GENETICS AND DISEASE

GENETICS AND DISEASE

ALFRED G. KNUDSON, Jr.
Department of Biology, City of Hope Medical Center, Duarte, California

THE BLAKISTON DIVISION
McGRAW-HILL BOOK COMPANY
New York Toronto Sydney London

GENETICS AND DISEASE

Library of Congress Catalog Card Number:

64-22460

35263

Preface

A characteristic of revolutionary change is that properties of a *new kind* emerge in the thing transformed, be it political, artistic, scientific, or other. The revolution in biology, which began during the misery of World War II, is no exception. In the space of a few years, biology was put on the same solid foundation of the molecular basis of matter that supports the great edifices of physics and chemistry; biology became a physical science.

Studies of human disease have made important contributions to this revolution; particularly outstanding are those on alkaptonuria, on the organism commonly causing lobar pneumonia, and on sickle-cell anemia. In turn the revolution has had a profound effect upon medicine. New explanations emerged suddenly for old diseases like the Down syndrome (Mongoloid idiocy). New therapeutic approaches have been pursued, as in phenylketonuria. New interpretations of the relationship of heredity and environment have been provoked by discovery of the protective effect against malaria offered by some disease-producing genes. Man's control of his own evolution is fast approaching possibility.

My purpose in writing this small volume on *Genetics and Disease* is to present these significant advances in terms of the human diseases familiar to the clinician, while suggesting at the same time that genetics contributes to unifying our concepts of disease. I have attempted to touch upon all categories ranging from congenital defects to the degenerative problems of the aged

and to show that genetic constitution plays a role in all disease. This approach has forced the omission of many disorders for which there is detailed knowledge and the inclusion of others where much remains to be learned.

The book is divided into eight chapters. The first three deal with genes, chromosomes, and genetic linkage. Certain congenital defects serve to illustrate the main points, with emphasis placed on the dynamic interaction of mutation and selection in the determination of the defects. The molecular events which mediate genetic function are the concern of the next two chapters. The chemical basis of congenital defects is discussed, as are therapeutic possibilities. Acquired disease as an interaction of the host with his environment is the subject of the last three chapters. Genetic constitution not only determines response to environmental agents but can be modified by them; the genetic apparatus itself undergoes irreversible change with time.

I hope this book will be of interest to students of biology and medicine, whether undergraduate or graduate. Clinical descriptions are included primarily for the benefit of preclinical medical students and students of biology. Hopefully, further reading will be encouraged; to that end, specific citations of pertinent literature are grouped at the end of each chapter and a general reading list appears following the last chapter.

I am deeply indebted to many colleagues for their assistance. Professors H. J. Muller, Ray D. Owen, and A. H. Sturtevant, and Drs. Marcel Baluda, William Kaplan, Vincent Marinkovich, Alexander Miller, Susumu Ohno, and Stanley Rappoport have each read extensive portions of the manuscript. Mr. Kurt Smolen, artist, and Mr. Richard Ray, photographer, have been particularly helpful in the preparation of illustrative material. I have a warm vote of thanks for Mrs. Patricia A. Ray, editorial assistant, and the several diligent typists who have labored on the manuscript. Finally, the patience and support of those with whom I worked in the Blakiston Division, McGraw-Hill, deserve special notice.

Alfred G. Knudson, Jr.

Contents

CHAPTER 1:	GENES AND CONGENITAL DEFECTS	1
CHAPTER 2:	CHROMOSOMES AND CONGENITAL DEFECTS	41
CHAPTER 3:	GENES, CHROMOSOMES, AND DISEASE	95
CHAPTER 4:	INBORN ERRORS OF METABOLISM	127
CHAPTER 5:	MOLECULAR DISEASE	157
CHAPTER 6:	INFECTION AND IMMUNITY	195
CHAPTER 7:	CANCER	235
CHAPTER 8:	DEGENERATIVE DISEASE AND AGING	261
APPENDIX:	GENERAL READING LIST	277
NAME INDEX		279
SUBJECT INDEX		289

1

Genes and Congenital Defects

MENDELIAN INHERITANCE

Man is a seeker of cause, and man as physician has been no exception. The nineteenth-century discoveries of the bacterial origin of several dreaded diseases were the first successes in the systematic investigation of the environment for causal agents. These demonstrations of the existence of specific causative agents laid the foundation for the science of etiology, or study of causes.

There was already much evidence that some diseases were inherited and apparently independent of environment. Several workers had even recognized the pattern of inheritance in hemophilia. Still there was no particle, or unit, of inheritance analogous to the bacterial units of infection. Actually such a unit had been discovered about the time that the great work on bacteria was being conducted. But the units, called *factors* by Gregor Mendel and genes by modern biologists, were not related to a visible physical entity, and the significance of Mendel's experiments was long unrecognized. The discovery of chromosomes and their orderly segregation during cell division led to a new receptivity to the concept of hereditary particles. Then in 1900 three investigators independently reported experiments which they believed pointed to the existence of hereditary units. Each of the three also reported his recent discovery that Mendel had preceded him by 34 years.

The principles which Mendel first propounded in 1866, and which have provided the foundation for the study of heredity and disease, were based upon his investigations of inherited characteristics of peas. He carefully avoided continuous variation in these experiments, selecting varieties that consistently differed from each other by a single distinct feature, such as flower color or appearance of the seed-coat. From the cross-pollination of two inbred strains he obtained a first generation (F_1) of plants each of which resembled one of the parental types. It did not matter which plant strain contributed the pollen. The F_1 plants pollinated among themselves produced a second generation (F_2) of plants of both original types, in the ratio of three of the type seen in the F_1 generation to one of the other (Fig. 1-1). Mendel concluded that hereditary factors exist in pairs, the members of a pair being either alike or different. One alternative form (*A*) is *dominant*, the other (*a*) *recessive* when both are present in the same plant. Such gene alternates are called *alleles*. A host is *homozygous* for a particular gene if identical alleles are present, *heterozygous* if they are different. Mendel's parental plants were homozygous (*AA* or *aa*), the F_1 plants were heterozygous (*Aa*), and the F_2 plants were a mixture of types (1*AA*:2*Aa*:1*aa*). The allelic

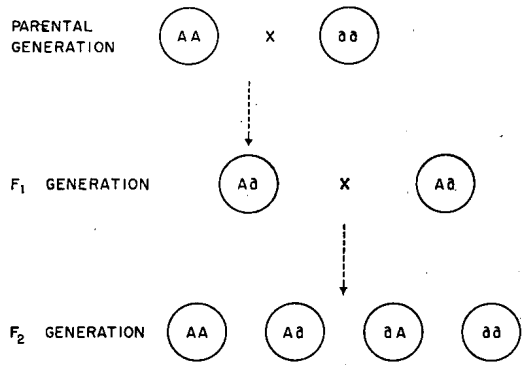

FIG. 1-1. Mendel's first law. The segregation of alleles of a gene.

composition (*AA*, *Aa*, or *aa*) of an organism is its *genotype;* its appearance, its *phenotype.* One phenotype may be produced by two different genotypes (*AA* or *Aa*).

This *segregation* of alleles typifies Mendelian inheritance and is sometimes referred to as *Mendel's first law of inheritance.* Many examples are found in plants and animals. In man chondrodystrophy is dominantly inherited, sickle-cell anemia, recessively. The blood group antigens A and B are *codominant,* each being expressed in the other's presence; and the O antigen is recessive to each. The A, B, and O antigens are determined by *multiple* alleles of a gene.

Mendel systematically studied the simultaneous inheritance of alleles of two different genes. In one critical experiment plants bearing seeds with smooth coats (*AA*) and yellow cotyledons (*BB*) were crossed with plants bearing seeds with wrinkled coats (*aa*) and green cotyledons (*bb*). The smooth and yellow (*AaBb*) F_1 seeds were grown to maturity and cross-pollinated back to the recessive (*aabb*) parental strain, producing approximately equal numbers of the four possible seed phenotypes: smooth yellow, smooth green, wrinkled yellow, and wrinkled green (Fig. 1-2). This *independent assortment* of genes, which is also observed in the great majority of human studies, is sometimes referred to as *Mendel's second law of inheritance.*

Mendel himself was aware of intermediate effects in some heterozygous plants. In fact, in his studies on flower color in beans he encountered a series of colors from white to reddish purple. He recognized that a spectrum of colors might be produced if color were determined by more than a single gene pair. Other workers subsequently extended these observations to the analysis of *continuous variation,* a phenomenon studied intensively by another biologist of the same century, Francis Galton, who was particularly interested in continuously varying human traits such as intelligence and height. Early in the present century such graded inheritance came to be explained as multifactorial and was first invoked for human traits by the

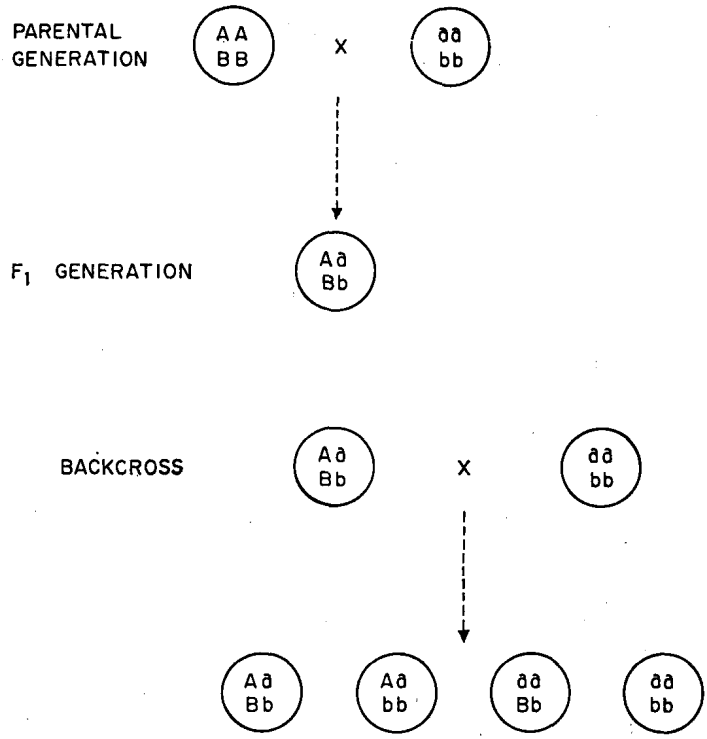

FIG. 1-2. Mendel's second law. The independent assortment of alleles of two different genes.

Davenports in 1910 as a result of their analysis of the skin color of the offspring of Negro-Caucasian matings.

The first example in man of disease caused by independent units of inheritance was that of alkaptonuria (Garrod, 1902), the recessively inherited condition which Garrod later made the cornerstone of his classic text "Inborn Errors of Metabolism." Other conditions were quickly recognized as Mendelian in their inheritance, and so was laid the foundation of the study of heredity and disease.

MENDELIAN INHERITANCE AND CONGENITAL DEFECTS

Dominantly Inherited Disease

The familiar form of dwarfism called achondroplasia, or chondrodystrophy, is a prime example of the Mendelian inheritance of disease. Affected individuals are small and disproportionate, with particularly short extremities (Fig. 1-3). All bones formed from cartilage are involved; proliferation of cartilage is greatly retarded in the metaphyses of long bones. The spine and ribs are affected, as is the cartilaginous base of the skull. Paraplegia and other neurological complications are common (Vogl, 1962). The mode of inheritance in chondrodystrophy is dominant (Mörch, 1940).

Chondrodystrophy is a *congenital defect*, a defect present at birth. Many chondrodystrophic dwarfs are stillborn or die in infancy; those surviving to adulthood produce fewer offspring than normal. This mortality and low fecundity generate a strong force of natural selection against affected individuals. About 80 per cent of the children born with this condition in one generation will not replace themselves in the next. If this selective force were the only one operating, the frequency of the disease would steadily decrease from one generation to the next. But this force is opposed by mutation.

The pedigree charts of families of chondrodystrophic dwarfs exhibit remarkable diversity, varying from isolated cases to large numbers of cases occurring over several generations. The index cases, called *propositi* or *probands*, in some instances live to produce children. Nearly always they choose unaffected mates. Although half of the children of such marriages are affected, as anticipated for dominant inheritance, only 20 per cent of the probands have an affected parent. Other cases probably arise by *mutation;* the gene which determines chondrodystrophy may suddenly appear in the population by the alteration, or mutation, of its normal counterpart. Each parent is normal, yet

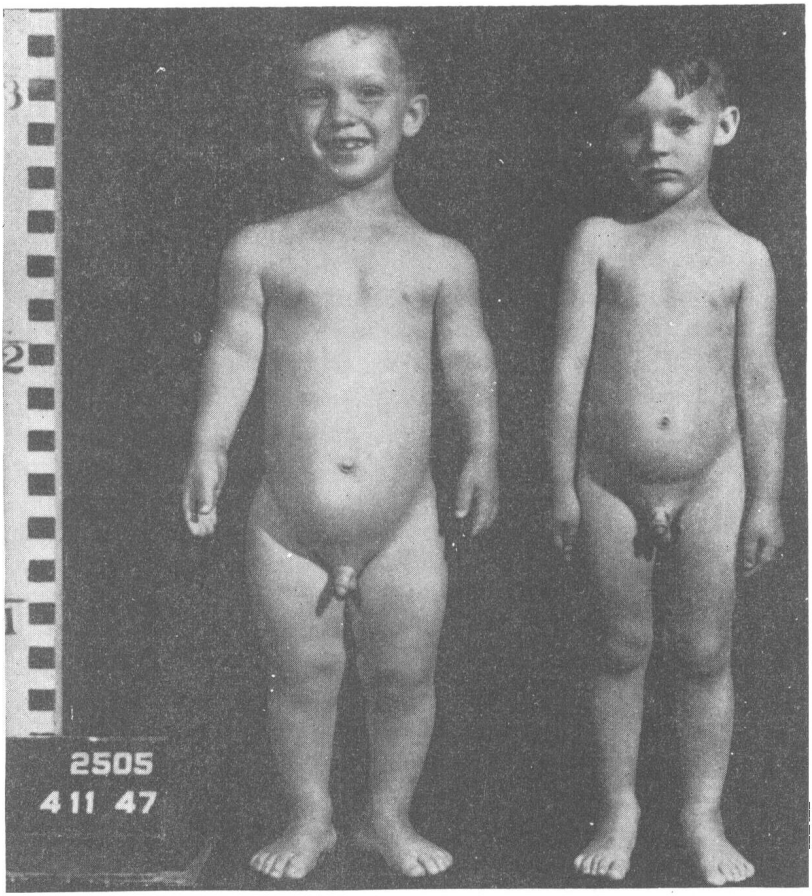

FIG. 1-3. Chondrodystrophy. An affected child (left) with a younger, normal child of approximately the same height, illustrating disproportionate growth. (*Courtesy Dr. George Donnell, Childrens Hospital of Los Angeles.*)

one offspring is not. A germ cell or its progenitor, which began with the normal allele, was changed by mutation into one bearing an abnormal allele.

While in every generation 80 per cent of the abnormal alleles are lost from the population at large by the process of selection,

this same number is replaced by the process of mutation. As long as these two forces are the only ones operating, and as long as they do not change in magnitude, the frequency of occurrence of the disease in a population will not change from one generation to the next. Such a population is in *genetic equilibrium* with respect to the gene in question.

From the frequency with which chondrodystrophy occurs, the frequency of the abnormal allele may be determined; and from the frequency of the abnormal allele, the rate of the process of mutation may be calculated. Two measurements of frequency may be reported for medical conditions; only one has meaning here. The frequency with which a condition is found at a given point in time in the general population is called its *prevalence;* its frequency of new occurrence among individuals of a defined population within a certain period of time is called its *incidence.* The birth incidence rate, the incidence among the children born within a given period of time, is the appropriate measurement here. From the incidence of the disease, denoted by the symbol x, we may directly calculate the *gene frequency* q of the abnormal allele. Since each chondrodystrophic individual is heterozygous, the frequency of the abnormal allele is equal to one-half the incidence of the disease;

$$q = \tfrac{1}{2}x \qquad (1\text{-}1)$$

The loss of abnormal alleles by selection is measured by the *coefficient of selection* s, which designates the fractional loss of affected individuals in going from one generation to another; for chondrodystrophy s is equal to 0.80. Since the loss of each affected individual results in the loss of one abnormal allele, the decrease in gene frequency per generation $(-\Delta q)$ is given by the equation

$$-\Delta q = \tfrac{1}{2}sx = sq \qquad (1\text{-}2)$$

On the other hand, the gain $(+\Delta q)$ of abnormal alleles by mutation is measured by the mutation rate μ, which designates the fraction of normal alleles changed to abnormal alleles per

generation. Since the frequency of normal alleles is $1 - q$, the gain of abnormal alleles by mutation is

$$+\Delta q = \mu(1 - q) \tag{1-3}$$

For populations in equilibrium there is no overall change in gene frequency, and q assumes a constant value, the equilibrium gene frequency $\hat{q}$. Under equilibrium conditions, $+\Delta q$ for mutation and $-\Delta q$ for selection must give a sum of zero

$$\Delta q = \mu(1 - \hat{q}) - s\hat{q} = 0$$

or when $\hat{q}$ is very small,

$$\mu = s\hat{q} \tag{1-4}$$

This equation has been used to estimate the mutation rate for chondrodystrophy. The incidence x is approximately 1 per 10,000 births, or 10^{-4} per birth. Therefore, the gene frequency q for the chondrodystrophy allele is 0.5×10^{-4}, or 5×10^{-5}. The coefficient of selection s being 0.80, the mutation rate μ is calculated to be 4×10^{-5}. This calculated value is in agreement with the direct measurement of mutation rate made by determining the number of mutant cases appearing in a large number of newborn infants. The mutation rate is of an order of magnitude (10^{-5}) commonly found among animals, plants, and microorganisms.

The mutations producing chondrodystrophy may not all give rise to the same allele; they may even occur in different genes. If they were identical, we would expect most affected children of an affected parent to die, just as most new mutant cases die. Actually this does not happen; the mortality rate for inherited cases is substantially lower than that for mutant cases. Although there are several explanations for this discrepancy, (Slatis, 1955; Stevenson, 1957), one possibility is that at least two mutant alleles exist, one compatible with long life. If this were the case, then the calculation of mutation rate above would apply to the total mutations of the gene in question to alleles which produce chondrodystrophy.

Equation (1-4) also tells us that the equilibrium frequency

$\hat{q}$ of the chondrodystrophy allele is a function of the mutation rate; rearranged it becomes

$$\hat{q} = \frac{\mu}{s} \tag{1-5}$$

A change in mutation rate will ultimately lead to a new equilibrium gene frequency. This is of considerable practical interest since Muller's discovery that irradiation can increase the spontaneous mutation rate (Muller, 1927). As can be seen by this relationship, a doubling of the mutation rate μ would result in a doubling of the equilibrium gene frequency $\hat{q}$ and hence of the incidence of the disease. The same result would obtain for other severe conditions that are completely dominant. This fact is basic to the evaluation of the hazards involved in the use of medical x-rays and of atomic energy, peaceful or otherwise. Central to the problem is the determination of the *doubling dose*, the amount of irradiation which will cause a doubling in the mutation rate. Many individuals will receive 1 to 10 roentgens (r) of irradiation before the end of the age of reproduction, but few will receive 100 r. Prevailing estimates of the doubling dose for mammals are in the range 30 to 50 r. In mice, the mutation rate is dependent not only upon the total dose but also upon its rate of delivery; the doubling dose from chronic exposure to radiation is probably much greater than the above value, which is for acute exposure (Russell, Russell, and Kelly, 1958).

The cause of spontaneous mutations is unknown. That background irradiation is responsible for a small percentage of them in man is indicated by the increase in mutation rate induced by irradiation. Experimental studies have revealed that a number of chemical agents are mutagenic, thereby introducing another parameter. Some of the operative environmental factors seem to produce a persistent effect. Parents of mutant cases are older on the average than parents of nonchondrodystrophic children. The ages of the fathers are disproportionately advanced, suggesting that the sperm cells of older men contain

the mutant alleles more often than do those of young men (Penrose, 1955).

Equation (1-5) states that gene frequency is inversely proportional to the coefficient of selection. If no affected individual bore children, then the value of s would become unity, and the equilibrium gene frequency would be determined solely by mutation rate:

$$\hat{q} = \mu \qquad (1\text{-}6)$$

This would represent the minimum to which the incidence could be reduced for any dominantly inherited condition. Under these conditions the dominant allele would behave like a *dominant lethal,* an allele lethal to the host in whose somatic cells it first appears. But sterilization of individuals with chondrodystrophy would decrease the incidence only from 5×10^{-5} to 4×10^{-5}. As it is, the gene is *semilethal.*

On the other hand, if the value for s for chondrodystrophy were reduced to one-half its present value, the incidence of the disease would double. In view of the astonishing medical progress occurring during the twentieth century, it may become possible to affect this value and so cause an increased incidence of the disease. As the value of s becomes very small, the value of $\hat{q}$ becomes large, and the conditions under which we derived our equations no longer pertain. However, the frequency of the gene would increase sufficiently to afford the chance of a mating between two affected individuals even on a random basis. In such an event, 25 per cent of the offspring would be normal, 50 per cent would be chondrodystrophic, and 25 per cent would be in a new category. What would be the phenotype of such a homozygous individual? The answer to this is not known, but he might be affected more severely than the heterozygote. Such was apparently the case when two individuals with the minor digital anomaly, brachymesophalangy, produced a grossly abnormal child with no fingers or toes, who died at the age of one year (Mohr and Wriedt, 1919). With respect to the severe abnormality, the causative mutant gene in this case could be called recessive, while with respect to brachymesophalangy it

could be called dominant. This illustration serves to emphasize that the terms dominant and recessive have meaning only in connection with phenotype.

Recessively Inherited Disease

Tay-Sachs Disease. Few diseases have clinical features so diagnostic as those of Tay-Sachs disease, or infantile amaurotic idiocy. Affected infants appear normal at birth, but during the first few months of life the parents and pediatrician begin to suspect that the baby is not as responsive to his surroundings as he should be. The suspicion that all is not well is confirmed when he reaches the age of six or seven months without being able to sit normally. From this time on the infant fails to acquire any new skills; he never learns to sit or stand but lies in his crib, often staring past his parent without social response. The slightest sound may elicit a generalized tremor, a sign called hyperacusis. His legs are held out straight, the feet are turned down, and his arms often assume the position seen with the tonic neck reflex, signs which betray impending decerebrate rigidity. His responses to visual stimuli gradually decrease and he becomes blind. During his second year, he becomes completely helpless as his head enlarges and he develops difficulty swallowing. Secretions begin to accumulate and threaten bronchopneumonia. Death usually occurs in the second, third, or fourth year of life.

The most pathognomonic clinical sign of Tay-Sachs disease was first described by the British ophthalmologist Tay. In the region of the macula lutea of each retina is a large white patch at the center of which is a brownish-red circular spot, a lesion designated as the cherry-red spot. Only two other clinical conditions ever manifest such a finding, and neither could be confused with Tay-Sachs disease. The finding is so universal that clinical diagnosis of Tay-Sachs disease should not be made in its absence.

The first pathological description was made by the American neurologist Sachs. The cerebral convolutions are usually prominent, and the brain is hard to the touch. The brain becomes