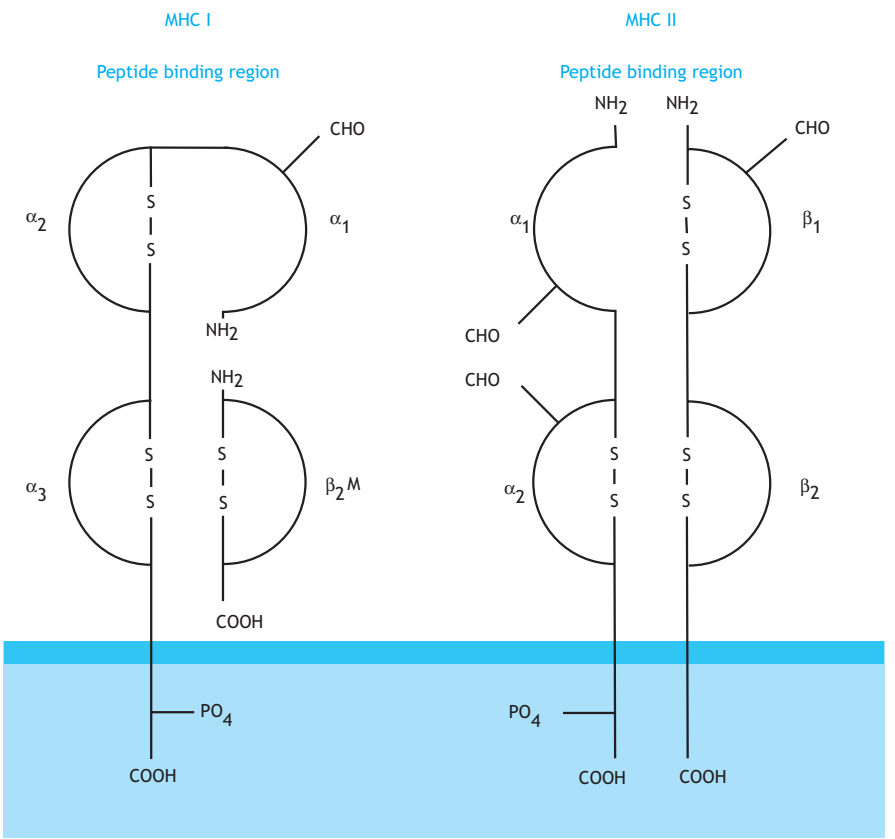


Figure 8–2. Structure of major histocompatibility complex (MHC) molecules. In class I MHC, specific polypeptide binding occurs in the groove between the α_1 and α_2 domains; β_2-microglobulin is an important part of this complex. In class II MHC, specific polypeptide binding occurs between α_1 and β_1 regions as shown, and β_2-microglobulin is not present.

d. The **distribution** of HLA haplotypes is **not random** (given the low likelihood of recombination in the cluster).
(1) Population-specific HLA haplotypes are common.
(2) For example, HLA A24 is found in Caucasians but not in Asians or Africans.

E. Class III

1. **Class III genes,** although found in the MHC cluster, **are *not* HLA genes.**
2. Complement components C2, C4 and B, and tumor necrosis factor (TNF) -α and -β are related to immune responses and defense.
3. All class III genes show **strong linkage to HLA genes.**

HEMOCHROMATOSIS (OMIM 235200)

- *The* HFE *gene is found in the class III region.*
- *Individuals with* HFE *mutations are at risk for iron accumulation and its associated toxicity; treatment is based on iron removal.*
- *Not surprisingly, the* HFE *gene can be traced by HLA linkage studies, but it also can be assayed directly.*

III. HLA–Disease Associations

A. Specific HLA alleles have been associated with many diseases. An obvious association is between HLA alleles and hemochromatosis (OMIM 235200), but this is due to the tight linkage (see preceding discussion) rather than any pathophysiologic connection.

B. In most cases, the pathophysiology has not been clarified by finding an association although some sort of immunologic relationship is suspected.

C. In some cases, finding a relationship with HLA can help clarify the diagnosis.

D. The association is usually expressed as **relative risk (RR)** expressed as RR = ad/bc, where a = the frequency of affected individuals with the given HLA allele, b = the frequency of affected individuals lacking the given allele, c = the frequency of the given HLA allele in *un*affected individuals, and d = the frequency of unaffected individuals lacking the given allele.

E. Selected examples of HLA–disease associations are shown in Table 8–1. Several features are common to these disorders.

1. The HLA associated risk is *relative* and thus *many* individuals carrying these (and other) HLA alleles do *not* develop these disorders.
2. Familial associations are recognized (see also Chapter 10) but the inheritance patterns are *not* clearly mendelian.
3. Penetrance is weak.
4. An association with **autoimmunity** is frequent.
5. Most of these disorders are **chronic** and, except for diabetes mellitus type 1, have a late onset and little effect on reproductive fitness.
6. Identifying an HLA association can at least *suggest* a basis for the disorder and can help distinguish different types of conditions with similar clinical presentations.
   - **a.** Approximately 95% of individuals with type 1 diabetes mellitus have either DR3 or DR4 (see Table 8–1).
   - **b.** No association with these haplotypes is seen for type 2 diabetes.

TECHNICAL ILLUSTRATION

- *Interpreting HLA associations can be difficult.*
- *The RR for an individual with HLA B27 to develop ankylosing spondylitis (AS) is ~90, but…*
- *HLA B27 is present in about 8% of Caucasians and…*
- *Only ~4% of individuals with HLA B27 will develop AS.*
- *Thus, HLA B27 alone is* not *sufficient to cause AS. Other contributing factor(s) have not been identified; however, rats transgenic for human HLA B27* do *develop a form of spondyloarthropathy, consistent with an important relationship.*

Table 8–1. Examples of HLA—disease associations.

Disease	HLA Allele	Relative Risk
Class I		
Ankylosing spondylitis	B27	90
Reiter syndrome	B27	37
Behçet syndrome	B51	16
Psoriasis vulgaris	Cw6	13
Class II		
Goodpasture syndrome	DR2	13
Sjögren syndrome	DQB1*0201	12
Myasthenia gravis	DR3	7
Alopecia areata	DQw7	6
Type I diabetes mellitus	DR4 DR3 DR3 or DR4	6 5 15
Graves disease	DR3	3

IV. Immunoglobulins

A. Structure

1. **Formation** of immunoglobulins (Ig) is complex, involving recombination, mutation and glycosylation.
2. The Ig molecule contains both **light** (short) and **heavy** (long) **chains** (Figure 8–3).
3. Each type of protein chain is based on the so-called **Ig domain.**
   a. The Ig domain is long, comprising ~100 amino acids, and includes a loop formed by disulfide (–S-S–) bonds between two cysteines.
   b. The Ig domain is evolutionarily old and appears in many proteins related to the **cell surface, defense,** and **cell-cell adhesion.**
   c. As shown in Figure 8–3, heavy chains contain four Ig domains; light chains, two.
   d. The Ig domain has a characteristic three-dimensional structure.

B. Heavy Chains

1. Heavy chains are encoded in a large (~1.2 Mb) region of **chromosome 14.**
2. There are **five major types** of heavy chains (designated **M, G, A, E,** and **D**); G and A are further divided into four and two subclasses, respectively.

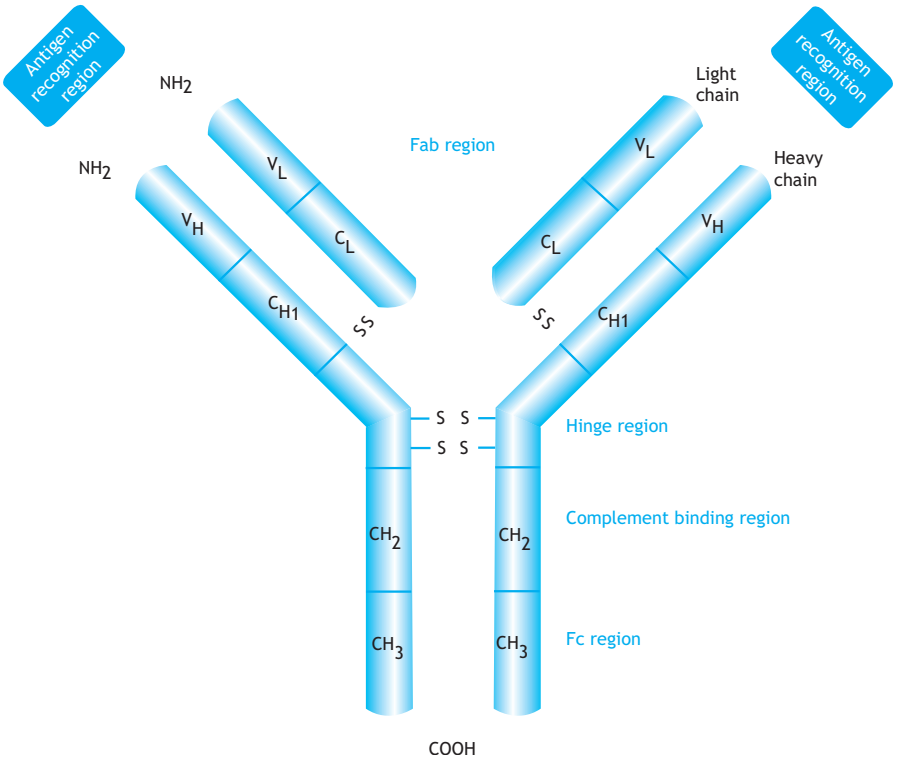


Figure 8–3. Simplified view of immunoglobulin G (IgG) molecule, showing two heavy and two light protein chains. Antigen recognition occurs at the *N*-terminal parts of the V_L and V_H regions.

3. Formation of a mature heavy chain involves *somatic* (as opposed to inherited) joining of coding sequences from V, D, and J gene regions in each primordial B cell. Later, the rearranged **VDJ gene** is joined with a **C region gene** to form the final protein.
   a. Joining of the VDJ regions is imprecise.
   b. Nucleotides are (apparently) randomly inserted into the joint between regions.
   c. Mutations occur within the J region.
   d. This gene reorganization occurs on either the maternal or paternal allele, and only a single rearranged heavy chain gene is expressed in a given B cell or its progeny (**allelic exclusion**).
4. Later selection of the C region gene determines the **isotype:** IgG, IgA, or IgE.

C. Light Chains

1. Light chains are of two types, κ or λ, encoded on **chromosomes 2 and 22,** respectively.
2. Genes for light chains contain V, J, and C regions (no D region).
3. **V-J rearrangement** occurs in immature **B cells.**

D. The Mature Immunoglobulin

1. The mature heavy and light chains pair to give the structure shown in Figure 8–3.
2. Due to all of the variations in forming and expressing a mature Ig, many types of antigens can ultimately be recognized.
3. Mature Igs are designated as IgM, IgG_1, and so on.
4. The encounter of a B cell with an antigen can lead to its growth as a **memory B cell** serving as a reserve source of its *specific* Ig.
5. Alternatively, following the encounter, the B cell can proliferate as a **plasma cell clone** and produce large amounts of antibody (Ig).

V. T-Cell Receptors

A. These receptors are located on the membranes of T cells and involve assembly of three domains: V, D, and J.

B. Somatic assembly of T-cell receptors does *not* include mutation (unlike Ig).

C. Most are heterodimers with α and β chains (designated **TCR α:β**), encoded on **chromosomes 14 and 2,** respectively.

D. Another type (TCR γ:δ) is seen less frequently.

VI. Ig Gene Superfamily

A. This superfamily includes a large array of structurally similar genes (Table 8–2).

Table 8–2. Immunoglobulin gene superfamily members.

General Category	Specific Example(s)
T-cell receptor components	TCR, CD3α and β
T-cell adhesion and related proteins	CD1, CD2, LFA3
T-subset antigens	CD4, CD8, CTLA4
Brain and lymphoid antigens	Thy1, MRC, Ox2
Immunoglobulin receptors	PolygR, Fcγ2b/γ1R
Nerve cell adhesion molecule (NCAM)	
Myelin protein (Po)	
Myelin-associated protein (MAG)	
Carcinoembryonic antigen (CEA)	
Platelet-derived growth factor receptor (PDGFR)	
Colony-stimulating factor 1 receptor (CSF1R)	
Basement membrane link protein (LINK)	

B. Members of this family are related at the DNA sequence level.

C. Evolutionary relatedness has maintained important topologic features for the proteins despite divergent function(s).

D. Only the Ig genes undergo **somatic mutation.**

VII. Features of Inherited Changes in Immune Function

A. The complexity of immune function offers multiple sites for inherited changes.

B. Consequences usually include increased **susceptibility to exogenous pathogens.**

C. Not surprisingly, many defects in immune function **present in childhood.**

1. For screening in infants, the level of **IgM** is particularly important to measure because it is a large complex that cannot cross the placenta and *must* be synthesized by the individual.
2. **IgG,** a relatively small molecule, crosses the placenta to the fetus in the third trimester; thus, IgG levels can be low in premature infants.
3. The duration of this maternally derived protection is limited, and IgG stores are usually exhausted by ~6 months of age.

D. **IgA deficiency** is the most common Ig deficiency but has multiple causes. Serum levels often rise slowly in normal individuals (sometimes well into adolescence) and so their measurements can be confusing.

IDENTIFYING IMMUNE DEFICIENCY DISORDERS

- *Single-gene disorders of immune and host defense can be grouped into several categories based on physiologic responses (Table 8–3).*
- *Identifying the physiologic deficiency can help distinguish the underlying disorder.*
- *An X-linked inheritance pattern can help distinguish some of these disorders.*
- *As a group, this entire category of illnesses has been the focus of considerable attention for treatment (see Chapter 12).*

CLINICAL PROBLEMS

A physician has just started work at a clinic that treats HIV-positive individuals, most of whom are immigrants from North Africa. The physician knows that ~5% of individuals have a severe reaction to the drug abacavir (Ziagen), which is recommended as a primary treatment of HIV, and studies from Australia have shown that this sensitivity is linked to HLA B5701. Unfortunately, the clinic population has a very low frequency of this HLA allele.

1. The physician would most likely conclude that

A. Abacavir sensitivity is unlikely in the clinic population.

B. The observed sensitivity may be related to another HLA marker that must be identified.

C. The molecular mechanism for sensitivity to the drug must be identified, as it may not be directly related to the HLA marker.

Table 8–3. Single-gene disorders of immune and host defense.

Physiologic Category	Map Location	OMIM	Comment
Combined Immunodeficiency			
Severe combined immunodeficiency (SCID)			SCID infants have complex problems with recurrent infections and reactions to live virus vaccines
SCIDX (Swiss type)	Xq13.1	300400	
Adenosine deaminase (ADA) deficiency	20q13.11	102700	
Purine nucleoside phosphorylase deficiency	14q13.1	164050	
Wiskott-Aldrich syndrome	Xp11.2	301000	Thrombocytopenia also is found
Ataxia-telangiectasia	11q22.3	208900	The immune deficiency is variable, but progressive cerebellar dysfunction and skin changes are prominent
T-Cell Dysfunction			
DiGeorge syndrome	22q11.2	188400	Also notable for dysmorphism and cardiac defects Many individuals have a deletion affecting several genes, but most of the clinical picture can be seen with mutation of *TBX1* gene alone Infections usually are chronic but may respond to treatment
Mucocutaneous candidiasis (multiple forms)			Chronic fungus susceptibilities are seen in all types
B-Cell Dysfunction			
X-linked hypogammaglobulinemia (Bruton)	Xq21.3	300300	Individuals often do well until they exhaust their supply of maternal antibodies at about 6 months of age, after which bacterial susceptibility arises
X-linked immunoproliferative syndrome	Xq25	308240	Viral susceptibility (especially to the Epstein-Barr virus) is prominent
Hyper-IgM-associated immunodeficiency	Xq26	308230	

Table 8–3. Single-gene disorders of immune and host defense. (cont.)

Physiologic Category	Map Location	OMIM	Comment
Dysfunction of Phagocytosis			
Chédiak-Higashi syndrome	1q42.1	214500	
Chronic granulomatous disease			Infections resolve slowly in all forms because of defective intracellular killing of bacteria
Cytochrome b α-subunit	16q24	233690	
Cytochrome b β-chain	Xp21.1	306400	
Myeloperoxidase deficiency	17q23.1	254600	Usually compatible with survival with at least some phagocyte function Affected individuals who also have diabetes can develop complications from fungal infections
Glucose-6-phosphate dehydrogenase (G6PD) deficiency	Xq28	305900	Some individuals with extremely low levels of G6PD lack adequate levels of intracellular NADPH (a product of the pentose phosphate pathway for which G6PD is the first enzyme) to support effective phagocyte function
Defects in Complement Protein(s) and Function			
Factor 3 deficiency Factor 5–9 deficiencies (multiple)	19p13.3	120700	Defects of most of the complement factors produce characteristic susceptibilities C3 deficiency causes susceptibility to encapsulated bacteria C5–9 deficiencies increase susceptibility to *Neisseria* spp
C1 inhibitor deficiency	11q11	106100	Angioedema and other problems with vascular permeability are prominent
Properdin deficiency	Xp11.4	312060	

D. It is worth trying the drug in the clinic population.

E. Renal clearance of the drug must be measured.

2. Synthesizing an immunoglobulin heavy chain gene

A. Involves information on three chromosomes

B. Incorporates sequences from six gene regions

C. Requires extensive rearrangement of the C region domain

D. More frequently involves the maternal sequences

E. May lead to a gene with frameshifts or stop codons

A man who hopes to donate a kidney to a relative with end-stage renal disease is being evaluated for donor compatibility. The evaluation shows that he is HLA DR4 positive. The man is 47 years old and has no personal or family history of diabetes.

3. Based on the evaluation results, the physician would most likely conclude that

A. The man will develop diabetes within the decade.

B. Other family members are at risk for diabetes.

C. The man should lose weight to reduce the likelihood of developing diabetes.

D. HLA DR4 and diabetes may not be linked in the man's family.

E. If the recipient is DR4 positive, the likelihood of developing diabetes is certain.

ANSWERS

1. The answer is C. The underlying molecular mechanism for sensitivity to the drug is an essential consideration in any population (see also Chapter 11). Because the basis for the aberrant response is unknown, one cannot conclude that the clinic population is not at risk (choice A) and a trial might have bad reactions (choice D). The original observation was only an HLA association, so whether another HLA marker might be linked in this population is unknown (choice B). Renal clearance of the drug (choice E) is not known to affect toxicity.

2. The answer is E. Some rearrangements lead to faulty genes due to imprecise joining of segments. Such recombinants will not be expressed as mature molecules. All Ig heavy chain genes are found on chromosome 14 and encompass four groups of sequences (choices A and B). The C ("constant") region sequences are not rearranged (choice C). The heavy chain can be derived from maternal or paternal sequences (choice D).

3. The answer is D. Recall that the association of HLA DR4 with diabetes is not causal. (And it is not known whether the DR3 allele is present; recall Table 8–1.) There may be no linkage with HLA DR4 and diabetes in this family; thus, no conclusions can be drawn for other family members, who may not even have been tested as potential donors (choice B). Although weight loss is generally beneficial for reducing the chance of developing diabetes (choice C), the patient is not identified as at increased risk and cannot be advised that he will develop diabetes within a specific time frame (choice A).

CHAPTER 9
GENETICS AND CANCER

I. Gene Changes

A. Genetic control of growth underlies tumor cell biology.

B. Changes in the integrity, function, and control of genes permit the cancer phenotype to develop and persist.

C. Identifying underlying gene changes can aid diagnosis, prognosis, and treatment strategies.

D. Inherited tumor syndromes show the effect(s) of mutations (see later discussion).

E. Genetic data show the complexity of cancer cell biology.

II. Chromosome Changes

A. **Aneuploidy** often is found in **late-stage tumors** and can be complex.

B. Large-scale, distinct chromosome changes are frequent and can be diagnostic.

1. The **Philadelphia chromosome (Ph^1)** is a **translocation** between **chromosomes 9 and 22** and a marker for chronic myelogenous leukemia (CML) (Figure 9–1 and Table 9–1).

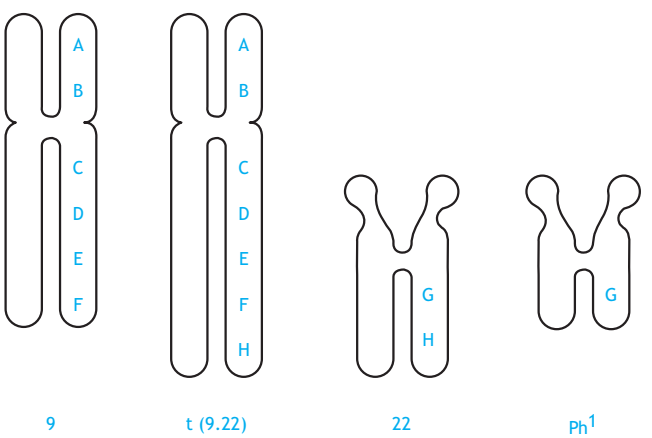


Figure 9–1. Diagram of a Philadelphia chromosome, a translocation between the distal long arms of chromosomes 9 and 22. What sort of translocation is this?

Table 9–1. Chromosome breakpoints and associated genes in malignancies.

Translocation	Location	Disease	Associated Gene(s)
SRC family			
t(9;22) [Ph^1]	9q34.1	CML/ALL	*BCR-ABL*[a]
t(1;7)	1p35–p34.3	T-ALL	*LCK*
t(2:5)	2p23	NHL	*ALK*
Serine protein kinase			
t(9;22)	22q11.21	CML/ALL	*BCR-ABL*[a]
Cell surface receptor			
t(7;9)	9q34.3	T-ALL	*TAN1*
Growth factors			
t(4;16)	4q26	T-NHL	*IL2*
t(5;14)	5q31.1	PreB-ALL	*IL3*
Mitochondrial membrane protein			
t(14;18)	18q21.3	NHL	*BCL2*
Cell-cycle regulator			
t(11;14)	11q13	CLL/NHL	*CCND1*
Myosin family			
inv(16), t(16;16)	16p13.13	AML_{M4Eo}	*MYH11*
Ribosomal protein			
t(3;21)	3q26	AML/CML_{blast} therapy-related myelodysplasia	*EAP (L22)*

SRC, a family of tyrosine protein kinases; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; PreB-ALL, Pre B-cell acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; AML_{M4Eo}, acute myelogenous leukemia subtype M4Eo; $CML_{blast,}$ chronic myelogenous leukemia subtype blast.

[a]The *BCR-ABL* gene is a chimeric gene formed by fusing the *ABL* (tyrosine kinase gene on chromosome 9) with *BCR* ("breakpoint cluster region"—serine-threonine kinase gene on chromosome 22). The fusion protein has different regulatory properties and is characteristic of malignant proliferation in CML (see text).

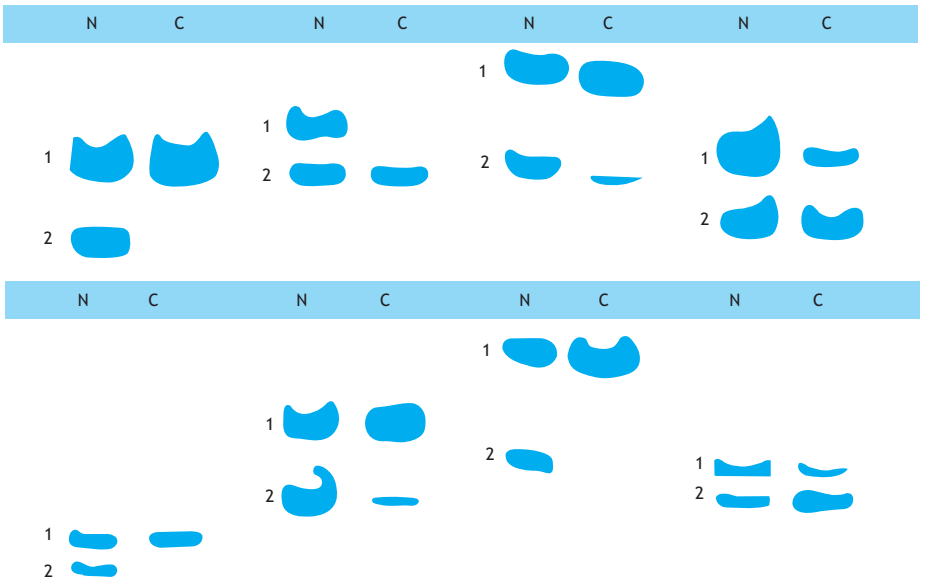


Figure 9–2. Southern blots showing loss of heterozygosity (LOH) in cells from colon tumors. Note different patterns in cells from different individuals. Such studies detect only changes that lead to altered mobility or disappearance of the specific DNA band; many point mutations (which also might be important in terms of tumor formation) will not be detected in this way. N, normal tissue; C, cancer. *(Modified from Fearon ER, Hamilton SR, Vogelstein B. Clonal analysis of human colorectal tumors.* Science *1987;238:193. Reproduced with permission from AAAS.)*

2. Molecular details listed in Table 9–1 have begun to assist drug and treatment design, as noted below.

C. Short-range changes often involve deletion or inactivation of single genes.

1. When an individual has two alleles at a locus (and is, by definition, heterozygous) deletion of one is detectable as **loss of heterozygosity** (LOH; recall Chapter 1).

a. Study of colon tumors at different growth stages has shown accumulation of LOH at different genes and chromosome regions (Figure 9–2).

b. Following LOH only one (apparently) intact copy of the gene remains.

c. In **autosomal dominant** (AD) tumor syndromes (eg, von Recklinghausen disease (VRNF, or neurofibromatosis 1), discussed in Chapter 3) one allele of the responsible gene is mutant by inheritance. Thus, losing the other allele removes all normal coding information.

TECHNICAL ILLUSTRATION

*The **Knudson hypothesis** holds that the appearance of a tumor in an individual who has inherited one mutation at a critical locus might reflect mutation (or loss) of the remaining allele. This notion of the need for two "hits" to inactivate a controlling gene has been confirmed in several AD tumor syndromes (Figure 9–3; see also Table 9–2).*

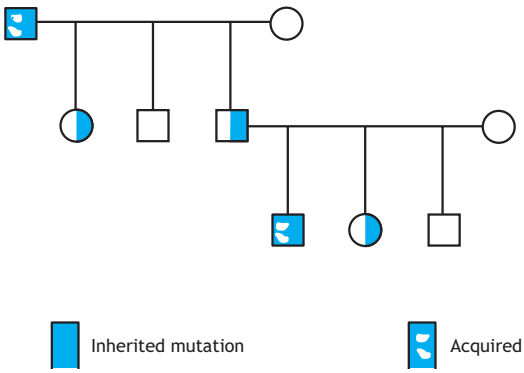


Figure 9–3. The Knudson hypothesis holds that two "hits" (mutations) are needed for tumorigenesis. When the first "hit" in one of a pair of autosomal genes is inherited, only a single "hit" in the other allele is needed to lead to tumor growth. Obviously, because one of a pair of genes is already mutated from the time of conception, the frequency of visible tumors is far higher than if independent mutations were needed in both. (Recall that the likelihood of having mutations in both genes is the product of the individual mutation frequencies.)

III. Gatekeeper Genes

A. Earlier called *tumor suppressor genes,* many **gatekeeper genes** were identified in tumor syndromes (eg, VRNF and others; see Table 9–2).

B. Some are relatively **cell-type specific** in their control (nerve, eye, colon, etc).

C. Given a relatively low mutation rate (~1 in 10^8 per cell per generation) and the presence of two gene copies (alleles) at each locus, *total* loss of gatekeeper function is unlikely because *both* alleles would need to be inactivated.

D. In tumor syndromes, however, as the Knudson hypothesis suggests, only a *single* event at the *remaining* functional allele would lead to loss of gatekeeper function.

Table 9–2. Autosomal dominant syndromes with gatekeeper gene mutations.

Syndrome	Protein	Tumor Cells	OMIM
Neurofibromatosis 1 (VRNF)	Neurofibromin	Schwann cells	162200
Neurofibromatosis 2	Neurofibromin-2	Cranial nerve VIII	101000
Retinoblastoma	RB protein	Retina	180200
Von Hippel-Lindau	VHL protein	Kidney and others	193300
Adenomatous polyposis coli	APC protein	Colon	175100

E. The frequency of allele loss might be expected to be higher in cell lines with rapid turnover (eg, bone marrow, skin, and gastrointestinal epithelia), because mitoses, replication, and opportunities for mutation are more frequent.

IV. Caretaker Genes

A. Integrity of genes and DNA is maintained by complex recognition and **repair enzyme** systems. Any change in their reliability can lead to a generalized increase in mutations (recall Chapter 1).

B. Due to **chronic, low frequency DNA damage** in everyone—resulting from drugs, radiation, or simply replication error(s)—the consequences of change in caretaker gene function can be widespread.

C. Reduced (or lost) caretaker gene function can lead to loss or dysfunction of gatekeeper genes with subsequent tumor development.

HEREDITARY NONPOLYPOSIS COLON CANCER (OMIM 120435)

- *The responsible gene (*MSH2*) is involved in repair of DNA base pair mismatches.*
- *Dysfunction of this gene reduces fidelity of repair and increases the mutation frequency for many genes, a state that has been called* ***genetic instability.***

V. Gene Analysis in Cancer

A. The variety of gene changes noted earlier implies that the biology of cancer cells will be deranged and complex.

B. Fluorescence in situ hybridization **(FISH)** and single nucleotide polymorphism **(SNP)** analysis can help interpret chromosome changes.

C. Detailed **molecular analysis** of DNA sequences at chromosome breakpoints, insertions, translocations, etc (eg, Ph^1) can identify the gene(s) involved (recall Table 9–1).

1. This can aid diagnostic precision.

2. Identifying responsible gene(s) or their change(s) may aid drug use and development.

3. Tumor-specific enzymes and other proteins can be clinical markers for the presence of the tumor and also important drug targets.

LEUKEMIA AND THE BCR-ABL PROTEIN (SEE TABLE 9–1)

- *The BCR-ABL protein is formed by fusing genes from chromosomes 22 and 9.*
- *It is an active tyrosine kinase and is central to the malignant phenotype.*
- *The drug* ***imatinib*** *(Gleevec) was designed as a tyrosine kinase inhibitor and has been useful in the treatment of tumors expressing this fusion protein in patients with* ***CML*** *and* ***acute lymphoblastic leukemia (ALL).***

D. **Microarray analysis** can measure the expression of thousands of genes simultaneously (see Chapter 1).

1. Although gene changes in tumors can be complex, expression of many (often most) genes usually is relatively stable (ie, their transcription patterns do not discriminate between tumor and normal cells).

2. Using **bioinformatics** it is theoretically possible to identify a relatively small number of genes (eg, < 100) whose expression patterns can classify certain tumors.
3. Details of the molecular biologic changes in individual tumors are valuable.
   a. Diagnostic classification is enhanced.
   b. Prognosis can be based on molecular changes by reference to databanks.
   c. Gene target(s) and response(s) at the cellular level can be monitored during treatment.
4. Gene studies will be even more useful for future diagnosis and treatment in oncology.

CLINICAL PROBLEMS

A 42-year-old woman has had three early miscarriages in the past 2 years. She and her husband recently underwent chromosome studies, and although both feel fine, the woman was told that she has a Philadelphia chromosome. She seeks advice from her physician.

1. Which of the following statements represents the most likely response?
   A. She should have family studies to determine whether this finding is present in close relatives.
   B. This is an indication for performing chorionic villus biopsy early in her next pregnancy.
   C. She should have a bone marrow study.
   D. The couple should consider in vitro fertilization.
   E. This result is likely to be an artifact.

Wilms tumor (OMIM 194070) accounts for ~8% of all childhood tumors. Siblings with Wilms tumor often have bilateral disease whereas most sporadic cases are unilateral. An earlier study showed that maternal alleles of markers on chromosome 11 were lost in 7/7 patients with sporadic tumors.

2. These observations indicate
   A. The Knudson hypothesis is not relevant to sporadic cases.
   B. A maternal mutation is the best explanation for the findings.
   C. A paternal mutation is the best explanation for the findings.
   D. More tumors must be studied because the data were biased.
   E. Sequencing is needed to clarify the gene change.

Martha is 31 and has come for a routine gynecologic visit. The physician has not seen her before and obtains a family history, which reveals several individuals with colon cancer in her mother's family (Figure 9–4). Martha's mother died in an accident at age 38 and was not known to have any health problems. Martha tells the physician she feels fine and, because her two older siblings have not been diagnosed with any health problems, has considered herself unlikely to be at risk for any problems.

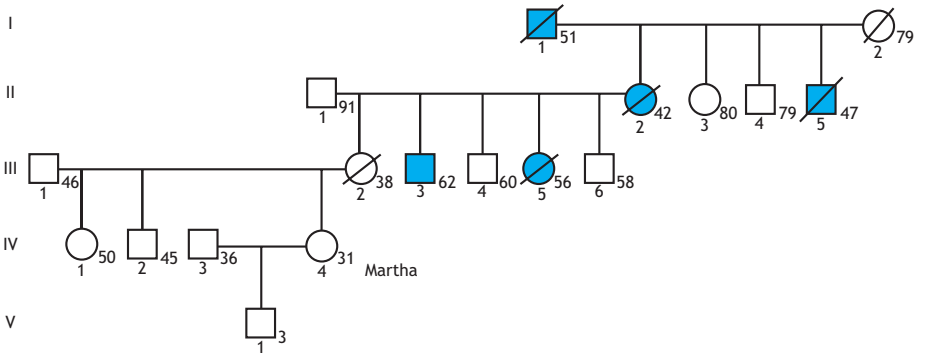


Figure 9–4. Pedigree for Question 3, showing family members with colon cancer.

3. The physician would most likely advise Martha that

A. The high frequency of colon cancer suggests that her maternal grandfather (II-1) is a carrier of this recessive trait.

B. Because her mother and uncles were not affected it is unlikely that she will develop colon cancer.

C. She should have a colonoscopy at age 50.

D. Her 60-year-old maternal uncle (II-4) should have a colonoscopy.

E. Her mother likely was a carrier for this trait, but her father (III-1) likely was not a carrier, and thus her siblings are unaffected.

ANSWERS

1. The answer is C. Assuming that the study was done in an experienced laboratory, this result strongly suggests a diagnosis of CML, and the patient needs further evaluation beginning with a bone marrow study. The physician may have made an early diagnosis that could improve the patient's treatment response. Study of her family members (choice A) is unlikely to be informative unless there have been fertility problems. Her miscarriages are likely too early for chorionic villus biopsy (choice B). In vitro fertilization (choice D) may not help if she has an abnormal cell line. Although the result may be an artifact (choice E), this is unlikely in an experienced diagnostic laboratory.

2. The answer is C. In essence, the data are LOH studies showing that loss of any maternal information for the gene (presumably normal, choice B) left the individuals with only a mutant *paternal* allele (hence, no intact copy of the gene). These findings are consistent with the Knudson hypothesis, with one inherited mutation and another created by LOH. The Knudson hypothesis specifically noted that sporadic tumors requiring "two hits" should be quite rare (and unilateral); thus, choice A is incorrect.

Although 7 cases is a relatively small number, there is no evidence for bias (choice D). Although potentially interesting, sequencing is not needed to establish the source of the mutation (choice E).

3. The answer is D. The kindred might show a recessive trait (choice A), but a dominant one is much more likely, placing II-4, the 60-year-old uncle, at 50% risk. Martha is at risk (choice B); she needs a colonoscopy now and should not wait to have a routine test at age 50 (choice C). Her father's status is not relevant to the segregation of an AD trait (choice E). The physician also needs to know what sort of colon cancer was present in family (ie, were polyps prominent?). Given the variability in phenotypic expression of AD traits (recall Chapter 3), it is difficult to predict age of onset of symptoms.

CHAPTER 10
GENETICS AND COMMON DISEASES

I. Genetic Variations Underlying Disease

A. Despite the importance of disorders due to single gene changes (and their emphasis in earlier chapters), most clinical problems have a more complex etiology.

B. There is striking variation among individual genes (eg, single nucleotide polymorphisms [SNPs], copy number variations [CNVs], and other polymorphisms; see Chapter 1).

C. Even apparently modest changes in gene structure (eg, SNPs) can affect ***control* of expression** (timing, volume, tissue distribution, etc) as well as details of three-dimensional protein structure, with implications for interactions with intra- and extracellular partners.

D. Expression levels of genes and proteins in single cells show impressive, apparently stochastic, variation(s) from cell to cell even *without* genetic differences, implying biochemical heterogeneity in any cell population.

E. Thus, the biologic substrate of *Homo sapiens* contains **widespread microheterogeneity** (much of which cannot currently be quantified), which achieves homeostatic stability at the level of the organism.

F. We may therefore consider any disease to result from interactions and derangements of **exogenous and endogenous factors** which, at a molecular level, may be ***unique* to the individual.**

G. Major clinical categories of disease must thus be viewed as the *sum* (or final common pathway) of many interacting factors (at least some being essentially random), although limited diagnostic, management, and treatment options often have led to their being considered homogeneous.

II. Epidemiologic Findings

A. Twin Studies

1. Studies of twins have helped show genetic contributions to common disorders (Table 10–1).

2. The distinction between disease frequency in monozygotic (ie, genetically virtually identical) versus dizygotic (ie, half of their genes in common) twins has been particularly valuable.

3. Even traits with relatively low concordance are still shared more frequently in monozygotic twins.

Table 10–1. Twin concordance data for common conditions.

	Percent Concordance	
Condition	**Monozygotic**	**Dizygotic**
Coronary artery disease	46	12
Hyperthyroidism	47	6.5
Congenital hip dislocation	41	2.8
Type I diabetes mellitus	56	11
Clubfoot	23	2.3
Pyloric stenosis	22	2

Data from Carter CO. The inheritance of common congenital malformations. *Prog Med Genet* 1965;4:59.

4. The number of finger ridges is determined before birth and, hence, is not very susceptible to environmental influences (Table 10–2).
5. Contrasting data are seen for coronary artery disease, a relatively late-onset problem with important environmental influences (Table 10–3).

B. Population Patterns of Disease

1. Concentrations of certain conditions within populations are recognized.
2. Some populations have high frequencies of **single gene variations** (see Chapter 4). Populations with prominent genetic disorders include
   a. Africans: sickle cell disease
   b. Caucasians: cystic fibrosis
   c. Northern Europeans: phenylketonuria (PKU)

Table 10–2. Finger ridge count correlations in family members.

Relationship	Correlation Coefficient	Correlation Expected
Monozygotic twins	0.95 ± 0.07	1.00
Dizygotic twins	0.49 ± 0.08	0.50
Sibling-sibling	0.50 ± 0.04	0.50
Parent-child	0.48 ± 0.04	0.50
Parent-parent	0.05 ± 0.07	0

Table 10–3. Ratios of death from coronary artery disease related to age of death of twin.

Age at Death (y)	Relative Risk	
	Men	**Women**
Monozygotic		
36–55	13.4	14.9
56–65	8.1	
66–75	4.3	3.9
76–85	1.9	2.2
≥ 85	0.9	1.1
Dizygotic		
36–55	4.3	2.2
56–65	2.6	
66–75	1.7	1.9
76–85	1.4	1.4
≥ 85	0.7	1.0

Reprinted with permission from Marenberg ME, et al. Genetic susceptibility to death from coronary heart disease in a study of twins. *N Engl J Med* 1994;330:1041.

   d. Mediterranean men: glucose-6-phosphate dehydrogenase (G6PD) deficiency

3. Other populations show divergent frequencies or characteristics of common disorders.

   a. In Hawaii, clubfoot is relatively frequent in Polynesians but rare in Chinese.

   b. Hypertension in young black men is clinically different from that seen in elderly Caucasian women, although actual pressure readings may be similar.

III. Threshold Model of Disease

A. There is a nearly infinite range of variation between and among populations in terms of environmental exposures, genetic variations, and details of individual biologic homeostasis; maladaptive perturbations of the latter are recognized as disease. Thus, a **threshold** model can be valuable (Figure 10–1).

B. As shown in Figure 10–1, distributions of individuals sharing susceptibilities (genetic or environmental) are shifted to the right, depending on common features.

C. This model leads to important predictions.

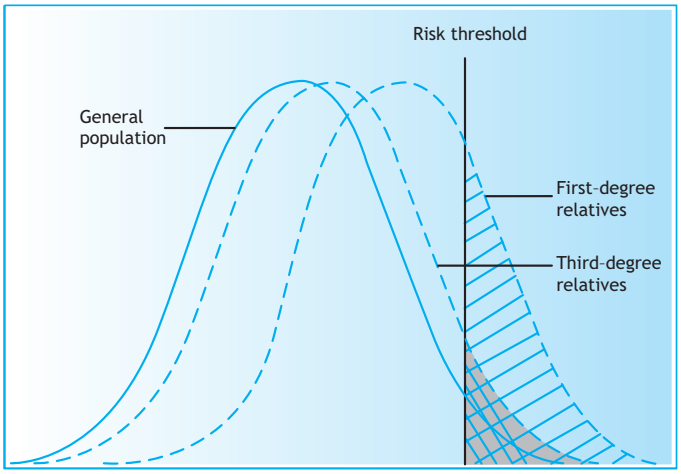


Figure 10–1. Distribution for a multifactorial trait consistent with a threshold model for common diseases. The dark vertical line indicates the risk threshold, and the distributions for different groups of relatives are shown, consistent with the notion that genetically closer relatives share more susceptibility genes. *(Adapted with permission from Fraser FC. The multifactorial/threshold concept: Uses and misuses.* Teratol *1976;14:267.)*

1. Different families can have different risks of recurrence (reflecting their own underlying variations).
2. Having more than one affected relative raises an individual's risk (Table 10–4).
3. Risk rises with parental consanguinity (increasing the likelihood of having gene variant(s) in common); the extreme example is seen for single autosomal recessive alleles in homozygotes.
   a. Populations isolated by geography (islanders), religion (old-order Amish, Parsi, Ashkenazi and Sephardic Jews), ethnicity, or a combination of these, can show increased frequencies of certain alleles.
   b. Such groups have passed through a genetic bottleneck with relatively little introduction of new gene variants from outside. Their genotypes are skewed toward the pattern(s) of founders of the group and, as described in Chapter 4, this has been called a **founder effect.**
4. Finding an individual with more severe changes implies a greater risk to relatives by suggesting a concentration of alleles conferring susceptibility in *that* kindred.
5. Relatives of an individual manifesting a very rare problem have a higher risk than they would if the problem were frequent.
   a. Frequently encountered problems are likely to reflect *multiple* different contributing factors (ie, many different paths to reach the same clinical end point).
   b. Rare problems may reflect the additive effects of relatively *few,* rare alleles that are more likely to be concentrated in a given family (especially if it is isolated).

Table 10–4. Frequencies of congenital malformations in relatives.

Congenital Finding	Incidence in General Population	Incidence Relative to General Population in			
		Monozygotic Twins	First-degree Relatives	Second-degree Relatives	Third-degree Relatives
Cleft lip, with or without cleft palate	1 in 1000	400×	35×	7×	3×
Clubfoot	1 in 1000	300×	20×	5×	2×
Neural tube defects	1 in 500		8×		2×
Hip dislocation (female)	1 in 1000	200×	40×	4×	1.5×
Pyloric stenosis (male)	1 in 500	80×	20×	5×	2×

Adapted with permision from Smith DW, Aase JM. Polygenic inheritance of certain common malformations. *J Pediatr* 1970;76:653.

6. In traits with a nonuniform sex distribution, relatives of an affected individual of the *less* frequently manifesting sex are more likely to have the problem.
- **a.** A larger contribution of risk-imparting alleles may be needed in an individual of the *less* frequently involved sex.
- **b.** This implies that the family may have a higher concentration of these alleles and, hence, a greater chance for their transmission.

INFECTIOUS DISEASES

- *A genetic susceptibility to several infectious diseases has been identified.*
- ***Duffy blood group*** *(OMIM 110700): An erythrocyte membrane antigen is needed for infection with vivax malaria. Large areas of western Africa are inhabited by individuals mutant for the gene (*DPG*) and, hence, negative for the antigen and resistant to infection.*
- ***Tuberculosis:*** *Susceptibility can be increased by a mutation in the IFN-γ receptor (OMIM 2099500).*
- ***Human immunodeficiency virus (HIV):*** *Table 10–5 lists loci affecting host susceptibility and the course of infection. The large number of these factors implies that a wide variety of outcomes might be expected in the presence of variant alleles at any or all of these loci. Therapeutic possibilities are suggested by some of these data.*

IV. Implications for Screening and Patient Care

A. Although many common disease categories (eg, hypertension, diabetes, obesity, heart disease, cancer, and dementia) are fundamentally heterogeneous, new analytical techniques based on molecular genetics have begun to reveal critical genes and variations.

1. Microarray technology permits simultaneous screening for > 10^5 SNPs located throughout the genome.

Table 10–5. Host genes affecting HIV infection.

Phase of Infection	Gene(s)	OMIM
Viral Entry		
Receptor	*CD4*	186940
Fusion co-receptor for M-tropic virus	*CCR2*	601267
	CCR5	601373
Fusion co-receptor for T-tropic virus	*CXCR1*	601470
	CXCR4	162643
Receptor for gp120 on		
Dendritic cells	*DCSIGN*	604672
Endothelial cells	*syndecans*	142460
Ligands for co-receptors (can prevent viral entry)		
CCR5	*CCL3*	182283
	CCL3L1	601395
	CCL4	182284
	CCL5	187011
CXCR4	*CXCL12*	600835
Viral Replication		
Transcription factors	*NFκB*	164011
	NFAT	600490
Replication factors	*APOBEC3G*	607113
	MURR1	607238
Host Immune Response		
KIR genes	*KIR3DS1*	604946
Inhibitory cytokines	*IL10*	124092
	IFNγ	147570

Table 10–5. Host genes affecting HIV infection. (cont.)

Phase of Infection	Gene(s)	OMIM
Chemokines to activate immune response	*CCL2*	158105
	CCL7	158106
	CCL11	601156
HLA factors		
Poorer prognosis	*B35, B53*	—
Protection	*B27, B57*	—

OMIM, Online Mendelian Inheritance in Man number; KIR, killer cell immunoglobulin-like receptor; HLA, human leukocyte antigen.

2. Comparing SNP variations with specific clinical end points is the basis for **genome-wide association (GWA)** studies.

B. Finding SNPs that vary with a clinical phenotype is the first step in identifying the specific gene (or variant) involved, and large populations can now be studied at reasonable cost to reveal reproducible associations.

C. Not surprisingly, GWA studies reveal variant(s) that *influence* risk but are not the only contributor(s) to the phenotype (Table 10–6).

1. Gene(s) or variant(s) noted in Table 10–6 were not necessarily suspected before GWA studies.

2. In some cases, only a *region* has been implicated (a specific change has not yet been defined).

3. The contributions of most individual gene(s) or variant(s) to specific disease risk(s) identified to date have been significant but often modest; note that risks in Table 10–6 apply to homozygotes. (Macular degeneration has been an important exception.)

D. There are likely to be many more loci influencing common diseases in different, possibly antagonistic, ways. Interactions of these loci with each other as well as with the environment will be complex.

1. The wide array of variation in susceptibilities and exposures means that an essential question in addressing *any* individual's illness is, "Why does *this* person have *this* problem *now?*"

2. The counterpart of the previous question is, "What treatment will be most effective for *this* problem in *this* individual?"

E. **Pharmacogenetics** (see Chapter 11) is becoming increasingly important, adding another level of complexity to care.

Table 10–6. Selected results of genome-wide association studies of common diseases.

Disorder	Gene or Variant Region	Chromosome	Increased Risk in Homozygotes
Macular degeneration	*CFH*	1q31	~600
Crohn disease	*IL23R*	1p31	~120
Prostate cancer	Rs447295 marker	8q24	30–90
	DG8S737 marker		
Coronary heart disease	*CDKN2A-CDKN2B* region	9p21	30–40
Type 2 diabetes	*CDKN2B* region	9p21	5–27 (for each variant)
	IGF2BP2 intron	3q28	
	CDKAL1 intron	6p22	
	HHEX region	10q24	
	SLC30A8 region	8q23	
	TCF7L2	10q25	
	KCNJ11	11p15	
	PPARG	3p25	

Based on data compiled from Science 2005;308:385,419,421; Science 2006;314:1461; and Science 2007;316:1331,1488; and from Cancer Res 2007;67:2944,2951,2905.

CLINICAL PROBLEMS

Researchers have been studying coronary artery disease among people living in a small town. The DNA sequence laboratory reports that haplotype Q is present in 50% of men who have had a heart attack earlier than age 60.

1. Which of the following statements represents the most likely response to this finding?

   A. The value is low; half of affected men do not share this haplotype.

   B. The value is high; the researchers can screen the population and predict 50% of the risk.

   C. Screening will not be helpful for older men.

D. Screening should be implemented in the local gynecology practice.

E. It will be important to know the haplotype frequency in the rest of the state.

A 29-year-old man seeks the counsel of his physician. He is being treated for hemochromatosis (OMIM 235200) and is doing well. His father, who has the same condition, has been treated with a good response for several years but recently was diagnosed with ankylosing spondylitis (AS).

2. Based on this information, the physician is most likely to conclude

A. The patient's mother should be tested for hemochromatosis.

B. There is no pathophysiologic connection between hemochromatosis and AS; hence the patient's risk for the latter is low.

C. The HLA B27 status of the patient's father should be determined.

D. Spinal radiographs should be obtained for this patient.

E. More aggressive iron-lowering treatment should be helpful for both the patient and his father.

A physician oversees the care of patients in a stroke clinic where 70% of patients are former smokers. Among these patients, the frequency of copy number variation (CNV) in gene Z is 40%. In nonsmokers with strokes, the CNV frequency is similar to that of the general population: 45%.

3. The physician is most likely to suspect that

A. CNV for gene Z is not a good discriminator in this population.

B. CNV is a risk factor for stroke in nonsmokers.

C. Low CNV predisposes to smoking.

D. Duration of smoking is the critical variable.

E. High CNV reduces the likelihood of smoking.

ANSWERS

1. The answer is E. The population frequency of this haplotype must be related to a larger population. The study group may have a founder effect and a skewed haplotype frequency without any relation to coronary disease, and thus the frequency cannot be considered exceptionally low (choice A) or high (choice B). More data are needed to justify studies of older men (choice C) or women (choice D).

2. The answer is C. AS has a recognized linkage association with HLA B27 (see Table 8–1), and the gene for hemochromatosis (*HFE*) is a type III gene in the major histocompatibility cluster on chromosome 6 (see Figure 8–1). The physician will be more confident of the father's diagnosis of AS if HLA B27 is found. Because of the tight linkage within this region, the presence of hemochromatosis in both father and son makes it more likely that the son would share the HLA B27 marker and be at increased risk of developing spondyloarthropathy. Despite absence of a direct pathophysiologic

connection between HLA B27 and AS, there is a relative risk of 90 for the latter when the former is found (choice B, recall Table 8-1). The mother's status (choice A) will not affect the son's diagnosis—the traits of interest have autosomal dominant inheritance. It is too early to obtain spinal radiographs in the asymptomatic son (choice D). There is no evidence that treatment of hemochromatosis has been inadequate in the two men (choice E).

3. The answer is A. In this case, the CNV is being compared with smoking (a well-known risk factor). A large population study might improve the odds but the small difference (40% vs 45%) is discouraging as a risk factor for stroke (choice B) or for smoking itself (choices C and E) for anyone in the population. These data offer no information regarding smoking duration (choice D) in this population.

CHAPTER 11
PHARMACOGENETICS

I. Overview

A. Drug metabolism depends on host biochemistry; hence, genetic variation(s) can affect responses to individual drugs.

B. **Pharmacogenetics,** as this area of study has been called, is an example of a **phenotype** (ie, the clinical response) dependent on *both* a **genotype** *and* exposure to an **exogenous trigger** (in this case, usually a drug).

1. Without exposure to the trigger the susceptibility imposed by the metabolic variation may never be recognized.

2. Once susceptibility has been identified, the individual and family members at risk can be tested, treated, or counseled.

C. Identifying variant drug response(s) may permit reduced toxicity, increased efficacy, and reduced cost.

II. Current Limitations and Recent Advances

A. Some inherited variations in drug response(s) have been recognized for many years, but most have not led to changes in drug use or routine testing.

B. More recently, drugs designed for distinct **cellular targets** have made interindividual difference(s) and their consequences more prominent.

C. Gene analysis in tumors already has identified specific molecular drug targets.

1. These are not necessarily inherited traits of the *patient* but, rather, reflect **individual genetic aspects of *tumor* biology.**

2. The multiple genetic changes found in tumors (see Chapter 9) underlie variable drug susceptibilities.

3. Microarray techniques can reveal critical gene changes (see Chapters 1 and 9).

DRUGS EXPLOITING UNIQUE TUMOR SUSCEPTIBILITIES

- ***Cetuximab*** *(Erbitux) targets* ***small-cell lung tumors*** *expressing EGF.*
- ***Trastuzumab*** *(Herceptin) was developed to treat* ***breast tumors*** *with HER gene expression*
- ***Imatinib*** *(Gleevec) is designed for tumors expressing the* ***BCR-ABL tyrosine kinase*** *(see Chapter 9).*

III. Treatment-related Issues

A. The recognition of variable drug response(s) to genetically targeted drugs has raised some concerns.

1. Testing may be **expensive** and **delay treatment.**
2. It may be difficult to justify the **cost of developing drugs** for individuals with **rare response patterns** (although some of these may fall into the "orphan" disease category; see Chapter 12).
3. **Liability** may develop if previously unrecognized adverse reactions are revealed.
4. Drug **doses may be inadequate;** for example, rapid clearance or inactivation of an antibiotic may lead to a poor clinical response and encourage development of resistant organisms.

B. Recognizing pharmacogenetic variation(s) will be an essential part of developing **"personalized medicine."**

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PD; OMIM 305900)

- *G6PD deficiency encompasses a group of X-linked variations predisposing individuals to **hemolysis** after taking drugs that cause **oxidative stress** (see also Chapter 5).*
- *Without exposure to these drugs, most men with the disorder have no problems (although all of their daughters will be carriers).*
- *These variations are sufficiently frequent (as high as 6×10^8 worldwide) to justify testing men from appropriate regions (eg, southern Italy, Southeast Asia) or ethnic groups.*
- *Once G6PD deficiency is identified, the man should carry a notice of his susceptibility.*

SUCCINYLCHOLINE INHIBITOR SUSCEPTIBILITY ("PSEUDOCHOLINESTERASE DEFICIENCY"; OMIM 177400)

- *Susceptibility to succinylcholine extends the duration of **respiratory** (and other) **muscle paralysis** following use of suxamethonium and related agents in **general anesthesia.***
- *Affected individuals usually are identified by their need for prolonged postoperative mechanical ventilation.*
- *This autosomal recessive trait usually is seen only in homozygotes (see Chapter 4).*
- *Individuals can be tested for variations expressed as a **dibucaine number** (Table 11–1). Family members at risk can be tested, although heterozygotes are usually asymptomatic.*

Table 11–1. Variations in pseudocholinesterase level.

Dibucaine Number	Frequency in Europeans	Enzyme Activity	Suxamethonium Sensitivity
80	95%	Normal	None
62	3.5%	Slight decrease	±
49	1 in 2500	Slight decrease	+++
22	1 in 11,000	Decrease	+++
0	1 in 170,000	Absent	++++

CLINICAL PROBLEMS

A 30-year-old Italian man comes to the walk-in clinic with abdominal pain. He recently returned from a 2-week trip visiting his family in Sicily. Three days ago, he visited another clinic, where he was given trimethoprim-sulfamethoxazole for cystitis, a problem that he has not had before. His urinary tract symptoms have largely resolved, although his urine has darkened.

1. Recognizing the problem, the examining physician would most likely

A. Recommend a steroid dose pack for 5 days.

B. Check a urine culture.

C. Obtain an abdominal ultrasound study.

D. Check a blood count.

E. Order liver function tests.

The safety committee for the anesthesiology department of a large metropolitan hospital is reviewing reports of two individuals with an extremely rare phenotype of malignant hyperthermia, a potentially life-threatening postoperative complication. Hyperthermia associated with mutations in the ryanodine receptor (OMIM 145600) cannot always be distinguished from other forms clinically, and multiple mutations have been discovered in the receptor. Consequently, diagnosis of this condition often has relied on muscle biopsy studies.

2. Which of the following approaches is most likely to reduce the incidence of this complication among patients undergoing surgery?

A. Maintain a lower temperature in the operating suite.

B. Improve monitoring of patients who receive anesthesia.

C. Screen all surgical patients for ryanodine receptor mutations by microarray.

D. Maintain a fresh supply of dantrolene to treat symptomatic patients.

E. Arrange for a study of receptor mutations to be undertaken in the community.

A physician has been asked to evaluate a possible outbreak of antibiotic-resistant tuberculosis in a village in northern India where several residents have died. An investigation by public health workers has shown that the drugs being administered are effective in vitro against the organism. The drug supply is fresh and is given to patients in the clinic under observation. It does not appear that new *Mycobacterium tuberculosis* strains have been introduced from elsewhere.

3. The physician is most likely to suspect which of the following as causative factors?

A. Treatment was probably given too late for the patients with advanced disease.

B. Contaminated milk is the source of new bacteria.

C. Drug turnover may be high in this population.

D. Malnutrition is the underlying problem.

E. Late monsoon rain has changed the local ecology.

ANSWERS

1. The answer is D. Because the patient is from a Mediterranean country, he has a high likelihood of having G6PD deficiency. It is likely that he has experienced a hemolytic episode after sulfonamide exposure. He needs hydration and observation until the hemolysis resolves and his urine clears. There is no indication of inflammation for which a course of steroids (choice A) would be needed. A urine culture (choice B) is likely to show no growth because he is taking an antibiotic. An ultrasound study (choice C) is not indicated unless the problem persists. The "dark urine" is due to bilirubin from hemolysis; there is no reason to suspect abnormal liver function (choice E). Follow-up observation is important, however, to be certain that the problem resolves and to offer counseling about the problem.

2. The answer is D. The rarity of the adverse reaction and the complexity and cost of screening make preemptive identification of susceptible individuals impractical now. The best use of resources is to have treatment readily available. Refer to the discussion of screening studies in Chapter 4. This reaction to anesthesia is not related to the ambient temperature (choice A). The two cases provide an opportunity to educate operating and recovery room staff about this problem but this will not, in itself, prevent a recurrence (choice B). Preoperative (choice C) or community (choice E) screening would be expensive and is impractical because multiple mutations are already recognized and more are likely to be found.

3. The answer is C. Local public health workers have already ruled out many of the possibilities and shown that the organism remains sensitive to the treatment recommended. Altered drug metabolism (eg, leading to rapid inactivation or excretion) in this isolated village population could explain what appears to be antibiotic resistance. The antituberculosis drug isoniazid has already been shown to have genetic variation in its metabolism. Because of the prominence of tuberculosis, the local clinic and public health workers are likely to be aggressive about early detection and treatment (choice A), which often can be helpful even if nutrition is limited (choice D). There is no evidence that other mycobacteria (for example bovine) have been introduced (choice B) or affected by the local environment (choice E), and the organism has been tested for sensitivity.

CHAPTER 12
GENETICS AND MEDICAL PRACTICE

I. Diagnosis

A. Before the diagnosis of a genetic (or any other!) disease can be made it must be considered in the differential diagnosis.

1. The **family history** (as reviewed in earlier chapters) often can generate suspicion.

a. The **pedigree** may reveal transmission of a trait.

b. Unfortunately, as noted earlier, there may be no information from the pedigree that directly suggests a genetic diagnosis. However, . . .

(1) The ***absence* of prominent findings** may eliminate some diagnoses and suggest considering less common ones.

(2) **Clues** such as **consanguinity** or membership in a relatively **isolated group** may suggest a recessive problem.

2. Details of the history or presentation may raise suspicion (Table 12–1).

II. Resources for Genetic Information

A. **Genetic databases** provide access to many details about rarely encountered problems.

1. **Online Mendelian Inheritance in Man** (OMIM; http://www.ncbi.nlm.nih.gov/omim) is coordinated through the National Center for Biotechnology Information (NCBI).

a. OMIM provides annotated data for known disorders and links to gene data, protein structure, and literature.

b. OMIM identifies each entry by a unique number (as used throughout this book).

2. Specialized textbooks (cardiology, endocrinology, etc) can provide valuable details.

a. *The Metabolic and Molecular Bases of Inherited Disease* (also available on CD-ROM) contains extensive discussions about most commonly recognized inherited conditions.

b. *Compendia* (also online) include photographs to aid identification of dysmorphic features and syndromes.

c. Chromosome interpretations are usually provided by cytogenetics laboratories; online sites and images provide references.

3. Commercial laboratories often can recommend appropriate tests and assist with their interpretation.

Table 12–1. Findings raising suspicion about genetic disease.

Component	Finding
Family history	Ethnicity Consanguinity Others with similar problems in family Health problems in multiple relatives, particularly if atypical Reproductive difficulties (infertility, habitual abortions, neonatal deaths) Delayed puberty, mental retardation, congenital malformations, neuromuscular disease
Personal history	Age of parents (particularly important if older) Poor childhood health Problems with wound healing or bleeding Drug reactions Special diet Fertility problems
Physical findings	Major malformation(s) Unusual stature Developmental delay Mental retardation Dysmorphic features Abnormal sexual development
Present illness	Exposure to teratogens or other drugs Atypical presentation of a common condition (eg, age of onset, unusual severity, management difficulties)

4. Research laboratories and genetics referral centers can offer evaluation and specialized experience.

B. **DNA sequence information** is becoming available.

1. The human genome sequence of 3×10^9 base pairs provides a basis for diagnostic studies.
2. Sequencing costs are falling (the current goal is the "$1000 genome").
3. Sequencing can provide an overwhelming amount of information.
   a. Computer analysis is needed to distinguish single nucleotide polymorphisms (**SNPs**), **mutations, deletions, insertions,** copy number variations (**CNVs**) and other changes as well as their potential significance.
   b. **Microarrays** can screen for changes in defined loci (see Chapter 1 and Figure 12–1).
4. The wide array of variations among individual genomes means that meaningful comparisons between individuals will require extensive comparative databases; these are being developed.

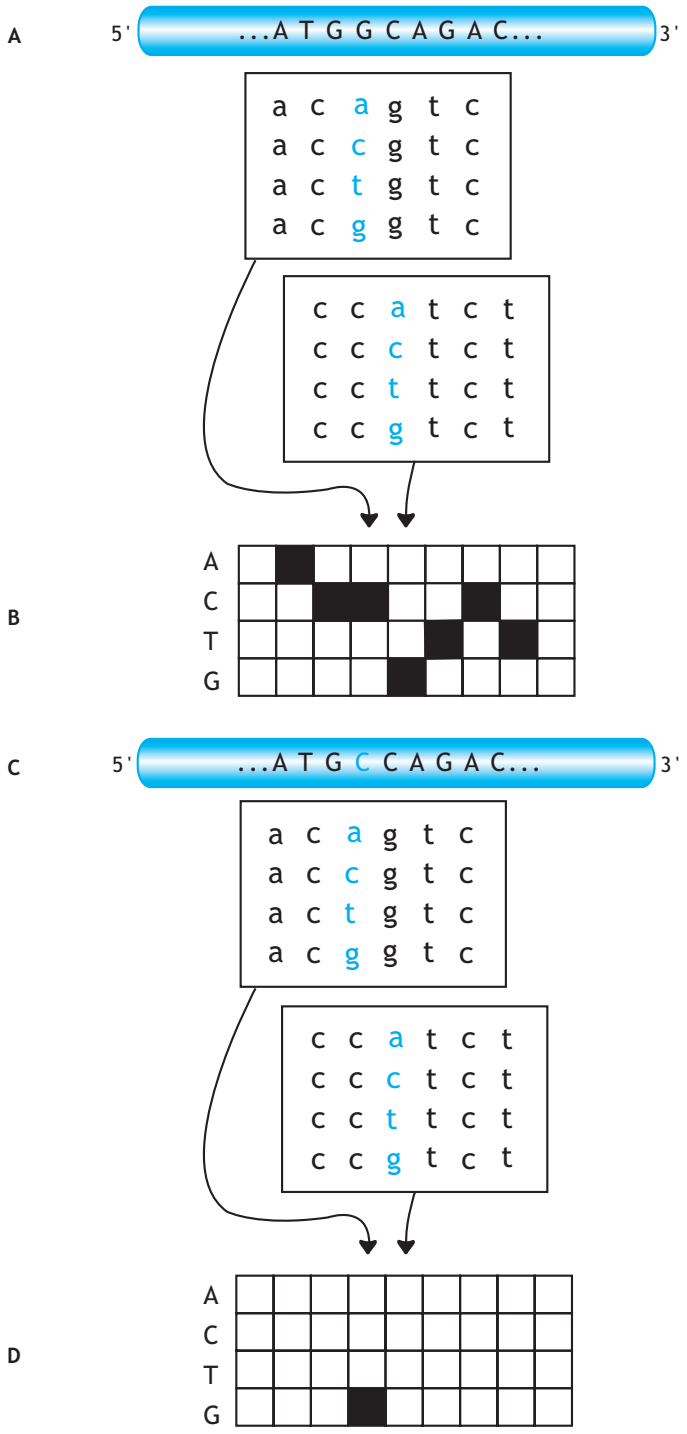


Figure 12–1. Microarrays permit multiplex gene analysis and also can provide sequence determination for known variants based on the assembly process described in Figure 1–9. In this example a single nucleotide sequence (***A***) is hybridized to a panel of four oligonucleotides, each varying at the same position. The next nucleotide is tested on another panel, staggered one position to the 3′ direction. The summary hybridization (***B***) identifies the complementary sequence. When a test sequence *(**C**)* with a single nucleotide change (G→C) is hybridized against the same panels, the pattern is remarkably different (***D***). By choosing oligonucleotide arrays to represent mutations and polymorphisms of interest, such an approach permits screening for thousands of changes in a single test. For simplicity, only short oligonucleotides have been shown; in practice, longer sequences are used. *(Modified from Chee M, et al. Accessing genetic information with high-density DNA arrays.* Science *1996;274:610. Reproduced with permission from AAAS.)*

III. Genetic Screening

A. Screening is being extended into larger populations.

1. Neonatal screening is now routine for many metabolic disorders (see Chapter 4).

a. Because screening is designed to have a sufficiently wide range of sensitivity to detect most abnormal individuals (ie, few false negatives) it also will identify some "normal" (or at least "atypical") individuals (ie, false positives).

b. Follow-up in referral laboratories and clinics must confirm and interpret screening data.

c. Tests must be relatively inexpensive (because they will be widely used).

d. Treatment should be available for the disorder (otherwise, there is little value in doing the test).

e. Screening can be directed to populations at increased risk. For example, it would not be useful to screen for sickle cell disease (OMIM 603903) in Caucasians or Tay-Sachs disease (OMIM 272800) in non-Jews.

2. Screening of family members can be valuable *if* the mutation is known; it may permit counseling or early preemptive treatment.

a. Identifying the **specific mutation** is essential, and the affected individual (**proband**) should be evaluated *first.*

b. Family studies can be particularly helpful in **pleiotropic autosomal dominant** (AD) conditions in which the clinical presentation may vary widely and identifying the risk can be lifesaving.

c. Screening may be useful for **preimplantation** or **prenatal studies** (see Chapters 1 and 2).

ACUTE INTERMITTENT PORPHYRIA (OMIM 176000)

- *This disorder involves an AD metabolic defect in heme synthesis.*
- *Patients experience attacks of paralysis, pain, or cognitive changes after exposure to drugs, hormones, and environmental agents.*
- *Kindred studies often identify individuals at risk who never have had an attack (in some families ~90% of those with the mutation are unaware of it), thus minimizing their chance of having an attack.*

3. Microarray-based assays reveal many changes.

a. The changes must be interpreted biologically and clinically.

b. They may be helpful in oncology for matching drugs to tumor characteristics, minimizing toxicity, and increasing effectiveness.

4. Screening may become helpful for **complex traits** (see Chapter 10).

a. Currently, the number of genetic contributions as well as their identity are unknown in most instances and may differ in different populations.

b. Studies of individuals with complex traits using SNPs, CNVs, and so forth, along with bioinformatics analysis may reveal a tractable number of informative markers (refer to Table 10–6 for several examples).

(1) **Marker pattern(s)** should, in theory, predict at least part of an individual's risk for common conditions.

(2) Identification of underlying genetic risk could enable clinicians to develop "customized" counseling and treatment strategies. This is the goal of **"personalized medicine"** and likely will become the basis for much future care.

B. **Confidentiality** will become even more important as broader screening approaches are introduced. For example, an individual's DNA sequence, once determined, will be a permanent part of his or her medical identity.

1. The sorts and amounts of data that can be generated for an individual are becoming enormous.

2. Such data must be readily available, and so portability and accessibility are essential.

3. The data likely will be stored electronically, possibly carried by the individual.

4. The basic data set could be updated and interpreted as new relationships, predispositions, susceptibilities, and other relevant factors are identified.

5. Insurability and employment concerns increase the importance of maintaining confidentiality while assuring data access.

IV. Treatment

A. Treatment of genetic diseases is becoming a realistic goal in many instances (Table 12–2).

1. Any genetic disease represents a distortion of an individual's homeostasis, and so the goal of treatment is restoring both immediate and long-term physiologic stability.

a. This does not always require complete correction of the underlying change.

b. This goal presents constraints and considerations different from much acute medical treatment (eg, trauma care).

2. Any treatment of genetic disease must consider the natural history of the distortions imposed by the mutation; some of these are not known initially.

a. Successful treatment of formerly lethal childhood conditions has led to a group of adult patients whose later complications have never been encountered (see Chapters 3–5).

b. Treatment response(s) may be constrained by other genetic variation(s) in the individuals (recall Chapters 9 and 11).

3. Effective treatment depends on understanding the detailed nature of the change(s) present; the most effective approaches have been based on understanding the molecular pathophysiology.

4. Treatments evolve with improved understanding, justifying judicious optimism.

5. Treatment that is effective in one set of physiologic circumstances may need modification in another (eg, diets of pregnant women with phenylketonuria differ from those used when they were children; recall Chapter 4).

6. The most important part of effective care for an individual with a genetic disorder is having the **correct diagnosis:** biochemical, molecular, chromosomal, or clinical. This also is essential for providing prognosis and counseling for the family and the individual.

B. **New approaches** to treatment hold great potential.

1. **Differentiated cell types,** including neuronal and myocardial precursors, are under study.

2. **Therapeutic cloning** may provide embryonic stem cells from the recipient.

a. Such cells should reduce problems with tissue rejection.

b. These cells could be targets for gene replacement prior to reintroduction (see below).

Table 12–2. Approaches to treatment of genetic diseases.

Treatment Approach	Example	OMIM
Modulation of Gene Function, Product, or Metabolism		
Hormone replacement	Thyroid hormones for congenital hypothyroidism prevent cretinism	
	Hormones can assist adolescent development in Turner syndrome (see Chapter 2)	
	Growth hormone replacement in appropriate children	
Metabolic modification	Heme derivatives (intravenous) reduce flux through the porphyrin synthesis pathway and control attacks in acute intermittent porphyria	176000
	Sodium phenylbutyrate provides an alternative route for nitrogen excretion in ornithine transcarbamylase deficiency	311250
	Glycine conjugates isovaleric acid in isovaleric academia, permitting its excretion	243500
Chemical limitation	Reducing phenylalanine in the diet of children with PKU permits growth while minimizing neuronal toxicity (see Chapter 4)	261600
	Eliminating galactose can prevent many problems in galactosemia	230400
	Chelating iron or copper can prevent systemic toxicity in hemochromatosis or Wilson disease, respectively (iron also can be lowered by phlebotomy)	235200 277900
Protein stabilization	High levels of vitamin B6 (as a cofactor) can stabilize some mutant forms of cystathionine β-synthase in homocystinuria, lowering blood levels of homocysteine and reducing vascular damage	236200
	Thiamine treatment can ameliorate some forms of maple syrup urine disease with mutations in the branched-chain α-ketoacid dehydrogenase complex	248600
Protein replacement[a]	β-Glucocerebrosidase for adult Gaucher disease reduces bone and hematologic complications	230800
	α-Galactosidase A reduces neuronal and vascular complications of Fabry disease	301500
	α-L-iduronidase has improved bone growth and somatic development in Hurler syndrome	607014
	Clotting factor VIII has reduced bleeding in hemophilia A (see Chapter 5)	306700

Table 12–2. Approaches to treatment of genetic diseases. (cont.)

Treatment Approach	Example	OMIM
	Immune globulins can enhance defenses in some individuals with defective immune function (see Chapter 8)	
Use of drugs	Hydroxyurea can increase fetal hemoglobin synthesis, in effect diluting the concentration of erythrocytes carrying sickle cell hemoglobin, and has been helpful in sickle cell disease (see Chapter 4)	603903
	β-Blockers can reduce stress on the ascending aorta, slowing development of aneurysms in Marfan syndrome	154700
	Losartan, a blocker of angiotensin II type 1 receptors, shows promise for reducing TGF-β signaling in Marfan syndrome	154700
Surgery		
Correction of complications	Replacing the aortic valve and ascending aorta in patients with Marfan syndrome can prevent (or acutely manage) dissection	154700
	Laminectomy can reduce spinal stenosis in achondroplasia	100800
	Neurofibromas can be excised in neurofibromatosis	162200
Preemptive removal of target organ(s)[b]	Colectomy can prevent colon cancer in individuals with APC	175100
	Mastectomy and oophorectomy in women with mutations in BRCA1 and BRCA2 can prevent breast and ovarian cancer	113705 600185
Transplantation		
Bone marrow[c]	Sickle cell anemia	603903
	Thalassemias	141900
Liver	Familial transthyretin amyloid neuropathy	176300
	Tyrosinemia	276700
	Maple syrup urine disease	248600
Kidney	Cystinosis	219800
	Polycystic kidney disease	173900

Table 12–2. Approaches to treatment of genetic diseases. (cont.)

Treatment Approach	Example	OMIM
Stem cells		
Umbilical cord[d]	Krabbe disease	245200
	Fanconi anemia	227650
	Blackfan-Diamond syndrome	105650
	Hurler syndrome	607014
Hematopoietic[e]	SCID	
	Fanconi anemia	227650
	Thalassemia major (and other hemoglobinopathies)	141900
Mesangioblast	Improves muscle function in a canine model of Duchenne muscular dystrophy	310200

PKU, phenylketonuria; TGF-β, transforming growth factor β; APC, adenomatous polyposis of the colon; SCID, severe combined immunodeficiency syndrome.
a Both natural and recombinant proteins may be given intravenously.
b In these and similar situations, the long-term natural history is unknown and surveillance is essential.
c Although potentially effective, this is not always the first choice for treatment.
d Often well-tolerated with relatively low tissue-type matching requirements.
e Bone marrow-derived cells have more stringent requirements for tissue matching.

c. Although not yet sufficiently reliable for widespread use, these techniques already have been used in the treatment of adenosine deaminase deficiency (OMIM 102700) and chronic granulomatous disease (OMIM 306400).

3. Gene replacement holds much promise.

a. Several criteria must be met for successful gene replacement.

(1) The required product must be produced by the recipient cells, ideally with repression or elimination of the mutant product, if it is present.

(2) The recipient gene should, ideally, persist in the recipient.

(3) The protocol must be reliable and consistent.

(4) In individuals with central nervous system involvement, the defect must be corrected there as well as in somatic tissues.

b. Viruses often have been proposed for introducing genes (Figure 12–2).

(1) The normal function of viruses is to introduce foreign genes into cells.

(2) The genetic material of many viruses becomes integrated into host cell DNA, permitting persistence in the progeny.

(3) Viruses often show restricted host range, permitting specific tissue delivery.

(4) Many details of viral molecular biology are known.

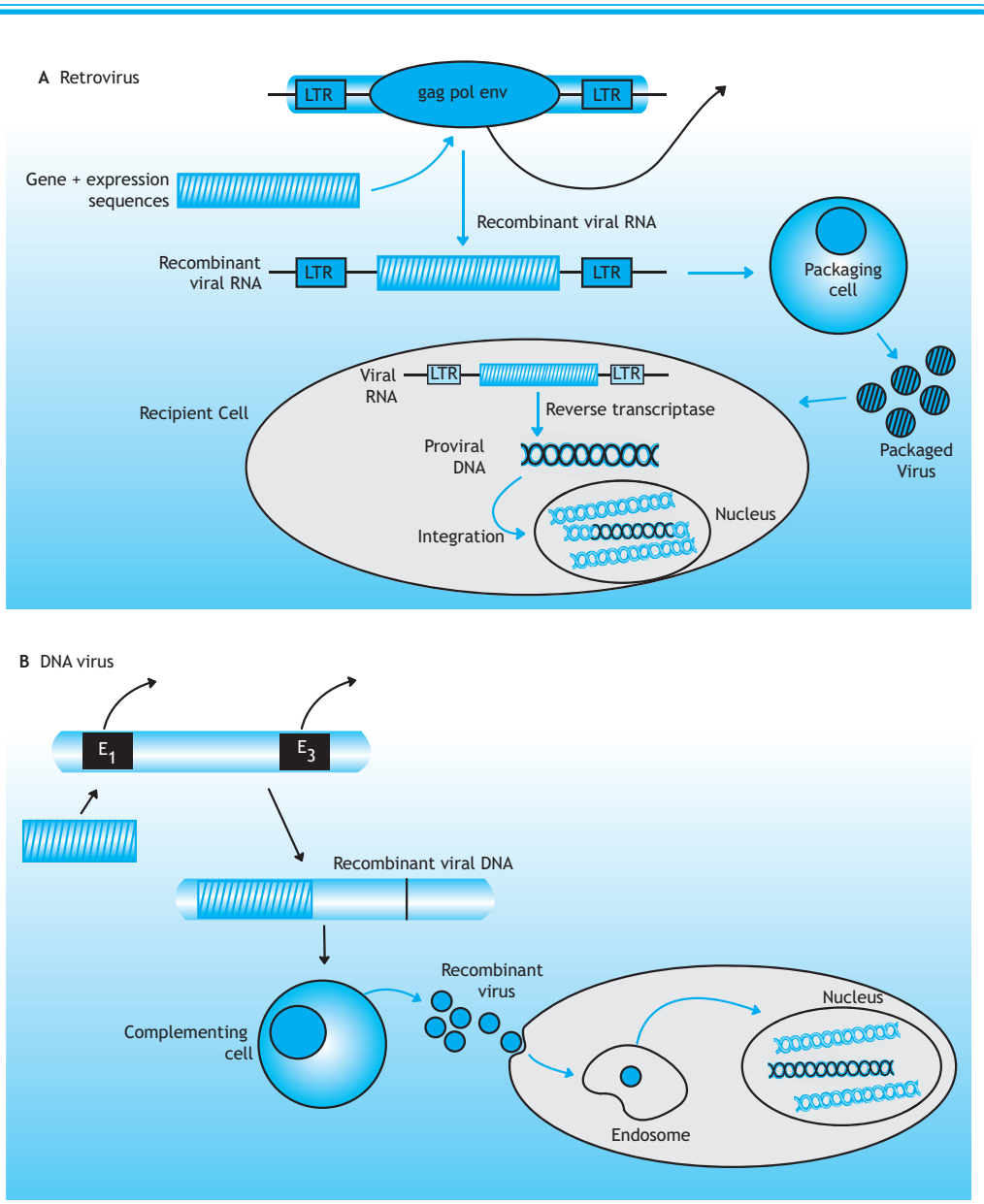


Figure 12–2. Strategies for introducing genes into cells using modified viruses. ***A.*** Retrovirus: the three genes of the virus are removed and replaced with the desired sequence. After entering the cell, viral reverse transcriptase synthesizes a double-stranded DNA copy that can be integrated into host cell DNA. ***B.*** DNA virus: a group of viral genes is replaced with the desired sequence. After the virus enters the cytoplasm, the DNA enters the nucleus where it persists extrachromosomally. Viruses used vary in genome size (adenovirus ~36 kb; HSV-1 ~150 kb).

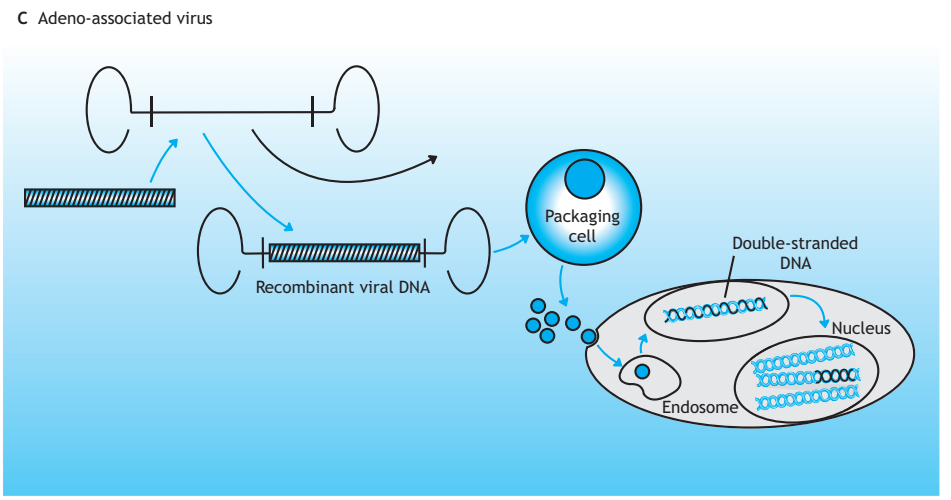


Figure 12–2. Strategies for introducing genes into cells using modified viruses. ***C.*** Adeno-associated virus: a small, single-stranded DNA virus forms a double-stranded DNA copy after infection. It can then enter the nucleus and integrate into cellular DNA.

(5) Recombinant viruses can be constructed, generally by replacing pathologic viral genes with the desired gene(s).
(6) Both **RNA-containing retroviruses** and **DNA-containing viruses** have been studied for use as vectors.

c. **Nonviral methods** for in vitro gene delivery also can be used.
(1) **Liposome-plasmid complexes** can be taken up by cells directly.
(2) **Complexes of DNA** with proteins that have cellular receptors can target the DNA to specific cells.
(3) **Direct DNA injection into cells** can work but often only transiently.
(4) **Stem cells** can be used (see Table 12–2).

d. Introducing **foreign DNA** into cells has both **theoretical and demonstrated risks,** generally related to the fate of the introduced DNA in the host genome.
(1) The new DNA may be inserted into a critical gene, effectively causing a new mutation.
(2) The DNA may insert near a gene that regulates cell proliferation, possibly causing a tumor (recall Chapter 9).

4. RNA interference (RNAi) may permit modulation of gene expression.

a. Small RNA molecules (usually ~20 nucleotides long) can control the steady state level of mRNAs by RNAi (recall Chapter 1).

b. So-called **microRNAs (miRNAs)** help regulate gene expression at the level of mRNA translation, a mechanism that may affect 30% of human genes.

c. Although their potential for inhibiting gene expression has been valuable in laboratory studies (RNAi can be used for virtually any gene) these RNAs have not yet been used to correct defective transcripts.
d. The specificity of miRNA is relatively low in mammalian cells; toxicity is possible.

5. The use of RNAi to **turn off expression** of a mutant gene could be valuable in AD conditions in which one copy of the gene remains normal.

FAMILIAL AMYLOID POLYNEUROPATHY (OMIM 176300)

- *This AD condition is caused by deposition of the met30 variant of transthyretin in nerves.*
- *Transthyretin is synthesized in the liver, and liver transplantation has been used to eliminate this source of the mutant protein (see Table 12–2).*
- *Affected individuals might benefit from RNAi-mediated reduction of synthesis of the met30 variant (these patients already have one copy of the normal gene).*

V. Prognosis

A. Prognosis for individuals treated for genetic diseases has unique aspects.
B. All treatments reviewed here affect only somatic tissues; the genotype is unaltered and the mutation can still be transmitted.
C. Individuals with genetic disorders often have lifelong problems because the mutation has fundamentally altered their basic biology.
D. The effect(s) of aging are poorly understood and may be unpredictable for individuals whose homeostasis is altered by a mutation.

DOWN SYNDROME

Improved care has led to longer survival of most individuals with Down syndrome, revealing the frequent early onset of Alzheimer-like dementia, which had not been noted earlier when longevity was limited.

MARFAN SYNDROME

Distal aortic dissections have developed in individuals with Marfan syndrome (OMIM 154700) years after repair of the proximal ascending aorta and aortic valve.

BREAST CANCER

Deciding about mastectomy and ovariectomy for women with BRCA1 *and* BRCA2 *mutations (OMIM 113705, 600185) is difficult in itself but, unfortunately, even having the surgery cannot assure the individual that other problems will not develop later.*

VI. Issues in Treatment of Genetic Diseases

A. Roles for caring individuals are more involved with complexities of individual biology.
1. Despite the excitement generated by new, apparently effective, treatments the responsibilities of physicians and coworkers are hardly easier.
2. Careful follow-up remains essential and must be done in the context of improved understanding of basic human biology and consequences of its perturbation.

Table 12–3. Sources of genetic disease support.

Genetic Alliance	Alliance of more than 600 genetic support and advocacy groups	(202) 966-5557 http://www.info@geneticalliance.org alliance@capaccess.org
MedHelp International	Search engine	(321) 259-7505 http://www.medhelp.org
National Organization for Rare Disorders (NORD)	Federation of voluntary organizations that help people with rare "orphan" diseases and the organizations that serve them	(800) 999-6673 http://www.rarediseases.org orphan@raredisease.org

3. As treatments alter basic cellular biochemistry the details will become more important and likely will only be revealed slowly.
4. Complex homeostatic systems cannot be expected to respond simply and consistently to short- or long-term intervention, particularly when the systems themselves have underlying variations (recall Chapter 10).
5. Ironically, these new challenges should make medicine even more effective and satisfying.

B. "**Orphan" disease support groups** provide a valuable resource.

1. Developing and introducing treatments for rare diseases is complex, expensive, and likely to be limited to small populations.
2. The **Orphan Disease Act** (1983) provides marketing protection for developing such treatments; many specialized treatments noted in this chapter are in this important category.
3. Many rare disorders have organized groups, often assembled by affected individuals and their families, that have been developed for social support and advocacy (Table 12–3).
   a. These groups often have practical experience with specific conditions.
   b. Such groups can support clinical studies.
   c. These groups can disseminate information to patients and families who might otherwise be isolated.

CLINICAL PROBLEMS

A 53-year-old woman has just been evaluated for colon cancer, and the examination and test results are normal. All of the other studies recommended for a woman of her age (mammogram, gynecologic evaluation, stress test, dermatology examination, routine lab studies, etc) have been performed, and she appears to be in good health. One month later,

she contacts the physician because her younger brother has had localized colon cancer detected after "some gene test." The family has Ashkenazi Jewish heritage. The woman also states that she is unwilling to consider a colectomy (see Chapter 3).

1. The physician would most likely advise that

A. Because her colonoscopy was clear, she is unlikely to develop colon cancer.

B. Because she is older than her brother and unaffected, no further studies are recommended.

C. Because she is unwilling to consider a colectomy, no further studies are recommended.

D. The woman should undergo the same genetic testing.

E. The woman's children should undergo the same genetic testing.

A treatment group of 40 individuals with adult Gaucher disease (OMIM 230800) has responded well to monthly infusions of β-glucosidase. After 10 years, 6 of the patients develop mild pancreatitis.

2. The physician who has been coordinating their care is most likely to suspect that

A. The dose schedule for the affected patients may have varied because they live farther from the treatment center.

B. The affected patients may be related.

C. The reaction to the exogenous enzyme in the affected patients includes cross-reactivity with pancreatic α cells.

D. The treatment is contributing to the problem and must be stopped.

E. The problem noted in the affected patients may develop in others.

3. In patients who undergo total colectomy for adenomatous polyposis coli (OMIM 175100)

A. Kidney stone risk is reduced by ~75%.

B. Gallstones will be a problem.

C. The likelihood of renal failure is increased.

D. Colon cancer will not develop.

E. The risks of hypertension and stroke are increased by ~30%.

ANSWERS

1. The answer is D. The patient's risk could be as high as 50% (for a dominant trait), and she must be followed more closely; thus, choice A is incorrect. If she has the gene change, she likely will need annual colonoscopy. AD traits have variable expressivity (recall Chapter 3), so her age (choice B) is not a deciding factor. Choice C, forgoing further studies, would not be recommended. In general, it is best to know her gene status to assist follow-up. Furthermore, new treatments may become available even if

colectomy is not considered. Her children may be at risk, but it would be inappropriate to study them (choice E) before her own status is known.

2. The best answer is E. The physician knows these people well and can exclude the explanations proposed in choices A through D. What is *not* known is what to expect in very long-term enzyme replacement; there are no helpful studies because the approach is new. The greatest likelihood of success will be in comparing these findings with those of other treatment centers. Often such data sharing is coordinated by the pharmaceutical company that produces the therapeutic agent.

3. The answer is D. Colectomy does not change the known risks for the conditions listed in choices A, B, C, and E. In addition, the long-term likelihood of developing tumors elsewhere is unknown.

APPENDIX
INDICATIONS FOR GENETIC CONSULTATION REFERRAL*

Table 1. Genetic consultation for preconceptional or prenatal patients.

Genetic consultation may be helpful for a prenatal or preconceptional patient who is or will be:	
Finding	**Reason to consider consultation**
Age 35 years or older at the time of delivery (for a singleton pregnancy)	Discuss testing options for identifying an age-related chromosome anomaly
Age 33 years or older at the time of delivery (for a twin pregnancy)	Discuss testing options for identifying an age-related chromosome anomaly
A close blood relative of her partner (consanguineous union)	Review pedigree and assess degree of relatedness; discuss potential additional fetal risks and testing options before and/or after delivery
Genetic consultation may be helpful for a prenatal or preconceptional patient who has:	
Finding	**Reason to consider consultation**
An abnormal first or second trimester maternal serum ± nuchal translucency screening test	Discuss risks to pregnancy and testing options
Exposure to a teratogen or potentially teratogenic agent during gestation such as radiation, high-risk infections (cytomegalovirus, toxoplasmosis, rubella), drugs, medications, alcohol, etc.	Discuss risks to pregnancy and testing options and rule out significant fetal ± maternal risks
A fetal anomaly or multiple anomalies identified on ultrasound and/or through echocardiography	Discuss risks to pregnancy and testing options
A personal or family history of pregnancy complications known to be associated with genetic factors such as acute fatty liver of pregnancy	Rule out significant fetal risks ± maternal risks, including a metabolic disorder

(continued)

*Prepared by the Professional Practice and Guidelines Committee of the American College of Medical Genetics.

Table 1. Genetic consultation for preconceptional or prenatal patients. (continued)

Genetic consultation may be helpful for either member of a couple with:	
Finding	**Reason to consider consultation**
A positive carrier screening test for a genetic condition such as cystic fibrosis, thalassemia, sickle cell anemia, Tay-Sachs, etc.	Discuss additional testing strategies and inheritance
A personal history of stillbirths, previous child with hydrops, recurrent pregnancy losses (more than two), or a child with sudden infant death syndrome (SIDS)	Rule out a chromosomal, metabolic, or syndromic diagnosis that may be associated with an unexplained neonatal death or SIDS
A progressive neurologic condition known to be genetically determined such as a peripheral neuropathy, unexplained myopathy, progressive ataxia, early onset dementia, or a familial movement disorder	Discuss a potential diagnosis, the differential diagnosis, inheritance, and testing options
A statin-induced myopathy	Discuss a potential mitochondrial disorder, inheritance and testing options
Genetic consultation may be helpful for either member of a couple with a family or personal history of:	
Finding	**Reason to consider consultation**
A birth defect such as a cleft lip ± palate, spina bifida, or a congenital heart defect	Discuss recurrence risks and testing options; discuss folate supplementation, if appropriate, for subsequent pregnancies
A chromosomal abnormality such as a translocation, marker chromosome, or chromosomal mosaicism	Discuss risks to the fetus and testing options
Significant hearing or vision loss thought to be genetically determined	Discuss risks to the fetus and testing options
Mental retardation or autism	Discuss risks to the fetus and testing options

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Table 2. Genetic consultation for adult patients.

Genetic Consultation may be helpful for an adult patient with a personal history of:	
Finding	**Reason to consider consultation**
Abnormal sexual maturation or delayed puberty	Rule out an intersex condition, chromosomal abnormality or syndromic diagnosis (e.g., androgen insensitivity, Klinefelter syndrome)
Recurrent pregnancy losses (RPLs) (more than 2)	Rule out a chromosomal rearrangement such as a balanced translocation or inversion; causes 5%–7% of RPLs
Tall or short stature for genetic background	Rule out a skeletal dysplasia, chromosomal or syndromic diagnosis (e.g., dyschondrosteosis, Klinefelter syndrome, Marfan syndrome)
One or more birth defects	Rule out a chromosomal or syndromic diagnosis (e.g., 22q deletion, Noonan syndrome); provide genetic counseling and discussion of preconception folate supplementation, if appropriate
Six or more café-au-lait macules >1.5 cm in diameter	Rule out neurofibromatosis type 1
Statin-induced myopathy	Rule out a mitochondrial disorder
Genetic consultation may be helpful for an adult patient with a personal or family history of:	
Finding	**Reason to consider consultation**
A cancer or cancers known to be associated with specific genes or mutations such as breast, ovarian, and colorectal in the context of a compelling family history; young age at onset, bilateral lesions, and familial clustering of related tumors	Rule out an identifiable mutation in a gene such as BRCA1, FAP, etc.; rule out a cancer syndrome (e.g., MEN2 or von Hippel-Lindau); discuss surveillance, treatment, testing options (if presymptomatic), and inheritance
Cardiovascular problems known to be associated with genetic factors such as cardiomyopathy, long QT, hyperlipidemia, etc.	Rule out a mutation in a causative or contributory gene; discuss surveillance, treatment, testing options, and inheritance
Suspected genetic disorder affecting connective tissue	Rule out a syndromic diagnosis (e.g., Ehlers-Danlos, Marfan syndrome, familial joint hypermobility); discuss surveillance, treatment, testing options, and inheritance
Hematologic condition associated with excessive bleeding or excessive clotting (as evidenced by recurrent deep vein thromboses or pulmonary emboli)	Confirm or rule out genetic condition (e.g., one of the hemophilias, von Willebrand, one of the genetic thrombophilias); discuss treatment, testing options, and inheritance

Table 2. Genetic consultation for adult patients. (continued)

Genetic consultation may be helpful for an adult patient with a personal or family history of:	
Finding	**Reason to consider consultation**
Progressive neurologic condition known to be genetically determined such as a peripheral neuropathy, unexplained myopathy, progressive ataxia, early-onset dementia, and a familial movement disorder	Confirm or rule out suspected diagnosis, discuss surveillance, treatment, testing options, and inheritance
Visual loss known to be associated with genetic factors such as retinitis pigmentosa, early-onset macular degeneration, and cataracts	Rule out a syndromic diagnosis (e.g., Stickler syndrome); discuss testing options, if applicable, and inheritance
Early-onset hearing loss	Rule out a syndromic or nonsyndromic genetic form of hearing loss; discuss surveillance, testing options, and inheritance
Recognized genetic disorder including a chromosomal or single gene disorder	Confirm the diagnosis; discuss prognosis, medical management, and inheritance
Mental illness such as schizophrenia, depression, bipolar disorder, etc.	Discuss diagnosis, inheritance, recurrence risks, and identify syndromes (e.g., 22q deletion), when possible
Genetic consultation may be helpful for an adult patient with a family history of:	
Finding	**Reason to consider consultation**
A close relative with a sudden, unexplained death, particularly at a young age	Rule out a genetic condition associated with this history, e.g., long QT, Marfan syndrome, and other cardiac conditions

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Table 3. Genetic consultation for pediatric patients.

Genetic consultation may be helpful for a neonate with:	
Finding	**Reason to consider consultation**
An abnormal newborn screening test	Rule out an inborn error of metabolism or other treatable condition; provide genetic counseling about recurrence risks
Congenital hypotonia or hypertonia	Rule out a chromosomal, metabolic, or syndromic diagnosis (e.g., Prader-Willi syndrome, congenital myotonic dystrophy, hyperekplexia).
Unexplained intrauterine growth retardation	Rule out a chromosomal or syndromic diagnosis (e.g., Russel-Silver syndrome, trisomy 18)
Genetic consultation may be helpful for a neonate, infant, or child with:	
Finding	**Reason to consider consultation**
A single major, or multiple major and/or minor anomalies	Rule out a chromosomal or syndromic diagnosis; provide genetic counseling for recurrence and possible preventive measures (e.g., folate supplementation in subsequent pregnancies)
Dysmorphic features that are not familial, especially if accompanied by developmental delay or mental retardation	Rule out a chromosomal or syndromic diagnosis (numerous conditions)
Failure to thrive	Rule out a chromosomal, metabolic, or syndromic diagnosis, or genetic condition (e.g., IGFIR mutations)
A known metabolic disorder or symptoms of a metabolic disorder such as intractable seizures, hepatosplenomegaly, acidosis, cyclic vomiting, persistent hypoglycemia, developmental regression, and unusual body odor	Diagnose an inborn error of metabolism; discuss treatment and management; provide genetic counseling
Abnormal brain MRI findings such as leukodystrophy, periventricular calcifications, unidentified bright objects, or a malformation	Rule out a chromosomal or syndromic diagnosis (e.g., neurofibromatosis, tuberous sclerosis); provide genetic counseling (e.g., some brain malformations such as Dandy-Walker malformation may be genetic)
An unusual growth pattern such as overgrowth, short stature, or hemihypertrophy	Rule out a chromosomal, syndromic, or metabolic diagnosis (e.g., Sotos syndrome, Beckwith-Wiedemann syndrome, Turner syndrome)

Table 3. Genetic consultation for pediatric patients. (continued)

Finding	Reason to consider consultation
Evidence of a connective tissue disorder such as extreme joint laxity, poor wound healing, or a marfanoid habitus	Rule out a connective tissue disorder, such as Ehlers-Danlos syndrome, Marfan syndrome
Congenital eye defects or blindness associated with problems such as microophthalmia, cataracts, megalocornea, retinitis pigmentosa, or cone-rod dystrophy	Rule out a syndromic diagnosis; provide genetic counseling for potentially hereditary ocular conditions
Significant hearing loss or deafness not secondary to recurrent otitis media	Rule out a syndromic form of hearing loss (e.g., Waardenburg syndrome) or identify a genetic form of nonsyndromic hearing loss
Cardiomyopathy not secondary to a viral infection	Rule out a mitochondrial disorder or other syndromic or metabolic diagnosis (e.g., carnitine deficiencies, Noonan syndrome, several forms of muscular dystrophy); provide genetic counseling for potentially hereditary forms of cardiomyopathy
Six or more café-au-lait macules >0.5 cm in diameter	Rule out neurofibromatosis type 1
Unusual skin findings such as multiple types of lesions, multiple lipomas, numerous hypo- or hyperpigmented lesions, and albinism	Rule out a chromosomal or syndromic diagnosis (e.g., chromosomal mosaicism, tuberous sclerosis, Cowden syndrome)
Born to a parent with a known chromosomal abnormality or rearrangement (balanced or unbalanced), especially if there are dysmorphic features and/or cognitive impairment	Rule out a chromosomal abnormality
Bilateral or multifocal malignancies such as retinoblastoma or Wilms tumor	Rule out a cancer syndrome or other chromosomal or syndromic diagnosis (e.g., aniridia-Wilms tumor caused by 11p13 deletion); provide genetic counseling for recurrence
Problems with clotting including disorders such as hemophilia and thrombophilia	Rule out an inherited clotting disorder as well as some syndromes (e.g., Noonan syndrome)
A recognized or suspected genetic syndrome including a chromosomal or single gene disorder	Confirm the diagnosis and discuss the prognosis, medical management, inheritance, and recurrence risks
A significant family history of medical or psychiatric conditions that puts the patient at risk of developing the same or similar disorder	Discuss diagnosis, inheritance, and possible testing options

Table 3. Genetic consultation for pediatric patients. (continued)

Genetic consultation may be helpful for a child with:

Finding	Reason to consider consultation
Unexplained mental retardation or global developmental disorder	Rule out a chromosomal, syndromic or metabolic diagnosis (e.g., fragile X, sex chromosome anomaly, some forms of mucopolysaccharidoses)
Autism or pervasive developmental disorder	Rule out a chromosomal or syndromic diagnosis (e.g., fragile X, Angelman syndrome, Rett syndrome)
Unusual behaviors, especially when associated with minor malformations and developmental delay or mental retardation	Rule out a chromosomal or syndromic diagnosis (e.g., Smith-Magenis syndrome, Lesch-Nyhan syndrome)
An immunodeficiency or significant immune problem	Rule out a syndromic diagnosis (e.g., 22q deletion) or genetic form of immunodeficiency (e.g., severe combined immunodeficiency syndrome)
Progressive muscle weakness that might be associated with a genetic disorder such as a form of muscular dystrophy, spinal muscular atrophy, or myotonic dystrophy	Confirm suspected diagnosis and provide genetic counseling
Other neurologic condition that might be associated with a genetic predisposition such as a peripheral neuropathy, unexplained myopathy, progressive ataxia, or any progressive neurologic disorder without a clear, nongenetic cause	Rule out a genetic diagnosis (e.g., spinocerebellar ataxia, Huntington disease), provide genetic counseling

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