Chapter 2

# Diagnostic Techniques in Medical Genetics

## CHAPTER OBJECTIVES

- ✓ Review pedigree analysis and its associated terminology.
- ✓ Discuss the methodology and applications for cytogenetic studies.
- ✓ Explain fluorescence in situ hybridization.
- ✓ Describe DNA analysis and biochemical analysis.

Because hereditary disorders can affect different organ systems as well as people of all ages, it is important for healthcare providers to be familiar with genetic testing methodology. These tests range from taking a thorough family history that includes several familial generations (i.e., pedigree), to DNA sequencing, to hybridization with specific probes. While it is impractical to construct a detailed pedigree with every patient visit due to time constraints, it is important to know how to map out a pedigree in case there is some concern about a specific disease within a family.

## Family History

Clinicians are well trained in the importance of taking a good family history and should at the very least ask about the medical history of all first-degree relatives (parents, siblings, and offspring) and, if possible, more distant relatives. Pertinent information includes age, sex, ethnicity, general health status, major illnesses, and cause of death. Once this information is obtained, it can be further analyzed utilizing a pedigree diagram to identify mode of inheritance for a disease process.

## Pedigree Analysis

A pedigree is a diagram representing the familial relationships among relatives. It can be used to analyze Mendelian inheritance of certain traits. The symbols have been standardized, in that females are represented by circles and males by squares (**Figure 2-1**). A diamond is used if the sex is unknown. In the case of a miscarriage, a triangle is used. Colored or shaded symbols show persons with the phenotype of interest, whereas heterozygous carriers of recessive alleles are depicted with half-filled symbols.

A mating between a male and a female is indicated by a single horizontal line that is then connected vertically with a second horizontal line below that connects the symbols for their offspring. Mating between related (**consanguineous**) individuals is indicated

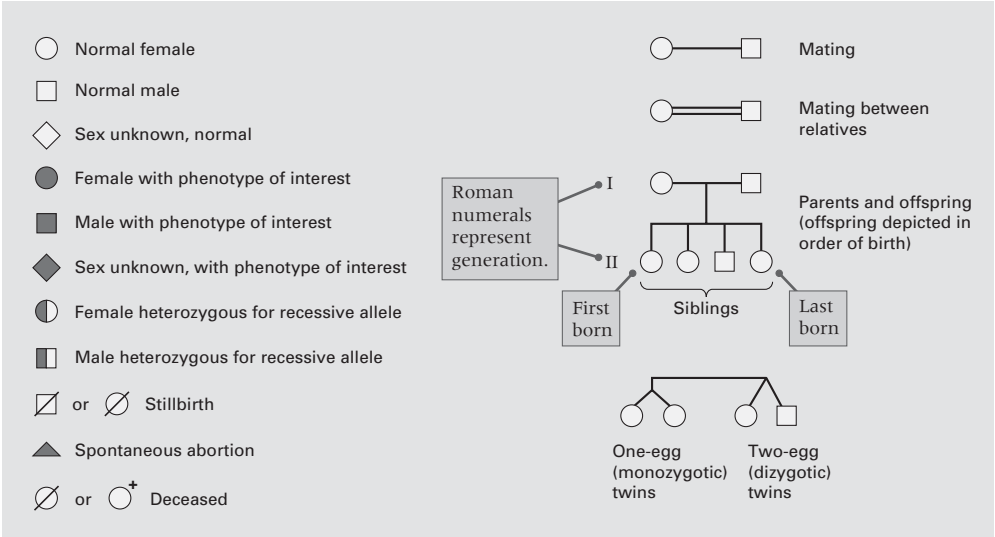



**Figure 2-1** Conventional symbols used in depicting human pedigrees.
*Source:* Bennett R, French K, Resta R, Doyle D. Standardized Human Pedigree Nomenclature: Update and Assessment of the Recommendations of the National Society of Genetic Counselors. *Journal of Genetic Counseling*. 17:424–433;2008. ©National Society of Genetic Counselors, Inc. 2008.

with a double horizontal line. The offspring, called **sibs** or **siblings**, are represented from left to right in order of birth; each row corresponds to a generation that is labeled with a Roman numeral.

**Figure 2-2** shows an example of a pedigree for a family in which some members have Huntington's disease (see Chapter 4). Within any generation, the individuals are numbered consecutively from left to right. The pedigree starts with the woman I-1 and the man is I-2. The man has Huntington's disease, as indicated by the shaded symbol. Because this disease is due to a dominant mutation, all affected individuals have the heterozygous genotype *HD hd*, whereas nonaffected people have the homozygous normal genotype *hd*

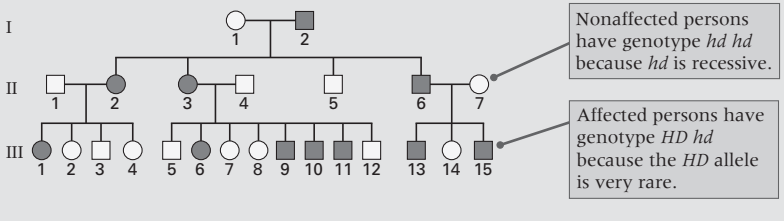



**Figure 2-2** Pedigree of a human family showing the inheritance of the gene for Huntington's disease. Females and males are represented by circles and squares. Shaded symbols indicate people affected with the disease.

*bd*. The disease has complete **penetrance**, which means the trait is expressed in 100% of persons with that genotype.

For example, in the case of a rare dominant allele with complete penetrance, the following characteristics are observed:

1. Females and males are affected equally.
2. Affected offspring typically have one affected parent, with the same likelihood ratio of the affected parent being the mother or the father.
3. Approximately 50% the siblings with the same parents are affected.

An example of a pedigree for a homozygous recessive allele is albinism (**Figure 2-3**). In comparison, inheriting a rare recessive allele with complete penetrance, would yield the following observed characteristics:

1. Females and males are affected equally.
2. Affected individuals would *not* have affected offspring.
3. Affected individuals typically have *no* affected parents.
4. Parents of those affected may be related.
5. Approximately 25% of siblings with the same parents are affected.

In the case of inheritance of a rare recessive trait, the mates of homozygous affected persons are usually homozygous for the normal allele, so all of the offspring will be heterozygous and not affected. Because it is more likely that a person will inherit only one copy of a rare mutant allele rather than two copies, heterozygous carriers of

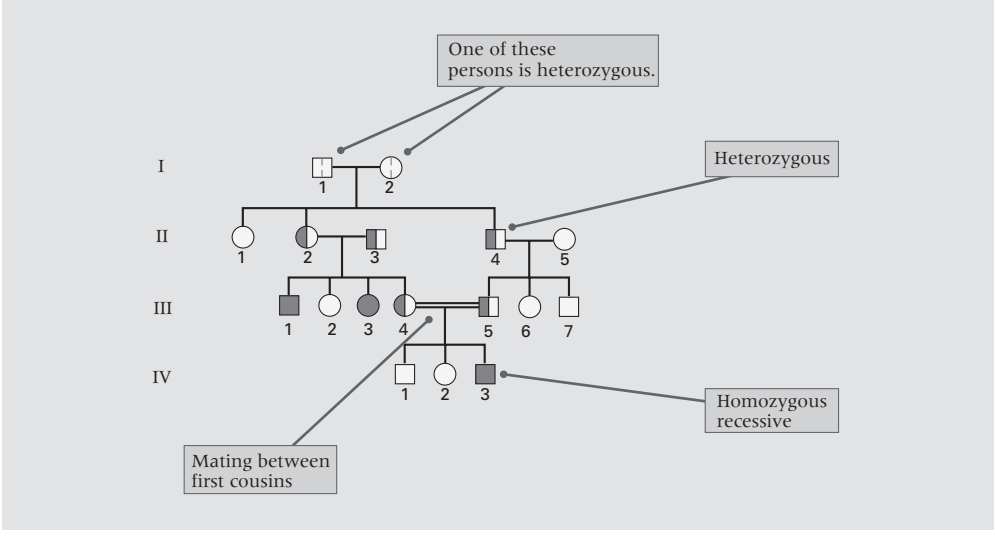



**Figure 2-3** Pedigree of albinism. With recessive inheritance, affected persons (filled symbols) often have unaffected parents. The double horizontal line indicates a mating between relatives—in this case, first cousins.

> mutant alleles are more common than homozygous affected individuals. Therefore, most homozygous recessive genotypes result from mating between heterozygous carriers in which each offspring has a 25% chance of being affected. This can especially occur if parents of affected individuals are related. (Hartl and Jones, 2005)

A rare recessive allele (i.e., albinism) is more likely to be expressed when mating between related heterozygous individuals occurs (Figure 2-3). The offspring resulting from this mating has a 25% chance of inheriting the homozygous recessive allele and will express the albino trait.

## Cytogenetic Studies

Cytogenetics is the study of chromosomes utilizing light microscopy. Chromosomal analysis is done by growing human cells in tissue culture, chemically inhibiting mitosis, staining, observing, photographing, sorting, and counting the chromosomes. Samples can be obtained from peripheral blood, amniotic fluid, trophoblastic cells from the chorionic villus, bone marrow, and cultured fibroblasts (usually obtained from a skin biopsy). In a **karyotype**, the chromosomes are rearranged systematically in pairs, from longest to shortest, and numbered from 1 (the longest) through 22 to represent the autosomes (**Figure 2-4**). The sex chromosomes are usually set off at the bottom right. The karyotype

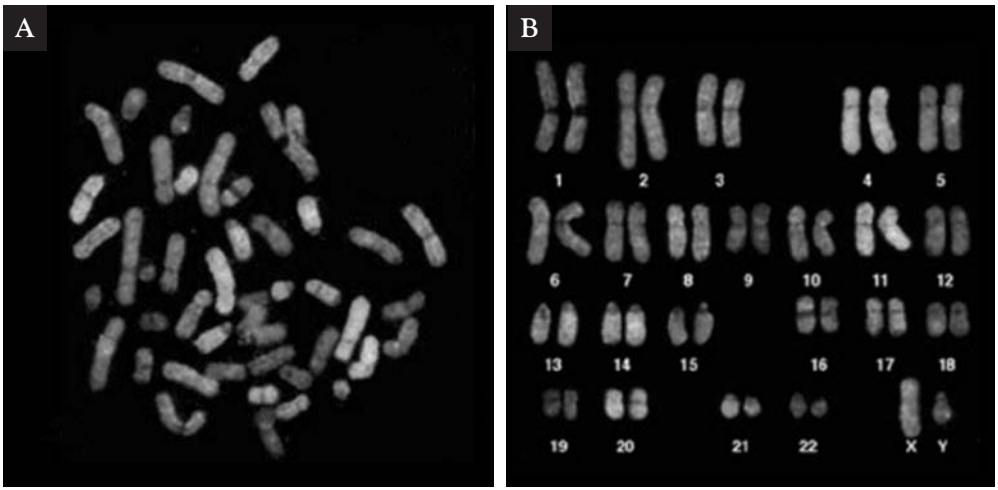



**Figure 2-4A, B** Human chromosome painting, in which each pair of chromosomes is labeled by hybridization with a different fluorescent probe. (A) Metaphase spread showing the chromosomes in a random arrangement as they were squashed onto the slide. (B) A karyotype, in which the chromosomes have been grouped in pairs and arranged in conventional order. Chromosomes 1–20 are arranged in order of decreasing size, but for historical reasons, chromosome 21 precedes chromosome 22, even though chromosome 21 is smaller.
*Source:* Courtesy of Johannes Wienberg, Ludwig-Macimillians-University, and Thomas Ried, National Institutes of Health.

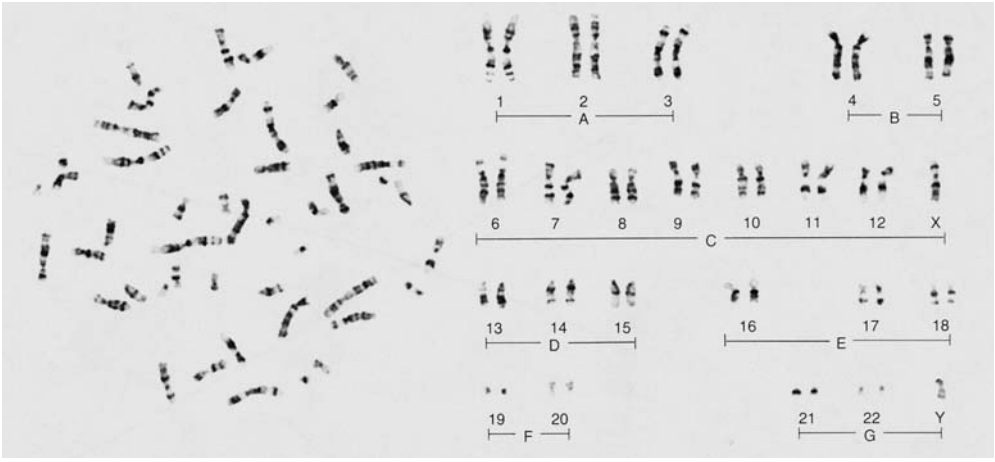
of a normal human female has a pair of X chromosomes instead of an X and a Y. **Chromosome painting**, as shown in Figure 2-4, helps to identify pairs of homologous chromosomes. The different colors are "painted" on each chromosome by hybridization with DNA strands labeled with different fluorescent dyes.

Another karyotype is shown in **Figure 2-5** with chromosome banding. These chromosomes have been treated with Giemsa stain, which causes chromosomes to exhibit transverse bands (G-bands) that are specific for each pair of homologs. These bands allow smaller segments of each chromosome arm to be identified. In addition to allowing the identification of autosomes and sex chromosomes, chromosomal abnormalities can be identified through this technique.

## Fluorescence in Situ Hybridization

Chromosome staining and painting provides a way to visualize banding patterns and pairs of homologous chromosomes. However, this interpretation can be rather difficult given that a "standard" karyotype reveals approximately 400 to 500 bands per set of haploid chromosomes. The development of fluorescence in situ hybridization (FISH) has made it easier to visualize and map chromosomal (gene) abnormalities.

"Fluorescent means emitting light that comes from a reaction within the emitter and "in situ" refers to the fact that this technique is done with the chromosomes, cells or tissue in place (in situ) on a microscope slide" (MedicineNet, 2010). A short sequence of nucleic




**(A)** Photograph of metaphase chromosomes **(B)** Karyotype

**Figure 2-5** A karyotype of a normal human male. Blood cells arrested in metaphase were stained with Giemsa and photographed with a microscope. (A) The chromosomes as seen in the cell by microscopy. (B) The chromosomes have been cut out of the photograph and paired with their homologs.

*Source:* Courtesy of Patricia A. Jacobs, Wessex Regional Genetics Laboratory, Salisbury District Hospital.

acid that matches a portion of the gene in question is labeled with a fluorescent dye and is referred to as a **probe**. The probe is then allowed to hybridize to suitably prepared cells or histological sections; hybrids are formed with complementary sequences of nucleic acids in a chromosome (**Figure 2-6**). Through nucleic acid hybridization, the degree of sequence identity can be determined and specific sequences detected and located on a specific chromosome (MedicineNet, 2010). This technique is frequently used to look for localization of genes on specific chromosomes.

**INDICATIONS FOR CYTOGENETIC ANALYSIS**

- Malformations associated with a particular syndrome or aberration
- Serious mental or physical developmental problems
- Maldefined genitalia (internal or external)
- Primary amenorrhea or delayed pubertal development
- Males with learning or behavioral disorders who are taller than expected
- Malignant or premalignant disease
- Parents of a patient with a chromosome translocation
- Parents of a patient with a suspected syndrome
- Couples with a history of multiple spontaneous abortions of unknown cause
- Infertility not caused by obstetric or urogenital problems
- Prenatal diagnosis

*Source:* Reproduced from Pyeritz RE. Medical Genetics. In Tierney L, et al. *Current Medical Diagnosis & Treatment*, 42nd ed. 2003.

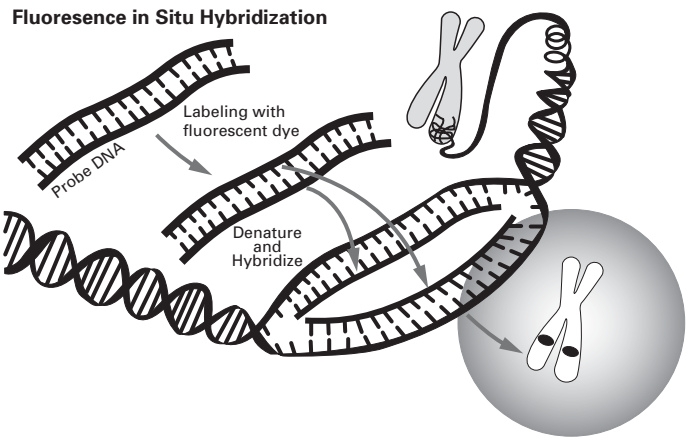



**Figure 2-6** Diagram showing fluorescence in situ hybridization.
*Source:* Courtesy of Fluorescence In Situ (FISH) National Genome Research Institute, National Institutes of Health. Available at http://www.genome.gov/10000206. Accessed January 16, 2010.

## DNA Analysis

Molecular genetics involves understanding the expression of genes by studying DNA sequences of chromosomes. Once a particular gene is shown to be defective in a given disease, the nature of the mutation can be elucidated by sequencing the nucleotides and comparing with that of a normal allele. Molecular testing is available for more than 1000 hereditary conditions and has had a significant impact on the diagnosis of Mendelian disorders.

Similar to the use of specific probes in a FISH analysis of chromosomal abnormalities, probes are used to identify specific genes that may be mutated in a certain hereditary disease. The probe may be a piece of the actual gene, a sequence close to the gene, or just a few nucleotides at the actual mutation. The closer the probe is to the actual mutation, the more accurate and the more useful the information. When even a minute amount of DNA from a patient (e.g., from a few leukocytes, buccal mucosal cells, or hair bulbs) is combined with the primers in a reaction mixture that replicates DNA—and after several dozen replication cycles are then performed via a process called **polymerase chain reaction (PCR)**—the region of DNA between the primers will be amplified exponentially. For example, the presence of early HIV infection can be detected after PCR amplification of a portion of the viral genome.

**EXAMPLE INDICATIONS FOR DNA ANALYSIS**

- Pre-symptomatic detection of Huntington's disease or adult polycystic kidney disease
- Screening for cystic fibrosis and thalassemias
- Screening for X-linked conditions such as Duchenne muscular dystrophy and hemophilia A and B
- Screening for familial polyposis coli

*Source:* Reproduced from Pyeritz RE. Medical Genetics. In Tierney et al. *Current Medical Diagnosis and Treatment*, 42nd ed. 2003.

## Biochemical Analysis

The primary goal of biochemical testing is to determine whether certain proteins are present or absent as well as to identify their characteristics and effectiveness in vitro. This kind of analysis is used to look for enzymatic defects, as these important catalysts are made of protein. For example, **phenylketonuria (PKU)** is an inherited disorder caused by the absence of or a defect in the enzyme **phenylalanine hydroxylase (PAH)**. In the absence of PAH, the amino acid phenylalanine accumulates and can lead to severe mental retardation. If this deficiency is diagnosed early in life, however, children can be placed on low-phenylalanine diets and mental retardation avoided. Based on this knowledge, all babies in the United States are screened for PKU.

Another disease process associated with a defective protein is **cystic fibrosis (CF)**. In this disease, a mutation in the **CFTR gene** disrupts chloride and water transport across membranes. The end result is production of thick and sticky mucus that obstructs the airways in the lungs and the ducts in the pancreas. In addition to breathing difficulty, people with CF have problems with nutrient digestion because the buildup of mucus prevents pancreatic digestive enzymes from reaching the intestine.

Both PKU and CF are examples of **inborn errors of metabolism**, which refers to an inherited defect in one or more enzymes. Currently, the state of Georgia screens newborns for 24 metabolic disorders plus sickle cell anemia. Not all states test for all of the same disorders in their screening of infants, however, and in some cases parents can refuse to have the tests done. For a more detailed listing and description of inborn errors of metabolism, refer to the United States National Newborn Screening Status Report at http://genes-r-us.uthscsa.edu/nbsdisorders.pdf.

## Chapter Summary

- A good family history should at the very least ask about the medical history of all first-degree relatives (parents, siblings, and offspring) and, if possible, more distant relatives.
- A pedigree can be used to analyze Mendelian inheritance of certain traits.
- Cytogenetics is the study of chromosomes utilizing light microscopy.
- Once a particular gene is shown to be defective in a given disease, the nature of the mutation can be elucidated by sequencing the nucleotides and comparing this sequence with that of a normal allele.
- The primary goal of biochemical testing is to determine whether certain proteins are present or absent as well as to identify their characteristics and effectiveness in vitro.

## Key Terms

**CFTR gene:** a gene that codes for a protein involved in chloride and water transport across membranes. In patients with cystic fibrosis, a mutation in this gene disrupts chloride and water transport across membranes. The end result is production of thick and sticky mucus that obstructs the airways in the lungs and the ducts in the pancreas.

**Chromosome painting:** use of differentially labeled, chromosome-specific DNA strands for hybridization with chromosomes to label each chromosome with a different color.

**Consanguineous:** mating between related individuals.

**Cystic fibrosis:** a congenital metabolic disorder, inherited as an autosomal recessive trait, in which secretions of exocrine glands are abnormal. Excessively viscid mucus causes obstruction of passageways (including pancreatic and bile ducts, intestines, and bronchi), and the sodium and chloride content of sweat are increased throughout the patient's life

**Inborn error of metabolism:** a genetically determined biochemical disorder, usually in the form of an enzyme defect that produces a metabolic block.

**Karyotype:** the chromosome complement of a cell or organism; often represented by an arrangement of metaphase chromosomes according to their lengths and the positions of their centromeres.

**Penetrance:** the proportion of organisms having a particular genotype that actually express the corresponding phenotype. If the phenotype is always expressed, penetrance is complete; otherwise, it is incomplete.

**Phenylalanine hydroxylase (PAH):** the enzyme that converts phenylalanine to tyrosine and that is defective in phenylketonuria.

**Phenylketonuria (PKU):** a hereditary human condition resulting from inability to convert phenylalanine into tyrosine. It causes severe mental retardation unless treated in infancy and childhood by a low-phenylalanine diet.

**Polymerase chain reaction (PCR):** repeated cycles of DNA denaturation, renaturation with primer oligonucleotide sequences, and replication, resulting in exponential growth in the number of copies of the DNA sequence located between the primers.

**Probe:** a labeled DNA or RNA molecule used in DNA-RNA or DNA-DNA hybridization assays.

**Sibling (sib):** a brother or sister, each having the same parents.

## Chapter Review Questions

1. Mating between related individuals, also known as _____, is indicated with a double horizontal line in a pedigree diagram.
2. Siblings of individuals who carry the recessive gene for albinism have a _____ percent chance of inheriting and being affected by this trait.
3. In a _____, the chromosomes are rearranged systematically in pairs, from longest to shortest, and numbered from 1 (the longest) through 22.
4. _____ with a fluorescent probe is one method used to assess the degree of sequence identity as well as detect and locate specific sequences on a specific chromosome.
5. In the absence of _____, the amino acid phenylalanine accumulates and can lead to severe mental retardation.

## Resources

*The American Heritage Dictionary of the English Language,* 4th ed. Boston: Houghton Mifflin; 2006.

Bennett RL, French KS, Resta RG, Doyle DL. Standardized Human Pedigree Nomenclature: Update and Assessment of the Recommendations of the National Society of Genetic Counselors. *Journal of Genetic Counseling.* 17:424–433; 2008.

Georgia Department of Human Resources. Newborn Screening for Metabolic and Sickle Cell Disorders Program. http://health.state.ga.us/programs/nsmscd/descriptions.asp.

Hartl, DL, Jones EW. *Genetics: Analysis of Genes and Genomes,* 6th ed. Sudbury, MA: Jones and Bartlett; 2005.

Hartl, DL, Jones EW. *Essential Genetics: A Genomic Perspective,* 4th ed. Sudbury, MA: Jones and Bartlett; 2006.

MedicineNet, Inc. Definition of Fluorescent in Situ Hybridization. Available at http: // www.medterms.com/script/main/art.asp?articlekey=3486. Accessed January 27, 2010.

National Newborn Screening and Genetics Resource Center. http://genes-r-us .uthscsa.edu/.

Pyeritz RE. Medical Genetics. In: Tierney LM Jr., McPhee SJ, Papadakis MA (Eds.), *Current Medical Diagnosis and Treatment,* 42nd ed. New York: McGraw-Hill; 2003, pp. 1643–1666.

*Stedman's Online Medical Dictionary.* http://www.stedmans.com/.