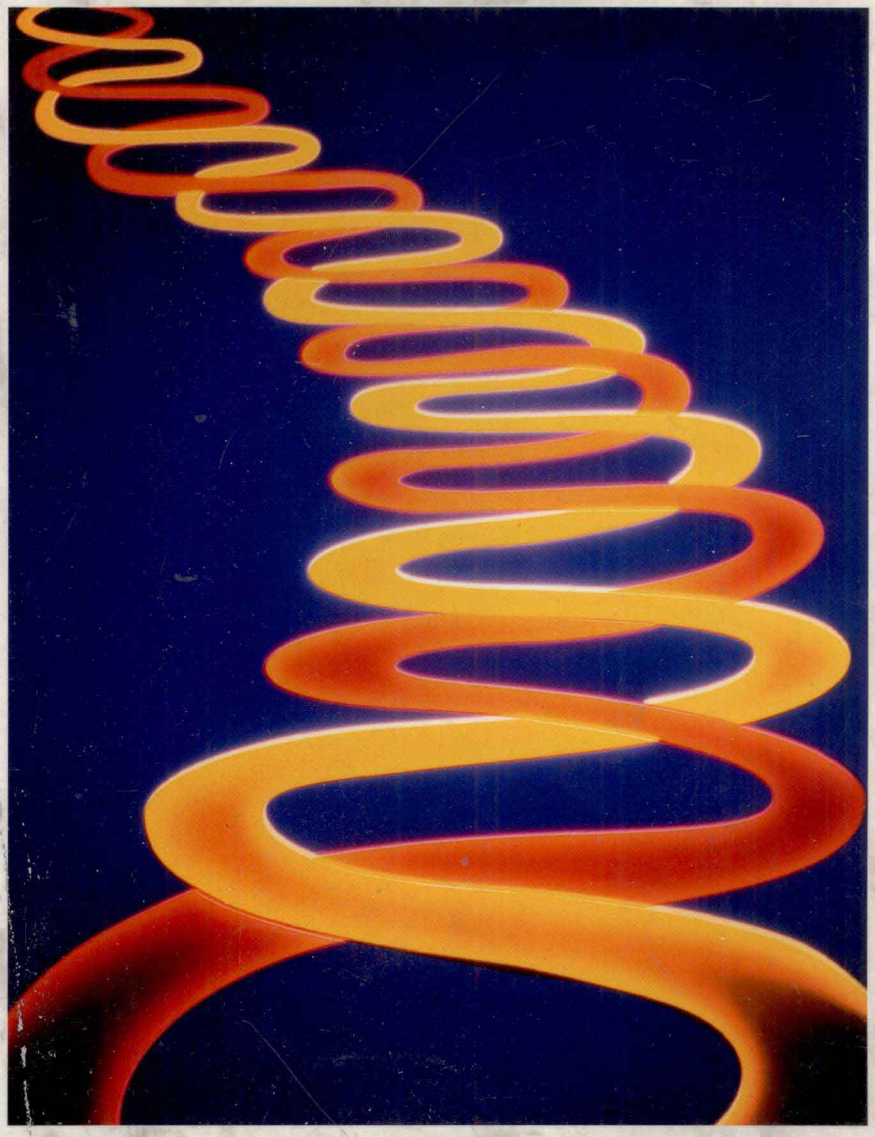
HUMAN GENETICS

A PROBLEM-BASED APPROACH



BRUCE R. KORF

Blackwell Science

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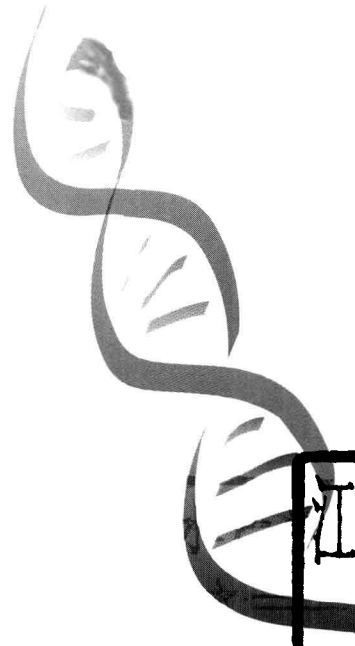

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Preface

Both medicine and genetics are undergoing revolutionary change, one in response to out-of-control costs and the other driven by unprecedented technical advance. New models of medical practice are evolving, with increasing attention to primary care and preventative medicine. Genetics will play a central role in this new approach, particularly as the genetic bases for common disease come to light. Today's medical students therefore face the dual challenge of mastering an ever complex body of knowledge along with the need to develop a set of problem solving skills that will foster career-long learning.

It has become clear that the traditional lecture-based medical curriculum is not well suited to these needs, opening the door to new approaches to medical education. The problem-based approach has been the mainstay of curriculum change at many schools, including here at Harvard Medical School. The first two years at Harvard Medical School now emphasize student-driven learning using a combination of lectures and problem-based tutorial exercises. Courses are taught in 6–12 week blocks, of which one is Genetics, Embryology, and Reproduction; this book is based on the genetics component of that course.

The initial impetus to write this book was to provide a set of clinical problems in genetics, but as the book evolved it became clear that there was another opportunity to change the way genetics is taught. If genetics is taught at all to medical students, it is usually done in an historical context, covering Mendelian genetics, cytogenetics, molecular genetics, etc. The power of modern molecular analysis, however, is that the mysteries of clinical experience are now being explained in precise molecular terms. Powerful concepts about the structure and function of the genome are emerging, and it is time to synthesize these into a new didactic model. Techniques such as pedigree analysis or gene cloning become the means; understanding basic ideas such as the mechanisms underlying inborn errors of metabolism are the ends.

I am indebted to students, teachers, and colleagues who have helped shape this work, some deliberately, others unwittingly. A series of first year Harvard Medical School classes have taught me what works and what does not. I have learned the culture of problem-based learning along with colleagues on the Genetics, Embryology, and Reproduction planning committee, including Philip Leder, Daniel Federman, Elizabeth Hay, Betsy Williams, Sohela Garib, and Elizabeth Armstrong. Innumerable colleagues have reviewed portions of the text and have provided illustrative material. The latter are acknowledged in figure legends; the former have included Alan Beggs, Peter Byers, Antonio Cao, Jonathan Fletcher, Uta Francke, Donald Johns, Mark Korson, Harvey Levy, Cynthia Morton, Richard Parad, Reed Pyeritz, Cliff Tabin, David Whiteman, Mary Ellen Wohl, and Joseph Wolfsdorf. Their many suggestions and corrections do not render the chapters immune from my further tampering, but have contributed enormously to maintaining accuracy and readability. Several editors at Blackwell Science deserve credit for stimulating me to undertake this project, providing vital feedback, and being patient. These include Victoria Reeders, Coleen Traynor, Kathleen Broderick, and Joy Ferris Denomme.

The processes of teaching and learning are inseparable. If the approach used in this book is successful, it will spawn a process of further evolution driven by feedback from those who use it.

B.R.K.

Introduction

The science of genetics is concerned with the mechanisms whereby biological traits are passed from generation to generation and expressed in the individual. Although its roots can be traced to the experiments of Mendel in the 1860s and the subsequent birth of cell biology, modern genetics is almost entirely a creation of the twentieth century. Genetics has a long tradition of merging clinical observation with basic laboratory investigation. This has culminated in the emergence of molecular biology, in which biological processes are understood from the level of the gene through proteins that direct the development and function of the organism. Recently, recombinant DNA technology has provided tools of enormous power, able to reveal the structure and function of genes. The entire human genome has been targeted for study, promising a revolution in understanding human development and physiology. Genetics is therefore playing an increasingly central role in medical science, and no physician can remain ignorant of its fundamental principles and approaches.

The teaching of genetics has focused traditionally on a number of distinct subject areas, such as mendelian genetics, cytogenics, molecular genetics, and population genetics. Although this approach may mirror the sequence in which our knowledge has emerged, a major opportunity to integrate concepts across disciplinary lines is lost. Indeed, the power of the modern molecular approach is best demonstrated by its ability to reveal the mechanisms underlying sometimes mysterious observations made in classic genetics.

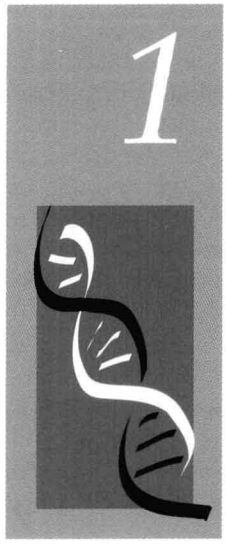
Consequently, this book will use a very different approach. Each of the 10 chapters herein focuses on a fundamental concept in genetics. The most basic, that a gene may direct the synthesis of an enzyme and that mutation can lead to deficiency of the enzyme and consequent disruption of a metabolic pathway, comprises the theme for Chapter 1. This chapter introduces the concept of autosomal recessive inheritance and the molecular basis for gene mutation. The mechanisms of dominant inheritance of mutations in genes that encode structural proteins are explored in Chapter 2. Chapter 3 then describes a new method for understanding the structure of genes based on knowledge of their location in the genome. X-linked genetic transmission is discussed next, in Chapter 4, along with the concept of X chromosome inactivation. This is followed, in Chapter 5, by a review of chromosomes and chromosomal anomalies. The inheritance of complex multifactorial genetic traits is considered in Chapter 6. Although this is a relatively unexplored area, it promises to be a source of major insight into the mechanisms of relatively common disorders such as heart disease and diabetes mellitus. A newly emerging field, inheritance through the mitochondrial genome, also is considered (Chapter 7). The study of mitochondrial DNA has led to the discovery of a new class of disorders of energy metabolism. The genetics of cancer and the notion of somatic genetic change are the subject of Chapter 8. Chapter 9 addresses the field of developmental genetics and, finally, Chapter 10 deals with population genetics.

Each chapter begins with a clinical case history, and details of the case unfold as the chapter proceeds. The cases will provide a driving force to introduce new concepts and maintain a link with their clinical application. Chapters end with another case that will continue the theme and introduce new ideas. Part of the challenge in teaching genetics is to provide a basis for mastery of new developments in a rapidly evolving field. Cases at the ends of chapters are structured to encourage self-directed learning.

Three basic themes may be discerned in the genetic approach to medicine, and these will compose the framework for each chapter. The first concerns the means by which genetic traits flow through families. This is most easily understood by the study of rare genetic variants resulting from changes in single genes. Increasingly, however, more complex and common traits are being scrutinized, leading to insights that may influence the health of large populations. The second theme concerns the flow of genetic information in the cell from DNA through RNA to protein. This provides the link from a heritable trait to a physiological event, and it is here that great advances are being made in diagnosis and management

of disease. The third theme involves the ethical context for human genetics. To the extent that the purview of genetics is now all of medicine, ethical issues in genetics are those of medicine itself. Yet genetics adds a dimension by taking into consideration health across generations. Attention to ethical issues must be integral to all aspects of medical education and is particularly important in the teaching of genetics.

Contents



1 Inborn Errors of Metabolism

OVERVIEW

Elucidation of the mechanism by which genes control biologic processes has been a major scientific advance in this century. The basic tenet—that genes consist of DNA and function to encode the structure of proteins—was established by the early 1950s. Since that time, attention has focused on the molecular mechanisms through which genes exert their effects. The flow of information from genes to proteins first came to light from the study of enzymes, proteins that catalyze biochemical reactions. In the 1940s, Beadle and Tatum demonstrated that strains of the fungus *Neurospora* that were unable to grow on media lacking specific nutrients were genetically unable to make the enzymes needed to synthesize those nutrients. Even before that, during the first decade of the twentieth century, Garrod had recognized genetic traits in families that he attributed to "inborn errors of metabolism," an inherited deficiency in the ability to carry out an essential biochemical reaction.

This chapter will explore the path from genes to enzymes. We will begin with the story of a child with albinism, one of the inborn errors of metabolism originally described by Garrod. After briefly describing the clinical entity and its biochemical basis, we will see how this disorder is genetically transmitted as an autosomal recessive trait. This will be the first of the basic modes of mendelian genetic transmission

to be considered. Returning to the biochemical basis of albinism, it will become apparent why genetic metabolic disorders tend to be inherited as recessive traits. Biochemistry, however, provides little insight into the mechanisms by which gene mutations occur and lead to deficient enzyme function. To understand this, it is necessary to isolate and purify the gene that encodes an enzyme—in this case, tyrosinase, the enzyme that is deficient in one form of albinism. Genes are isolated by a process of *cloning*, the basic approach to which will be described, as will the means by which a cloned gene is characterized.

How does this help us to better understand a disorder such as albinism? We will see how gene mutations are identified in affected individuals and how this knowledge provides the basis for genetic testing of affected individuals and their family members. Although such testing is of limited value in families with albinism, it is of great importance in caring for those with other inborn errors, some of which have devastating effects on health. From diagnosis we will turn to treatment, looking at medical treatments, both established and experimental, and at the prospect of genetic therapy. A personal statement by an individual affected with albinism is provided in the Perspective. This chapter will close with the story of another inborn error of metabolism, which considers some of the public health issues raised by newborn screening.

1.1 Oculocutaneous Albinism

Ben and Linda are concerned that their 1-year-old daughter Katie is having a problem with her eyes. Katie seems to have difficulty focusing on objects, and her eyes tend to jiggle back and forth when she moves them. Also, although she responds to light and has learned to smile responsively, Katie does not seem to recognize her parents from across the room. Ben and Linda bring Katie to her pediatrician, who refers her to an ophthalmologist. The ophthalmologist notes that Katie has blue eyes and that there is no pigment in her retina. A dermatologist then is asked to see Katie, and the dermatologist is impressed by her very fair skin and white hair (Figure 1-1). It has been a source of amusement to her parents that Katie has a much fairer complexion and lighter hair than anyone else in the family, but they have assumed that her hair would darken as she gets older. The dermatologist plucks a few scalp hairs and sends them for assay of tyrosinase activity (Figure 1-2). No enzyme activity is found, confirming a clinical diagnosis of oculocutaneous albinism.

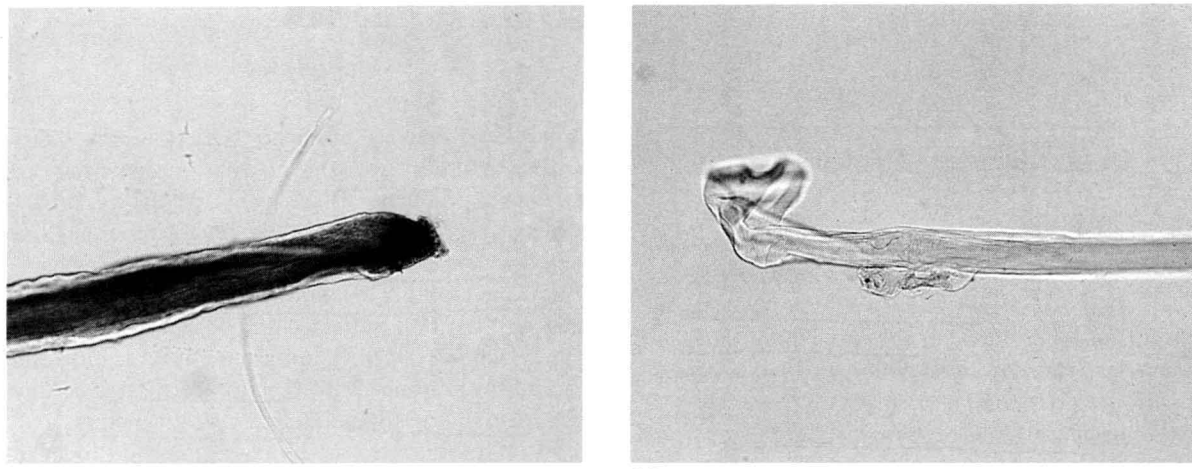
FIGURE 1-1 Child with oculocutaneous albinism, with white hair, fair skin, and eyes that are sensitive to light.

Oculocutaneous albinism (OCA) is characterized by total or nearly total absence of pigmentation. The major pigment in the body is melanin, a complex molecule synthesized by melanocytes in organelles called *melanosomes*. The biochemical pathway leading to melanin begins with the amino acid tyrosine (Figure 1-3). This is first hydroxylated to dihydroxyphenylalanine (DOPA), and then DOPA is oxidized to DOPA quinone. Both reactions are catalyzed by the enzyme tyrosinase. Deficiency of tyrosinase activity is responsible for the most common form of OCA. Affected individuals cannot form DOPA or DOPA quinone and therefore cannot synthesize melanin. They have normal numbers of melanocytes, but the melanocytes are not pigmented (Figure 1-4). The standard tyrosinase assay is performed by incubating freshly plucked hair bulbs in the presence of tyrosine. Normally this results in generation of melanin, but no melanin is produced in the absence of tyrosinase activity.

Melanin, the major pigment of hair and skin, provides protection from the damaging effects of ultraviolet light. Persons with albinism are extremely sensitive to sunburn and have a high risk of developing skin cancer. They must avoid exposure to direct sunlight by wearing a hat and long clothing and using sunscreen.

Ben and Linda have heard of albinism and can now understand why Katie has fair skin and white hair. They assume that this will not cause medical problems. They have difficulty understanding why Katie's vision is abnormal, however.

Melanin is also present in the eye. Iris pigmentation gives rise to eye color, so persons with albinism have blue or gray irises. Pigment is normally present in the retina and is important for vision. Lack of this pigment leads to very poor visual acuity (20/200 or worse) and extreme sensitivity to light. Poor visual acuity makes it difficult to focus on objects at a distance, causing the eyes to jiggle back and forth (a condition referred to as *nystagmus*) as they try to achieve visual fixation. For unknown reasons, absence of tyrosinase activity also results in aberrant migration of nerve fibers along the optic pathways during embryonic development. This leads to defective stereoscopic vision in persons with albinism.



(a) (b)

FIGURE 1-2 Hair bulb test for oculocutaneous albinism. Hair bulbs are incubated in presence of tyrosine; melanin is produced by normal hair (**a**) but not hair from a person with oculocutaneous albinism due to tyrosinase deficiency (**b**). (Photograph courtesy of Dr. William Oetting, University of Minnesota.)

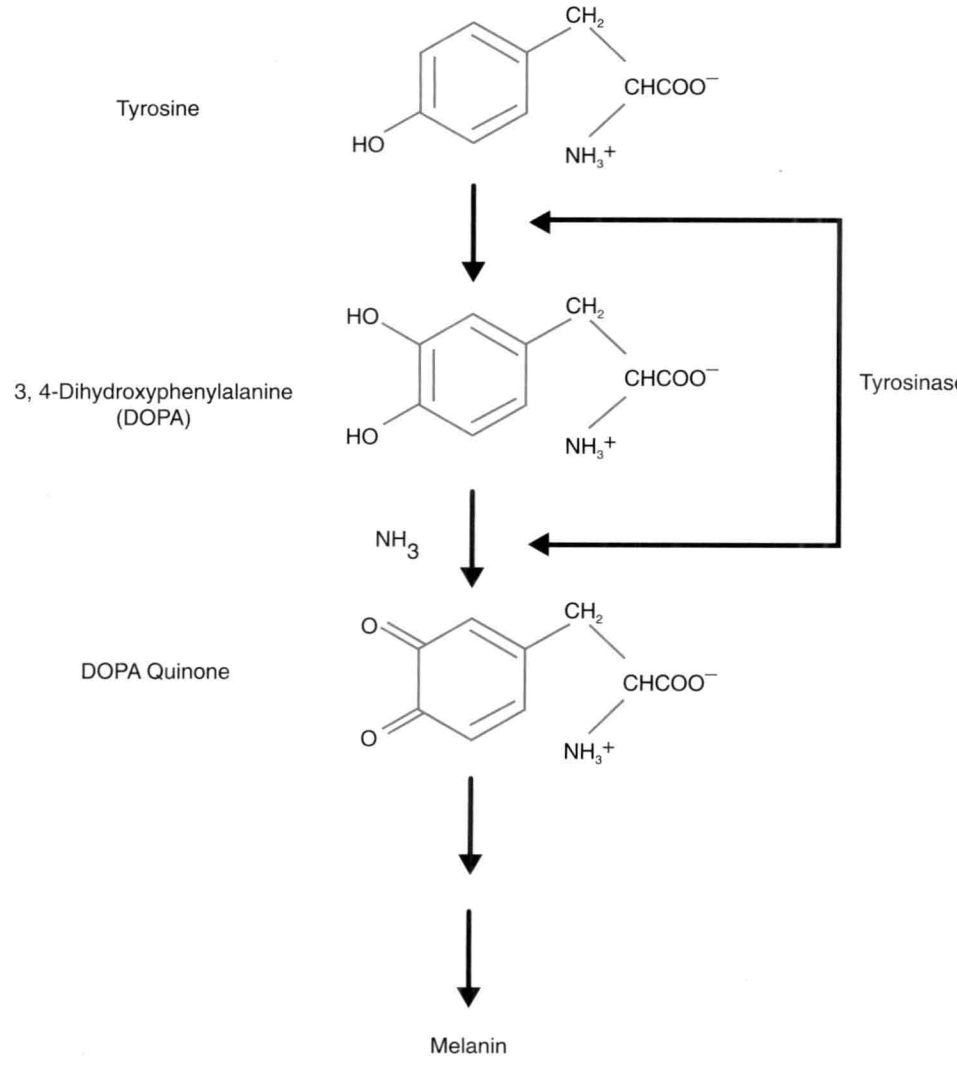


FIGURE 1-3 The first two steps in melanin biosynthesis, catalyzed by the enzyme tyrosinase.

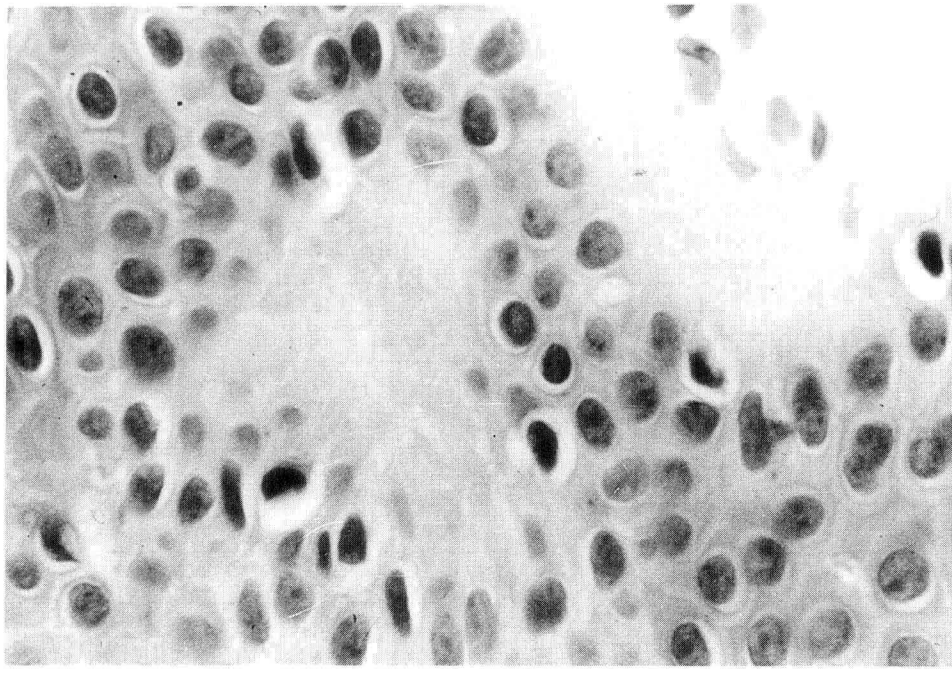


FIGURE 1-4 Photomicrograph of skin from individual with oculocutaneous albinism. No pigment granules are seen in melanocytes (cells with halolike region surrounding nucleus). (Courtesy of Dr. Cynthia Magro, Pathology Services, Boston.)

Oculocutaneous Albinism

- Lack of pigmentation
- Fair skin and hair
- Decreased visual acuity
- Lack of stereoscopic vision

1.2 Autosomal Recessive Genetic Transmission

Ben and Linda are referred to a geneticist, which surprises them because neither of them has albinism and there is no known history of albinism in either of their families. They have two other children, Jamie, a 3-year-old boy, and Susan, a 5-year-old girl (Figure 1-5). Both have light brown hair and normal vision. The geneticist takes a complete family history. Ben and Linda are a bit put off when asked if they might be related to one another, which they are not (Ben's ancestry is Irish and German, and Linda's is Italian).

OCA is inherited as an **autosomal recessive** trait. An affected child such as Katie is homozygous for a mutation in the gene encoding tyrosinase. Her

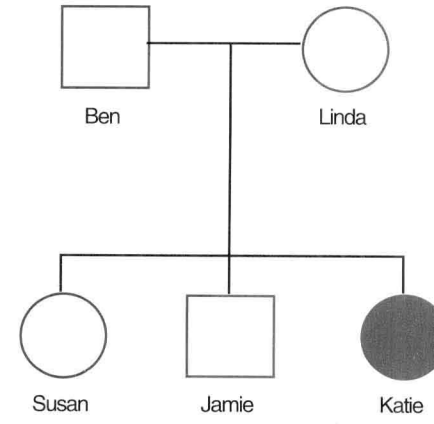


FIGURE 1-5 Family pedigree for Ben and Linda.

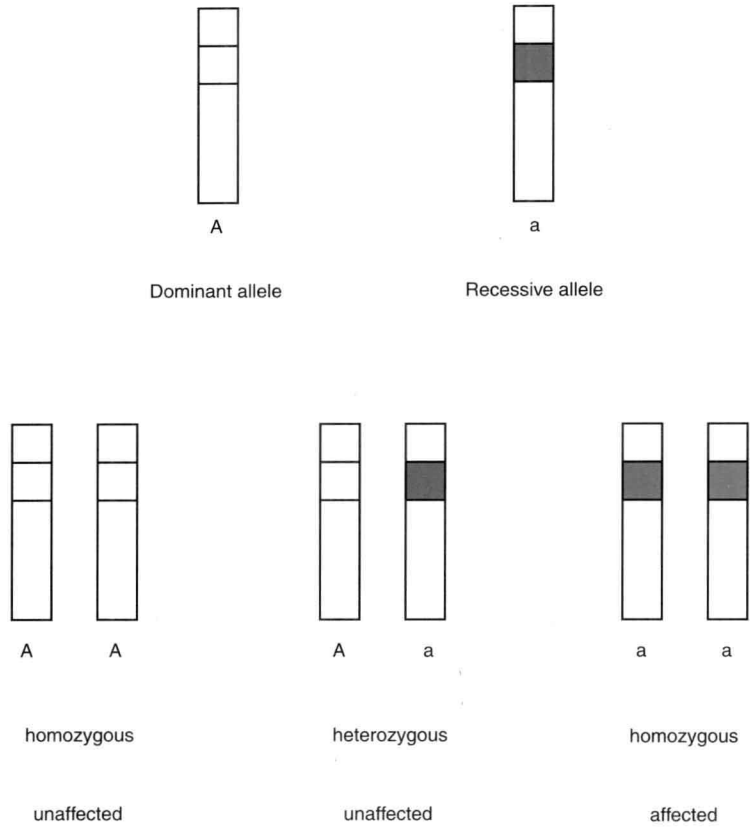


FIGURE 1-6 Genotype and phenotype associated with gene locus with dominant allele *A* and recessive allele *a*.

parents are heterozygous carriers for the mutation but do not manifest any clinical signs of OCA. It is not expected that there would be a family history of OCA in prior generations, although it is likely that Ben and Linda have many relatives who are also OCA carriers. Because tyrosinase mutant alleles are relatively rare, it is unlikely that other members of the family who might be carriers also have partners who are carriers.

The foundation for our modern understanding of genetic segregation is that a diploid organism contains two copies of every gene (excepting those carried on the sex chromosomes). One copy of each gene is inherited from each parent. These gene copies separate during the formation of haploid germ cells and are reunited at the time of fertilization. The individual copies of a particular gene are called *alleles*. The genetic constitution of an individual with respect to a particular trait is referred to as **genotype**, whereas the corresponding physical manifestation of the trait is the **phenotype**. In human genetics, traits can be transmitted as **autosomal** or **sex-linked**, **dominant** or **recessive**. Gender is determined by the sex chromosomes: A man has an X and a Y chromosome, and a woman has two X chromosomes. Genes located on a sex chromosome are said to be *sex-linked*, whereas the non-sex-linked genes are referred to as *autosomal*.

For an autosomal recessive trait to be expressed, both copies of a gene pair must be present in an altered (**mutant**) form. A person who expresses the phenotype of an autosomal recessive trait is said to be **homozygous** for the mutant gene (Figure 1-6). Autosomal recessive traits arise in children of phenotypically normal parents when both parents are carriers—that is, each parent has one normal (**wild-type**) allele and one mutant allele. The parents are said to be **heterozygous**. Each parent has a 50% chance of transmitting the wild-type or the mutant allele to a germ cell, and therefore each child has a

one in four chance of inheriting the gene mutation from both parents and being affected by the disorder (Figure 1-7). Heterozygous carriers do not manifest features of the genetic trait.

The wild-type allele is said to be *dominant* to the mutant; the mutant allele is *recessive*. Recessive traits tend to occur among children in a sibship but not in other members of a family (Figure 1-8). The parents are heterozygous but do not manifest the mutant phenotype. Other members of the parents' families (e.g., their siblings, parents) may also be carriers but, if the mutant allele is rare in the population, it is unlikely that other relatives will be homozygous.

The chances that both members of a couple carry the same rare mutant allele are increased if they are related to one another (i.e., they are **consanguineous**) and hence have inherited the same rare allele from a common ancestor (Figure 1-9). That is why Ben and Linda were questioned about the possibility of their being relatives. The frequency of consanguinity tends to be increased in families with rare autosomal recessive disorders, but it is important to remember

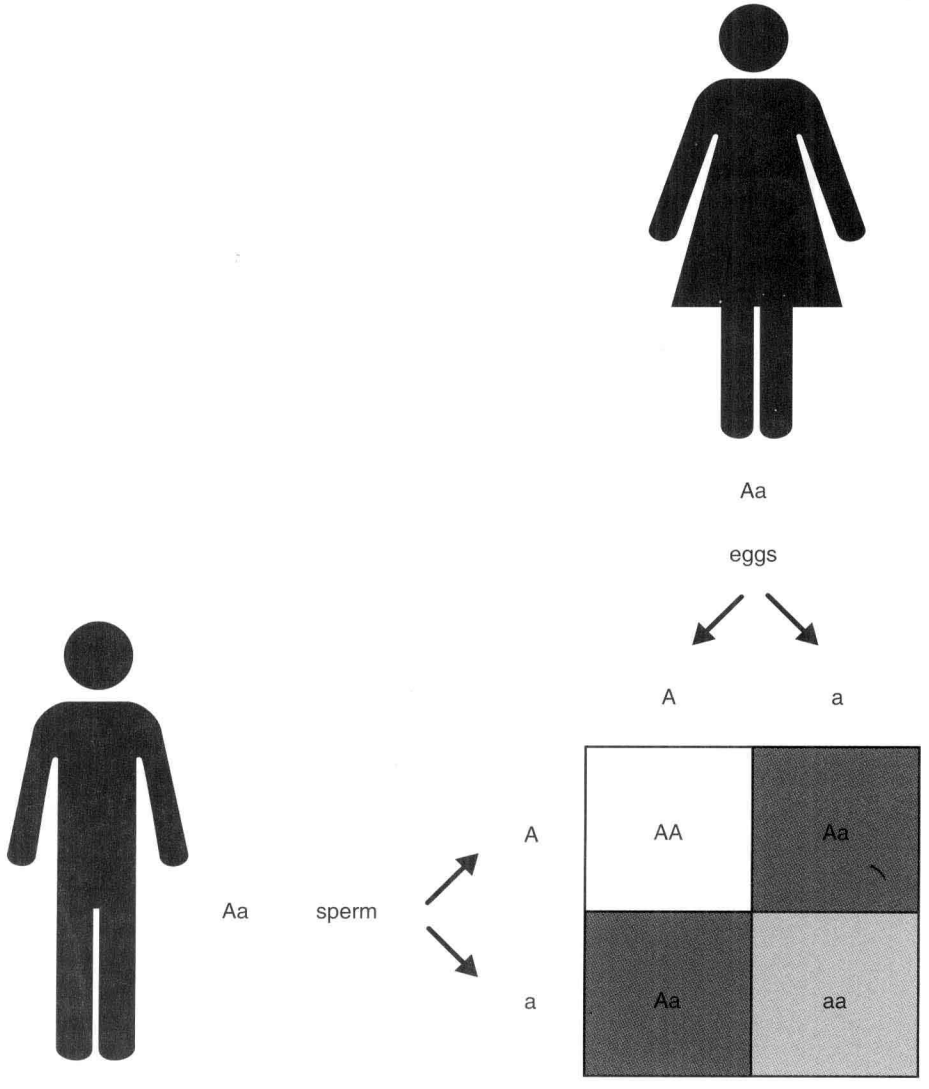


FIGURE 1-7 Segregation of dominant and recessive alleles in family in which both parents are heterozygous.