Autosomal Dominant and Recessive Inheritance

- Many important and well-understood genetic diseases are the result of a mutation in a single gene.

- The 2005 on-line edition of Mckusick's *Mendelian Inheritance in Man http://www.ncbi.nlm.nih.gov/Omim* lists nearly 15,000 single genes and nearly 8000 **single-gene,** or **monogenic,** traits defined thus far in humans.

- Of these 23,000 genes and traits, nearly 21,000 are located on autosomes, more than 1,200 are located on the X chromosome, and 59 are located on the Y chromosome.

BASIC CONCEPTS OF FORMAL GENETICS

Gregor Mendel's Contributions

- Monogenic traits are also known as mendelian traits, after Gregor Mendel.

- Mendel studied seven traits in the pea, each of which is determined by a single gene. These traits included attributes such as height (tall versus short plants) and seed shape (rounded versus wrinkled).

- The variation in each of these traits is caused by the presence of different alleles at individual loci.

- Two central principles emerged from Mendel's work. The first is the **principle of segregation**, which states that sexually reproducing organisms possess genes that occur in pairs and that only one member of this pair is transmitted to the offspring.

- The prevalent thinking during Mendel's time was that hereditary factors from the two parents are "blended" in the offspring.

- The principle of segregation states that genes remain intact and distinct. An allele for "rounded" seed shape can be transmitted to an offspring in the next generation, which can, in turn, transmit the same allele to its own offspring.

- Mendel's principle of independent assortment was his second great contribution to genetics. This principle states that genes at different loci are transmitted independently.

- The principle of independent assortment dictates that the allele transmitted at one locus ("rounded" or "wrinkled") has no effect on which allele is transmitted at the other locus ("tall" or "short ").

- Mendel's experiments also showed that the effects of one allele may mask those of another.

- He performed crosses (matings) between pea plants homozygous for the "tall" gene (i.e. having two identical copies of an allele which we will label *H)* and plants homozygous for the "short" gene (having two copies of an allele labeled *h)*.

- Mendel found that the offspring of these crosses, even though they were heterozygotes, were all tall.

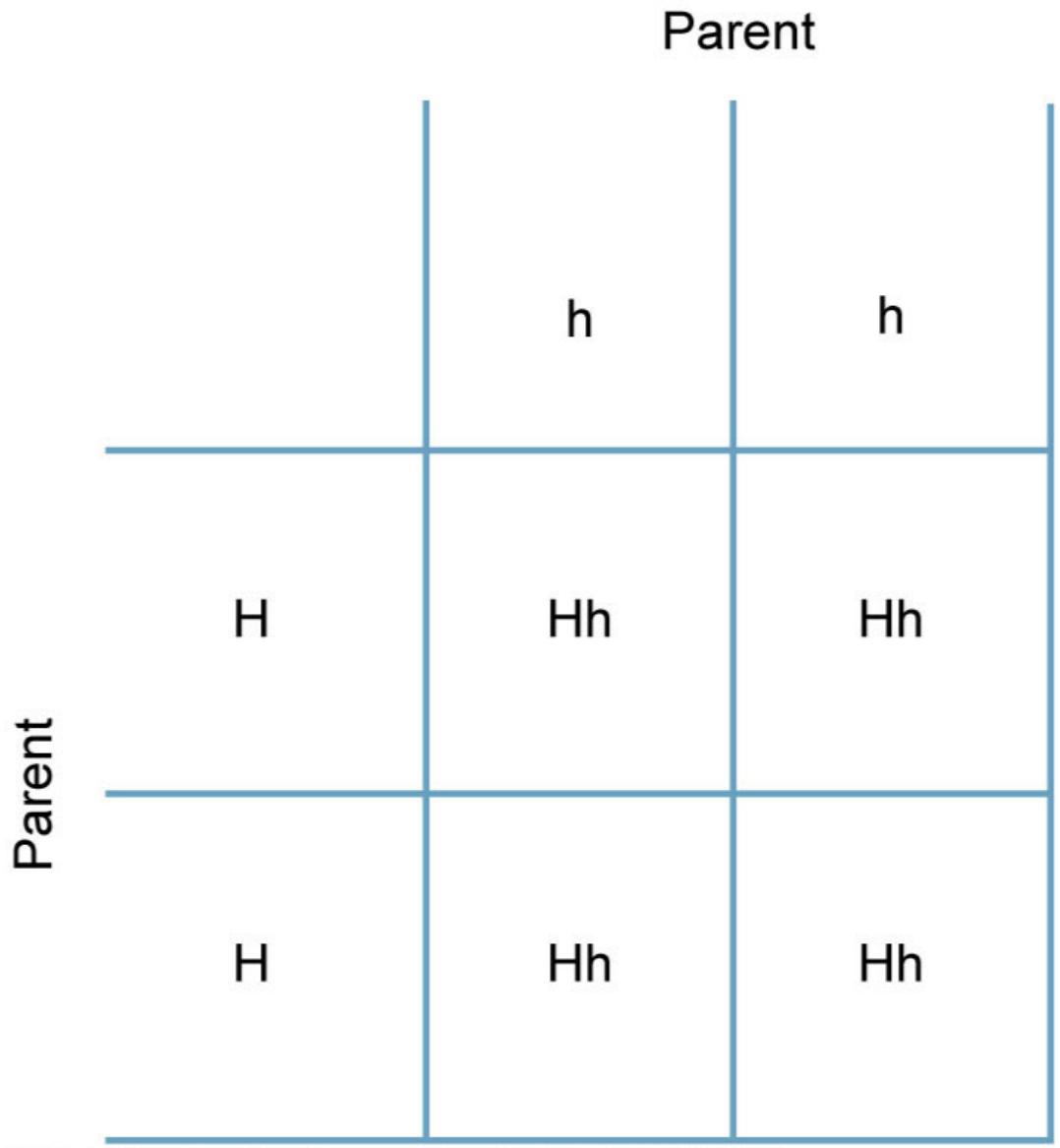


Fig. 3-1. Punnett square illustrating a cross between *HH* and *hh* homozygote parents.

- In heterozygotes, the consequences of a recessive allele are hidden. Whereas a dominant allele exerts its effect in both the homozygote *(HH)* and the heterozygote *(Hh),* the presence of the recessive allele is detected only when it occurs in homozygous form *(hh).*

- Thus, short pea plants can be created only by crossing parent plants that each carry at least one *h* allele.

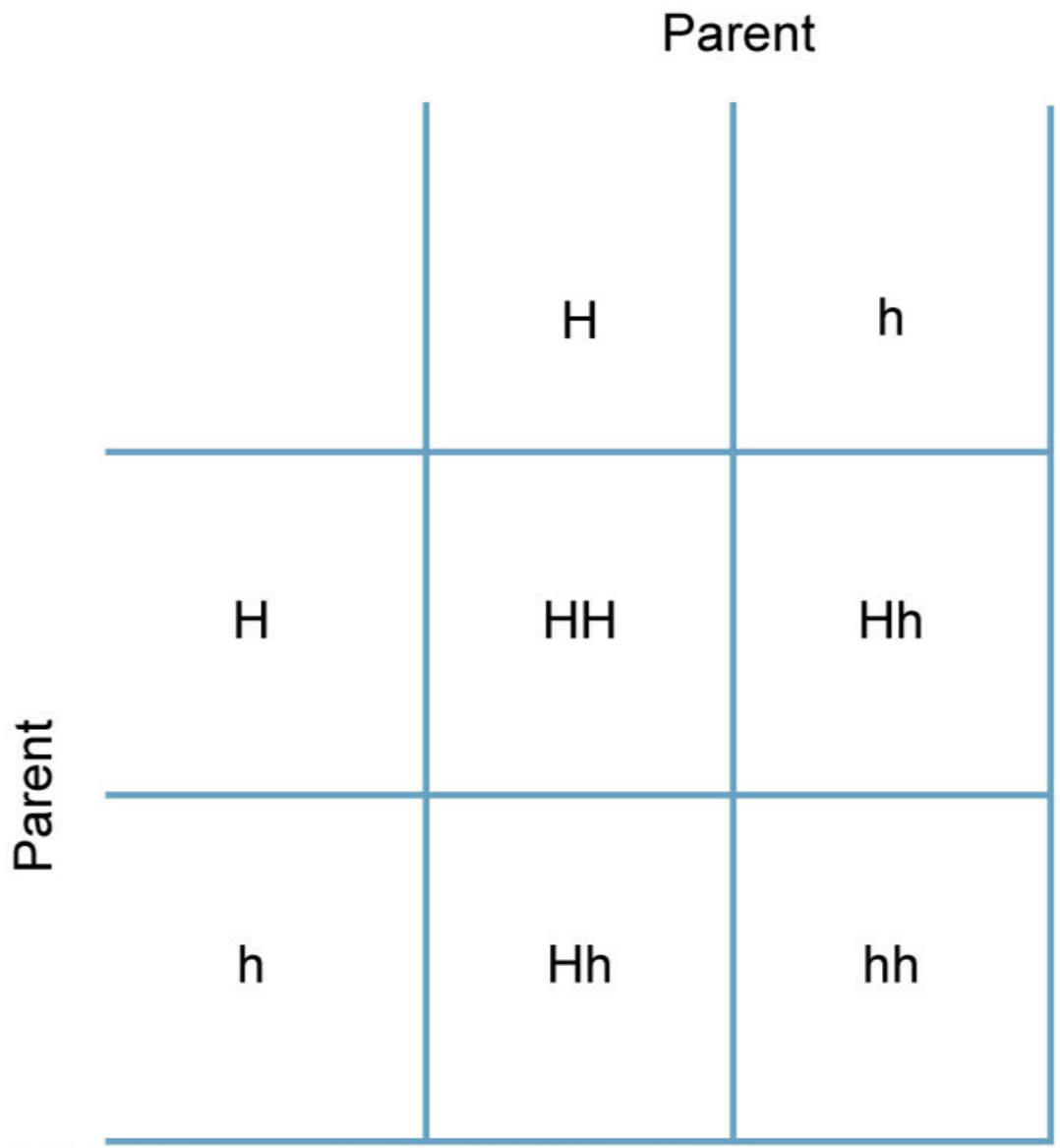


Fig. 3-2. Punnett square illustrating a cross between two *Hh* heterozygotes.

- The principle of segregation describes the behavior of chromosomes in meiosis.

- When Mendel performed his critical experiments, he had no direct knowledge of chromosomes, meiosis, or genes.

- Although his work was published in 1865 and cited occasionally, its fundamental significance was unrecognized for several decades. Yet Mendel's research, forms the Foundation of much of modern genetics.

Basic Concepts of Probability

- Risk assessment is an important part of medical genetics. For example, the physician or genetic counselor commonly informs couples of their risk of producing a child with a genetic disorder.

- In order to understand how such risks are estimated, some basic concepts of probability must be presented.

- A probability is defined as the proportion of times that a specific outcome occurs in a series of events.

- Since probabilities are proportions, they lie between 0 and 1, inclusive.

- During meiosis, one member of a chromosome pair is transmitted to each sperm or egg cell. The probability that a given member of the pair will be transmitted is ½, and the probability that the other member of the pair will be transmitted is also ½.

- Since this situation is directly analogous to coin tossing, in which the probabilities of obtaining heads or tails are each *1/2,* we will use coin tossing as our illustrative example.

- When a coin is tossed repeatedly, the outcome of each toss has no effect on subsequent outcomes. The events are thus said to be independent.

- Even if we have obtained ten heads in a row, the probability of obtaining heads or tails on the next toss remains 1/2.

- Similarly, the probability that a parent will transmit one of the two alleles at a locus is independent from one reproductive event to the next.

- The independence principle allows us to deduce two fundamental concepts of probability, the multiplication rule and the addition rule.

- The multiplication rule states that if two trials are independent, then the probability of obtaining a given outcome in both trials is the product of the probabilities of each outcome.

- For example, we may wish to know the probability that an individual will obtain heads on both tosses of a fair coin. Because the tosses are independent events, this probability is given by the product of the probabilities of obtaining heads in each individual toss: 1/2 X 1/2 : 1/4. Similarly, the probability of obtaining two tails in a row is ½ * ½ = ¼.

- The multiplication rule can be extended for any number of trials.

- Suppose that a couple wants to know the probability that all three of their planned children will be girls. Since the probability of producing a girl is approximately 1/2, and since reproductive events are independent of one another, the probability of producing three girls is 1/2 X 1/2 X 1/2 = 1/8.

- If the couple has already produced two girls and then wants to know the probability of producing a third girl, it is simply ½.

- The addition rule states that if we want to know the probability of either one outcome or another, we can simply add the respective probabilities together.

- For example, the probability of getting two heads in a row (1/2 X 1/2, or 1/4) or the probability of getting two tails in a row (1/4) is given by adding the two probabilities together: 1/4 + 1/4 = 1/2.

- As another example, imagine that a couple plans to have three children, and they have a strong aversion to having three children all of the same sex. They can be reassured somewhat by knowing that the probability of producing three girls (1/8) or three boys (1/8) is only 1/4 [1/8 + 1/8). The probability that they will have some combination of boys and girls is thus 3/4, since the sum of the probabilities of all possible outcomes must add to 1.

Gene and Genotype Frequencies

- The prevalence of many genetic diseases can vary considerably from one population to another.

- For example, cystic fibrosis, a severe respiratory disorder, is quite common among Caucasians, affecting approximately 1/2,500 births. It is rare in Asian populations, affecting only 1/90000 births.

- Sickle cell disease is common among African-Americans, affecting approximately 1/600 births. Yet it is almost never seen among individuals of northern European descent.

- The concepts of genotype frequency and gene frequency help us to measure and; understand population variation in disease genes.

- If we have typed 200 individuals in a population for the MN blood group.

- In the MN system the effects of both alleles can be observed in the heterozygote. M and N are thus said to be codominant: the heterozygote can be distinguished from both homozygotes. Any individual in the population can have one of three possible genotypes : he or she could be homozygous for M (genotype MM), heterozygous (MN), or homozygous for N (NN).

- After typing each person in our sample, we find the following distribution of genotypes: MM, 64; MN, 120; NN, 16. The frequency of MM is 64/200 (= 0.32), the frequency of MN is 120/200 (= 0.60), and the frequency of NN 16/200 (= 0.08). The sum of these frequencies must, of course, equal 1.

- The gene frequency for each allele, M and N, can be obtained here by the process of gene counting. Each : MM homozygote has two M alleles, while each heterozygote has one M allele. Similarly, NN homozygotes have two N alleles, and heterozygotes have one N allele.

- In the sample measured here, there are:

(64 X 2) + 120 = 248 M genes

(16 X 2) + 120 : 152 N genes

→ In total, there are 400 genes at the MN locus (i.e., twice the number of subjects, since each has two alleles).

→ To obtain the frequency of M, we then calculate 248/400 = 0.62. The frequency of N, 152/400, is 0.38. The sum of the two frequencies must equal 1.

The Hardy-Weinberg Principle

- If we imagine a locus that has two alleles, labeled A and a. Suppose that we know the frequency of allele A, which we will call p, and the frequency of allele a, which we will call q.

- If we wish to determine the expected population frequencies of each genotype, AA, Aa, and aa assuming that individuals in the population mate at random with regard to their genotype at this locus (random mating = panmixia), so the genotype has no effect on mate selection.

- If men and women mate at random, then the assumption of independence is fulfilled. This allows us to apply the addition and multiplication rules to estimate genotype frequencies.

- If we suppose that the frequency, p, of allele A in our population is 0.7. Then 70% of the sperm cells in the population must have allele A, and 70% of the egg cells must have allele A.

- As p and q must sum to 1, then 30% of the egg and sperm cells must carry allele a (ie,= 0.30).

- Under panmixia, the probability that a sperm cell carrying A unites with an egg cell carrying A is given by the product of the gene frequencies:

$p*p= p^2 = 0.49$ (multiplication rule). This is the probability of producing an offspring with the AA genotype.

- Using the same reasoning, the probability of producing an offspring with the aa genotype is given by $q*q=q^2=0.09$.

What about the frequency of heterozygotes in the population? There are two ways a heterozygote can be formed.

- Either a sperm cell carrying A can unite with an egg carrying a, or a sperm cell carrying a can unite with an egg carrying A.
- The probability of each of these two outcomes is given by the product of the gene frequencies, pq.
- To know the overall probability of obtaining a heterozygote (i.e., the first event or the second), we can apply the addition rule, adding the probabilities to obtain a heterozygote frequency of 2pq.
- These operations are summarized in Fig. 3-29.

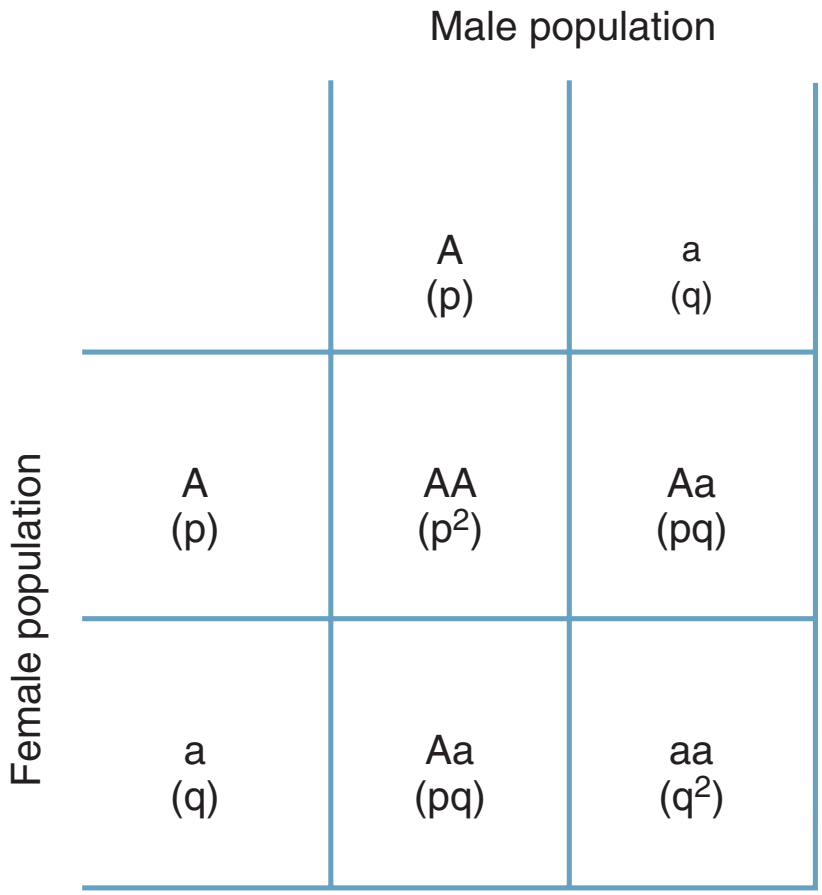


FIG 3-29 The Hardy–Weinberg principle. The population frequencies of genotypes *AA, Aa,* and *aa* are predicted on the basis of gene frequencies *(p* and *q).* It is assumed that the gene frequencies are the same in males and females.

The Concept of Phenotype

- The term ” genotype " has been defined as an individuals genetic constitution at a locus.

- The phenotype is what we actually observe physically or clinically. Genotypes do not uniquely correspond to phenotypes.

- Two different genotypes, a dominant homozygote and a heterozygote, may have the same phenotype. An example would be cystic fibrosis.

- Conversely, the same genotype may produce different phenotypes in different environments. An example of this is the recessive disease phenylketonuria (PKU), seen in approximately 1/10,000 Caucasian births.

→ Mutations at the locus encoding the metabolic enzyme phenyalanine hydroxylase render the homozygote unable to metabolize the amino acid phenylalanine.

→ While babies with PKU are normal at birth, their metabolic deficiency produces a buildup of phenylalanine and various toxic metabolites.

→ This is highly destructive to the central nervous system, and it eventually produces severe mental retardation.

→ It has been estimated that untreated PKU babies lose, on average, one to two lQ points per week during the first year of life.

→ Thus, the PKU genotype can produce a severe disease phenotype.

→ It is easy to screen for PKU at birth, and the disease can be avoided by initiating a low-phenylalanine diet within 1 month after birth.

→ The individual still has the PKU genotype, but the phenotype of mental retardation has been profoundly altered by environmental modification.

→ This example shows that the phenotype is the result of the interaction of genotype and environmental factors.

→ It should be emphasized that ”environment” can include the genetic environment (i.e., genes at other loci whose products may interact with a specific gene or its product).

Basic Pedigree Structure

- The pedigree is one of the most commonly used tools in medical genetics.
- It illustrates the relationships among family members, and it shows which family members are affected or unaffected by a genetic disease.
- Typically, an arrow denotes the proband, the first individual diagnosed in the pedigree. The proband is sometimes also referred to as the index case or propositus (proposita for females).
- When discussing relatives in families, one often refers to degrees of relationship.

→ First-degree relatives are those who are related at the parent-offspring or sibling (brother and sister) level.

→ Second-degree relatives are those who are removed by one additional generational ”step" (e.g., grandparents and grandchildren, uncles/aunts and nieces/nephews).

→ Third-degree relatives would include, for example, first cousins, great-grandchildren, and so on.

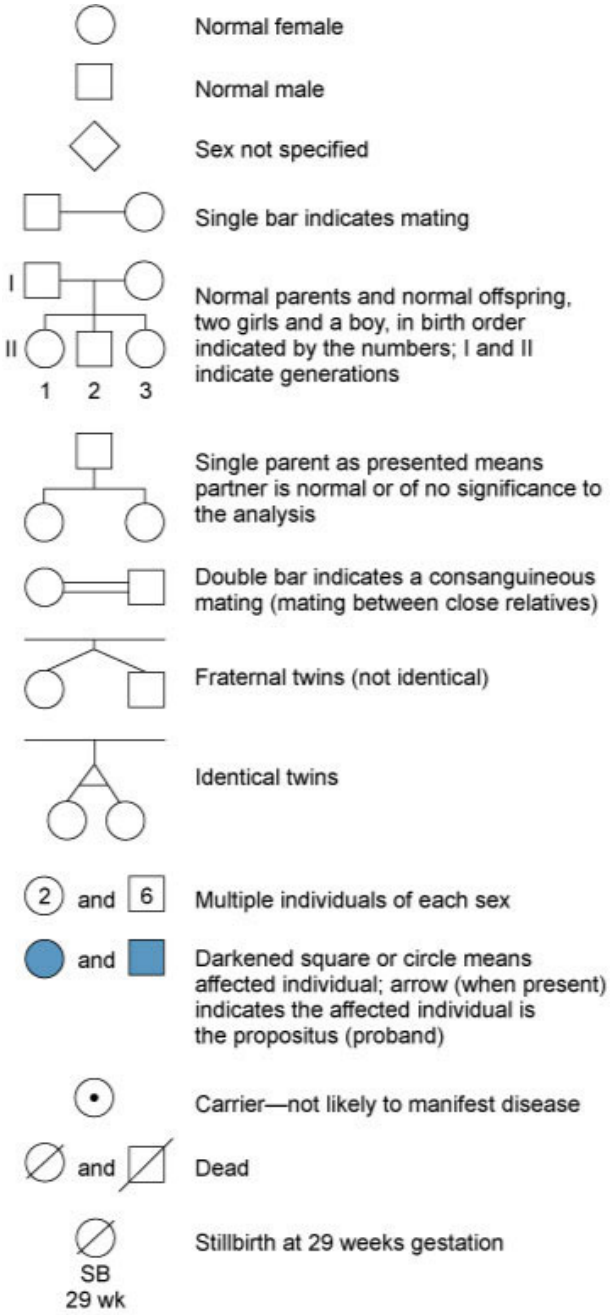


Fig. 4-1. Basic pedigree notation. For further details, see Bennett et al: J Genet Counsel 2008; 17:424-433.

AUTOSOMAL DOMINANT INHERITANCE

Characteristics of Autosomal Dominant Inheritance

- Currently, there are more than 4,400 known autosomal dominant traits, most of which are rare diseases.
- Each autosomal dominant disease is rather rare in populations, however, with the most common ones having gene frequencies of about 0.001.

- Matings between two individuals both affected by the same autosomal dominant disease are thus uncommon. Most often, affected offspring are produced by the union of a normal parent with an affected heterozygote.

- The affected parent can pass either a disease gene or a normal gene to his or her children. Each event has a probability of 0.5. Thus, on the average, half of the children will he heterozygotes and will express the disease, while half will be normal homozygotes.

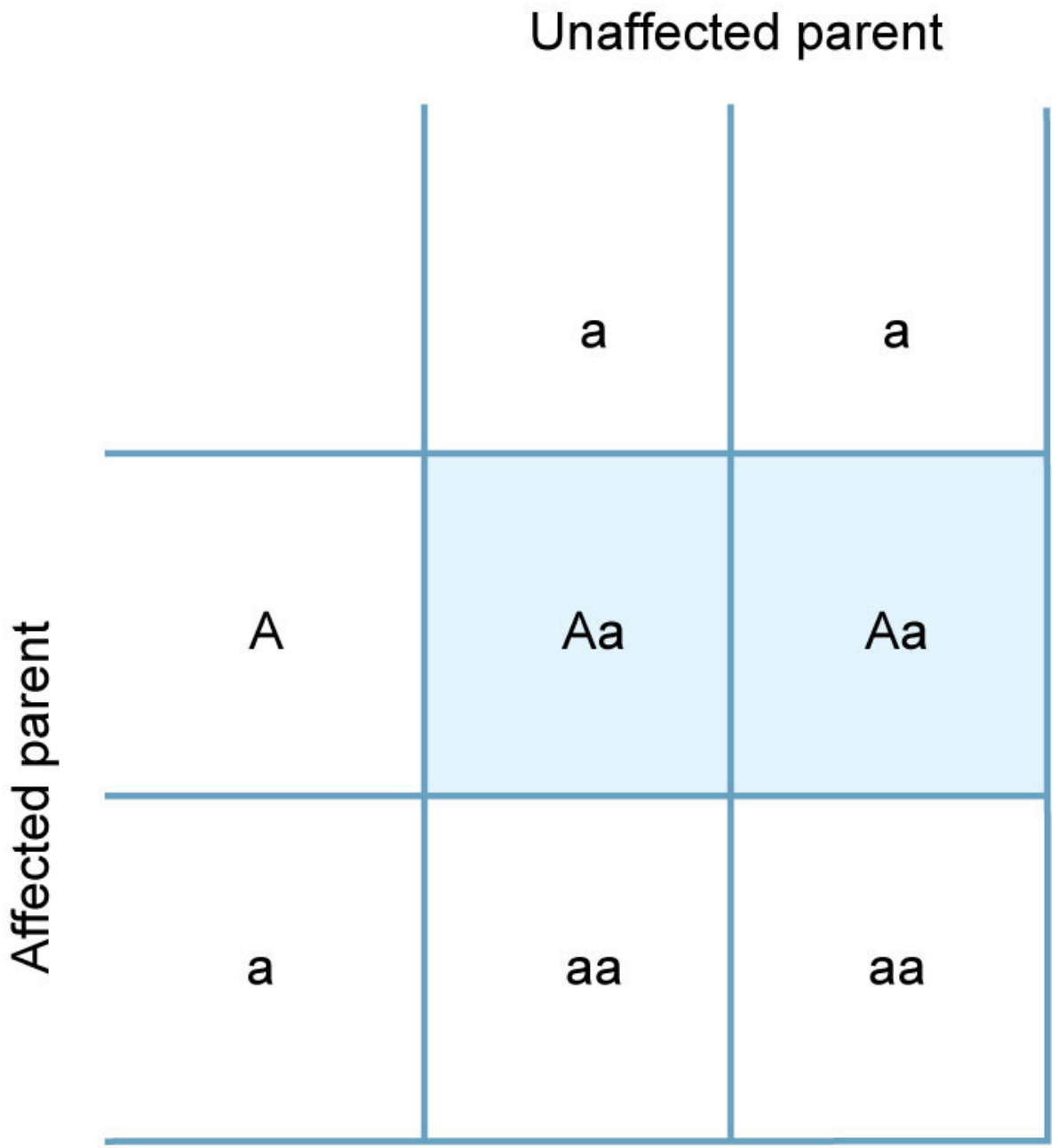


Fig. 4-2. Punnett square illustrating the mating of an unaffected individual (*aa*) with an individual who is heterozygous for an autosomal dominant disease gene (*Aa*). The genotypes of affected offspring are shaded.

- Postaxial polydactyly, the presence of an extra digit next to the fifth digit, can be inherited as an autosomal dominant trait.

- Let A symbolize the gene for polydactyly, and let a symbolize the normal allele. The pedigree illustrates several important characteristics of autosomal dominant inheritance.

→ First, the two sexes exhibit the trait in approximately equal proportions and males and females are equally likely to transmit the trait to their offspring. This reflects the fact that this is an autosomal disease (as opposed to a disease caused Y and X chromosome mutation, in which these proportions typically differ).

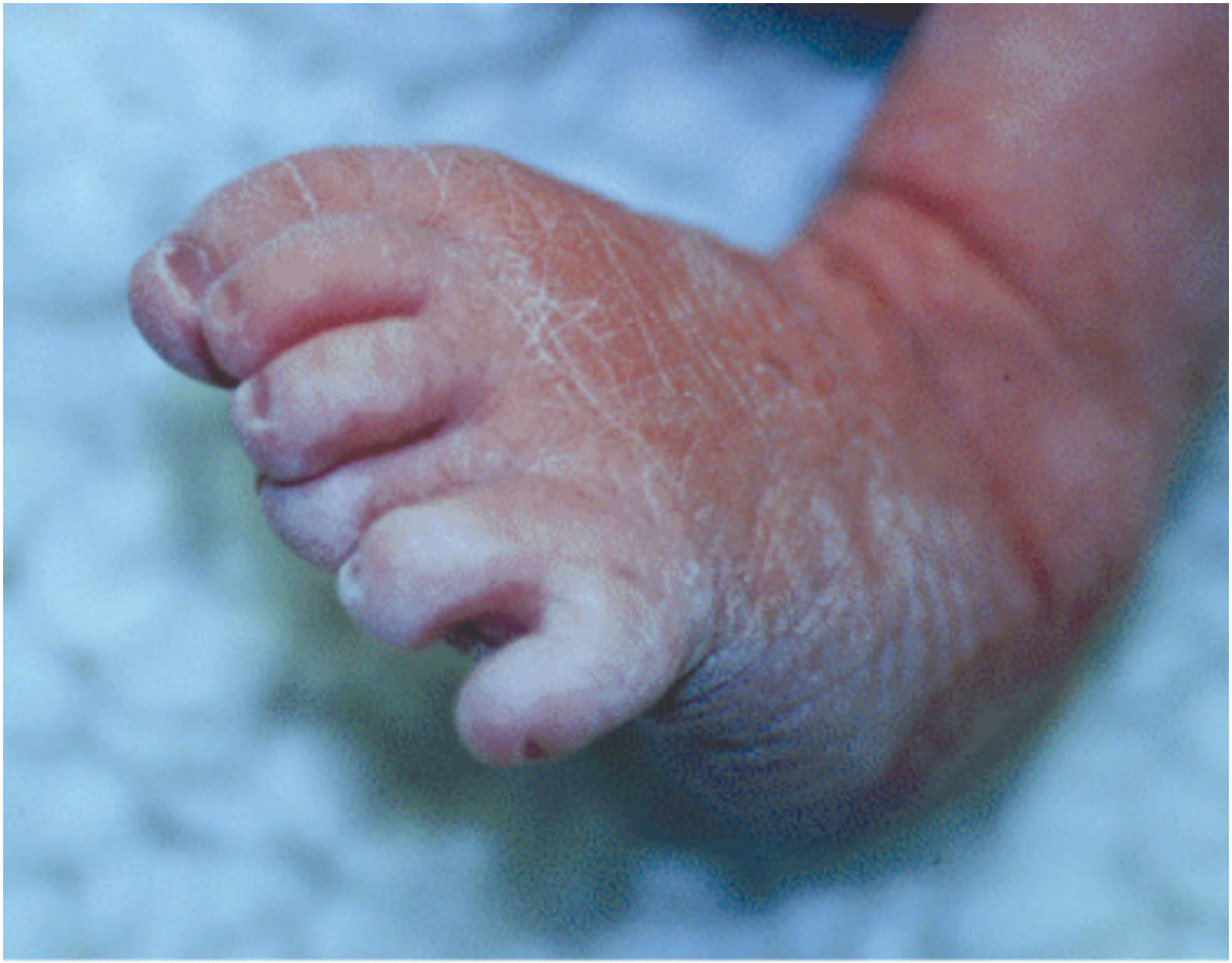


Fig. 4-3. Postaxial polydactyly. An extra digit is located next to the fifth digit.

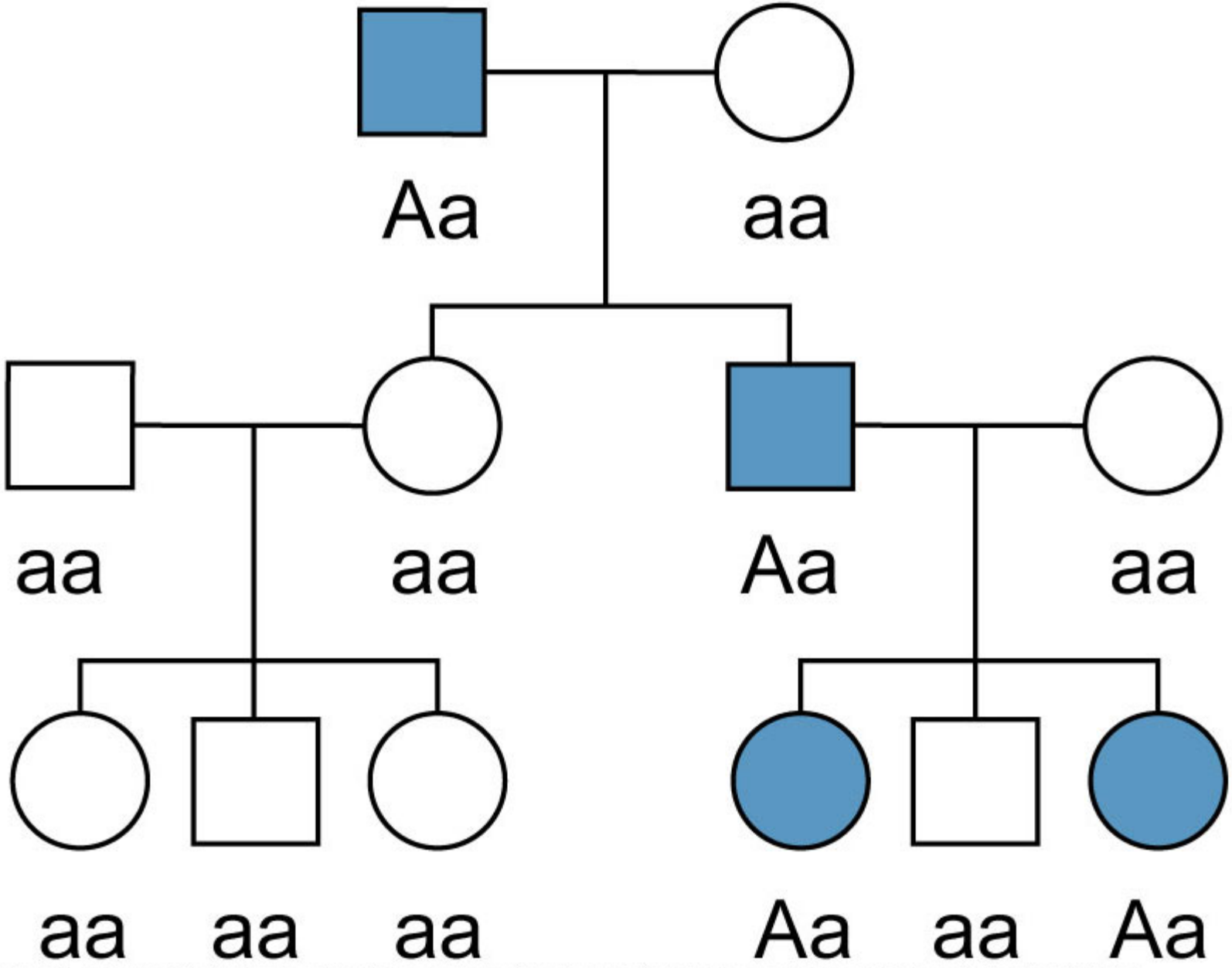


Fig. 4-4. Pedigree illustrating the inheritance pattern of postaxial polydactyly, an autosomal dominant disorder. Affected individuals are represented by shading.

→ Second, there is no skipping of generations: if an individual has polydactyly, one parent must also have it. This leads to a vertical transmission pattern, in which the disease phenotype is usually seen in one generation after another. Also, if neither parent has the trait, none of the children have it.

→ Third, father-son transmission of the disease gene is observed. Although father-son transmission is not required to establish autosomal dominant inheritance, its presence in a pedigree excludes certain other modes of inhentance (particularly X-linked inheritance).

→ Finally, an affected heterozygote transmits the trait to approximately half of his or her children. However, since gamete transmission, like coin tossing, is subject to chance fluctuations, it is possible that all or none of the children of an affected parent will have the trait.

Recurrence Risks

- Parents at risk for producing children with a genetic disease are often concerned with the question: What is the chance that our future children will have this disease?

- When one or more children have already been born with a genetic disease, the parents are given a recurrence risk. This is the probability that subsequent children will also have the disease.
- If the parents have not yet had children, but are known to be at risk for having children with a genetic disease, an occurrence risk can be given.

- When one parent is affected by an autosomal dominant disease (heterozygote) and the other is normal, the occurrence and recurrence risks for each child are 1/2. It is important to keep in mind that each birth is an independent event, as in the coin-tossing examples.

- Thus, even though parents may have already had a child with the disease, their recurrence risk remains 1/2. Even if they have had several children, all affected (or all unaffected) with the disease, the law of independence dictates that the probability that their next child will have the disease still ½.

AUTOSOMAL RECESSIVE INHERITANCE

- Autosomal recessive diseases are fairly rare in populations.
- Heterozygous carriers for recessive disease genes are much more common than affected homozygotes.

- The parents of individuals affected with autosomal recessive diseases are usually both heterozygous carriers.

- As the Punnett square demonstrates, one fourth of their offspring will be normal homozygotes, half will be phenotypically normal heterozygous carriers, and one fourth will be homozygotes affected with the disease (on average).

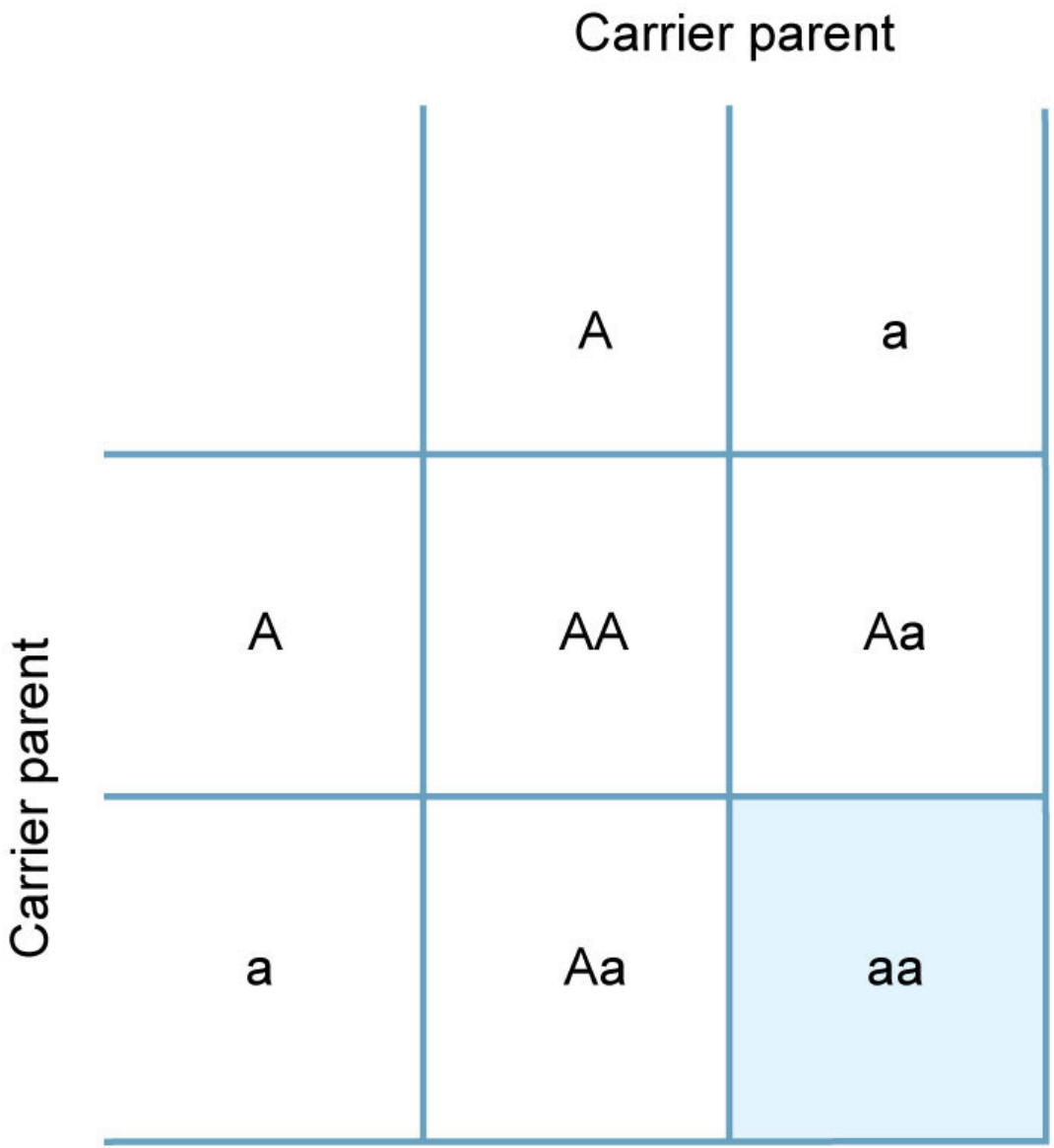


Fig. 4-5. Punnett square illustrating the mating of two heterozygous carriers of an autosomal recessive gene. The genotype of the affected offspring is shaded.

Characteristics of Autosomal Recessive Inheritance

- A pedigree showing the inheritance of an autosomal recessive form of albinism that results from mutations in the gene that encodes tyrosinase, a tyrosine-metabolizing enzyme.
- The resulting tyrosinase deficiency creates a block in the metabolic pathway that normally leads to the synthesis of melanin pigment. Consequently, the affected individual has very little pigment in the skin, hair, and eyes.
- Because melanin is also required for the normal development of the optic fibers, albinos may also display nystagmus (rapid uncontrolled eye movement), strabismus (deviation of the eye from its normal axis), and reduced visual acuity.
- The pedigree demonstrates most of the important criteria for distinguishing autosomal recessive inheritance.

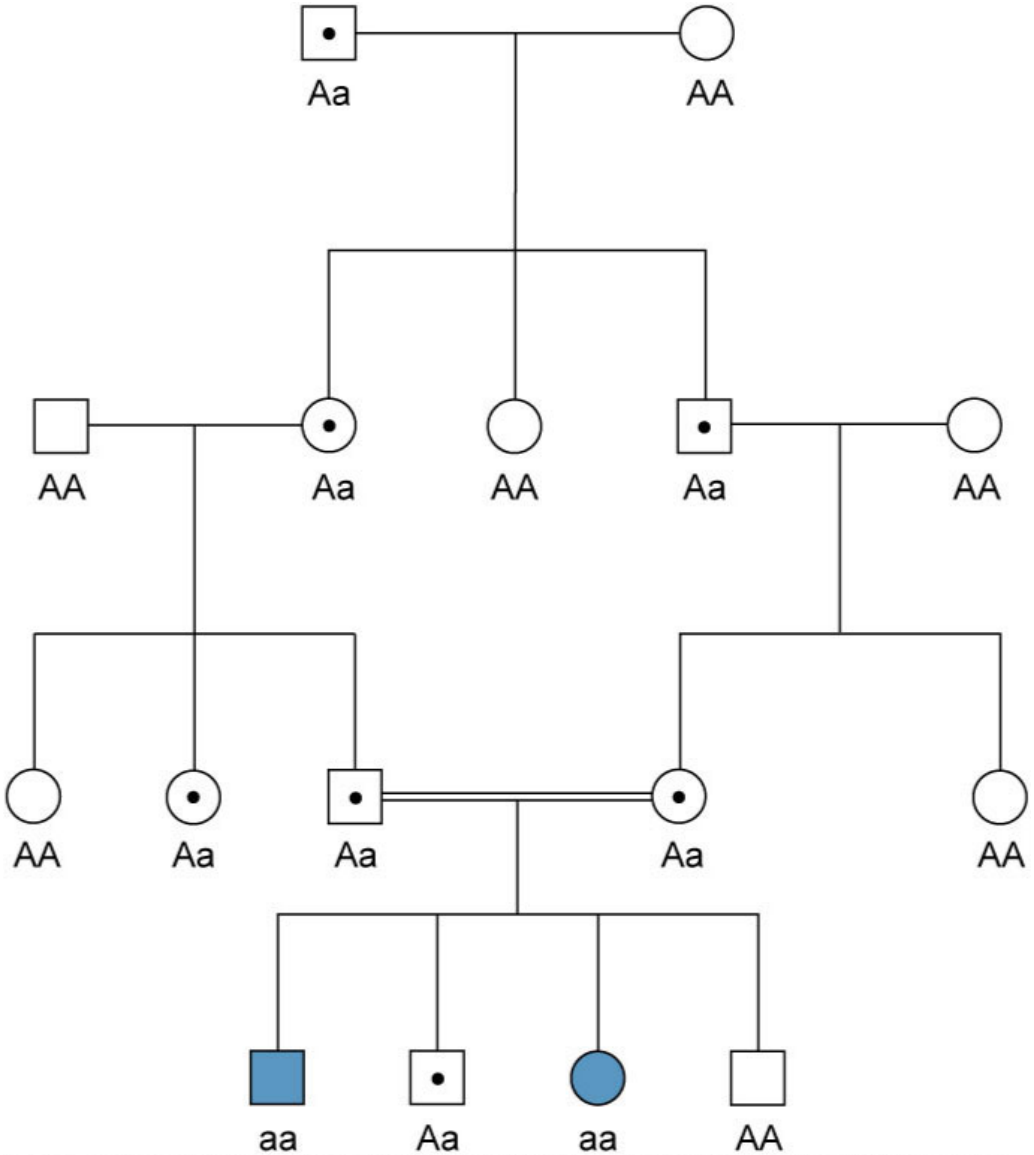
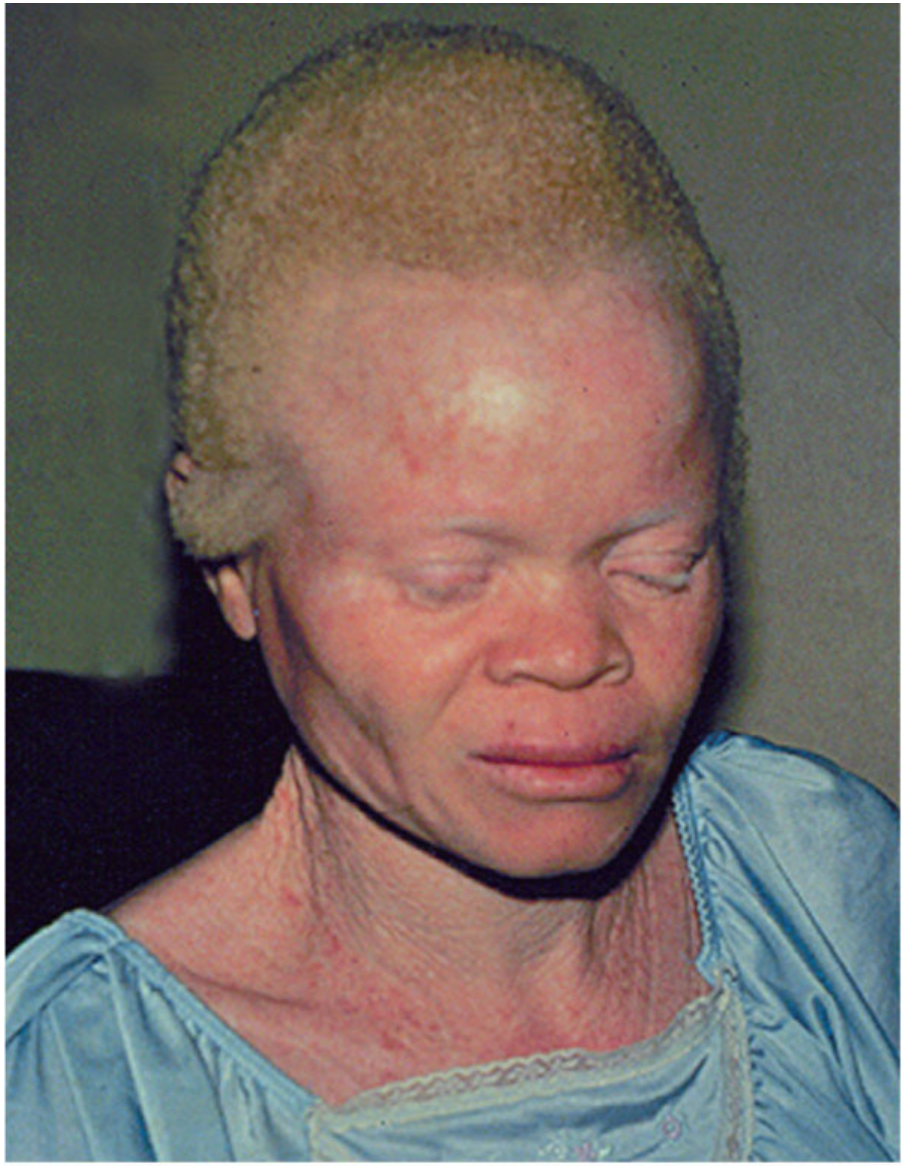


Fig. 4-6. Pedigree showing the inheritance pattern of tyrosinase-negative albinism, an autosomal recessive disease. Consanguinity in this pedigree is denoted by a double bar connecting the parents of the affected individuals.



(Courtesy of Dr. Phil Fischer, Mayo Clinic.)

Fig. 4-7. An African woman with oculocutaneous albinism, illustrating a lack of pigmentation in the hair and skin. She is looking away from the camera because her eyes are more sensitive to light than are those of persons with normally pigmented retinas.

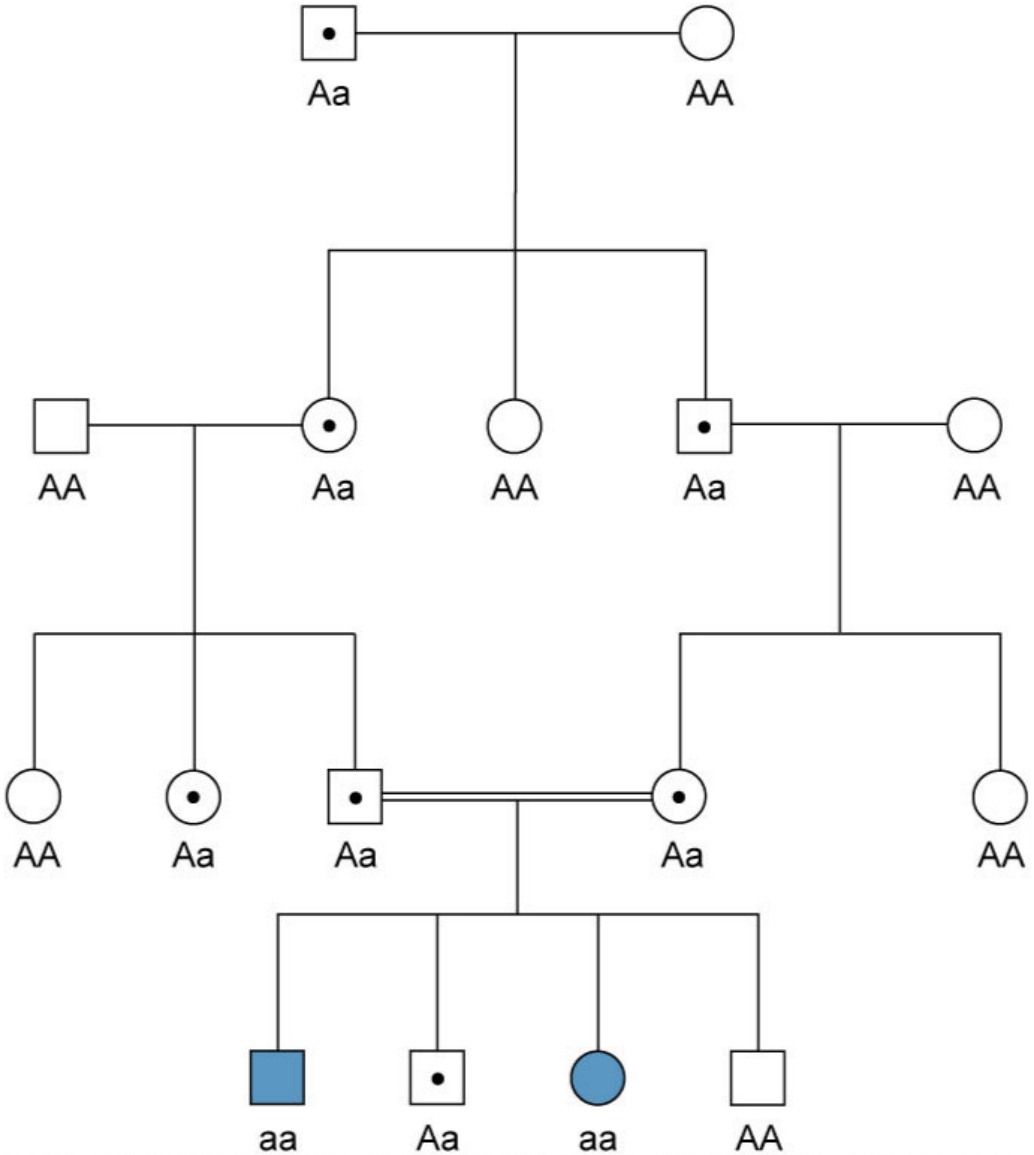


Fig. 4-6. Pedigree showing the inheritance pattern of tyrosinase-negative albinism, an autosomal recessive disease. Consanguinity in this pedigree is denoted by a double bar connecting the parents of the affected individuals.

→ First, unlike autosomal dominant diseases, in which the disease phenotype is seen in one generation after another, autosomal recessive diseases are usually seen in one or more siblings but not in earlier generations.

→ Second, as in autosomal dominant inheritance, males and females are affected in equal proportions.

→ Third, on average, one fourth of the offspring of two heterozygous carriers will be affected with the disorder.

→ Finally, consanguinity is present more often in pedigrees involving autosomal recessive diseases than in those involving other types of inheritance.

- The term consanguinity (Latin, ”with blood") refers to the mating of related individuals. It is often a factor in recessive disease because related individuals are more likely to share the same disease genes.

TABLE 4-1 A Comparison of the Major Attributes of Autosomal Dominant and Autosomal Recessive Inheritance Patterns

ATTRIBUTE	AUTOSOMAL DOMINANT	AUTOSOMAL RECESSIVE
Usual recurrence risk	50%	25%
Transmission pattern	Vertical; disease phenotype seen in generation after generation	Disease phenotype may be seen in multiple siblings, but usually not in earlier generations
Sex ratio	Equal number of affected males and females (usually)	Equal number of affected males and females (usually)
Other	Father-to-son transmission of disease gene is possible	Consanguinity is sometimes seen, especially for rare recessive diseases

Recurrence Risks

- The most common mating type seen in recessive disease involves two heterozygous carrier parents.

→ This reflects the relative commonness of heterozygous carriers and the fact that many autosomal recessive diseases are severe enough that affected individuals are less likely to become parents.

→ The punnett square demonstrates that one fourth of the offspring from this mating will be homozygous for the disease gene and therefore affected. The recurrence risk for the offspring of carrier parents is then 25%.

- Occasionally, a carrier of a recessive disease gene mates with an individual homozygous for the disease gene. In this case, roughly half of their children will be affected, while half will be heterozygous carriers. The recurrence risk is 50%.

→ Because this pattern of inheritance mimics that of an autosomal dominant trait, it is sometimes referred to as quasi-dominant inheritance.

→ With extended studies of pedigrees, in which carrier matings are observed, it can be distinguished from true dominant inheritance.

- When two individuals affected by a recessive disease mate, all of their children must also be affected.

→ This observation helps to distinguish recessive from dominant inheritance, since two parents both affected by a dominant disease will nearly always both be heterozygotes and thus one fourth of their children will be unaffected.

FACTORS THAT MAY COMPLICATE INHERITANCE PATTERNS

New Mutation

- If a child has been born with a genetic disease and there is no history of the disease in the family, it is possible that the child is the product of a new mutation.

→ The gene transmitted by one of the parents underwent a change in DNA resulting in a mutation from a normal to a disease-causing allele.

→ The recurrence risk for the parents' subsequent offspring is not elevated above that of the general population.

→ The offspring of affected child may have a substantially elevated occurrence risk.

- It is estimated that 7/8 of all cases of achondroplasia are due to new mutations.

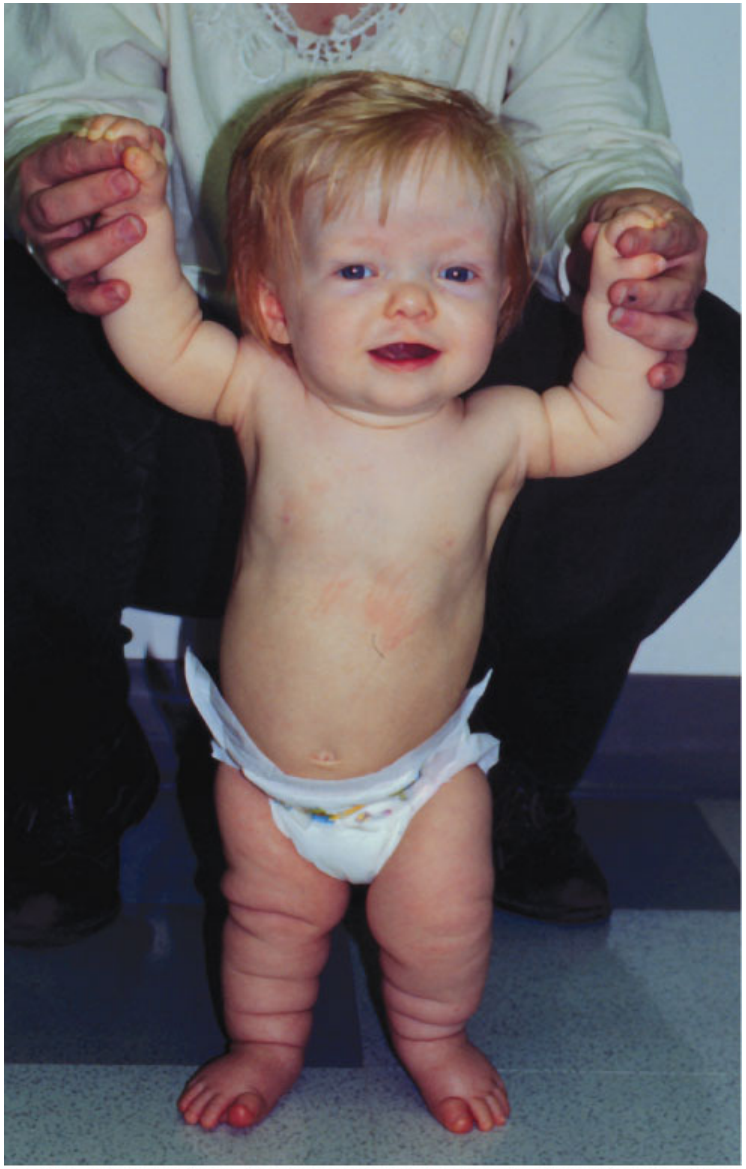


Fig. 4-8. Achondroplasia. This girl has short limbs relative to trunk length. She also has a prominent forehead, low nasal root, and redundant skin folds in the arms and legs.

Germline Mosaicism

- Germline mosaicism occurs when all or part of a parents germ line is affected by a disease mutation but the somatic cells are not.
- It elevates the recurrence risk for future offspring of mosaic parent.

→ During the embryonic development of one of the parents, a mutation occurred that affected all or part of the germline but few or non of the somatic cells of the embryo.

→ Osteogenesis imperfecta type II, Neurofibromatosis type I, DMD, Hemophilia A are examples.

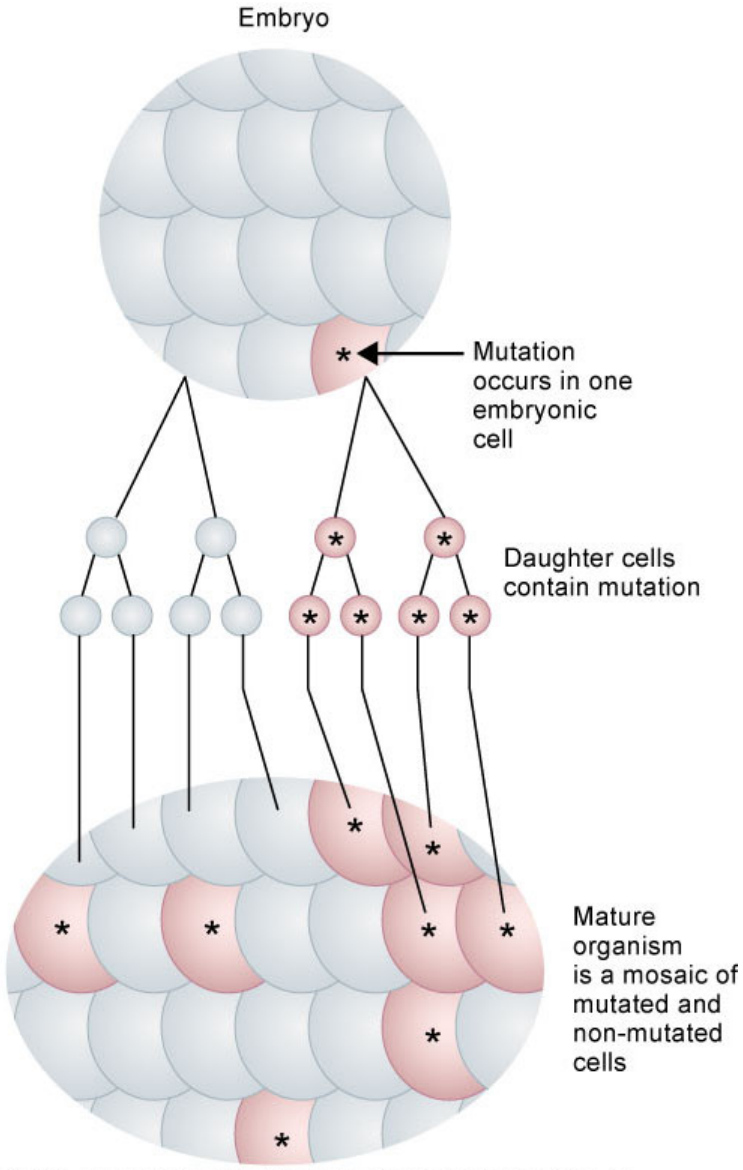


Fig. 4-9. A mutation occurs in one cell of the developing embryo. All descendants of that cell have the same mutation, resulting in mosaicism. If the first mutated cell is part of the germline lineage, then germline mosaicism results.

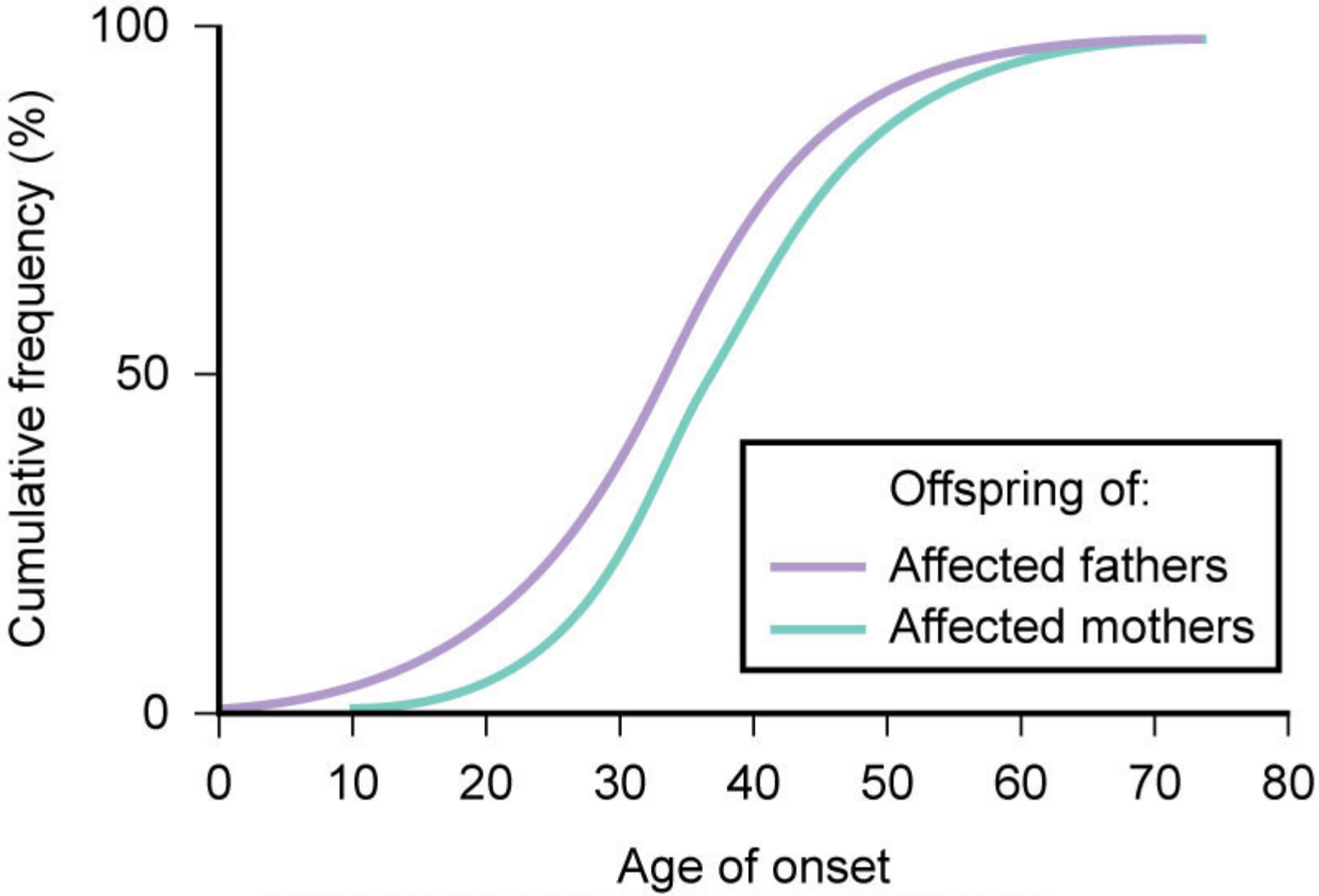
Delayed age of onset

- While some genetic diseases are expressed at birth or shortly afterward, many others do not become apparent until well into adulthood.
- One of the best-known examples is Huntington's disease, a neurological disorder whose main features are progressive dementia and increasingly uncontrollable movements of the limbs.

→ Symptoms are not usually seen until age 30 or later. Thus, those who develop the disease have often had children before they are aware that they carry the gene.

→ If the disease were present at birth, nearly all affected persons would die before reaching reproductive age, and the frequency of the gene in the population would be much lower.

→ Delaying the age of onset thus reduces natural selection against a disease gene, increasing its frequency in a population.



(Data from Conneally PM: Huntington disease: Genetics and epidemiology. Am J Hum Genet 1984;36:520.)

Fig. 4-11. Distribution of the age of onset for Huntington disease. The age of onset tends to be somewhat earlier when the affected parent is the father.

Reduced Penetrance

- an individual who has the genotype for a disease may not exhibit the disease phenotype at all, even though he or she can transmit the disease gene to the next generation.

- Retinoblastoma, a malignant eye tumor, is a good example of an autosomal dominant disorder in which reduced penetrance is seen.

→ Family studies have shown that about 10% of the obligate carriers of the retinoblastoma susceptibility gene do not have the disease. The penetrance of the gene is then said to be 90%.

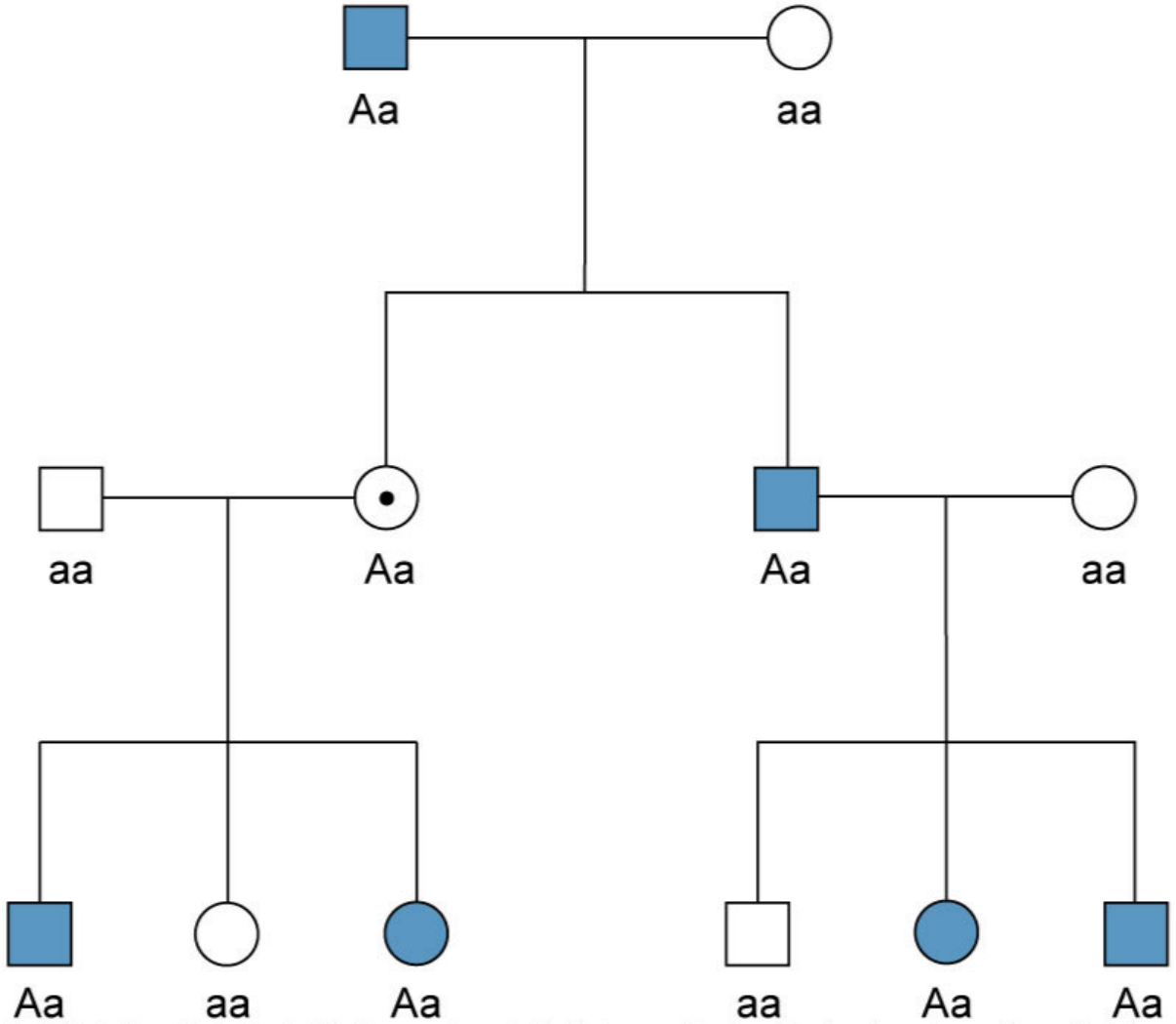


Fig. 4-10. Pedigree illustrating the inheritance pattern of retinoblastoma, a disorder with reduced penetrance. The unaffected obligate carrier, denoted by a dot, has the same genotype as the affected pedigree members.

Variable Expression

- Here, the penetrance may be complete, but the severity of the disease can vary greatly.
- A well-studied example of variable expression in an autosomal dominant disease is neurofibromatosis type 1.

→ A parent with mild expression of the disease -so mild that he or she is not aware of it- can transmit the gene to a child who may have severe expression.

→ Variable expression provides a mechanism for disease genes to survive at higher frequencies in populations.

Pleiotropy and Heterogeneity

- Genes having more than one discernible effect on the body are said to be pleiotropic.
- A good example of a gene with pleiotropic effects is given by Marfan syndrome. This autosomal dominant disorder affects the eye, the skeleton, and the cardiovascular system.

→ All of the observed features of Marfan syndrome are due to unusually stretchable connective tissue.

→ Mutations in the gene encoding fibrillin, a component of connective tissue, are responsible for the multiple defects seen in this disorder.

Pleiotropy and Heterogeneity

- Just as a single gene may have multiple effects, a single disease phenotype my be caused by mutations at different loci in different families.
- The causation of the same disease phenotype by mutations at distinct loci is termed locus heterogeneity.

→ Adult polycystic kidney disease (APKD), an autosomal dominant disorder in which a progressive accumulation of renal cysts is seen.

→ APKD can be caused by mutations in genes on either chromosome 16 (PKD1) or chromosome 4 (PKD2).

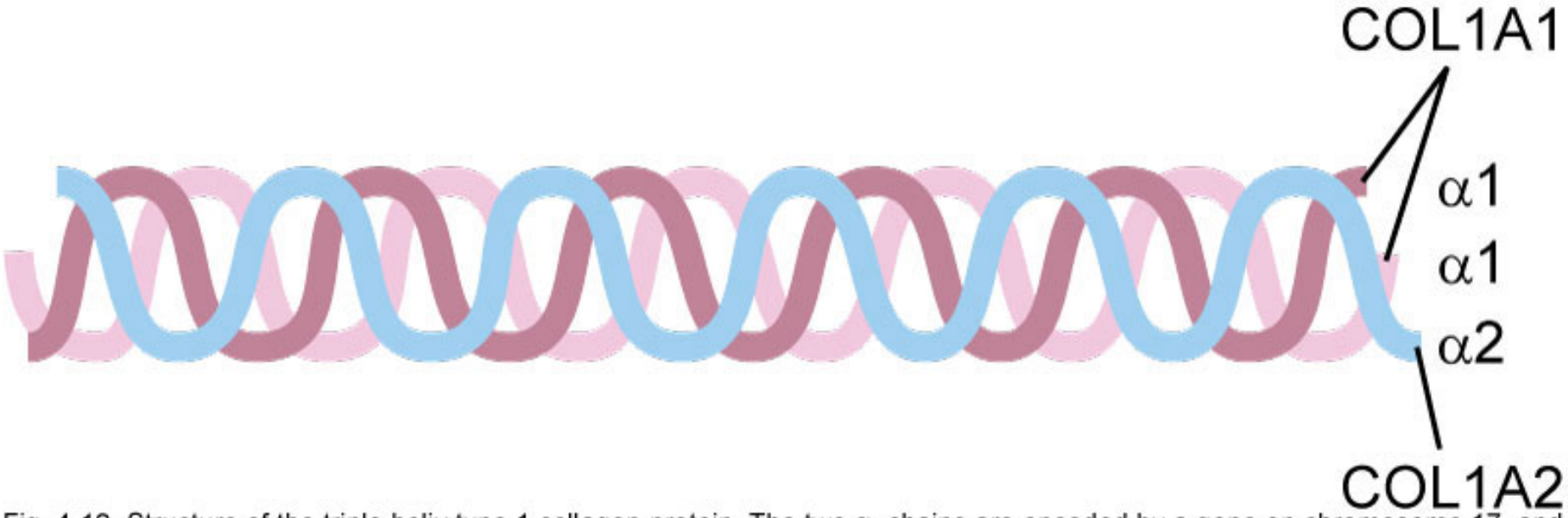


Fig. 4-12. Structure of the triple helix type 1 collagen protein. The two α_1 chains are encoded by a gene on chromosome 17, and the α_2 chain is encoded by a gene on chromosome 7.

Genomic Imprinting

- Mendel's experimental work with garden peas established that the phenotype is the same whether a given allele is inherited from the mother or the father.

→ this principle does not always hold

→ A striking example is provided by a deletion of 3 to 4 Mb on the long arm of chromosome 15.

→ When this deletion is inherited from the father, the offspring manifest a disease known as Prader-Willi syndrome. The disease phenotype includes short stature, hypotonia, small hands and feet, obesity, mild to moderate mental retardation, and hypogonadism.

→ When the deletion is inherited from the mother, the off-spring develop Angelman syndrome, which is characterized by severe mental retardation, seizures, and an ataxic gait. Both diseases are seen in about 1/15,000 individuals, and in both about 70% of cases are caused by chromosome deletions.

- The differential activation of genes, depending on the parent from which they are inherited, is known as genomic imprinting.

Anticipation and Repeat Expansion

- some genetic diseases seem to display an earlier age of onset and/or more severe expression in the more recent generations of a pedigree.

→ This pattern was termed anticipation, and it has been the subject of considerable controversy and speculation.

→ The number of these repeats is strongly correlated with severity of the disease.

- Unaffected individuals typically have 5 to 30 copies of the repeat.
- Those with 50 to 100 copies of the repeat may he mildly affected or have no symptoms.
- Those with full-blown myotonic dystrophy may have anywhere from 100 to several thousand copies of the repeat sequence.

→ The number of repeats often increases with succeeding generations: a mildly affected parent with 80 repeats may produce a severely affected offspring with more than 1,000 repeats