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CHAPTER 6

Clinical Genetics for the Dental Practitioner

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CHAPTER OUTLINE

The purpose of this chapter is twofold: to review genetic principles and to mention a few examples of the influence of genetic factors on major craniofacial, oral, and dental conditions. As the basis for relatively rare developmental dysplasias, diseases, and syndromes that show a genetic cause or marked genetic influence becomes known, increasing attention is being paid to those genetic factors that influence (or are associated with) more common conditions. An increased appreciation of how genetic factors interact with environmental (nongenetic) factors to influence growth and pathology will lead to an increased understanding of pathogenesis and the recognition that some groups or individuals may be more susceptible, or that they may respond differently to treatment.[1] Further information may be found online in the Genetics Home Reference Your Guide to Understanding Genetic Conditions at http://ghr.nlm.nih.gov.

REVIEW OF GENETIC PRINCIPLES

The *genome* contains the entire genetic content of a set of chromosomes present within a cell or an organism. Within the genome are genes that represent the smallest physical and functional units of inheritance that reside in specific sites (called loci, or locus for a single location). A *gene* can be defined as the entire DNA sequence necessary for the synthesis of a functional polypeptide molecule (production of a protein via a messenger RNA intermediate) or RNA molecule (transfer RNA and ribosomal RNA). *Genotype* generally refers to the set of genes that an individual carries and, in particular, usually refers to the specific pair of alleles (alternative forms of a particular gene) that a person has at a given location (locus) of their total collection of DNA, called their *genome*. In contrast, *phenotype* is the observable properties and physical characteristics of an individual, as determined by the individual's genotype and the environment in which the individual develops over a period of time.

Remarkable advances in the biochemical techniques that are used to study cell molecular biology and DNA have taken researchers to the threshold of understanding the regulation of cell functions. To illustrate, not so long ago DNA analyses were performed on minute amounts (picograms) of DNA. This limitation was necessary because there was so little DNA available for study in samples. When the DNA polymerase enzyme was discovered that could replicate DNA through the polymerase chain reaction and make it by the gram, this sample problem disappeared. This advance facilitated completion of the human genome project, which resulted not only in definition of a single human genome sequence composed of overlapping parts from many humans, but also in an expanding catalogue of more than 1 million sites of variation in the human genome sequence. These variations (or polymorphisms) may be used as markers to perform genetic analysis (including analysis of genetic-environmental interaction) in human beings.[2] The genome varies from one individual to the next, most often in terms of single base changes of the DNA, called *single nucleotide polymorphisms* (SNPs, pronounced "snips"). The main use of this human SNP map will be to determine the contributions of genes to diseases (or nondisease phenotypes) that have complex, multifactorial bases.[3]

CELL DIFFERENTIATION AND DEVELOPMENTAL BIOLOGY

It is fascinating that a single fertilized ovum contains within itself the potential for development of the incredibly complicated human organism. Cellular differentiation is a critical component of this developmental process, and aside from the development of antibody diversity, typically occurs in the absence of genetic alteration or mutation. Different types of cells gain their specific identities by using a particular subset of the approximately 30,000 or more genes present within the genome. The types of polypeptides that a cell can synthesize include enzymes, which catalyze various activities of cellular metabolism and homeostasis; structural proteins, which form the intracellular and extracellular scaffolding or cellular matrix; and regulatory proteins, which convey signals from the outside of the cell to the nucleus and modulate or control specific gene expression. In a developing embryo, cells reside in a three-dimensional environment and are responsive to signals from themselves (autocrine), from nearby sources (paracrine), and from anatomically distant sources (endocrine). Many of these signals are mediated by soluble molecules (either peptide or nonpeptide in origin) that bind to specific receptors (proteins) that are present on the surface or on the inside of cells. In addition to signals from soluble factors, cells can respond to cell-to-cell or cell-to-extracellular matrix signals.[4]

The action of "turning on" or "turning off" specific genes, referred to as *regulation of gene expression*, is carefully orchestrated and remains a critical element in determining cell specificity and tissue morphogenesis. Transcription factors bind to DNA and either facilitate or suppress initiation of gene transcription, the most common control point of gene expression. In the development of the craniofacial complex there is increasing evidence for the role of homeobox-containing gene families that encode transcription factors. These then are critical for the control of complex interactions between genes that are subsequently expressed during development.[5]

In summary:

1. The genetic message lies in the DNA itself, which is coded and transmitted from cell generation to cell generation when these DNA molecules are replicated (or duplicated).

2. A given cell type and function is defined by what specific RNA molecules are made from the DNA master. These RNA molecular copies direct protein synthesis in the cell.

3. Transcription factors determine which genes are expressed through the production of the RNA and subsequent protein.

4. Development occurs through the action of specific transcription factors and other regulators of protein production on specific genes that need to be expressed next in time.

CHROMOSOMES

DNA is grouped into units called *chromosomes*. Humans have 46 chromosomes that contain an estimated 30,000 genes, including numerous duplicates. Of the 46 chromosomes, the sex chromosomes are the X and Y, with the remaining 44 chromosomes referred to as *autosomes*. Each autosome has a paired mate that is referred to as its *homologue*. Therefore, with the exception of some of the genes on the X and Y chromosomes in males, there are at least two copies of each gene unless a piece of DNA is deleted. Thus the human chromosome complement consists of 23 pairs of chromosomes (one pair of sex chromosomes and 22 pairs of autosomes).

One area of special interest to the clinician is cytogenetics, the study of chromosomes. This interest has been stimulated by the development of techniques in which cells are grown in culture and the chromosomes are examined under a microscope for changes in size, shape, and fine structure. This is called *karyotyping*. Fig. 6-1 shows the karyotypes of a normal human male and female. By applying this technique, Lejeune and colleagues demonstrated that the fundamental cause in Down syndrome is the presence of an extra specific chromosome (number 21) in the affected individual's karyotype.[6] When an entire extra chromosome is present, the condition is called a *trisomy* of the chromosome in question, for example, trisomy 21 for Down syndrome. Fig. 6-2 shows the karyotype of a male who has Down syndrome. The extra chromosome in the group of number 21 and number 22 chromosomes is readily apparent.

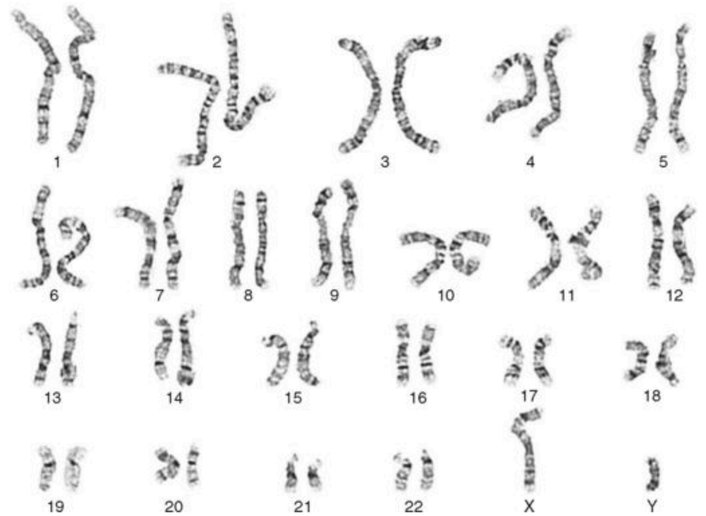


Figure 6-1 Banded karyotypes of a normal human male *(left)* and female *(right)*. Group designations according to Denver nomenclature are also indicated.

(Courtesy Cytogenetic Laboratories, Indiana University School of Medicine.)

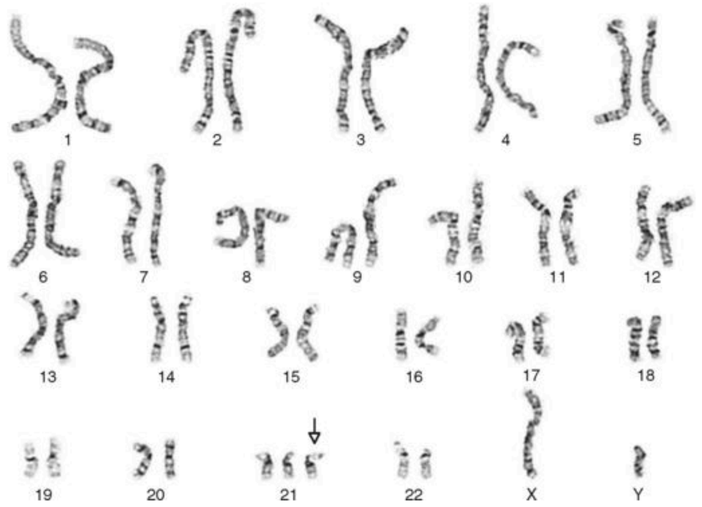


Figure 6-2 Banded karyotype of a male with trisomy of chromosome 21 (Down syndrome).

(Courtesy Cytogenetic Laboratories, Indiana University School of Medicine.)

Since this report in 1959, many disease states have been shown to be associated with an incorrect chromosome complement. By using this approach with considerable refinement, it was shown that alterations in the fine structure of chromosomes, as well as in their number, could be present. Monosomy of an autosome, or a missing autosomal chromosome, had not been believed to be compatible with life, but several monosomies in live-born children have now been reported. Monosomy of the sex chromosomes can be compatible with life and typically affects development of both internal and external sex organs of the individuals. The best known example of this is Turner syndrome, which occurs in approximately 1 in every 5000 live female births. These persons are phenotypic females who are usually missing one of the X chromosomes and are chromosomally designated as 45, X. Other aberrations of the X chromosome may also cause Turner syndrome. Affected individuals are typically short of stature, lack secondary sex characteristics, and are sterile. The Turner syndrome karyotype is shown in Fig. 6-3. Table 6-1 lists common chromosomal aberrations that produce clinical disease, including examples of translocations (the attachment of a broken piece from one chromosome to another, but not homologous, chromosome) and deletions (the absence of a piece of chromosome).

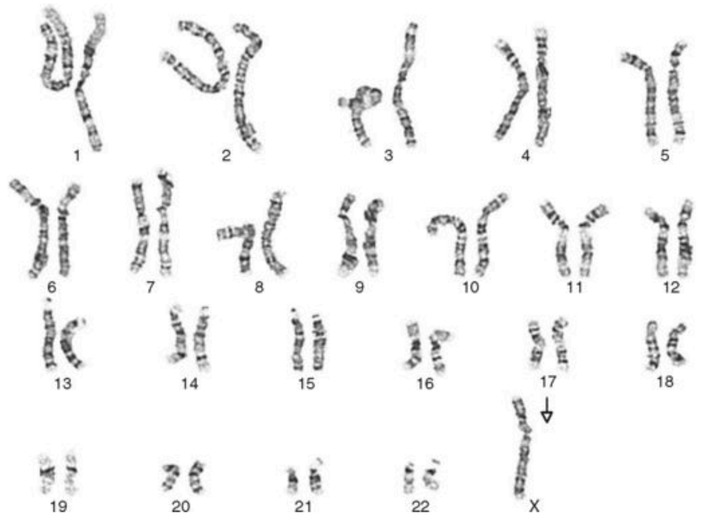


Figure 6-3 Banded karyotype of a female with missing X chromosome (Turner syndrome).

(Courtesy Cytogenetic Laboratories, Indiana University School of Medicine.)

Table 6-1 Common Chromosomal Aberrations

Type	Specific Alteration	Clinical Result
Aneuploidy	Trisomy 21	Down syndrome
	Trisomy 18	Edwards syndrome
	Trisomy 13	Patau syndrome
	Extra X chromosomes	In females: XXX, XXXX, XXXXX syndromes
		In males: Klinefelter syndrome—XXY, XXXY, and XXXXY
	Monosomy, autosomal	Usually nonviable
	Monosomy, X chromosome	In females: Turner syndrome, 45,X
		In males: nonviable, 45,Y
Translocation	14/21, 21/21 or 21/22	Translocation carrier (normal phenotype) or Down syndrome
Deletion	Ring chromosome	Variable
	Short arm chromosome No. 5	Cri du chat syndrome
	Philadelphia chromosome (No. 22)	Chronic myeloid leukemia

Chromosome abnormalities are an important cause of spontaneous abortion. About 15% of all recognized pregnancies end in spontaneous abortion, and the incidence of chromosome abnormalities in such abortions is greater than 50%. Only 0.3% to 0.5% of all live-born infants have a chromosome abnormality that is detectable with standard microscopic karyotyping. Microdeletions and microduplications of DNA, not visible by routine chromosome karyotype analysis, are a major cause of human malformation and mental retardation. A complementary analysis called *comparative genomic hybridization* (CGH) or *array comparative genomic hybridization* (arrayCGH or aCGH) can improve the diagnostic detection rate of these small chromosomal abnormalities. This technique attains such a high-resolution screening by hybridizing differentially labeled test and reference ("normal") DNAs to arrays consisting of thousands of genomic clones. In this way, relatively

small differences between the test and reference DNA sequences may be discovered and investigated further if indicated.[7,8]

HEREDITARY TRAITS IN FAMILIES

Heritability is the proportion of the total phenotypic variance in a sample that is contributed by genetic variance.[9] On an individual basis for a binary trait (i.e., a disease or trait that an individual either has or does not have), heritability is not the proportion of disease or the trait attributable to, or caused by, genetic factors. For a quantitative trait, heritability is not a measure of the proportion of an individual's score attributable to genetic factors.[10] A trait with a heritability of 1 is said to be expressed without any environmental influence, whereas a trait with a heritability of 0.5 has half its variability (from individual to individual) influenced by environmental factors and half by genotypic factors. Values greater than 1 may occur because the methodology provides an estimate of heritability under several simplifying assumptions that may be incorrect.

There is the common perception that knowing a trait's heritability will somehow affect how a patient should be treated (e.g., for malocclusion) or that it will define the limits of tooth movement or the manipulation of jaw growth. This is not true. The ability of the patient to respond to changes in the environment (including treatment), which has nothing to do with heritability, defines these limits. Heritability estimates imply nothing about trait size or treatment limits based upon a presumed genetic "predetermination."[11] Even so, the estimation of heritability can provide an indication of the relative importance of genetic factors on a trait in a group at that time. Confirming that there is a certain degree of genetic influence on a trait is a preliminary step to performing further specific genetic linkage studies (using DNA markers) to determine areas of the genome that appear to be associated with the characteristics of a given trait.[12]

When hereditary traits in families are to be studied, it is convenient to think of three classes of genetically influenced traits:.(1) monogenic, (2) polygenic, and (3) multifactorial. Recently the polygenic and multifactorial classes have often been combined into what are referred to as *complex traits* rather than Mendelian traits.[13] Monogenic traits are produced and regulated by a single gene locus. Usually they are relatively rare in the general population (occurrence in fewer than 1 per 1000 individuals). However, if the appearance of an affected person is striking, there may be instant recognition of the disease, as with patients having albinism, achondroplasia, or neurofibromatosis. Monogenetic conditions often occur in families and show transmission characteristics of the Mendelian (dominant or recessive) traits.

Polygenic traits, too, are hereditary and typically exert influence over common characteristics such as height, skin, and intelligence. This influence takes place through many gene loci collectively asserting their regulation of the trait. Although each gene involved has a minimal effect by itself, the effect of all the genes involved is additive. The associated phenotype is rarely discrete and is most commonly continuous or quantitative. Because these traits show a quantitative distribution of their phenotypes in a population, they do not show Mendelian inheritance patterns. It is important to note that the very nature of their influence (multiple genes each with a small additive effect) dictates that their environment may readily influence them. Monogenic traits are not readily amenable on a large scale to environmental modification, although there can be variation, presumably secondary to other genetic and environmental factors. By contrast, one can easily think of a dozen environmental factors known to influence height and intelligence quotient.

Finally, multifactorial traits or conditions are influenced by multiple genes but differ significantly from polygenic traits in that the influence is achieved through an interaction of multiple genes and environmental factors, and occurs when a liability threshold is exceeded. Although typically the number of genes involved is many, occasionally a few genes, sometimes only two or three, influence the trait. The effect of these genes on the phenotype is therefore a net effect, not necessarily a simple additive one. Furthermore, phenotypic expression approaches that of a discrete Mendelian trait and therefore cannot be readily classed as a quantitative trait. Likewise, the effect of a gene influencing the phenotype may not be as great as that of a gene associated with a monogenic trait, but the gene may be referred to as having a major effect. Among the well-known hereditary types of conditions designated as multifactorial are many of the severe nonsyndromic congenital malformations such as cleft lip and palate (CLP), neural tube defects such as spina bifida-anencephaly, and hip dislocation. Multifactorial complex inheritance is discussed later.

The investigation of human heritable traits usually involves the observation of specific features in a family and the study of that family's pedigree. The affected individual in a family who first brings that family to the attention of the geneticist is called the *proband* or *propositus*. This individual is the index case. Brothers and sisters of the proband are *siblings* or sibs. Thus a sibship consists of all the brothers and sisters in a nuclear family unit (parents and their offspring). The clinical appearance in an individual of a given trait, such as eye color or height, is that individual's phenotype, whereas the specific genetic makeup that influences or is associated with the phenotype is the genotype.

In an earlier section, the point is made that the human chromosome complement has 22 homologous pairs of autosomes and one pair of sex chromosomes. Because of homologue pairing (excluding the X and Y chromosomes in the male), there are at least two copies of each gene, one located at the same position (locus) on each member of the homologous pair. Genes at the same locus on a pair of homologous chromosomes are *alleles*. When both members of a pair of alleles are identical, the individual is *homozygous* for that locus. When the two alleles at a specific locus are different, the individual is *heterozygous* for that locus.

A gene that results in the expression of a particular phenotype in single dose (i.e., heterozygous) is a dominant gene. If the gene must be present in double dose (homozygous) to express the phenotype, it is a recessive gene. It is actually the phenotype that is dominant or recessive and not the gene itself. The terms *dominant gene* and *recessive gene*, though, are commonly used to describe these types of inherited traits in families.

Construction of a pedigree, which is a shorthand method of classifying the family data, conveniently summarizes the family data for the study of inherited traits. The symbols used in constructing a pedigree are shown in Fig. 6-4. The observable inheritance patterns followed by such monogenic traits within families are determined by (1) whether the trait is dominant or recessive, (2) whether the gene is autosomal (on one of the autosomes) or X linked (on the X chromosome), and (3) the chance distribution in the offspring of those genes passed from parents in their gametes (sperm and ova). Pedigree construction is a valuable tool for the clinician who is concerned with the diagnosis of and counseling regarding hereditary

traits. Every dentist should be able to construct and interpret a pedigree, because it is a certainty that patients will come to the dentist's office with heritable oral diseases that need diagnosing before treatment is begun.

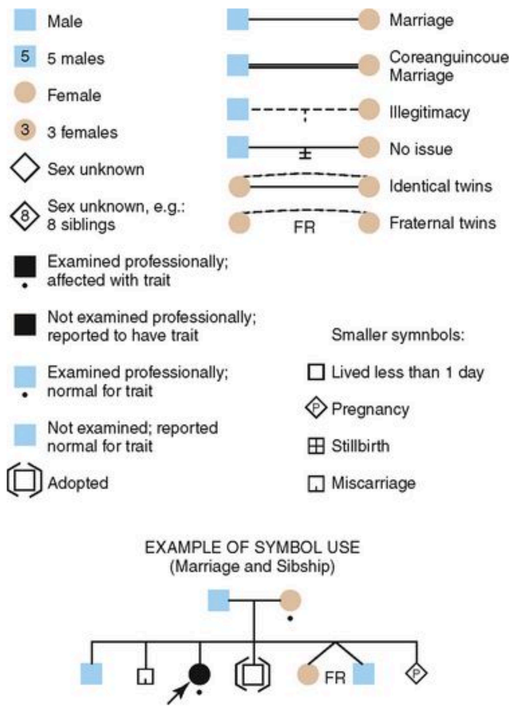


Figure 6-4 Pedigree symbols used in family studies.

The simple patterns of monogenic inheritance seen in families are described in the following discussion. Because all the Mendelian modes of inheritance are found in the amelogenesis imperfecta (AI) disorders, these are used to illustrate basic genetic principles.

DEVELOPMENTAL BIOLOGY OF ENAMEL

For a review of how molecular biologists are studying the genetic factors involved in dental development, there is a paper by Tucker and Sharpe.[14] The two developmentally different cell layers involved in dentinogenesis, inner enamel epithelium (enamel) and neural crest (dentin), are separated by an extracellular matrix.[15] Specific tooth development is then mutually dependent on reciprocal cell-to-cell signaling between these two developmentally different cell layers.[16] The genes involved in the development of these tissues are candidates for DNA mutation analysis, especially if they are in a chromosome location that has been associated with or linked to an inherited defect of enamel or dentin. The most intriguing dental research today is (1) the attempt to localize the genes for these proteins to specific loci and (2) the biochemical identification of a specific defect in the protein that prevents it from functioning normally. The following is a discussion of genetic principles best exemplified by the heritable disorders of enamel. Further discussion of the molecular basis of the heritable disorders of dentin and enamel appears in Chapter 7.

Based on the clinical appearance, radiographic characteristics, and microscopic features, oral pathologists have recognized three major types of inherited enamel defects: hypoplasia, hypocalcification, and hypomaturation.[17] These terms also provide the general description of the disease phenotypes. For example, in type 1, enamel hypoplasia, the enamel is hard and well calcified but defective in amount, so the teeth appear small. Two types of deficient enamel phenotypes are seen: generalized (all the enamel) and localized (pits and grooves in specific areas). Type 2, hypocalcification disorders, are those in which the enamel matrix is so drastically altered that normal calcification cannot occur, with the result that the clinical phenotype is a soft, mushy enamel that easily wears away. Type 3 defect, hypomaturation, involves the process of maturation of the enamel crystal. This occurs after an essentially normal enamel matrix has been established. The enamel is of normal thickness (not hypoplastic) and relatively normal hardness (slightly hypocalcified) with reduced radiographic density and discoloration.

From this collection of enamel diseases we can now draw out four examples of AI that illustrate the four major Mendelian modes of inheritance: autosomal dominant (AD), autosomal recessive (AR), X-linked dominant (XLD), and X-linked recessive (XLR).

One characteristic of inherited dental defects is that both dentitions (primary and permanent) are affected. Occasionally, the defect is expressed differently in the two dentitions, as in the case of dentin dysplasia type II.[18] However, it is much more common to see the same clinical and radiographic picture in both dentitions. Both dentitions are affected in the AI disorders.

AUTOSOMAL DOMINANT INHERITANCE

From pedigrees such as that shown in Fig. 6-5, the following criteria for AD inheritance may be deduced:

1. The phenotype occurs in successive generations, that is, it shows vertical inheritance.

2. On the average, 50% of the offspring of an affected parent will also be affected.

3. Normal parents have normal offspring. The following causes of exceptions to this rule are worth noting:
   - Nonpenetrance of the trait (defined later).
   - A mutation in either the sperm or egg.
   - Germinal mosaicism. This is an increasingly invoked explanation for this situation. In this case, one of the parents is mosaic in the germ cell line and the sperm or eggs are of two types-one cell line with and one cell line without the mutation. Chance determines which sperm cell line will be selected. However, as the molecular basis of genetic traits becomes evident, mutation analysis may show that a parent believed to not be affected may actually also have the somatic mutation found in affected children.
   - Nonpaternity. Although this is not strictly a genetic problem, the illegitimacy rate in the U.S. population is high enough to make this a likely explanation when a normal couple has a child affected with a completely penetrant dominant trait.

4. Males and females are equally likely to be affected.

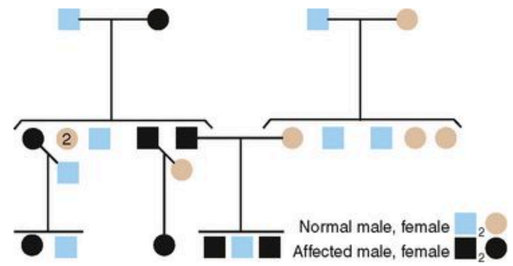


Figure 6-5 Autosomal dominant inheritance in hypocalcified amelogenesis imperfecta.

The hypocalcification type of AI provides an excellent example of AD inheritance. For diagnosing this trait, several criteria are employed. First, enamel matrix is susceptible to abrasion. The clinical picture is typical-gross accumulation of plaque on teeth that are hypersensitive because of the exposed dentin. Second, radiographs show enamel of varying thickness interproximally but with a Swiss cheese appearance because of loss of mineral. Thus severe abrasion of this soft enamel is common.

AUTOSOMAL RECESSIVE INHERITANCE

Recessively inherited traits require that both genes of a given pair at a single locus code for defective proteins. Thus, of the two alleles at this genetic locus for AI, both must be mutants to show the trait. The following three gene pairs are recognized: AA—normal; Aa—heterozygote, showing an unaffected phenotype; aa—homozygous-affected. The most common genetic situation producing an affected child is that in which both parents are heterozygous at this genetic locus (Fig. 6-6).

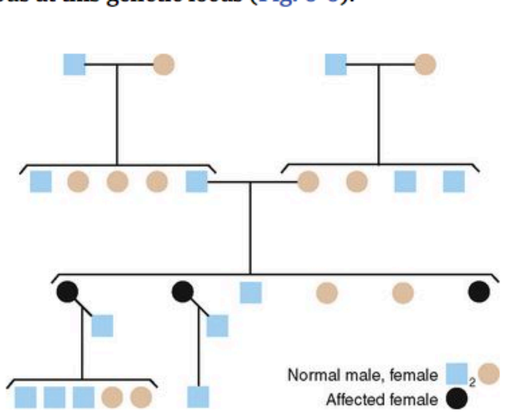


Figure 6-6 Autosomal recessive inheritance in pigmented amelogenesis imperfecta.

The following significant points about recessive inheritance must be noted:

1. The concept of a gene carrier is used here. The carrier is heterozygous for a recessive gene, and this single gene has only subtle, if any, expression. Parents of an affected child are typically heterozygous (carriers) and are then interpreted as being normal. Sometimes the carrier state can be detected, as in the case of phenylketonuria or Tay-Sachs disease. In these conditions a test is available for carrier identification to detect the presence of the single mutant gene. This greatly improves the precision of genetic counseling.

2. The rarer the recessive gene, the more likely that normal parents who have an affected child will be blood relatives. This is a consanguineous mating. Given that both parents who produce an AR-affected child are heterozygotes, it is easy to see that only one out of the four possible combinations of parents' genes results in the homozygous-affected genotype. Hence, the recurrence risk for an affected child in this case is 25%. Note that transmission of the phenotype in a pedigree is horizontal (typically present only in sibs) and not vertical as with a dominant trait.

Of several AR types of AI, the one chosen for discussion here is the pigmented hypomaturation form. In this instance, the genetic defect probably lies in the protein needed in late tooth development to produce mature, hard, and dense enamel. The defective enamel present is softer than normal but not nearly as soft and easily abraded as in the hypocalcification defect. Remarkably, a brown pigment is found in these outer layers of enamel that are formed last, imparting a dark brown, unsightly appearance that necessitates restorative treatment. A pedigree illustrating AR inheritance of this hypomaturation defect is shown in Fig. 6-6.

X-LINKED OR SEX-LINKED INHERITANCE

Genes on the sex chromosomes are unequally distributed to males and females. This inequality is the result of the following facts: (1) males have one X and one Y chromosome, whereas females have two X chromosomes and (2) the genes active on the Y chromosome are essentially concerned with the development of the male reproductive system. For these reasons, then, males are hemizygous for X-linked genes, meaning that they have only half (or one each) of the X-linked genes. Because females have two X chromosomes, they may be either homozygous or heterozygous for X-linked genes, just as with autosomal genes.

Interesting genetic combinations are made possible by the male hemizygous condition. Because only one gene locus of each kind in the X chromosome is represented in the male, all recessive genes in single dose express themselves phenotypically and thereby behave as though they were dominant genes. On the other hand, X-linked recessive (XLR) genes must be present in double dose (homozygous) in females to fully express themselves. Consequently, full expression of rare XLR diseases in practice is restricted to males and is seen infrequently in females.

To this point we have considered heritable defects in two of the three major types of enamel disorders. The third type-AI, hypoplastic type-shows both autosomal and X-linked modes of inheritance, but only one X-linked type is described here.

X-LINKED DOMINANT

Fig. 6-7 is the pedigree of a family with an X-linked dominant (XLD) form of AI, hypoplastic type.[19] The clinical features are diagnostic and in some females can be quite striking.

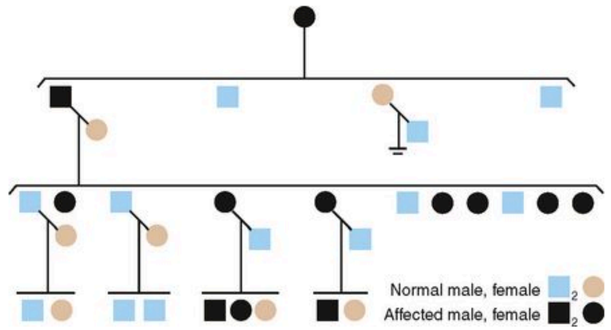


Figure 6-7 X-linked dominant inheritance in hypoplastic amelogenesis imperfecta.

Once again, both dentitions are affected similarly. The surface defect has been described as being granular, lobular, or even pitted. Conceivably, all these different forms of expression are the result of the action of a single gene (or at least its alleles). The enamel is hard but because of its thinness is more susceptible to fracture and abnormal wear. Under the appropriate conditions, this trait resembles a hypocalcification defect. However, radiographs quickly resolve this diagnostic problem and show enamel of normal density but with greatly reduced thickness.

X-LINKED RECESSIVE

A pedigree of a family with the X-linked recessive form of enamel hypomaturation is shown in Fig. 6-8. The genetic criteria for diagnosing an XLR trait are summarized as follows:

1. Because the gene cannot be passed from father to son, affected fathers almost never have affected sons. A son could be affected if the mother is a carrier of the XLR trait.

2. All daughters of an affected male receive his X-linked genes. Therefore affected males transmit the trait to their grandsons if they are affected through their daughters.

3. The incidence of the trait is much higher in males than in females. This is typified by the disease hemophilia, which is also caused by an XLR gene.

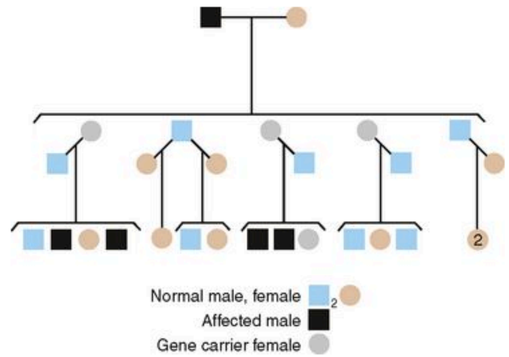


Figure 6-8 X-linked recessive inheritance in hypomaturation amelogenesis imperfecta.

The clinical features of XLR hypomaturation type AI are most striking. The enamel has a somewhat reduced hardness but is not soft. However, the crowns of the teeth look like mountains with snow on them. Hence the name given has been "snow-capped teeth." Radiologically the enamel is hypomature; it shows a lack of contrast between enamel and dentin even though the enamel is of normal thickness.

It should be noted that heterozygous females occasionally show significant clinical expression of a single XLR gene. The reason for this apparent contradiction is the process of X-inactivation, termed *lyonization* after geneticist Mary Lyon. This occurs only in females. All normal female cells have two X chromosomes, but most of the genes on one of the two X chromosomes are inactivated approximately at the blastula stage of development. This has the effect of making the total number of active, X-linked genes about the same in both males and females. If the female is heterozygous for an X-linked trait, two populations of cells result. One cell population has genes on one X chromosome that are active, while the other cell population has genes on the other X chromosome that are active. When by chance the X chromosome with the deleterious gene is active in a significant proportion of the cells, its expression may be observed in that female. Chance dictates that this imbalance does not occur frequently, but because all females are, by definition of lyonization, mosaic with regard to X-linked traits, phenotypic expression of heterozygous genes may occur in them.

The previous statements concerning the distribution of XLR genes in males and females apply equally as well to XLD genes. The principal difference lies in the fact that when the gene is dominant more females than males will show the trait (see pedigree in Fig. 6-8). Because all XLR genes behave as dominant genes in males, no new criteria are made for their inheritance in males. The following criteria distinguish an XLD trait in families:

1. Affected males must transmit the trait to all of their daughters (as with XLR traits), but all of them are affected, because fathers give their X chromosome to their daughters and their Y chromosome to their sons.

2. Affected males cannot transmit the trait to their sons (just as with XLR traits).

3. Heterozygous females transmit the trait on the average to 50% of their children of both sexes, whereas homozygous-affected females will have only affected children. The latter situation is exceptionally rare for a dominant trait and is practically never observed. Thus all females affected with a dominantly inherited X-linked trait are considered to be heterozygotes until proven otherwise.

Two points are emphasized here. First, transmission of XLD genes by females follows a pattern indistinguishable from that of autosomal transmission. Thus these two types of dominant inheritance can be differentiated only by observation of the offspring of affected males. Second, it was noted that XLR disorders are much less common in females than in males. The reverse is true for XLD traits. An XLD trait should appear about twice as often in females as in males, because females have twice as many X chromosomes as males.

VARIATION IN GENE EXPRESSION

The patterns of inheritance shown in traits determined by genes at a single locus are usually easy to recognize. However, many factors may modify the expression of a gene in a family in such a way that a typical monogenic pattern of inheritance is not discernible. Two concepts related to modification of gene action are discussed here: penetrance and expressivity.

PENETRANCE

When a person with a given genotype fails to demonstrate the phenotype characteristic for the genotype, the gene is said to show reduced penetrance. This is a situation most commonly seen with dominant traits. Dentinogenesis imperfecta, an AD trait, is practically 100% penetrant, because all individuals who carry that gene show its phenotype. On the other hand, osteogenesis imperfecta shows incomplete penetrance, because pedigree studies demonstrate individuals who must carry the gene but who do not appear to be affected. Another relevant example is found in the CLP trait. Consider the following family history: a grandfather and his grandson both have CLP but the boy's mother (also the grandfather's daughter) does not. The probability is very high that her son's cleft liability came from his grandfather and therefore was passed through the mother without being expressed as an overt cleft. Possibly the subtle action or predisposition of a clefting gene or genes may be found using measurements of facial structures, or variation in other structures such as the orbicularis oris muscle may be identified. This could increase the power of linkage analysis of the predisposing genotype. With the spectacular advances in the understanding of the human genome, we may be able to locate a gene that regulates clefting before its action at the molecular level is known or how it shows this action as a clinical trait.

EXPRESSIVITY

If a single gene trait can show different phenotypes in the affected members of kindred, it shows variable expressivity. Osteogenesis imperfecta also provides an illustration of variable gene expression. The cardinal signs of this disease are (1) multiple fractures, (2) blue sclera, (3) dentinogenesis imperfecta, and (4) otosclerosis, which results in a hearing deficit. Affected persons in a single family may show any one or a combination of these signs, which illustrates the considerable variation in gene expression. The minimum expression of the gene observed in a family might then be only a blue color to the sclera, which could be unnoticed by the clinician. In this case, highly variable gene expression may fade into nonpenetrance.

The craniosynostosis syndromes are AD traits associated with single gene mutations. They also provide good examples of how, even with the strong influence of a single gene, the phenotype can vary markedly. Although it was once thought that a particular mutation in a given gene would always result in a specific syndrome, several identical mutations in the fibroblast growth factor receptor 2 (FGFR2) gene have been found in patients diagnosed with the three different clinical craniosynostosis syndrome entities of Crouzon, Pfeiffer, and Jackson-Weiss syndrome.[20,21]

Another example of the individual variability of these single gene mutation autosomal dominant phenotypes occurred when two individuals in the same family had the classic phenotypes of Pfeiffer and Apert syndromes. In addition, seven other family members had unusually shaped heads and facial appearance reminiscent of Crouzon syndrome.[22] The phenotype may be so variable that this individual may appear to be clinically normal, yet have the same gene mutation associated with Crouzon syndrome in three of his children and two of his grandchildren. Only through the analysis of radiographic measurements was a minimal expression of features suggestive of Crouzon syndrome evident.[23]

EPIGENETICS

The influence of one or more modifying genes through their protein products in reducing or enhancing the effect of another gene has been referred to as *epigenetics*, but is now commonly referred to as *epistasis*. Now *epigenetics* refers to changes in gene expression that are inherited but not caused by alteration in the sequence of the gene. Examples of epigenetics include gene expression that is altered by methylation or acetylation, and by inhibition of messenger RNA expression by interfering RNA or microRNA binding.[24] Although monozygotic (identical) twins are epigenetically indistinguishable during the early years of life, older monozygous twins exhibit remarkable differences in their overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation, which can create differences in gene expression between the twin pairs.[25] These epigenetic factors can help explain the relationship between an individual's genetic background, the environment, aging, and disease. It can do so because the epigenetic state varies among tissues and during a lifetime, whereas the DNA sequence remains essentially the same. As cells adapt to a changing internal and external environment, epigenetic mechanisms can "remember" these changes in the normal programming and reprogramming of gene activity.[26] This is leading to a new way of thinking on how the genome and environment interact, with a tremendous impact on the study of developmental biology, cancer, and other diseases.

MULTIFACTORIAL INHERITANCE

The following features typify multifactorial inheritance, in contrast to monogenic inheritance: (1) multiple genes (polygenes) at different loci are involved in expressing the phenotype, and (2) the phenotype produced is a summation of the effects of polygenes interacting with their environment. The phenotypic result is often a continuously varying spectrum of that trait (e.g., height) rather than presentation as a discrete (trait present or absent) phenotype.

Many common diseases, such as dental caries, have continuous variation with no sharp distinction between normal (average) and abnormal (extremes). However, there may be a specific measurement point beyond which that disease is arbitrarily regarded by the clinician as abnormal.

Multifactorial inheritance is troublesome to analyze genetically; in fact, geneticists often arrive at a diagnosis of multifactorial inheritance for a given trait only after the monogenic forms of inheritance have been considered and found to be unlikely. Certain techniques for studying it have been developed. The simplest is the method of resemblance between relatives, which states that the more closely related two individuals are, the more closely they resemble each other concerning the specific trait in question. It is important to stress, though, the continuous phenotypic variation that is characteristic of inheritance patterns resulting from polygenes.

This issue of continuous variation is emphasized because the most common diseases with which the dentist must deal (i.e., periodontal disease, dental caries, and malocclusion) are multifactorial traits. Only the extremes of variation are readily apparent to the dentist, such as in the child with rampant caries or the adult who is caries free. In this latter instance, if one did not understand the concept of multifactorial inheritance, one might conclude that such individuals represent a discrete phenotype influenced by a single gene in a Mendelian manner. This is frequently not the case.

A most important feature of traits produced by polygenes is that they are susceptible to environmental modification. A phenotype resulting from the concerted action of 100 genes is much more likely to be altered and modified by the existing environment than a trait controlled by only one or even several genes. Even so, this does not mean that a trait resulting from only one or even several genes cannot be influenced by environmental factors. The change in phenotype depends on the individual's ability to respond to the environmental factor, which may be heavily influenced by the same gene(s) originally influencing the phenotype or by other genes.

An example of a polygenic trait that is markedly influenced by environmental factors is dental caries, which is the interaction product of three essential factors: a cariogenic diet, a caries-producing bacterial flora, and a susceptible tooth. These three factors encompass a variety of biologically complicated entities, such as saliva, plaque, tooth matrix formation, and crystallization. It should be easy to see that the development of these complex elements must involve a great number of genes. Environmental modification, such as properly timed systemic fluoride supplementation, produces a considerable alteration in the phenotype without changing the genetic constitution of the individual. The reader can probably think of additional environmental modifications that can produce a greatly altered dental caries experience without changing an individual's genes. Some conditions that are attributed to a multifactorial inheritance

because they tend to occur in particular families may be greatly influenced by a gene or genes that predispose to the condition, depending on what other genetic or environmental factors are involved.

MULTIFACTORIAL (COMPLEX) INHERITANCE IN HUMAN DISEASES

For many common disorders, such as diabetes and hypertension, and even for the major common congenital malformations (i.e., spina bifida, hydrocephalus, and CLP), there is a definite familial tendency. This is shown by the fact that the proportion of affected near relatives is greater than the incidence in the general population. However, this proportion is much lower than what is expected for a monogenic trait, and the explanation most commonly offered for major congenital malformations is that they are multifactorial traits. As previously stated, one definition of a complex trait is that it represents the summation of the effects of many genes (polygenes) interacting with the environment, which is why it has also been termed multifactorial. Environment is defined as those nongenetic circumstances that render an individual more or less susceptible to a disease state. In contrast to so-called simple monogenic traits, whose characteristics have been summarized in preceding paragraphs, multifactorial complex diseases show the following characteristics:

1. Each person has a liability for a given disease, and that liability represents a sum of the genetic and environmental liabilities.

2. The multifactorial-threshold model is a mathematical way of expressing these liabilities. For polygenic traits, the model is simply a gaussian curve. As already noted, for multifactorial traits, a threshold must be added to allow the continuous polygenic model to be used in describing noncontinuous or discrete traits. For many human congenital malformations, a multifactorial model with threshold is appropriate for describing discrete traits such as CLP. Such a threshold means that all persons with sufficient gene dosage and environmental interaction will be above the threshold of expression and show the cleft lip. Those with less will not show a cleft lip. A graphic representation of this idea is shown in Fig. 6-9.

3. Because of the differing dosage of polygenes in groups that show a specific phenotype (e.g., CLP), the overall incidence of this trait will vary in near relatives of those affected. For example, a dominantly inherited trait has a gene dosage of 1 in 2 (50%). Assuming that several polygenes may be involved in CLP, this figure decreases at least 10-fold to about 1% to 5%. The incidence in a random population is even lower, or about 1 per 1000. Therefore increasing gene dosage for a multifactorial complex trait in a family is associated with an increased incidence of that trait in near relatives of the affected individuals. The nature of this system with a threshold permits large numbers of persons at risk for showing that phenotype (CLP) to carry the liability for clefting without expressing it clinically. Based on current research findings regarding traits that are multifactorial with a threshold, it appears that it will be difficult to relate these mathematical observations to cellular biologic function.

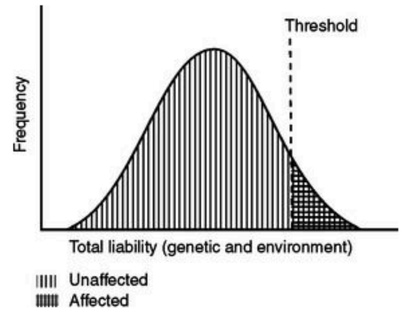


Figure 6-9 Multifactorial model for inheritance of cleft lip and palate.

Toward the end of the nineteenth century, Galton recognized that twins could be useful for evaluating the nature-nurture argument that was raging at that time. Interest in the twin method for study of the relative importance of heredity and environment in humans has been increasing. One explanation for this interest is that many human traits are complex, are susceptible to environmental modification, and therefore are difficult to study by conventional methods. The twin method allows an approach to the study of such traits and is based on the principle that human twins are of two basic types: monozygotic (or identical) twins, resulting from a single ovum fertilized by a single sperm, and dizygotic (or fraternal) twins, resulting from fertilization of two ova by two sperm. It is axiomatic that monozygotic twins have identical genotypes, whereas dizygotic twins are no more closely related to each other than are any two siblings.[27] It also follows that differences between monozygotic twins result from environmental differences (although as previously mentioned, epigenetics could be a factor), whereas those between dizygotic twins result from differences in both heredity and environment.

To use the twin method, one must distinguish between the two types of zygosity. If both twins are identical for the trait in question (regardless of their zygosity), they are described as *concordant*. If they are unlike for the trait, they are *discordant*. Such intrapair differences are usually expressed in percentage figures for a group of twins being evaluated. For example, monozygous twins show a 33% concordance for CLP, whereas dizygous twins show only a 5% concordance.

Another method to estimate the heritability of a trait and to evaluate evidence of linkage of a phenotype with DNA polymorphisms is by sib-pair analysis. Heritability estimates can be generated from within- and between-sibship variance quantified by generalized linear models, with confounding factors controlled for where indicated. Polymorphic DNA markers may be tested for genetic linkage (proximity) to a gene influencing a particular phenotype by testing whether the magnitude of the phenotypic difference between two siblings is correlated with the alleles they share that are identical by descent (IBD). An allele is considered to be IBD if both members of a sibling pair inherited the same marker allele from the same parent. If a marker is linked to a gene contributing to the phenotype in question, then siblings with a similar (if quantitative) or the same (if discrete) phenotype would be expected to share more alleles IBD, whereas siblings with widely differing phenotypes would be expected to share few if any alleles IBD near any gene(s) influencing the phenotype.[28] In addition, another method of looking for DNA markers is the linkage disequilibrium or association analysis. In its simplest terms, this refers to a nonrandom association of alleles at two or more loci. It was found that some sections of DNA do not tend to change through generations, in what are called *haplotype blocks*. Because of this, testing one SNP within each block for significant association with a disease or trait is possible when an influencing locus for that disease or trait is located in or at least close to that haplotype block.[29] Partly because these haplotype blocks occur in populations, these analyses

can be performed on unrelated individuals (affected and controls), as opposed to linkage analyses.

However, if the control individuals are from a different genetic background than the affected individuals, then there may be a bias. One way to deal with that problem is to use the quantitative transmission disequilibrium test. This analysis calculates the difference between the value of the quantitative trait in the offspring and the average value of the quantitative trait in all offspring in all families studied, while simultaneously considering the allele transmission from parent to offspring.[30] Thus, whereas the sib-pair linkage analysis involves two or more siblings, the quantitative transmission disequilibrium test involves trios of parents and one or more siblings.

INFLUENCE OF GENETIC FACTORS ON MAJOR CRANIOFACIAL, ORAL, AND DENTAL CONDITIONS

GENETICS AND DENTAL CARIES

It is clear from many dietary studies that variation in susceptibility to dental caries exists even under identical, controlled conditions.[31] This implies that, because of genetic differences, certain environmental factors are potentially more cariogenic for some people than for others. This is not to say that dental caries is an inherited disease; rather, genetic influences may modify the overt expression of this disease in the individual.

Fifty years ago, dental caries was presented to dental students as a disease that was so common that more than 99% of the general population was afflicted by it. Although it is still recognized as a common disease, the use of systemic and topical fluorides and persistence by organized dentistry to bring about changes in dietary habits and oral hygiene practices have contributed significantly to a remarkable decrease in the prevalence of this disorder, especially noted in children. Currently, it is not unusual for a prepubertal-age child to be caries free. However, there is individual variation in caries that is not fully explained by hygiene or fluoride exposure.

Three essential interacting elements comprise the model system for dental caries that is most commonly used to discuss its etiology. These factors are microorganisms, substrate (fermentable carbohydrates), and host factors, such as tooth anatomy. It is in the last area of the host factors that genetics exerts a major influence on dental caries initiation.

Several investigators have studied the genetic aspects of dental caries in humans, using both the twin and the family pedigree approaches. Because dental caries is an age-dependent process, much of the reported data cannot be compared because of age differences in the various population groups studied. Nevertheless, the family observations by Klein and Palmer[32] and Klein[33] are worth noting. Their findings indicated that children have a caries experience remarkably similar to that of their parents when the susceptibility of both parents is the same (either high or low). When caries susceptibility of the two parents is dissimilar, however, the children's susceptibility tends to be more like that of the mother than that of the father. This finding was particularly evident in daughters.

Because dental caries is an infectious communicable disease, however, familial clustering may to some degree reflect familial environmental contact, with transmission of cariogenic bacteria to children at certain ages. Li and Caufield found that mothers are the principal source of mutans streptococci to their infants, with a greater rate of transmission to female than male infants.[34]

The more common a genetic trait is, the more difficult it is to demonstrate its genetic character. Several authors have attempted to do this for dental caries by the study of twins. Book and Grahnen attempted to maximize differences in caries experience within families by selecting caries-free 20-year-old men and comparing caries experience within their families.[35] Results showed that parents and siblings of caries-free propositi had significantly lower rates of decayed, missing, and filled teeth than the control families. The authors concluded that the observed differences are hereditary and probably polygenic in nature.

Studies of twins by Dahlberg and Dahlberg,[36] Mansbridge,[37] Horowitz and colleagues,[38] Caldwell and Finn,[39] and Bretz and colleagues[40] indicated that genetic factors make a significant contribution to individual differences in caries susceptibility. However, most authors agree that this genetic component of dental caries is overshadowed by the overall effect of environmental factors in most subjects. Although it appears that genetic factors significantly contribute to the colonization of specific oral bacteria,[41] or the levels of *Streptococcus mutans* specifically,[42] the conclusion from clinical twin and familial correlation studies and estimation of heritability studies regarding the degree of genetic influence on caries may be confounded by familial factors such as oral hygiene habits, diet, and the already mentioned transmission of cariogenic bacteria within the family.

A review of inherited risks for susceptibility to caries found evidence of an association between altered dental enamel development in defined populations and an increased risk of caries, as well as a relationship between host immune complex genes and different levels of cariogenic bacteria and enamel defects.[43] This is further supported by the finding of a significant interaction between tuftelin SNP genotypes and *S. mutans* levels,[44] and variation in the amelogenin gene and caries susceptibility.[45] Thus the individual's genotype may influence the likelihood of intraoral colonization of cariogenic bacteria, which further exemplifies the complexity of caries development. Genetic studies on well-characterized populations with clearly defined caries experience will help define those host factors that have the greatest influence on the incidence of caries.[35]

For example, the first genome wide association (GWAS) study on human caries suggested that loci for low caries susceptibility were located on chromosomes 5q13.3, 14q11.2, and Xq27.1, whereas loci for high caries susceptibility were located on chromosomes 13q31.1 and 14q24.3.[46] Further work to define the genes involved, which may for example be related to saliva flow, plaque formation, and diet preferences, are underway. Bretz and colleagues found that genetic factors contributed independently to both dental caries and sucrose sweetness preference,[47] although it is likely that an increased preference for sweets would affect the caries rate.

The foundation for looking for different individual susceptibilities to caries is based upon animal studies. Hunt and colleagues succeeded in establishing caries-resistant and caries-susceptible strains of rats using inbreeding techniques.[48] Although the resistant strain was challenged by oral inoculation of cariogenic bacteria, the resistant phenotype was maintained. These were the first studies to confirm the presence of important genetic elements influencing dental caries susceptibility.

From this information, it is clear that heredity plays an

important but complex role in the cause of dental caries. Studies done largely since the 1970s have examined the influence of saliva proteins on dental plaque formation. A group of saliva proteins designated as the proline-rich proteins (PRPs) because of their high content of the amino acid proline have been linked to early plaque and pellicle formation.[49,50] PRPs closely resemble enamel matrix proteins in both composition and structure, which accounts for why they bond so tightly to hydroxylapatite crystals. Furthermore, the ability to produce these several types of PRPs is inherited as a group of autosomal codominant traits. At least eight different polymorphic PRPs are known, and all these proteins are coded for by a block of genes called the salivary protein complex, located on the short arm of human chromosome 12.[50,51] These polymorphic acidic PRPs in saliva are encoded at two loci, PRH1 and PRH2. Pa, Db, and PIF are alleles at the PRH1 locus,[52] and PRH2 codes for Pr.[53] Only a few studies have attempted to associate these salivary protein phenotypes with oral disease states. Yu and colleagues reported significant association between Pa1 and Pr22 and an increase in dental caries scores in the permanent teeth of children 5 to 15 years of age.[54] This result suggested that persons with either or both of these two genotypes (Pa1 and/or Pr22) may be at significant risk for increased susceptibility to dental caries, whereas the allelic genes, Pa- and Pr11 or Pr12, appear to confer caries resistance. The mechanism of action could be related to the formation of a caries-susceptible plaque. Zakhary and colleagues found that presence or absence of the Db allele of PRH1 may affect caries; in their study, all caucasians had significantly greater *S. mutans* colonization than did African-Americans, but only Db-negative caucasians had significantly more caries.[55]

In summary, susceptibility to human dental caries is influenced to a significant but variable degree by genetic factors in most individuals.[56] This genetic influence control is undoubtedly complex in nature and strongly implies considerable environmental influence. However, there are likely individuals in whom the genetic susceptibility is markedly greater than that of most of the population. Specific types of dental caries susceptibility representing the extremes of variation of this trait may ultimately prove to be monogenic or major gene traits, but at present the evidence is insufficient for a clear statement of such inheritance.

GENETICS AND PERIODONTAL DISEASE

The periodontal disease state is often described as a local inflammatory disease with possible underlying systemic factors. This disease is so widespread in human populations and has such widely varying clinicohistopathologic features that it seems certain that multiple diseases with multiple causes are being lumped together as a single entity. Periodontists suggest that there is evidence for the existence of several variant types of periodontal disease generally subclassified by the age of onset, severity of bone loss, oral hygiene status, and presence or absence of local factors. One might visualize a continuum of disease expression ranging from a localized gingivitis to a generalized periodontitis with severe bone and tooth loss. Such a complex disease shows both inflammatory and degenerative pathologic features.

It is easy to understand why genetic studies of this common problem have been neglected. As is true for dental caries, periodontal disease is common, occurs with a continuum of expressivity, and is greatly influenced by environmental conditions, such as diet, occlusion, and oral hygiene habits. All of these features fit the description of a complex type of disease or at least of disease susceptibility.

Most genetic studies of a trait make use of families with multiple affected individuals or twins. A carefully designed study of twins with periodontal disease by Ciancio and colleagues was reported in 1969.[57] Using the Ramfjord index, which evaluates gingival inflammation, calculus formation, tooth mobility, and tooth loss in all four quadrants of the mouth, the authors examined seven monozygotic and 12 dizygotic pairs of teenaged twins. They concluded that there was no evidence in these twins for significant heritability of any of these dental parameters.

Alternatively, Michalowicz and colleagues published a large study of adult twins (mean age, 40 years) of which there were 63 monozygotic and 33 dizygotic pairs.[58] Using elements of the Ramfjord index as criteria for diagnosis, heritability estimates were calculated. The authors state that from 38% to 82% of the periodontal disease identified in these twins was attributable to genetic factors.

Investigation by Kornman and colleagues into the association of different polymorphisms of inflammation-mediating genes and periodontal disease in adult nonsmokers indicated interleukin 1α and 1β (IL-1α and IL-1β) genotype may be a risk factor.[59] The IL-1β polymorphism was IL-1b 13953 and the IL-1α polymorphism was IL-1α −889. Nonsmokers aged 40 to 60 carrying the "2" allele (in either homozygous or heterozygous state) at both loci were observed to have nearly 19 times the risk of developing severe periodontitis as did subjects homozygous for the "1" allele at either or both of these loci. However, this association has been seen in other,[60] but not all populations.[60-63] Greenstein and Hart noted that the relationship of specific IL-1 genotypes and the level of crevicular fluid IL-1β is not clear, and that the ability of the genetic susceptibility test for severe chronic periodontitis based on the finding of Kornman and colleagues to forecast which patients will develop increased bleeding on probing, periodontitis, or loss of teeth or need for dental implants is ambiguous.[64] This illustrates the complexity of genetic association studies, and genetic counseling is based upon a marker that accounts for only a portion of phenotypic variation.[65,66]

Early-onset periodontitis has been the subject of most family studies. Because several forms of early-onset periodontitis (e.g., localized prepubertal periodontitis, localized juvenile periodontitis [JP], and generalized JP) can be found in the same family, the expression of the underlying genetic etiology appears to have the potential to be influenced by other genetic factors.[67]

Progress has been made in the study of rare genetic conditions or syndromes that can predispose to periodontal disease or have periodontal disease as a relatively consistent component of their pleiotropic effect. For example, leukocyte adhesion deficiency (LAD), type I and type II, are autosomal recessive (AR) disorders of the leukocyte adhesion cascade.[68] LAD type I has abnormalities in the integrin receptors of leukocytes resulting from mutations in the b2 integrin chain (ITGb2) gene leading to impaired adhesion and chemotaxis, which results in an increased susceptibility for severe infections and early-onset (prepubertal) periodontitis.[69,70] LAD type II is also an AR disorder secondary to mutation in the SLC35C1 gene encoding a GDP-fucose transmembrane transporter (FucT1) located in the Golgi apparatus. The infectious episodes and the severity are much milder than those observed in LAD type I, and the only persistent clinical symptom is chronic severe periodontitis. The exact defect in the system is absence of the stalyl Lewis x (SleX)

structure antigens, which are important ligands for the selectin on the leukocyte, which leads to a profound defect in leukocyte rolling, the first step in the adhesion cascade. This causes a marked decrease in chemotaxis accompanied by pronounced neutrophilia. Apart from the leukocyte defect, these patients suffer from severe growth and mental retardation and exhibit the rare Bombay blood group type.[68]

Ehlers-Danlos syndrome (EDS) is a collection of 10 types distinguished on the basis of clinical symptoms and inheritance pattern. In addition to consistent early-onset periodontal disease, patients with EDS type VIII have variable hyperextensibility of the skin, ecchymotic pretibial lesions, minimal bruising, minimal to moderate joint hypermobility of the digits, and cigarette paper scars. Inheritance is AD. Early-onset periodontal disease may also be found in patients with EDS type IV. These individuals are usually characterized by type III collagen abnormalities with hyperextensibility of the skin, ecchymotic pretibial lesions, easy bruisability, cigarette paper scars, joint hypermobility of digits, pes planus, and, of greatest concern, arterial and intestinal ruptures. Like type VIII, type IV also has AD inheritance.[71] The presence or absence of type III collagen abnormalities has been taken to be a differentiating factor between the two types, with EDS type IV showing abnormal type III collagen. The considerable overlap in phenotype of these two types warrants careful family and clinical evaluation, and biochemical studies of collagen when a patient with features of EDS and periodontal disease is evaluated.[72]

Chédiak-Higashi syndrome has frequently been linked with severe periodontitis.[70] This rare AR disorder is characterized by oculocutaneous hypopigmentation, severe immunologic deficiency with neutropenia and lack of natural killer cells, a bleeding tendency, and neurologic abnormalities. It is caused by mutations in the CHS1/LYST gene.[73]

Papillon-Lefèvre syndrome and Haim-Munk syndrome are two of the many different types of palmoplantar keratoderma, differing from the others by the occurrence of severe early-onset periodontitis with premature loss of the primary and permanent dentition. Haim-Munk syndrome is characterized in addition by arachnodactyly, acroosteolysis, and onychogryphosis.[74] Hart and colleagues[75] have shown that both of these AR syndromes are due to different mutations in the cathepsin C (CTSC) gene. A possible role for a mutation in this gene has also been reported in patients with generalized nonsyndromic aggressive periodontitis.[76]

EARLY-ONSET PERIODONTITIS

Early onset of periodontitis may occur in the primary dentition (prepubertal periodontitis), may develop during puberty (JP), or may be characterized by exceedingly rapid loss of alveolar bone (rapidly progressive periodontitis). Along with hypophosphatasia, prepubertal periodontitis appears to be the most commonly encountered cause of premature exfoliation of the primary teeth, especially in girls.[71]

JP has the following features:

1. An early onset of the breakdown of periodontal bone. This bone loss is of two types: chronic periodontitis in a generalized form affecting any dental area, and a localized form in which the molar or incisor regions of bone are the most severely affected.

2. Bone destruction that is rapid and vertical, with specific microorganisms associated with the periodontal lesion.

3. Familial aggregation, especially in the molar and incisor types. It seems probable that the generalized and localized types represent two different aspects of the same disorder; this discussion considers them as a complex entity called *familial JP*.

At least three different modes of inheritance have been proposed for JP. The early reports of Benjamin and Baer, and later that of Melnick and colleagues, offered support for an XLD trait.[77] This conclusion was based on the observation that twice as many females were observed to be affected as males (see preceding criteria for XLD inheritance). However, Hart and colleagues have shown that females are twice as likely as males to seek treatment.[78] When this biased ascertainment is considered, the male-to-female ratio is essentially unity. Saxen demonstrated that a clinical phenotype found in Finland closely resembling JP (if not actually JP) showed an AR mode of inheritance.[79] This disorder may be peculiar to Finland. Boughman and colleagues reported linkage of a gene in a single large family for an AD form of JP on chromosome 4 to another dental trait (dentinogenesis imperfecta, Shields type III).[80] Hart and colleagues, in a study of 19 unrelated families, strongly excluded linkage between an early-onset periodontitis susceptibility gene and chromosome region 4q12-q13 assuming locus homogeneity.[81] They concluded that the previous report of linkage was a false-positive, or that there are two or more unlinked forms of JP, with the form located in 4q12-q13 being less common. Dominant inheritance is probable for the type most prevalent in this country (Fig. 6-10).

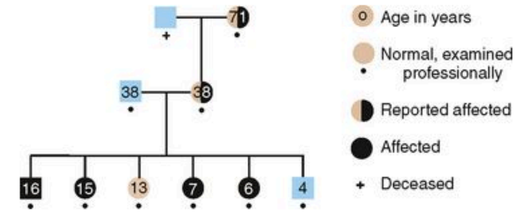


Figure 6-10 Pedigree of family with juvenile periodontitis, a dominant trait.

Evaluation of the same IL-1α and IL-1β polymorphisms found by Kornman and colleagues[59] to be associated with periodontitis in adult nonsmokers was performed in black and white families with two or more members affected with early-onset periodontitis by Diehl and colleagues.[82] Interestingly, they found the IL-1 alleles associated with high risk of early-onset periodontitis to be the ones suggested previously to be correlated with low risk for severe adult periodontitis. They concluded that early-onset periodontitis is a complex, oligogenic disorder (i.e., involving a small number of genes), with IL-1 genetic variation having an important but not exclusive influence on disease risk.

GENETICS OF MALOCCLUSION

The study of occlusion pertains to relationships between teeth in the same dental arch, as well as between the two dental arches when the teeth come together. Many factors are involved in the definition of normal occlusion. Some of the most important orofacial parameters of occlusion are airway function, soft tissue anatomy and function, size of the maxilla, size of the mandible (both rami and body), arch form, anatomy of teeth (including malformation), congenitally missing teeth, and rotation of teeth. All of these important elements must be included in the concept of occlusion.

Malocclusion is perhaps somewhat easier to define. One may simply say that malocclusion is a significant deviation from normal

occlusion. However, this description is useful only if one considers the multiple aspects implicit in such a definition. Normal occlusion and malocclusion are dynamic concepts that involve the interrelationships of many factors, not a few of which have been shown to be influenced by genetic factors. For example, in a study of the association of the Pro561Thr (P56IT) variant in the growth hormone receptor (GHR) gene with craniofacial measurements on lateral cephalometrics radiographs by Yamaguchi and colleagues, those who did not have the GHR P56IT allele had a significantly greater mandibular ramus length (condylion-gonion) than did those with the GHR P56IT allele in a normal Japanese sample of 50 men and 50 women.[83] The average mandibular ramus height in those with the GHR P56IT allele was 4.65 mm shorter than the average for those without the GHR P56IT allele. This significant correlation between the GHR P56IT allele and shorter mandibular ramus height was confirmed in an additional 80 women.

Theoretically, there are two general ways in which predisposing or causative factors for malocclusion could be due to heritable characteristics.[84] One would be inheritance of a disproportion between the size of the teeth and the jaws, resulting in crowding or spacing, whereas the other would be inheritance of a disproportion in the position, size, or shape of the mandible and maxilla. Genetic influences on each of these traits are rarely due to a single gene, which would be necessary for malocclusion to be due to the simple inheritance of discrete skeletal and dental characteristics. Instead they are often polygenic with the potential for environmental influence. Part of the practice of orthodontics is to use environmental (i.e., nongenetic) influences for the correction of malocclusion. Dental anthropologists would say that malocclusion is uncommon in pure racial populations. However, it has been debated whether this is due to the lack of procreation with other populations or the less refined diet often eaten by these typically isolated groups.

The experiments of Stockard and colleagues in dogs have been cited as evidence that crossbreeding among inbred strains increases the incidence of malocclusion.[85] However, the anomalies they produced may have been due in part to the influence of a major gene or genes that have been bred to be part of specific breeds. It seems improbable that racial crossbreeding in humans could resemble the condition of these experiments and thereby result in a synergistic increase of orofacial malrelations. An exception may occur on an individual basis in the child of an individual a dominant trait or syndrome that results in a malocclusion child. Depending on whether it is autosomal or X-linked, the dominant gene, if transmitted to an offspring, may also affect the offspring's occlusal development in a similar fashion as in the affected parent.

Studies of occlusion in twins have also been made. Lundstrom performed an intensive analysis of specific dentofacial attributes in twins and concluded that heredity played a significant role in determining the following characteristics: tooth size, width and length of the dental arch, height of the palate, crowding and spacing of teeth, and degree of overbite.[86] Kraus and colleagues made a cephalometric study of triplets in an attempt to assign an inherited basis to specific craniofacial morphologic features.[87] The authors looked at the lateral profile of the head and cranial vault, the outline of the calvaria, the cranial base, and the facial complex, which included both the upper and lower face and the maxillomandibular relationship. In addition, they selected 17 individual measurements of single portions of a given bone (e.g., posterior border of ramus). They concluded that morphology of an individual bone is under strong genetic control but that the environment plays a major role in determining how various bony elements are combined to achieve a harmonious or disharmonious craniofacial skeleton. This observation at least partly explains the remarkable differences sometimes seen in the facial patterns of identical twins and emphasizes the important role of environment in their development.

Class III malocclusion morphology is heterogeneous, with varying incidence among different ethnic groups, and various facial patterns may as a composite result in the condition.[88,89] There is a strong heritable component in class III malocclusion in general, with modes of inheritance being reported to be polygenic,[90] autosomal dominant in a Libyan sample,[91] and autosomal dominant with incomplete penetrance with a multifactorial component in a Brazilian sample.[92] The variation in ethnic incidence may also reflect variation in genes involved in these groups as suggested by the finding of linkage to chromosomes 1p36, 6q25, and 19p13.2 in Korean and Japanese patients primarily with mandibular prognathism,[93] and to 1p22.1, 3q26.2, 11q22, 12q13.13, and 12q23 in Colombian patients primarily with maxillary hypoplasia.[94]

Harris has shown that the craniofacial skeletal patterns of children with class II malocclusions are heritable and that there is a high resemblance to the skeletal patterns in their siblings with normal occlusion. From this he concluded that the genetic basis for this resemblance is probably polygenic. Interestingly, Harris used the family skeletal patterns as predictors for treatment prognosis of the child with a class II malocclusion.[95]

King and colleagues noted that many studies that which estimate heritability of craniofacial structures may have a bias because they have generally involved subjects who had not undergone orthodontic treatment, and that often subjects judged to have an extreme malocclusion were excluded. They found that, in contrast to the relatively high heritability of cephalometric variables and low heritability of occlusal variables in subjects with naturally occurring good occlusion, the heritability estimates for craniofacial skeletal variables in subjects with overt malocclusions were significantly lower and the heritability estimates for occlusal variations were significantly higher. This observation supports the idea that everyone does not react to specific environmental factors in the same manner, although those who are related are more likely to react in a similar fashion. To quote King and colleagues[96]:

> We propose that the substantive measures of intersib similarity for occlusal traits reflect similar responses to environmental factors common to both siblings. That is, given genetically influenced facial types and growth patterns, siblings are likely to respond to environmental factors (e.g., reduced masticatory stress, chronic mouth breathing) in similar fashions. Malocclusions appear to be acquired, but the fundamental genetic control of craniofacial form often diverts siblings into comparable physiologic responses leading to development of similar malocclusions.

Although we have some information about genetic influence on specific traits (e.g., missing teeth, occlusal patterns, tooth morphology, and even mandibular prognathism), these cases are exceptions, and we do not have sufficient information to make accurate predictions about the development of occlusion simply by studying the frequency of its occurrence in parents or even siblings. Admittedly, family patterns of resemblance are frequently obvious, but predictions must be made cautiously because of the genetic and environmental variables and their interaction, which are unknown and difficult to evaluate.

Currently, the results of studies on the genetic and

environmental factors that influence the development of malocclusion are representative of the samples studied, not necessarily of any particular individual. In addition, the extent that a particular trait is influenced by genetic factors may have little if any effect on the success of environmental (treatment) intervention. Even so, it may be that genetic factors that influenced a trait will also influence the response to intervention to alter that trait, or other genetic factors may be involved in the response. Therefore the possibility of altering the environment to gain a more favorable occlusion theoretically exists even in individuals in whom the malocclusion does have a relatively high genetic influence. However, the question of how environmental and genetic factors interact is most relevant to clinical practice, because it may explain why a particular alteration of the environment (treatment) may be successful in one compliant patient and not in another.[97]

Multiple factors and processes contribute to the individual response to treatment. Some patients exhibit unusual untreated growth patterns, treatment outcomes, or reactions to medications linked to polymorphic genes. Analysis of overall treatment response requires a systems analysis using informatics for integration of all relevant information. The influence of genetic factors on treatment outcome must be studied and understood in quantitative terms for it to be applied effectively for each patient. Conclusions from retrospective studies must be evaluated by prospective testing to truly evaluate their value in practice. Genetic studies are necessary to further the evidence base for practice. Only then will we begin to truly understand how nature (genetic factors) and nurture (environment factors, including treatment) together affect treatment of our patients.[1]

EXTERNAL APICAL ROOT RESORPTION

Basic descriptors of root resorption are based on the anatomic region of occurrence; that is, designations are *internal root resorption* and *external root resorption* (cervical root resorption and external apical root resorption [EARR]). EARR is a frequent iatrogenic outcome associated with orthodontic treatment and may also occur in the absence of orthodontic treatment.[98,99] Although orthodontic treatment is associated with some maxillary central incisor EARR in most patients, and more than one third of those treated experience greater than 3 mm of loss, severe EARR (>5 mm) occurs in 2% to 5%.[100,101]

Currently, there are no reliable markers to predict which patients will develop EARR nor how severe EARR will be following orthodontic tooth movement,[102] although the shape of the root does appear to be associated with the likelihood of EARR and is best examined on periapical rather than panoramic radiographs.[103] Even when duration of treatment is a factor, it along with several significant dentofacial structural measurements (e.g., overjet) do not account for enough of the observed variability to be useful as predictors of EARR by themselves.[101]

Although orthodontic tooth movement, or *biomechanics*, has been found to account for approximately one tenth to one third of the total variation in EARR,[104-106] Owman-Moll and colleagues showed that individual variation overshadowed the force magnitude and the force type in defining the susceptibility to histologic root resorption associated with orthodontic force.[107] Individual variations were considerable regarding both extension and depth of histologic root resorption within individuals, and these were not correlated to the magnitude of tooth movement achieved.[108]

The degree and severity of EARR associated with orthodontic treatment is multifactorial, involving host and environmental factors,[109] with genetic factors accounting for at least 50% of the variation overall and approximately two thirds of the variation seen in maxillary central incisor EARR.[110,111] In addition, studies in a panel of different inbred mice also supported a genetic component involving multiple genes in histologic root resorption.[112,113]

A polymorphism in the IL-1β gene in orthodontically treated individuals account for 15% of the variation in maxillary central incisor EARR. Individuals homozygous for the IL-1β allele 1 have a 5.6-fold (95% confidence interval, 1.9 to 21.2) increased risk of EARR greater than 2 mm compared with individuals who are not homozygous for the IL-1β allele 1.[114] The potential for IL-1β to have an effect on root resorption was supported by the increase in orthodontically induced histologic root resorption in the absence of IL-1β cytokine in a knockout mouse model,[115,116] and a P2rx7 knockout mouse model,[117,118] because a lack of the P2rx7 receptor results in a lack of interleukin-1b. In both of these mouse knockout models, there was no difference at baseline between the wild-type ("normal") and knockout mice histologic root resorption, whereas the application of force resulted in a significant increase in histologic root resorption in the wild-type mice. There was in addition a significant ($P < .02$) increase in histologic root resorption in both types of knockout mice with force applied over the force applied wild-type mice. Thus there was a significant interaction between the genotype and environment (orthodontic force) on histologic root resorption.

Although IL-1β is the first genetic marker suggested to be associated with EARR, it accounts for too small an amount of the total variation to be predictive. Additional genetic studies such as the one that found genetic linkage for EARR with a marker on chromosome 18 near the RANK gene are needed to determine what other genes influence EARR.[119] Other candidate genes include P2RX7, the genes for other proteins involved in the maturation and release of IL-1β, and those involved in the RANK/RANKL/OPG pathway of osteoclastogenesis.[116] Future estimation of susceptibility to EARR will likely require the analysis of a number of genes, root morphology, dental and facial measurement values, and the amount of tooth movement planned for treatment.

GENETICS OF CLEFT LIP AND PALATE

Studies of the CLP phenotype in twins indicate that monozygous twins have a 35% concordance rate, whereas dizygous twins show less than 5% concordance.[120] Information from two sources (families and twins) then establishes a genetic basis for CLP, but despite many extensive investigations, no simple pattern of inheritance has been demonstrated. This has led to proposal of a variety of genetic modes of inheritance for CLP, including dominance, recessiveness, and sex linkage, and has led ultimately to the documentation of modifying conditions that may be present, such as incomplete penetrance and variable gene expressivity.[121] There are three important reasons for the failure to resolve the question of a hereditary basis for clefts: (1) some clefts are of a nongenetic origin and should not be included in a genetic analysis; such cases are seldom recognized and are difficult to prove; (2) individuals who have increased genetic liability for having a child with CLP often fail to be recognized, but because they do not have CLP themselves they cannot be identified with certainty; this latter

situation defines the problem of nonpenetrance for genes that control CLP[122]; and (3) CLP, although sometimes appearing to be relatively simple in origin, is undoubtedly a complex of diseases with different etiologies lumped together because of clinical disease resemblance (they all show clefting).

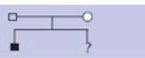
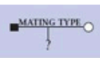
There are two clearly recognized groups of etiologically different clefts—cleft lip either with or without cleft palate (CL(P)) and isolated cleft palate (cleft palate only, or CPO). These two entities, CL(P) and CPO, occur as single cases in a family and as multiple cases in a family. In the former they are called *sporadic*, and in the latter they are called *familial* or *multiplex*. Some researchers refer to multiplex cases as those individuals with findings in addition to an oral cleft, even if a specific syndrome is not recognized. It should also be noted that the CPO that occurs without a cleft of the lip is different from the palatal cleft that occurs as a part of CL(P). The embryology and developmental timing are both different, and CPO is more commonly part of a syndrome than is CL(P). CPO is less common, with a prevalence of approximately 1 per 1500 to 2000 births in caucasians, whereas CL(P) is more common, 1 to 2 per 1000 births. The prevalence of CPO does not vary in different racial backgrounds, but the prevalence of CL(P) varies considerably, with Asian and American Indians having the highest rate and Africans the lowest. There are also gender ratio differences, with more males having CL(P) and more females having CPO. Except in a small number of syndromes such as Van der Woude syndrome, families with one type of clefting segregating in the family do not have the other cleft type occur at a rate higher than the population prevalence.

When all potential study groups for CL(P) and CPO are considered, the minimum number is six: three subgroups for CL(P) and three for CPO. These three for each type of cleft are the sporadic and the familial groups, and a group of syndromes that feature CL(P) and/or CPO. Approximately 30% of CL(P) and 50% of CPO patients have one of more than 400 described syndromes.[123]

As noted earlier, it is probable that minor and subtle facial changes are more likely to produce the best-correlated phenotype needed to pinpoint the cleft genotype. Part of the reason for this view is the suspicion that certain facial shapes are more predisposed to developing CL(P) than others[124,125] and that subepithelial defects of the upper lip musculature are part of the phenotypic spectrum of oral clefts and may represent an occult, subclinical manifestation of the anomaly.[126] Although this approach seems best for producing an accurately generated clefting phenotype, further study is needed of the developmental anatomy of the head and face.

Recurrence risks for CL(P) and CPO have been reported in which penetrance was not considered. These data are frequently cited by genetic counselors as illustrating the lesser risk associated with a multifactorial than with a monogenic trait. These data consider CL(P) and CPO without regard to the three groups discussed previously. These data, therefore, are average risk figures (Table 6-2).

Table 6-2 Recurrence Risks for Cleft Lip with or without Cleft Palate (CL[P]) and Isolated Cleft Palate (CP)

			
Fogh-Andersen[76,121]	4%	2%	14%
CL(P)	12%	7%	17%
CP*			
Curtis and Walker[130]	4%	4%	19%
CL(P)	2%	6%	14%
CP*			
Curtis, Fraser, and Warburton[131]	4%	—	17%
CL(P)	7%	—	15%
CP*			

*Isolated cleft palate data were obtained from families with cleft individuals, in addition to the immediate family unit.

The published data on nonsyndromic cleft populations comes from around the world (Japan, China, Hawaii, Denmark, Sweden, Great Britain, and North America). These studies make it clear that both CL(P) and CPO are heterogeneous diseases. That is, there are multiple causes for the single phenotypes, CL(P), and CPO. To summarize the generally accepted hereditary basis for CL(P) and CPO: Single, nonsyndromic cases of CL(P) and CP, or sporadic clefts, are believed to be the result of a complex interaction between multiple genetic and environmental factors. Hence, their etiology is multifactorial in the true sense of the word, and the chance that these multiple factors would interact to produce a cleft phenotype in relatives is small, probably less than 1%.

The ?other nonsyndromic group consists of multiple cases of clefts that occur in a single family. These are called familial (or multiplex) and have been viewed by researchers as the "true" genetic cases. Familial occurrences of CL(P) and CPO seem most likely to be accounted for by the action of a single major gene, but the influence of multifactorial (complex) trait factors is difficult to rule out. Thus we are left with the idea that both multifactorial and single major gene elements may have a role in producing sporadic and familial cases of CL(P) and CPO. For an overview of genetic factors in orofacial clefting, the reader is referred to the papers by Lidral and colleagues and Vieira and colleagues.[123,127]

An example of an environmental (dietary) factor that is associated with a decrease in neural tube defects such as spina bifida, as well as orofacial clefting, is the maternal intake of folate (folic acid), now a common component in prenatal vitamins. To be effective, such vitamins or other dietary supplements must be used at least around the time of conception because of the embryologic timing of neural tube closure, and lip and palate formation. Because of the public health importance and critical need before a woman may realize that she is pregnant, folic acid fortification of grains in the United States became mandatory January 1, 1998, specifically to reduce the occurrence of neural tube defects, which has happened. This has also to a lesser degree reduced the occurrence of orofacial clefting. Interestingly, however it did not decrease the occurrence of orofacial clefting in the children whose mothers smoke cigarettes, a risk factor associated with an increase in the occurrence of orofacial clefting.[128]

Although some genetic and environmental risk factors for CL(P) have been identified, many nonsyndromic clefts are not linked to any of these factors. Furthermore, there is a paucity of information available on the long-term consequences for children born with CL(P) or CPO. To address these concerns the National Center on Birth Defects and Developmental Disabilities at the Centers for Disease Control and Prevention conducted a workshop entitled "Prioritizing a Research Agenda for Orofacial Clefts." Experts in the fields of epidemiology, public health, genetics, psychology, speech pathology, dentistry, health economics, and others participated in this workshop to review the state of knowledge on orofacial clefts, identify knowledge gaps that need additional public health research, and create a prioritized public health research agenda based on these gaps. Their report is recommended to the reader as an excellent summary of the current knowledge and future research priorities for orofacial clefting.[129]

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