Appendixes

Appendix A
Basic Tenets of Human Genetics

Basic Human Genetics

The chemical bearer of genetic information is DNA, which takes the structural form of a double-stranded helix (figure A-l). DNA is composed, in part, of four chemical subunits called bases. These four bases—guanine (G), adenine (A), thymine (T), and cytosine (C)-are the coding units of genetic information. The bases normally pair predictably-A with T, and G with C (figure A-2)—to form the DNA double helix structure. The order and organization of the base pairs determines all genetic information. Genes, which are segments of DNA, come in many different sizes ranging from 1,000 to 1 million base pairs in length. A mutant gene results in an altered amino acid sequence, which can alter the protein in such a way that clinical symptoms arise.

Genes are organized in microscopically visible bundles called chromosomes (figure A-3). The chromosomal constitution of each individual is derived equally from mother and father. In humans, 23 chromosomes are contributed by each parent in the gamete (egg or sperm), resulting in a total of 46 chromosomes-22 pairs of autosomes and 1 pair of sex chromosomes (two X chromosomes for females and an X and a Y for males). The entire complement of genetic material in this set of chromosomes, about 3.3 billion base pairs-50,000 to 100,000 structural genes—is called the human genome. Only about 2 percent of the genes have been identified along the chromosomes.

The physical location of a gene on a chromosome is called its locus. Some genes have been mapped and cloned and can be identified directly at their locus. But it is the exception that the direct link between one gene, one locus, and one disease can be made. Most diseases are multifactorial and polygenic; i.e., several genes in combination with specific environmental factors act together to produce the disease state.

Because chromosomes come in pairs, there are two copies of a gene at each locus, one inherited from each parent. Different "versions" of a gene at a particular locus are called alleles. When two or more alleles of a gene are found at a particular locus in the population, the genetic variants are referred to as polymorphisms. Polymorphisms (i.e., common genetic differences between people) play an important role in diagnosing genetic diseases.

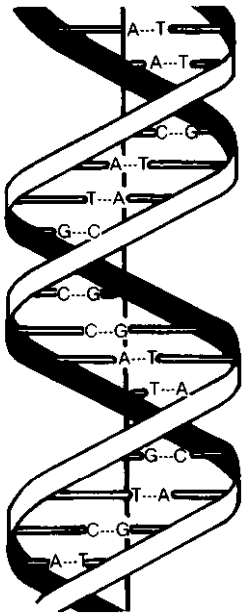
The term 'mutation' refers to a change in the sequence or number of nucleotides in a gene. Mutations arise through a number of mechanisms: through environmental agents as mentioned previously, or through normal cellular processes. Not all mutations cause disease, although that is the reference in which the term is commonly used. Mutations that form in germline cells that produce eggs and sperm are inherited by offspring, whereas those that occur in somatic cells remain only in the descendants of those cells in the affected individual. This distinction is critical in distinguishing between monitoring and screening. Genetic screening can detect traits that are caused by mutations in both somatic **and** germ cells, whereas monitoring generally detects mutations that have occurred in that population of somatic cells being tested and cannot be extrapolated to other tissues.

Genetics and Disease

The link between familial factors and certain health conditions has long been recognized. A useful classification of the genetic basis of disease is disorders caused by:

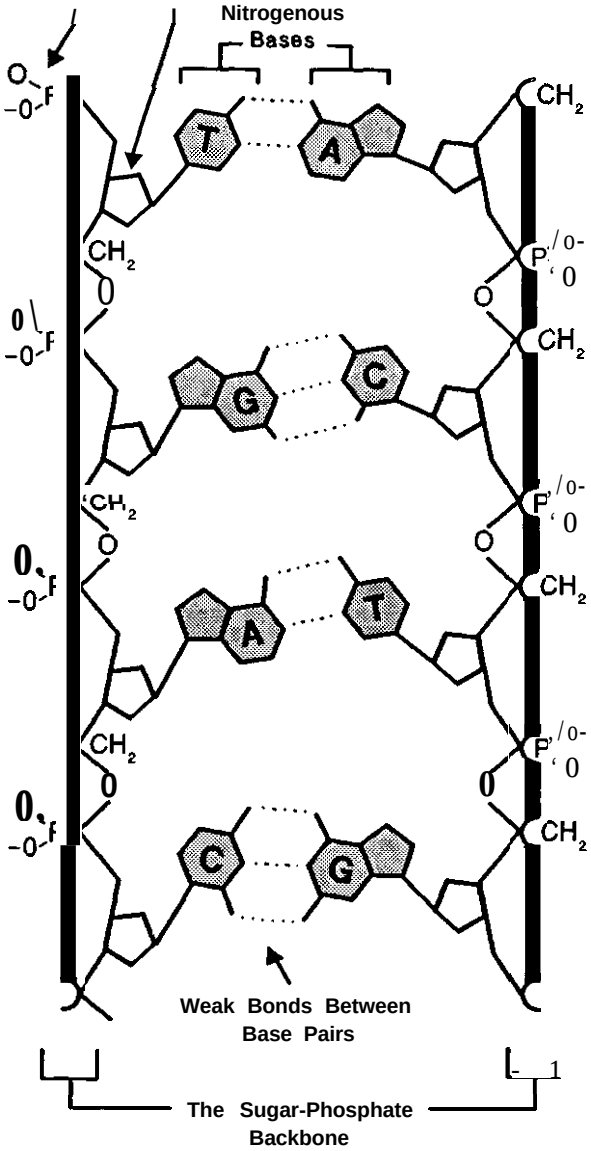
. a single mutant gene (monogenic);
. chromosomal aberrations;
- genetic predisposition combined with environmental interaction (multifactorial); and
- changes in the somatic cells (cancer, aging, autoimmune disease, and some congenital malformations).

Figure A-I—The Structure of DNA



SOURCE: Office of Technology Assessment, 1990.

Figure A-2—DNA Base Pairing



The four bases of DNA, guanine (G), adenine (A), thymine (T), and cytosine (C) form the four letters of the genetic code. The pairing of the four bases is A with T, and G with C, It is the sequence of bases along the strand of DNA encodes the genetic information.

SOURCE: Office of Technology Assessment, 1990.

Single Gene Disorders

A gene can be altered by a "point" mutation (i.e., a change in only a single base pair) resulting in an altered protein or function for which it codes. Thus, a gene can have several functional and dysfunctional variants, all of which are referred to as alleles. Each individual carries two sets of genes, one from each parent. If the two members of a pair of genes are the same, the individual is considered to be homozygous for that locus. If the two members of the pair are different, the individual is said to be heterozygous for the locus. Such an individual has the trait, but not necessarily the disease. Heterozygotes for certain traits, therefore, make two kinds of gene products from their two genes. If only one functional gene is needed to produce sufficient protein activity, the individual will be normal for that trait and the mutant allele will be considered recessive. If, however, the one mutant allele is sufficient to produce a defect in the individual, the trait is considered dominant.

Most often, single gene mutations, whether transmitted recessively or dominantly, cause a defect in enzymes that may cause "inborn errors" of metabolism and transport. These errors result from lack of a functional enzyme and may result in:

- diseases resulting from absence of the end product;
- diseases resulting from the accumulation of substrates or metabolizes;
- diseases resulting from interference with regulatory mechanisms; and
- diseases where there is an inborn error of membrane transport.

Single gene mutations can also cause amino acid substitutions that produce "hemoglobinopathies' (hemoglobins with abnormal functions). Sickle cell anemia is a common form of hemoglobinopathy. The mutant gene in this disease causes an abnormality in hemoglobin which distorts the red blood cells, resulting in small blood vessel blockage and subsequent oxygen deficit.

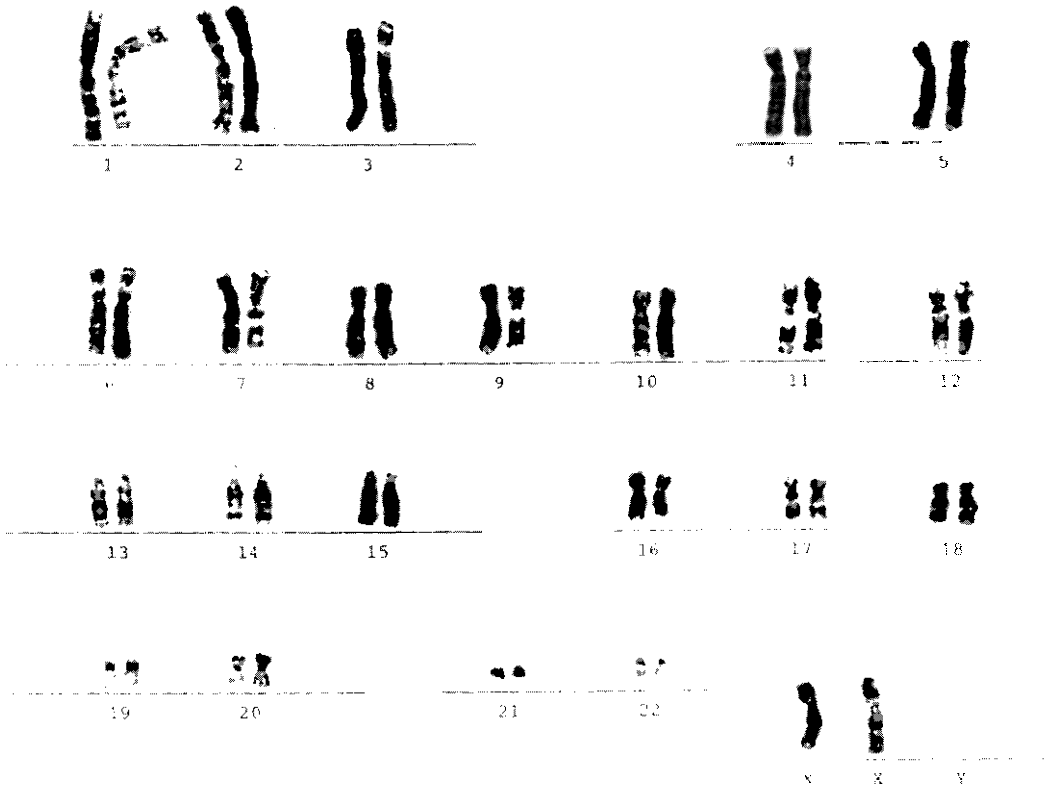
Some 400 gene products of the approximately 3,000 known single gene disorders have been identified (5). The nature of the mutations involved, however, has only been determined in 45 of these disorders (see table A-l). Better understanding of the nature of the mutation leads to increased capabilities for diagnosis, both prenatally and in children and adults and, in a few disorders, has resulted in improved treatment for affected individuals.

Critical to diagnosis and treatment has been understanding the mode of inheritance of the mutant gene. The three modes of inheritance are discussed below. Figure A-4 illustrates the various modes of inheritance for single gene disorders. The figure represents a pedigree or family tree, the standard tool used by geneticists tracking the history and mode of inheritance of genetic disease in families.

Autosomal Dominant Inheritance

If the mutant gene is located on one of the autosomes (not a sex chromosome) and it is dominant, every affected

Figure A-3-Chromosome Complement of a Normal Human Female



SOURCE: The Genetics & IVF Institute, Fairfax, VA.

individual has an affected parent, except for cases arising from a fresh mutation. If that affected individual has an unaffected spouse, each of their children will have a 50 percent chance of inheriting the mutant gene and having the disease. Offspring of affected persons who have not inherited the mutant gene will not have affected offspring.

Examples of genetic disease inherited in this manner are Huntington's disease, adult polycystic kidney disease, and Marfan syndrome. Except for eases where the disease has appeared as a fresh mutation in an individual, families with dominantly inherited genetic disease are more likely to know they are at risk because of the appearance of the disease in preceding generations. More problematic are those diseases with a late onset, such as Huntington's, because individuals might not know they are affected until they already have children.

Autosomal **Recessive Disorders**

Recessive deleterious genes produce disease only in the homozygote, that is, persons with two copies of the mutant gene. Affected individuals have received one mutant gene from each parent. Most often both parents are asymptomatic heterozygotes, unaware that they carry the mutant allele until they either produce an affected offspring or undergo testing for heterozygote or 'carrier' status. There is infrequently a family history of the disease because of the small likelihood that two individuals heterozygous for the same trait would meet and have children. Recessive disorders are more common than dominant disorders. Even if both parents are carriers of the trait, each child has a 1 in 4 chance of inheriting the mutant gene from both parents, producing the homozygous or disease state. Statistically, each child also has a 50 percent chance of inheriting one mutant gene from either parent, thus, becoming a carrier. Each child also has a 25 percent chance of inheriting the normal gene from both parents.

Examples of some common autosomal recessive disorders are cystic fibrosis, sickle cell anemia, phenylketonuria, and beta-thalassemia.

Sex Linkage

Theoretically, if the mutant gene is on one of the sex chromosomes, the X or the Y, the pattern of inheritance differs. In reality, the Y chromosome is very small and contains few genes. There are no known diseases trans-

Table A-I-Some Known Hereditary Disorders for Which Gene Has Been Cloned

Acatalalasemia	Hereditary congenital hypothyroidism
Alpha 1 -Antitrypsin deficiency	Hers' disease (glycogen storage disease VI)
Alpha-Thalassemia	Homocystinuria
Antithrombin III deficiency	Hypobetalipoproteinemia, premature atherosclerosis
Argininosuccinic aciduria	Isolated familial growth hormone deficiency
Atransferrinemia	Lecithin-cholesterol acyltransferase disease
Beta-thalassemia	Lesch-Nyhan syndrome
C2 deficiency	Lipid adrenal hyperplasia deficiency
C3 deficiency	McArdle's disease
C4 deficiency	Medium-chain acyl-CoA dehydrogenase deficiency
Carbamylphosphate	Mucopolysaccharidosis VII
Chronic granulomatous disease	Ornithine transcarbamylase deficiency
Citrullinemia	Osteogenesis imperfecta
Color blindness	Phenylketonuria
Congenital adrenal hyperplasia	Phosphoglycerate kinase deficiency
Diabetes mellitus due to abnormal insulins	Porphyria cutanea tarda
Duchenne muscular dystrophy	Propionic acidemia type I
Dysfibrinogenemias	Propionic acidemia type II
Dyslipoproteinemia	Pyruvate carboxylase deficiency
Ehlers-Danlos syndrome type IV	Renal tubular acidosis with osteopetrosis
Ehlers-Danlos syndrome VII A2	Retinoblastoma
Elliptocytosis-1	Sandoff's disease
Elliptocytosis-2, spherocytosis	Severe combined immuo-deficiency from adenosine deaminase deficiency
Fabry's disease	Sickle cell anemia
Factor VII deficiency	Tay-Sachs disease
Factor X deficiency	Thrombophilia from plasminogen activator deficiency
Factor XIII deficiency	Thrombophilia from plasminogen variant
Familial hypercholesterolemia	Thrombophilia from protein C deficiency
Familial hypoparathyroidism (one form)	Triosephosphate isomerase deficiency
Fructose intolerance	Tyrosinemia type II
Fucosidosis	X-linked icthyosis
Gaucher's disease	von Willebrand's disease
Glucose-6-phosphate dehydrogenase deficiency	
Gyrate atrophy	
Hemophilia A	
Hemophilia B	

SOURCE: S.E. Antonarakis, "Diagnosis of Genetic Disorders at the DNA Level," New *England Journal of Medicine* 320:153-163, 1989.

mitted via the Y chromosome. The X chromosome, however, is much larger and contains numerous genes, many of which are known to cause disease when in the mutant form. Genes on the X chromosome can also be dominant or recessive, but the fact that females have two X chromosomes, and males have only one X and one Y leads to differences in the patterns of inheritance. Figure A-4 illustrates the characteristic pedigree for X-linked disease, usually being transmitted by an asymptomatic female to each of her sons with a 50 percent probability. Daughters of the unaffected carrier mother each have a 50 percent chance of also being unaffected carriers. Sons who do not inherit the abnormal gene are unaffected and cannot transmit the gene.

Examples of X-linked disorders are Duchenne muscular dystrophy and hemophilia.

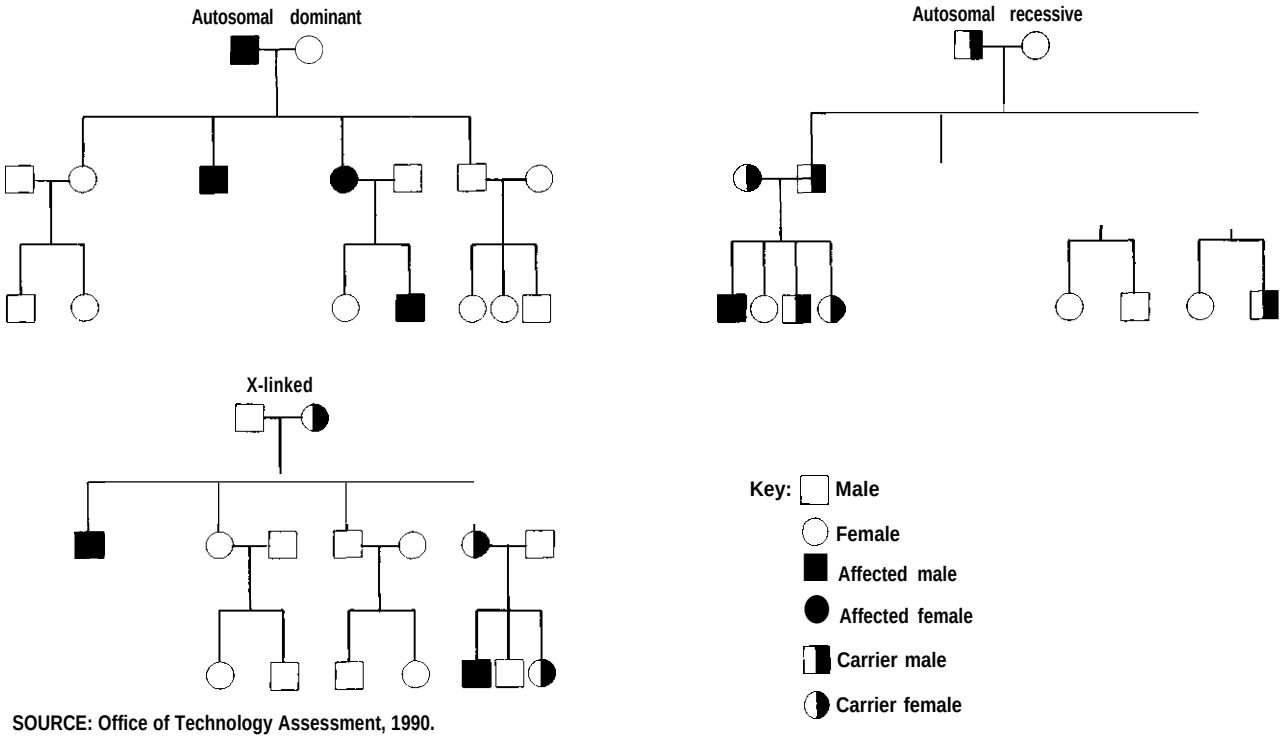
Confounding Factors in Mendelian Patterns

Not all mutant genes display the regularity of transmission implied by the previous discussion. Several irregularities can complicate accurate genetic diagnoses and are described in the following sections.

Mutation-As mentioned earlier, for two reasons, family history does not usually forewarn individuals that they are at risk for genetic disease. First, many diseases are transmitted in an autosomal recessive manner, meaning the chances are very low that two carriers for the same disease will meet, procreate, and have an affected child. Second, naturally occurring mutation rates exist for all diseases. For example, "Fragile X Syndrome and Duchenne muscular dystrophy have high mutation rates which explains why these diseases persist despite the fact that those who have them rarely procreate.

Variable Expressivity--Variable expressivity is the term used for the variation in severity of effects produced by the same genes in different people. The physical presentation of effect of a gene is referred to as the "phenotype." Thus, one genotype often gives rise to a range of phenotypes in different individuals. For example, the neurofibromatosis gene may cause multiple disfiguring tumors in one patient but only skin discoloration and a few insignificant tumors in another patient.

Figure A-4-Modes of Inheritance of Single Gene Disorders



SOURCE: Office of Technology Assessment, 1990.

Heterogeneity-The same or similar physical characteristics can be produced by different genes or loci. Thus, several different genotypes can produce similar phenotypes. In most genetic diseases, heterogeneity appears to be the rule rather than the exception.

Penetrance—To further complicate matters, a gene that expresses itself clinically in one person can produce no detectable effect in another. The failure to reach the "clinical surface' in an individual is called "nonpenetrance." The gene is silent. At the population level, the gene is said to have reduced penetrance. Reduced penetrance is most easily detected in the case of dominant traits, when an individual who must carry the mutant genes, based on pedigree or DNA analysis, does not have the disease or trait.

Phenocopies--Sometimes the effect of a gene is simulated by an environmental agent in an individual not carrying the mutant gene. For example, congenital deafness can be caused by a recessive gene or by the drug streptomycin.

Multifactorial Inheritance

Combinations of genes encode complex aspects of the human phenotype, such as the immune response and cholesterol metabolism. Defects in one or more of these genes can cause diseases that may be exacerbated by environmental factors such as viruses, chemicals, and radiation; thus the term "multifactorial disease. " Multifactorial diseases are far more common than single gene disorders. They include coronary artery disease, diabetes mellitus, multiple sclerosis, schizophrenia, epilepsy, allergic rhinitis, asthma, some forms of arthritis, and some forms of emphysema, to name a few (4).

Identification of the genes that make an individual "susceptible" to disease state is especially compelling because of the possibility of prevention. An individual identified to be at risk can avoid known exogenous risk factors such as diet or infectious agents, or watch for the developments of symptoms for treatment at an early stage.

Chromosomal Disorders

Sometimes genetic disease is caused by structural aberrations in the chromosomes. Chromosomal aberrations (CAs) are gross structural changes visible under the light microscope that arise from errors in cell division. During the two types of cell division in humans —mitosis and meiosis—' 'mistakes" can occur resulting in too much, too little, or rearranged chromosomal material in the daughter cells. Errors in the number or structure of chromosomes can result in maldevelopment of the fetus and subsequent disorders in liveborn infants.

In some cases, CAs can be caused by environmental agents such as radiation, chemicals, or viruses (2,3,6,8). These environmental insults have been associated less

with errors in number than with breakage of the chromosomes. Such breakage can interfere with the genetic signals necessary for normal cell growth and repair (e.g., it is well-documented that large doses of ionizing radiation cause chromosomal breakage correlated with certain forms of cancer) (1,7).

Errors in chromosome number and structure can present clinical disorders in a variety of forms, from before conception to the advanced stages of disease. The spontaneous frequency of CAs (both structural and numerical) in newborns is about 6 per 1,000. Chromosomal analysis of human spontaneous abortuses shows that about 50 percent are chromosomally abnormal. In addition, persons exposed to ionizing radiation and certain chemicals have increased frequencies of CAs in their lymphocytes. Many forms of cancer are associated with increased frequencies of aberrations. And, several human hereditary conditions, such as ataxia telangiectasia and Fanconi's anemia, are associated with increased frequencies of CAs as well as increased incidence of cancer.

Genetic evaluation at the chromosomal level, rather than the biochemical or molecular level, is referred to as "cytogenetics. Cytogenetic approaches to genetic screening are most reliably used in prenatal diagnosis for women of advanced maternal age, and for the diagnosis of certain forms of cancer and certain hereditary traits. The use of cytogenetic tests for monitoring populations exposed to genotoxic agents and ionizing radiation is discussed in chapter 4.

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