GENETIC INFLUENCES IN CARIES AND PERIODONTAL DISEASES

Thomas M. Hassell
Department of Periodontology, College of Dentistry, University of Florida, PO Box 100434, Gainesville, Florida 32610
Emily L. Harris
Kaiser Permanente Center for Health Research, Portland, Oregon 97277

ABSTRACT: Deciphering the relative roles of heredity and environmental factors ("nature *vs.* nurture") in the pathogenesis of dental caries and diseases of the periodontium has occupied clinical and basic researchers for decades. Success in the endeavor has come more easily in the case of caries; the complex interactions that occur between host-response mechanisms and putative microbiologic pathogens in periodontal disease have made elucidation of genetic factors in disease susceptibility more difficult. In addition, during the 30-year period between 1958 and 1987, only meager resources were targeted toward the "nature" side of the nature/nurture dipole in periodontology. In this article, we present a brief history of the development of genetic epistemology, then describe the three main research mechanisms by which questions about the hereditary component of diseases in humans can be addressed. A critical discussion of the evidence for a hereditary component in caries susceptibility is next presented, also from a historical perspective. The evolution of knowledge concerning possible genetic ("endogenous", "idiotypic") factors in the pathogenesis of inflammatory periodontal disease is initiated with an analysis of some foreign-language (primarily German) literature that is likely to be unfamiliar to the reader. We identify a turning point at about 1960, when the periodontal research community turned away from genetics in favor of microbiology research. During the past five years, investigators have re-initiated the search for the hereditary component in susceptibility to common adult periodontal disease; this small but growing body of literature is reviewed. Recent applications of *in vitro* methods for genetic analyses in periodontal research are presented, with an eye toward a future in which persons who are at risk—genetically predisposed—to periodontal disease may be identified and targeted for interventive strategies. Critical is the realization that genes and environment do not act independently of each other; the appearance or magnitude of heritability may differ with various environments.

Key words: genetics, heredity, caries, periodontitis, susceptibility, host response, twins.

"There is no escape from the conclusion that nature prevails enormously over nurture...
My fear is that my evidence proves too much, and may be thus discredited...
(because) it seems contrary to all experience that nurture should (count) for so little."

— Sir Francis Galton, Fellow of the Royal Society, 1876

Historical Perspective

Practitioners and scientists today have at their disposal a great wealth of knowledge about the etiology and the pathogenesis of dental caries and periodontal diseases. The dentist who notes that a child's dental status mirrors that of the parent may ask why it is so. The answer to that question can be found in the history of the epistemology of the knowledge base itself, and provides a fascinating rampage through the evolution of the science of the cell and the gene.

There were several crucial milestones. Aristotle (384-322 BC) and Paracelsus (1493-1541 AD), in antiquity and during the Renaissance, respectively, agreed that "all animals and plants, however complicated, are constituted of a few elements which are repeated in each of them" (DeRobertis and DeRobertis, 1987). The term "cell" was first used by Robert Hooke (1635-1703) as he examined the texture of cork using magnifying lenses. van Leeuwenhoek (1632-1723) first observed that cells have nuclei. Mathias Schleiden (1804-1881) and Theodor Schwann (1810-1882) established the definitive cell theory of life. Rudolf Virchow (1821-1902) stated, "*Omnis cellulae e cellula*" (All cells arise from pre-existing cells), thus

establishing the process of cellular division as the central phenomenon in reproduction, and as the basis for the persistence and transfer of species characteristics. James Watson (1928-) convinced Francis Crick (1916-) in 1951 that knowledge of the three-dimensional structure of DNA would make its role in heredity apparent, and most college freshmen today are aware of the chain of scientific events that was set into motion by a one-page paper in *Nature* in 1953.

Charles Darwin (1809-1882) set sail on his five-year voyage aboard the *Beagle* at age 22, with absolutely no training in science, and with no reason whatever to call into question the then-accepted view that the species of plants and animals alive on earth were as they had always been since the creation. In his attempt to reconcile the many contradictions in nature that he encountered on his voyage, Darwin came upon the idea that species do not remain immutable but change into other species and diverge, and that one species can give rise to two or more. In 1836, 23 years before the publication of his *On the Origin of the Species*, Darwin wrote, "Animals, our fellow brethren in pain, disease, death, suffering and famine—our slaves in the most laborious works, our companions in our amusements—they may partake of our origin in one common ancestor—we may be all netted together." He noted that, since Neolithic time, cultivation of plants and breeding of animals were commonplace, and that the results were generally predictable, and were the result of selection, namely, selecting parental lines to enhance quality of offspring.

Gregor Mendel (1822-1884) was born in Heinzendorf, in what is now The Czech Republic. As a monk in the garden of the Augustinian monastery at Brünn, he grew his pea plants from 1856 through 1865, and published his results in the *Proceedings of the Brünn Society for Natural History* in 1866. The paper was read by few and apparently understood by no one until at least 1900, long after Mendel's death. In short, Mendel appears to have been a man of many and considerable talents whose major work was not appreciated and who undoubtedly came to regard himself as a scientific failure (Goodenough and Levine, 1974).

Francis Galton (1822-1911) was the son of the daughter of Erasmus Darwin, and was thus Charles Darwin's cousin. Galton's IQ was estimated to be over 200. He established fingerprinting as an infallible means of human identification. He coined the term "eugenics" (in 1883), and campaigned vigorously for the establishment of a national eugenics program to foster healthiness and brilliance and to suppress sickliness and stupidity. The basis for these efforts was Galton's own research on the inheritance of talent. It is virtually certain that Galton never read Mendel's 1866 publication, yet in 1875 he sketched the essence of genetic theory, using the terms "latent" and "patent" to describe what we now refer to as genotype and phenotype. He is considered the father of the twin paradigm for ascertainment of the relative roles of inheritance and environment ("nature *vs.* nurture") in disease susceptibility. He liked to relate an anecdote from nature to support his refutation of the role of parental nurturing in human development: "Consider the history of the cuckoo, which is reared exclusively by foster mothers. It is probable that nearly every young cuckoo has been brought up in a family whose language is a chirp and a twitter. But the cuckoo cannot or will not adopt that language, or any other of the habits of its foster parents. The cuckoo leaves the nest as soon as able, to find its own kin, identifying henceforth with them. The earliest instructions ("nurture", environment) from the alien foster parents are utterly neglected..."

J. Mengele studied twins in Frankfurt, under one of Europe's foremost geneticists, Otmar Freiherr von Verschuer, in 1937, and later at Auschwitz (Mitscherlich and Mielke, 1949).

Definitions

Heredity is the transmission from parent to offspring of certain characteristics and thus describes the process whereby humans, mice, flies, plants, fungi, bacteria, viruses, and all forms of living things reproduce themselves, or at least something unmistakably like themselves (Hayes, 1968). "Life" involves not only growth, organization, and continuity of existence over millennia, but also change and the capacity for modification of form in response to environmental changes; this latter is subsumed under the rubric of evolution.

Genetics is the study and analysis of heredity in all of its aspects and complexities. Genetics is therefore the most fundamental biologic science; it is the focal point upon which all other aspects of biology must necessarily converge (Hayes, 1968).

Astoundingly meager progress was made toward our understanding of the mechanisms of evolution and heredity during the almost 2,000 years that separated Aristotle's world from that of Darwin and Mendel. This was due to numerous factors, of course, including plagues, wars, famines, the Dark Ages, religious zealots, and other cataclysms, but also to the complexity of the system (life itself) and the paucity of tools for dissecting it.

To study genetics is to involve oneself in the critical evaluation of character similarities and character differences between parents and offspring, and the way in which such characteristics are inherited. Critical to this mindset is the fact that what is inherited is not the character itself but the *potential to express it*; further, such potential can be substantially influenced by the environment to which the organism is exposed (Hayes, 1968).

From a teleological point of view, diseases have been likened to bioassay systems by means of which

nature judges the relative desirability of a given genotype in a given environment. According to the concept proposed by Martin and Hoehn in 1974, if a particular mutation results in lethality in environment A, it will be eliminated on the basis of natural selection in favor of a more competitive genome. Quite a different outcome may ensue, however, in environment B. Because all phenotypes result from the interactions between genes and environment, genetic analysis makes it possible to understand deviations from the normal, *i.e.*, pathological conditions. In Fig. 1, from the classic paper entitled "Genetics and Human Disease" (Martin and Hoehn, 1974), pathologic change is classified graphically according to the relative contributions of genes and environment to the disease phenotype. For example, the disease retrolental fibroplasia requires premature birth and high oxygen concentration for its development, an unusual environment indeed. While, in such an environment, a broad expanse of genotypes will express the disease, high-risk and low-risk genotypes may exist. On the other hand, Down's syndrome demands a very specific genotype (trisomy 21), and is expressed in all environments. Suxamethonium sensitivity requires both a high-risk genotype and exposure to an unusual environment (the presence of suxamethonium, a muscle relaxant). Atherosclerosis may affect many different genotypes in many different environments, but the severity of the disease is influenced by both environmental and genetic risk factors. With specific reference to caries and periodontal disease, genetic pathology will be concerned with the mechanisms by which both high-risk and low-risk genotypes (shaded areas in Fig. 1) influence the development of the disease in a given environment, *e.g.*, in the presence of putatively pathogenic micro-organisms.

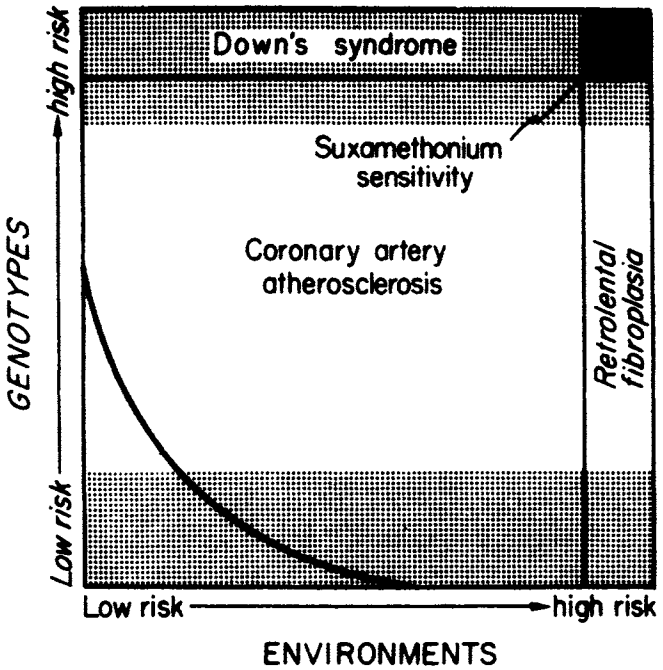

Figure 1. Schematic and quantitatively arbitrary classification of some prototype human diseases, based on the relative contributions of environment and heredity to the expression of the phenotypes. See text for descriptive explanations [from Martin and Hoehn, 1974, with permission].

Introduction

The fact that many traits or characteristics of clinical interest are familial ("run in families") has been recognized since the earliest days of dentistry and medicine. Until quite recently, however, it has been most expeditious to concentrate on environmental factors that contribute to disease, paying relatively little attention to individual differences either in disease susceptibility or in the traits that relate to the disease process (Osborne, 1963). For example, it is known that tobacco smoke contains some 4,700 chemical compounds, over 40 of which are acknowledged carcinogens (Bartecchi *et al.*, 1995); and yet, not all smokers develop bronchogenic carcinoma. Thirty years ago, Tokuhata (1964) studied lung cancer in several thousand smokers, non-smokers, and their relatives, and provided an instructive and revealing schematic (Fig. 2) implicating a "familial factor" in the pathogenesis. The diagram shows the synergistic interaction between the familial factor and the tobacco factor. Individuals who have both such characteristics are subjected to a dramatic increase in the pathogenesis of the disease, which the author referred to as a "booster effect". Ten years later, Kellermann *et al.* (1973) identified one "familial factor" in lung cancer etiology, when they were able to ascertain three separate groups in the general population, having low, intermediate, and high inducible aryl hydrocarbon hydrolase (AHH) activities. (AHH is a mixed-function oxidase whose inducibility is under the control of a single genetic locus [Gielen *et al.*, 1972] and which is responsible for conversion of some potential carcinogens to the active carcinogenic form.) Kellermann and co-workers published data demonstrating that susceptibility to bronchogenic carcinoma is associated with the higher activity of the hydrolase; not a single case of lung cancer was found in the low-inducibility group. Thus, even though exposed to potential carcinogens (*e.g.*, in tobacco smoke), persons in the latter group will be less likely to transform potential carcinogens into the reactive (epoxide) forms. Quite recently, Rennert *et al.* (1990) have discussed the likely role of differing genetic susceptibility to explain the low inci-

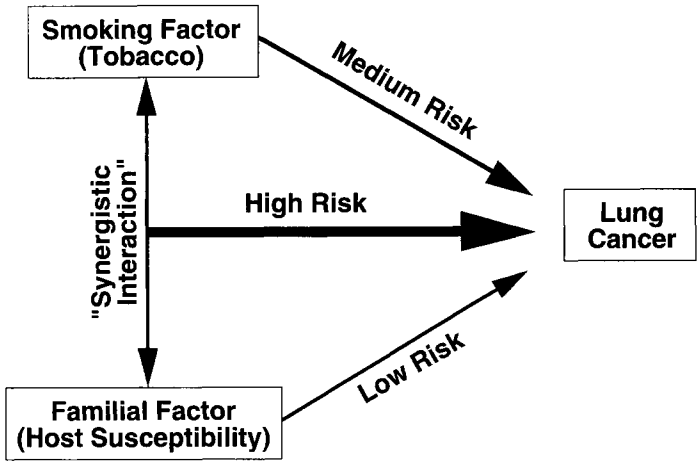

Figure 2. Schematic expression of possible relationships among lung cancer, tobacco smoke, and host genetic susceptibility. See text for discussion [adapted from Tokuhata, 1964].

TABLE 1

Heritability Estimates for Some Common Diseases [from summary data published by McKusick, 1978]

Disease/Disorder	Population Frequency (percent)	Heritability Estimate
Varicose veins	2	1.0
Epilepsy	1	0.75
Schizophrenia	1-2	0.7-0.8
Peptic ulcer	4	0.37
Hypertension	2	0.76
Bronchial asthma	4	0.80
Diabetes—early onset	0-1	0.6-0.7
Diabetes—late onset	1-2	0.3-0.4
Ischemic heart disease	3	0.6-0.7
Allergy—all forms	15-30	0.4-0.5

dence of lung cancer in some ethnic groups where smoking frequency does not differ from that of other, disease-prone, groups in the same geographic area.

In many less complicated diseases, the relative roles of environment and heredity have been well-established for decades. For example, variation in risk of having varicose veins has no environmental component; in the presence of the genotype, this esthetically unpleasant condition is expressed regardless of the environment. On the other hand, there is virtually no genetic component in, for example, bee stings, to which every individual is equally susceptible if exposed to the hive. Table 1 presents some of the pathological entities whose "heritability" is known. In its simplest form, heritability is normally expressed as a percentage, which tells the proportion of the variation in disease susceptibility that can be attributed to heritable characteristics; the remainder is attributable to environmental factors. Because the environment may change over time or differ among populations, and because genes may be expressed only at certain ages, heritability is not one number. The study population must be considered when heritability estimates are interpreted. The heritability of a trait can be calculated from population or family studies, but the more common method is *via* measurement of the concordance rates for the character in question in sets of identical and non-identical human twins (see complete discussion below); for example, the concordance rate for varicose veins is 1.0 (100%) in identical twins, but considerably less in fraternal twins, the latter being no more similar genetically than any sibling pair. Fraternal twins will have shared a common *in utero* environment and are age-matched in terms of environmental experiences. On the other hand, the concordance rates for both types of twins will be identical for a "disease" such as bee stings, which is totally environmentally determined; regardless of one's genotype, if he/she is in the vicinity of a beehive at the wrong moment, a bee sting is likely! One must appreciate, however, that concordance rates cannot be interpreted directly as "heritability", because population frequency and population risk must be factored in as well. (See Vogel and Motulsky, 1979, for an in-depth discussion of the heritability index.)

Deciphering the Nature-Nurture *Ratio in Dentistry*

At a symposium on genetics in 1974 (later published in *Dental Clinics of North America*, 1975), Niswander elegantly described three methods which can be applied to get a better understanding of the genetic basis, the genetic component, of dental diseases. All three of the methods relate to studies of humans, not to animal research or to *in vitro* studies (the latter will be addressed later in this article). Niswander emphasized that while most dental disorders (*e.g.*, caries, malocclusion, periodontitis) "seem to have a recognizable component of genetic variation, in none do genes with major effect(s) appear [at this time] to be of very great importance."

Population Studies

It is sometimes possible to approach the genetics of human disease at the population level. Studies of groups of individuals with differing levels of inbreeding—for example, children of marriages between cousins—and, conversely, studies of outbred or hybrid populations (*e.g.*, offspring of interracial marriages) offer opportunities for

genetic studies. Two excellent examples of this approach can be found in the 1958 study of the effects of inbreeding on the oral conditions of Japanese children, in which almost 7,000 children were examined (Schull and Neel, 1965), and the orodental examinations of almost 10,000 children in Hawaii by Chung and co-workers (1970, 1971, 1977a,b) and Chung and Niswander (1975).

In the Japanese endeavor, significant consanguinity effects were detected for occlusal defects, enamel hypoplasia, and gingivitis, which suggests that recessive genes play a role in these conditions. The findings for gingivitis were especially indicative of a greater susceptibility to infection in the children of inbreeding, due either (1) to greater sensitivity to bacterial substances, (2) to an oral milieu more favorable to bacterial colonization or proliferation, or (3) to impairment of the host-defense mechanisms of the inbred child. In the extensive Hawaiian studies by Chung *et al.* (1970, 1971, 1977a,b), gingivitis was scored on anterior teeth, and an index of oral hygiene was also used. Even after the effects of oral hygiene (and some other environmental variables as well) were factored out, a significant association was found between gingivitis and racial intermixture. As shown in Table 2 (adapted from Niswander's 1975 depiction of the Hawaiian data), the children's gingivitis scores correlated positively with the score of the parental race having the lower mean periodontal index score. Niswander states that this would indicate the participation of recessive genes in increasing risk of periodontal disease (Niswander, 1975). While these two landmark studies demonstrate nicely the power of population studies for detecting genetic effects in dental disease susceptibility, particularly autosomal recessive effects, the huge numbers of clinical examinations that have to be performed make this approach less attractive.

Family Studies

Chronic diseases that have high population frequencies tend to aggregate in families. Yet today, the precise reasons for such familial clustering remain largely unclear, although the genetic mechanism for commonly occurring chronic diseases is generally acknowledged to have a complex etiology (Potter, 1990; Nishimura *et al.*, 1990). In its traditional and most restrictive form, the family method for ascertainment of information about the heritability of certain characteristics consists of pedigree analysis. Usually, this method is applicable only to those circumstances wherein discrete and/or easily definable traits result from simple genetic mechanisms and exhibit straightforward Mendelian segregation (Fisher, 1918; Osborne, 1963). Unfortunately, any type of familial environmental factor that may account even partly for resemblance between family members is precluded, and thus any observed positive parent-offspring or sibling-sibling covariance has to be interpreted as evidence for a purely genetic mechanism (Morton and Rao, 1978). This can be particularly troublesome and misleading in studies of dental disorders such as caries or chronic adult periodontitis, which are known to be influenced more or less dramatically by within-family factors such as diet, oral hygiene, etc. (Potter, 1990). The family method is generally effective in linkage studies as well as in studies designed to identify the carrier state (Osborne, 1963). Using newer statistical methods for segregation analysis, one can take into account environmental factors such as diet and oral hygiene (Bonney, 1986). The basic premise of a pedigree analysis is illustrated in Fig. 3, which depicts a family that was plotted after a dentist detected severe periodontal destruction around the mandibular incisors and all first molars in the female proband (P), an 18-year-old. This pedigree was published in 1952 by Dr. Gustav Korkhaus, Director of the Dental Clinics at the University of Bonn, Germany. We believe this to be the earliest report of a genetic component in localized juvenile periodontitis (Korkhaus referred to it as "frühform Paradentopathie"). In the United States, the pedigree/family method of analysis was subsequently applied in studies of localized juvenile periodontitis—for example, early on by Melnick *et al.* (1976) and subsequently by others. Furthermore, developments in the 1980s and 1990s included advances in segregation analysis, which rendered the method of pedigree analysis more robust (see, *e.g.*, Marazitta *et al.*, 1994).

Twin Method

Identical and fraternal (monozygous and dizygous) human twins provide a unique resource for studies of the origin and natural history of various diseases, because their genetic similarities are well-defined and easily understood: MZ twins share 100% of their genes; DZ twins share, on average, 50%, as do ordinary siblings. Introduction of the twins technique for studying disease pathogenesis is credited to Sir Frances Galton (1876), generally regarded as the father of the twin method. Actually, one may question in hindsight whether Galton even realized the distinctively unique classes of twinning (monozygous and dizygous), but despite this ignorance, his paradigm represented the advent of a new way of studying inheritance. Siemens (1924) improved the twin method by three fundamental additions: (1) improvement of sampling bias by increasing sample size, thus decreasing the errors inherent in small population sizes; (2) developing a reliable method for zygosity determination, thus improving validity; and (3) including dizygous twins in each study as the ideal control group. The twin method has been used in all areas of medical research (as reviewed by Haseman and Elston, 1970; Christian and Norton, 1977; Nance, 1979; Hrubec and Robinette, 1984). The basic purpose for a twin study is to detect hereditary variability, and secondarily to ascertain genet-

TABLE 2

Mean Scores for Gingivitis in Offspring of Various Intraracial and Interracial Marriages

Race (Mother)	Race (Father)	Mean Gingivitis Score* of Offspring
Caucasian	Caucasian	0.35
Hawaiian	Hawaiian	0.52
Japanese	Japanese	0.20
Hawaiian	Caucasian	0.34
Caucasian	Hawaiian	0.31
Japanese	Caucasian	0.18
Caucasian	Japanese	0.26

*Gingivitis scores were extracted from the first three levels of the Periodontal Index (Russell, 1956), which was used in this study of 9,912 children aged 12-18 years (Chung *et al.*, 1970) [adapted from the summary reported by Niswander, 1975].

hereditary variability, and secondarily to ascertain genetic-environmental interactions. The twin paradigm will not provide any information concerning genetic mechanisms, but it is the most exacting, precise, and efficient means to test for the presence of genetic variability.

Monozygous twins result from a single fertilized ovum which, after several divisions, proceeds to develop into two distinct individuals; such individuals are genetically identical and always of the same sex, with male twins and female twins occurring at about a 50:50 ratio. Two separately fertilized ova that are brought to term during the same pregnancy result in the birth of dizygous twins, approximately half of which are male-female pairs (Gittelsohn and Milham, 1965). In the Western hemisphere, twins have accounted for about 12 of each 1,000 births, but the incidence of twinning appears to be increasing, a fact that may be due to increased use of assisted reproductive technologies and the older age of pregnant women (Millar *et al.*, 1992). Dizygotic twinning is higher in Blacks but lower in Asians in comparison with Caucasians (Strandskov and Edelen, 1946; Imaizumi and Inouye, 1979).

For use of the twin model in genetics research, positive ascertainment of zygosity is extremely important. A simple method that has, for the most part, stood the test of time involves a single question, first suggested and utilized by Swedish researchers: "When you were children, were you as alike as two peas in a pod?" (Cederlöf *et al.*, 1961). More extensive questionnaires of confusibility and similarity, completed by twins or their parents, provide accurate zygosity information for over 90% of twin pairs, excluding those pairs (*ca.* 5%) that remain unclassified (Nichols and Bilbro, 1966). More or less extensive questionnaires have been proposed for this purpose (Jablon *et al.*, 1967; Torgensen, 1979). If the 90% level of accuracy is unacceptable, more reliable methods are available, but they are expensive, involving collection of blood for immunologic typing of 22 or more blood antigens; one or more discrepancies between twin pair members indicates a dizygotic pair (Lykken, 1978). Other means, such as fingerprinting (Slater, 1963) or physical measurements (Osborne and DeGeorge, 1959), have not come up to the blood antigen's virtual 100% accuracy in zygosity determination. Anonymous DNA markers or DNA "fingerprinting" may provide simpler and more accurate methods of zygosity determination than traditional blood antigen typing (Akane *et al.*, 1991; Erdmann *et al.*, 1993). Questionnaire methods of zygosity determination are typically used in large studies in which there is no in-person contact, such as with survey studies and data linkage studies; comparison of questionnaire results with blood typing for a subsample provides validity information. Zygosity is usually assessed through blood typing in smaller studies that involve examinations.

There are several limitations of the twin method in genetic research. The twin method presupposes that both prenatal and postnatal environments for MZ and DZ twins are similar; additionally, it assumes that the environmental covariances of MZ and DZ twins are similar. Allen (1976) suggested that these assumptions represent an inherent weakness of the human twin method. Assuming similar environmental covariance between MZ and DZ twins, any differences in co-twin similarity between MZ and DZ twin pairs must be attributed to a genetic component. But environmental differences between the two classes of twins do occur, which may be reflected by lower birth weights and an increased incidence of congenital anomalies in MZ twins (Myrianthopoulos, 1978). Indeed, if one member of an MZ twin pair is smaller at birth, that individual will continue to lag behind in growth as well as in intelligence into adult life (Babson and Phillips, 1973). The most important limitations of the twin method continue to be the relative rarity of twins, the biases that can occur in the recruitment and selection of twins for study, the unique gestational and social experiences of twins, and the failure of conceptual models to deal effectively with interactions between genotype and environment (Allen 1965; Vogel and Motulsky, 1979, 1986; Hrubec and Robinette, 1984). Effective methods for reconciling various models of genotypic and environmental components of twin studies have been reviewed recently by Neale and Cardon (1992).

The evaluation of data collected from studies of human twins is, on the face of it, simple and straightforward, although sometimes fraught with difficulties of interpretation due to some of the reasons stated in the

previous paragraph. Here we will present only the most basic formulae to give the reader a fundamental idea of how the twin method works in practice. Those interested in a deeper understanding of twin data evaluation, statistical handling, and some of the potentially important nuances of twin data are referred to the excellent treatises by Haseman and Elston (1970), Christian *et al.* (1974), Christian and Norton (1977), Nance (1979), and Neale and Cardon (1992). Under the simplest assumptions, P = G + E, where P is a quantitative phenotype or underlying disease risk, G is the genotype, and E is the environment. According to this model, phenotypic variance equals genotypic variance plus environmental variance, where environmental variance includes that shared by family members, that unique to the individual, and variation in measurement. If variation in risk of disease or in a quantitative trait is completely due to genetic differences (*i.e.*, 100% heritability), then the correlation in risk of disease (or in the quantitative trait) for identical twins will be 1.0; that is, they will be identical phenotypically. Under 100% heritability, the correlation for fraternal twins would be 0.50, the same as for any sibling pair. A high degree of similarity for identical twins is an indication of an important contribution by genetic variation. For example, the finding that when one member of an MZ pair is affected by Huntington's disease, the other is certain to be affected by it in time is very important to the understanding of the pathogenesis of this disease (Reed and Chandler, 1958; discussed by Hrubec and Robinette, 1984). Thus, if only *one* MZ twin pair were found to be discordant for Huntington's disease, the present etiologic concept would be drastically altered (Hrubec and Robinette, 1984).

Heritability of disease risk or a quantitative trait can be detected by comparison of the variance within identical twin pairs to the variance within fraternal twin pairs; the F statistic provides a simple test for the presence of genetic variation (Christian *et al.*, 1974). An upper-limit estimate for heritability of disease risk (or of a quantitative trait) is simply the intraclass correlation coefficient for identical twins. If the assumptions of the twin model are met, heritability can be estimated by comparing the intraclass correlation coefficients for identical and fraternal twins. The most commonly used formula is: $2(r_{MZ}-r_{DZ})$. Note that we have used the term "disease risk" rather than "disease". Concordance rates (probability of developing disease if one twin has a disease) are typically used to measure similarity in twin studies. However, concordance rates are not directly interpretable as estimates of heritability, as are intraclass correlation coefficients. Concordance rates must be referenced to disease risk in the population to estimate the degree of clustering within twinships (or within families). Concordance rates and population risk together can be used to estimate correlation in underlying risk of disease (Vogel and Motulsky, 1979). These correlations can then be used to estimate genetic variance or heritability, just as one would for quantitative traits. Both parametric and nonparametric tests can be used to test for the presence of genetic variance (Haseman and Elston, 1970). Patterns of MZ and DZ twin correlations can reveal some things about the nature of genetic and environmental influences. Assume, for example, that co-twins are more similar than two individuals chosen at random:

Pattern of Correlations	Most Likely Explanation
$r_{MZ} = r_{DZ}$	shared environment
$r_{MZ} = 2r_{DZ}$	additive genetic variance
$r_{DZ} < r_{MZ} < 2r_{DZ}$	shared environment plus additive genetic variance
$r_{MZ} > 2r_{DZ}$	• dominant genetic variance • gene-gene interaction • gene-environment interaction • shared environment for MZ > DZ

The patterns of similarity for MZ and DZ twins can be more formally assessed by path analysis (Neale and Cardon, 1992). In addition, one can estimate the similarity of twin pairs while simultaneously accounting for other factors (Muñoz *et al.*, 1986).

Population studies, family studies, the twin model, and variations of the twin paradigm (*e.g.*, the twin half-sib method described by Potter, 1990) have all been used in dentistry to evaluate the inheritance potential of numerous orofacial traits (see Table 3). Significant and major genetic effects have been reported for the mechanisms that influence intra-alveolar dental development (Green and Aszkler, 1970), mesiodistal tooth dimension (Osborne *et al.*, 1958; Horowitz *et al.*, 1958b), buccolingual tooth dimension (Potter *et al.*, 1976), morphologic variation in permanent molar teeth (Biggerstaff, 1970), the Carabelli cusp trait (Biggerstaff, 1973; Townsend and Martin, 1992), cleft lip/palate (Fogh-Anderson, 1942; Cronin and Hunter, 1980), as well as various other aspects of the craniofacial complex (reviewed by Nakata, 1985). The influence of heredity in the etiology of dental caries and several of the periodontal diseases will be reviewed in what follows.

DENTAL CARIES

The question of a possible true genetic predisposition toward dental caries has piqued the minds of dental investigators for decades (Siemens and Hunold, 1924; Bachrach and Young, 1927; Dahlberg and Dahlberg, 1942; Horowitz *et al.*, 1958a,b; Mansbridge, 1959). Excellent reviews of genetic studies of dental caries were pub-

lished in 1963 (Finn and Caldwell) and in 1972 (Zengo and Mandel), with more recent work being presented by Boraas *et al.* (1988). Although the pathogenesis of the caries process is rather well understood today, and although it is quite more complex than was believed in the early days of dental research, for the sake of simplification we can presuppose that the caries attack rate in humans is a consequence of at least five distinctly separate traits or attributes (expanded from Osborne, 1963): (1) the density or structural integrity of the dental enamel, (2) topical and/or communal water fluoridation, (3) the composition of the secretions of the salivary glands, (4) nutrition and day-to-day dietary habits, and (5) personal and professional oral hygiene. The latter could be considered to include the spectrum of oral bacterial flora. Numbers (1) and (3) are obviously the most likely candidates for direct genetic control; (4) and (5) less so, at least directly; and (2) must be viewed as "purely environmental". Well-controlled laboratory studies in albino rats established early on that inheritance of susceptibility to dental caries was a viable hypothesis (Hunt *et al.*, 1944).

Applying the family/population method of analysis, Klein (1946) examined 5,400 individuals who were members of 1,150 different families, and demonstrated that the amount of dental disease (*viz.*, caries, "DMF") that appeared in the offspring was quantitatively related to that which had been experienced by their parents. He concluded: "...(this) makes it difficult to exclude the view that dental disease susceptibility in children involves strong familial vectors [*sic*] which very likely have a genetic basis, perhaps sex-linked."

In the first sentence of their landmark paper on "Clinical and Genetical Studies of Dental Caries", Böök and Grahnén (1953) stated: "Dental caries is *the* major disease of dentistry." While their pre-fluoridation statement may have little validity today, it nevertheless inspired them to carry out an exceptional clinical study in Lund, Sweden. They were responding to the conclusions of the classic "Vipeholm Study" (Gustafsson *et al.*, 1952), which established the role of between-meal sucrose intake as a major cause of dental caries. Their premise was that since the results of the Vipeholm Study were based on "mentally defective individuals—predominantly...low grade imbeciles and idiots—", they might not apply to the general population. As their study population, Böök and Grahnén adroitly selected the parents and siblings of intellectually normal, middle-class individuals who were highly resistant to dental caries (decay-free persons). The 53-page article by Böök and Grahnén (1953) is remarkable in its depth and breadth of investigative zeal. The authors could detect no environmental factor that could explain the differences in caries susceptibility between caries-free and caries-prone individuals/parents/siblings. Böök and Grahnén concluded that "genetic factors play an appreciable part in determining individual resistance against dental caries". Apparently disappointed with their findings, the authors ended their article thus: "From a practical odontological point of view the results are of minor importance."

Working at Columbia University, Horowitz *et al.* (1958a,b) brought some clarity to the question of caries susceptibility in their study of human twins. They were bothered by the discrepancy between the previous results of studies of adults and juveniles, primarily twin studies, and concluded that "in all probability" a hereditary factor in dental caries experience cannot be readily measured until eruption of the permanent teeth is essentially complete. Introducing the term Caries Experience Rate (CER) into the dental literature, Horowitz *et al.* demonstrated a genetic component of variability in caries incidence in adults who were otherwise in good systemic health, and claimed, therefore, to have found a definitive hereditary factor in susceptibility to caries.

Working separately in Edinburgh, Scotland, but at the same period of time, Mansbridge (1959) examined 232 like-sex twin pairs in schools, and reported that the resemblance in caries experience between monozygous twins was greater than between dizygous twins. He carried his study a step further, also, by including unrelated pairs of children as the control group, and found that these subjects exhibited less resemblance in caries experience than either type of twin. These findings were corroborated by Finn and Caldwell in Alabama (1963), who also detected differences between smooth-surface and pit-and-fissure caries lesions, indicating that the smooth-surface lesion may be under more strict genetic control.

Realizing that dental caries is a pathologic entity that results from the interaction of endogenous and exogenous traits, Goodman *et al.* (1959) studied 38 like-sexed MZ and DZ twin pairs in Michigan in an attempt to relate tooth decay to other factors that might be under genetic control. They reported significant heritability for the presence of several oral micro-organisms, including Streptococci, and also for salivary flow rate, salivary pH, and salivary amylase activity. Aside from hereditary factors relating directly to enamel constitution, Goodman and co-workers thus established other genetically influenced factors as operative in caries etiology.

Fairpo (1979), working in Leeds (England), expanded on previous studies of caries susceptibility by greatly increasing the "N" in the twin study population, and by evaluating total caries experience in a racially discreet, age-matched sample. The results carried previous conclusions (Finn and Caldwell, 1963; Akhmedov, 1973) into both the permanent and the deciduous dentitions, implicating a strong genetic factor in caries susceptibility.

The studies of Chung *et al.* (1970, 1971) and Chung

and Niswander (1975) have been mentioned earlier in this paper, namely, the dental examinations of almost 10,000 schoolchildren in Hawaii in 1967-68. The essence of the caries data was the finding that any racial effects, when evident, were additive for dental caries. Thus, in the absence of maternal influence or other factors, the indication is that cariogenic factors act primarily in an additive mode when one considers racial crossings.

In his 1972 speech at the annual meeting of the Swiss Society for Odontology in Lausanne, Hans Mühlemann presented a philosophical view when considering the scientific evidence about caries (and periodontal diseases) in humans from the genetic point of view (Mühlemann, 1972): "Dental caries is a polyfactorial entity. Could caries not therefore also have a polygenic heritability? One gene could influence the resistance of enamel by determining its chemistry or its morphology, another gene could control the composition of saliva, which could influence partly the oral flora; a third gene could determine eating habits; a fourth could influence one's characteristic personal view of or approach to oral hygiene at home. Given this, is a clean genetic analysis possible in man?" Mühlemann reviewed the literature up to 1972 exhaustively (his paper in the *Schweizerische Monatsschrift für Zahnheilkunde* contained over 100 citations), but for reasons that are difficult to comprehend, he came to the conclusion that "...hereditary factors are of minor pathogenic importance in comparison with environmental influences...Bad teeth are not inherited from parents...". It is unclear how he could deny the preponderance of the scientific evidence for *strong* genetic control that had emerged from previous twins studies, family studies, and population studies.

During the most recent 15-year period (1980-1995), very little about caries and genetics has appeared in the literature, except, that is, for the major contribution by Boraas and co-workers in 1988. They performed a six-year retrospective study of a large cohort (N = 97) of adult twins (the mean age was 40.6 yr) who had been raised apart since birth, and a control group of dizygous twins also raised apart. This is a powerful method, because the effects of common environment are eliminated; thus, the intraclass correlation coefficient between monozygous twins becomes a direct measure of heritability (Bouchard, 1984). Remarkably, of the 17 orofacial parameters studied, 15 were associated with highly significant within-pair resemblance in monozygous twins reared apart. Table 4 shows the significance of MZ and DZ correlation coefficients for some of the measured parameters. This study has provided new and convincing evidence for a marked genetic component to dentate status and dental caries experience. It also provides strong support for the earlier studies that had implicated hereditary contributions to tooth size, dental malalignment, occlusion, and tooth morphology.

TABLE 3

Some Examples of Orofacial and Orodental Characteristics that have been Evaluated for Genetic Contribution Using the Human Twin Model

Trait	Year	Author(s)
Dental caries	1958a	Horowitz *et al.*
Anterior tooth size	1958	Osborne *et al.*
Salivary microflora	1959	Goodman *et al.*
Caries	1963	Finn and Caldwell
Jaw size	1964	Lundström
Premolar morphology	1969	Wood, Green
Palatal size	1970	Riquelmi, Green
Carabelli cusp	1973	Biggerstaff
Occlusal variation	1980	Corruccini, Potter
Occlusion	1981	Potter *et al.*
Hypodontia	1982	Markovic
Tooth dimensions	1985	Sharma *et al.*
Gingival fibroblast proliferation rate	1989	Cockey *et al.*
Phenytoin-induced gingival overgrowth	1990	Hassell *et al.*
Periodontal indices	1991b	Michalowicz *et al.*
Saliva proteins	1994	Rudney *et al.*

PERIODONTAL DISEASES

While the hereditary basis for susceptibility to dental caries is rather well-founded, the situation *vis-à-vis* chronic inflammatory periodontal disease is considerably less so. This has been due not to any lack of investigative enthusiasm over the past eight decades, but rather to the relative complexity of the disease, continually emerging new knowledge about its pathogenesis, vagueries of clinical diagnosis and statistical quantitation, and the profession's own nomenclature for classifying these diseases, which keeps evolving even today (Ranney, 1993).

Three comprehensive reviews of genetic risk factors in periodontal disease have appeared very recently (Michalowicz, 1993, 1994; Hart, 1994). The reader is referred to these excellent papers. Here, we will assess several selected aspects of heredity in periodontology, which should complement other recent reviews, including an earlier treatise by Sofaer (1990).

The Multifactorial Nature of Periodontal Disease

Periodontal disease results from the interaction among several classes of factors, including exposure to oral bacteria, response to the bacteria (promoting or inhibiting

TABLE 4

Significance of Intertwin Resemblance (Concordance) for Various Orodental Characteristics*

Characteristic	MZ	DZ
Number of teeth present	$p < 0.001$#	NS†
Percent of teeth restored	$p < 0.001$	NS
Percent of surfaces restored	$p < 0.001$	NS
Percent of teeth carious	$p < 0.001$	NS
Percent of surfaces carious	$p < 0.001$	NS
Tooth size	$p < 0.001$	NS
Malalignment of teeth	$p < 0.009$	NS
Intercanine arch width	$p < 0.001$	$p < 0.01$
Intermolar arch width	$p < 0.001$	$p < 0.05$
Overbite/overjet	NS	NS

*Adapted from the original data of Boraas *et al.*, 1988.

#Significance of within-pair data.

†NS = not significant.

colonization and growth), and tissue structure (facilitating or protecting against bacterial colonization and growth and the resultant tissue damage)(Taubman *et al.*, 1984). These factors are themselves influenced by many factors, including the host's immune system and personal oral hygiene (Lang *et al.*, 1995), or other habits, such as smoking (Bergström and Preber, 1994). Models of periodontal disease risk may differ slightly in their conceptualization, but all include oral bacterial composition, oral hygiene or preventive behaviors, tooth or tissue structure, adequacy of immune response, lifestyle (diet, smoking), psychological factors (stress and reaction to stress), and socio-economic factors (Johnson *et al.*, 1988; Beck, 1994; Clarke and Hirsch, 1995). To various extents, genetic variation determines individual differences in many of these factors. Experiences or exposures shared by family members may also affect these risk factors for periodontal disease. When considering genetic influences on periodontal disease or periodontal measures, it is important to consider genetic factors in a broad sense—to measure adequately and include risk factors that may have a genetic component or cluster in families for non-genetic reasons.

The potential importance of genetics and heredity to clinical dental practice in general and alveolar bone pathology in particular was noted early on; Praeger (1924a,b) stated that such knowledge must influence the therapy and the prognosis of oral diseases. He also applied to the oral cavity the earlier conclusions of Siemens (1924) and Siemens and Hunold (1924), who had noted "...an increase of ideotypic [genotypic] diseases from generation to generation". Praeger studied periodontal disease in numerous sets of twins, and although he acknowledged that his sample size was small, he nevertheless concluded: "Das Zwillingsmaterial...gibt immerhin einen Fingerzeig für die Bedeutung erblicher Faktoren bei der Alveolarpyorrhoe" ("The twins material does nonetheless provide hints about the importance of heritable factors in alveolar pyorrhea"). That other clinicians and educators were having similar thoughts during the 1920s (*e.g.*, Polevitzky, 1929) can be found in a series of (undocumented but interesting) quotations provided by Korkhaus in his extensive 1952 treatise:

- (Moral, 1924) "Pedigree analyses reveal again and again that blood relatives often suffer from pyorrhea."
- (Sachs, 1925) "I have often observed that the parents of children with advanced pyorrhea lost their teeth early."
- (Reinmoller, 1925) "The susceptibility to true alveolar pyorrhea is due to a specific lack of host resistance which is most likely not acquired, but inherited."
- (Boenheim, 1928) "Inheritance plays a massive role in periodontitis. I once saw a mother of 8 who had periodontal disease, and so did 4 of her children."

During the 1930s, Hruska (1939) traveled extensively in Europe collecting data about the geographic (and racial) distribution of periodontal diseases. In 1939, he published his claim that periodontal diseases were based on inherited "factors"; then he pursued his studies of racial and geographic distribution of the disease (mainly by measuring and comparing tooth mobility) for a decade (Hruska, 1951). Also during the 1930s, two doctoral candidates at the University of Münich were pursuing clinical research projects targeted toward elucidating hereditary factors in periodontal disease. Dickmann (1935) performed a study of periodontal disease in 47 families and came to the conclusion that a dominant form of Mendelian inheritance was involved. Kalewe's (1937) doctoral dissertation was based on a study of periodontal destruction in monozygous twins. Although the thesis itself has been lost, the abstract was maintained on microfilm; it concludes, "It has been possible to demonstrate a genetic factor that is associated with the severity of periodontal destruction."

Additional reports, mainly in the German-language literature, appeared during the 1940s concerning various aspects of heredity and periodontal diseases (Roccia, 1940; Noack, 1940; Beyer, 1941; Parma, 1942; Euler, 1945; Schudel, 1948). These studies, which have been reviewed by Gorlin *et al.* (1967), were for the most part observational/descriptive in nature, with no control groups, but were ambitious for the time, detailing possible age effects on disease incidence and severity (Beyer, 1941; Euler, 1945), and detection of probands for pedigree

analyses (Roccia, 1940). Parma (1942) examined the periodontal conditions of a group of overweight subjects ("pyknic") and compared them with slender individuals ("leptosomic"), but found no differences. Schudel (1948) detected more severe periodontal destruction in men who were prematurely gray or who were balding, both of which conditions were assumed at the time to be hereditary. Of note is that the 1940 study by Noack is the only one in the literature in which concordance rate for periodontal disease in monozygous twins was *not* found to be higher than that in dizygous twins.

European investigators continued to work on the "racial question" into the 1950s. Leguay and Mantelin (1952) examined periodontal conditions among North African Berbers, while Rainowa (1955) was studying Bulgarian farmers and city-dwellers. In the latter work, urban individuals were found to exhibit almost 20% less disease than inhabitants of rural areas. Family studies continued also: Rojahn (1952) performed clinical and radiographic examinations of members of 32 families (N = 133), *differentiating between gingivitis and periodontitis*, and reported that in 87% of cases, if an offspring manifested disease, so did at least one of the parents. Rojahn concluded from this that "endogenous as well as exogenous factors" are responsible for periodontal infections. Working in post-war Bonn, Germany, Korkhaus (1952) studied periodontal disease using both the family pedigree method and the human twin paradigm. Of 13 sets of MZ twins over the age of 25, Korkhaus detected moderate to severe periodontitis in four sets, with both co-twins affected and concordant for pocket formation, recession, subgingival calculus accumulation, tooth mobility, and suppuration. Korkhaus acknowledged that his sample size was very small, but suggested that his preliminary findings should serve as the stimulus for expanded studies of adult twins in periodontology, including dizygous twins as a control group. Almost 40 years went by before this advice was heeded! (Cockey *et al.*, 1989; Michalowicz, 1991a,b, 1992, 1994; Hassell *et al.*, 1994). Korkhaus also did some pioneering genetics research with family pedigree analysis in cases of what we today term early-onset periodontitis (he called it "frühform Paradenopathie"). (American periodontics textbooks as late as the middle 1960s made no mention of a hereditary component in LJP.) We have reproduced here one of Korkhaus's pedigrees (Fig. 3); it is, we believe, the first such study ever pubhshed. Korkhaus (1952) concluded: "This first ever reported familial occurrence of a rare periodontal disorder is likely a hint that a critical factor in the pathogenesis of many periodontal diseases is anchored in the idiotype [genotype]."

Reiser and Vogel (1958) were interested in subgingival calculus formation as an etiologic factor in periodontal disease. They studied over 150 sets of MZ and DZ twins: Ninety-four percent of the MZ twins were concordant for subgingival calculus (either presence or absence), while for DZ twins the figure was 75%. The difference is statistically significant and indicates that heredity plays an important role in calculus accumulation.

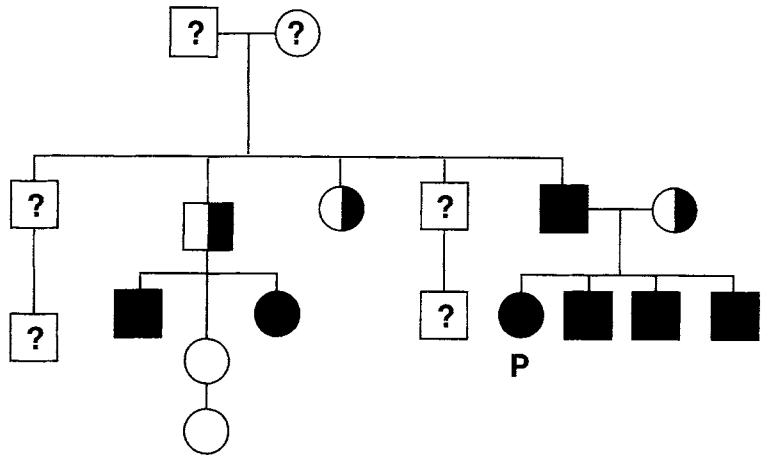

Figure 3. Pedigree of an extended family in which a type of early- onset periodontitis was detected in an 18-year-old female [redrawn from Korkhaus, 1952]. P = proband, ● = affected female, ◑ = non-affected female, ■ = affected male, ◨ = non-affected male, ? = unknown if affected or not (subject unavailable for examination), and ◨ / ◑ = minor clinical signs, but not definitive diagnosis of periodontitis.

A Fork in the Road

And now we come to what could be considered as a major turning point in periodontal research. During the late 1950s, at the National Institutes of Health in Bethesda, Maryland, a team of investigators (Baer and Bernick, 1957; Baer and Lieberman, 1959, 1960; Baer *et al.*, 1961) performed a series of breeding experiments with several strains of mice, working on the hypothesis that susceptibility or resistance to periodontal disease is influenced in an important way by genetic factors. They demonstrated that significant differences exist between mouse strains *vis-à-vis* susceptibility or resistance to alveolar bone destruction; for example, the inbred strain STR/N was very susceptible, while strain DBA/2JN was quite resistant. Baer and his co-workers reported on cross-breeding of the STR/N and DBA/2JN strains, with evaluation of almost 7,000 teeth in *ca.* 1,200 offspring (from the original crosses, and from repeated F1 and F2 back-crosses). They concluded that the STR/N and DBA/2JN strains of mice differed at many genetic loci, each of which evidently has a small effect on periodontal disease (susceptibility). The difference between strains was attributed to a maternal extra-chromosomal influ-

ence that could be passed through at least two generations. In the experiments, it was also possible to detect that environmental variation affected *expression* of the alveolar bone destruction trait. Finally, the authors were able to rule out any effect of contamination, contagion, or mutual contact among the mice as a factor in the differences in periodontal destruction observed between strains and among offspring.

Looking back now over the 35 years since the papers of Baer *et al.*, a time during which major (and not altogether successful) efforts with massive Federal and private financial support have been pursued in attempting to decipher the *microbiology* of periodontal diseases, one wonders why the clear evidence for a major *genetic* component presented by Baer and his colleagues in an elegant animal model was not seized upon by the scientific community. Indeed, after the 1961 report by Baer *et al.*, there was very little interest expressed in pursuing hereditary aspects of periodontal disease susceptibility. The STR/N strain of mouse, known then to be highly susceptible to periodontal disease, was exploited almost not at all, except for one paper in the German-language literature (Rateitschak and Reimers, 1969), which again concluded that periodontal destruction in this strain could be explained only by a genetic mechanism and not by local factors. A rat model of periodontal destruction was used by Hefti (1987) in his studies of microbiologic and other environmental factors; however, he concluded that while his model could be used to study the pathogenesis of bacterially induced inflammatory reactions, it differed considerably in several respects from the histologic picture of periodontitis in humans. Were there, in those years immediately after the Baer *et al.* publications, sentiments about the non-applicability of animal models to the human condition? Did the thalidomide debacle of the early 1960s (in which the drug had no teratogenic effect in the animal model—rabbit—but caused phocomelia in human offspring) contribute to the cessation of research with animals and genetic models for periodontal disease? Or was it the exciting work emerging from the Scandinavian universities, led initially by Waerhaug (1952) then by Löe and coworkers (1965), demonstrating a major role for bacteria in dental plaque as elicitors of the disease, that led researchers away from the study of hereditary factors and toward the study of local (environmental) factors? According to Löe (1993), the discipline of periodontology was in disarray at about that time; confusion and crisis reigned in the late 1950s; a single paradigm for research into the causes of periodontal disease was lacking. Professional and academic insecurity was fed by contradictions, half-truths, an informational morass, and unsuccessful (empirical) treatment strategies. The opportunity to seize upon the microbiologic character of the disease process was appealing, it was approachable with the technical means of the time, and it offered to bring concordance between theory and practice. Genetics was abandoned. Microbiology reigned, and continues to (*e.g.*, Preus *et al.*, 1994).

Gorlin *et al.* (1967) resurrected the concept in 1967 with a review of the older literature, but did not offer much enthusiasm for pursuing genetic research in periodontology by describing it as "extremely complex" and "extremely difficult" and "of not much practical importance". Ciancio and co-workers made another effort in 1969, by studying periodontal conditions in MZ and DZ twin pairs, but reported to have detected no evidence to support the concept that probing depths or recession or gingival scores or calculus or plaque scores are inherited. However, only 26 twin pairs were evaluated (7 MZ and 19 DZ), and the mean age of their twin sample was 15 years (range = 12-17)—*not* an ideal group in which to study periodontal disease!

The Herculean efforts of the group headed by Chung (1977a,b), mentioned earlier in this paper, also provided no encouragement for further study of a genetic basis for periodontal disease, since their path analysis of 241 families "failed to detect significant heritability". Again, however, this was a study of *children*. The 1990 paper by Sofaer reviewed the mostly negative previous findings, pointing out also that if only 10-20% of most populations are at risk for periodontal disease, then studying it in general populations may well mask any hereditary component of susceptibility.

Back on Solid Ground: Periodontal Disease *Associated with Genetic or* Familial Conditions

By examining associations between periodontal disease and specific medical conditions or syndromes, and learning about the underlying causes of those associations, it is possible to discover specific, possibly inherited, characteristics that affect periodontal disease risk or severity. While such characteristics may be clearly deficient or abnormal in rare conditions, less drastic alterations in structure or function may explain a portion of the interindividual variability in periodontal health in the general population. Diverse findings from various chromosomal abnormalities, genetically inherited monogenic syndromes, and some rare familial conditions provide substantial support for the role of a single gene or genes of major effect for periodontal disease etiology, rather than a multifactorial concept.

Chromosomal abnormalities

It has long been recognized that individuals with Down syndrome, who are trisomic for chromosome 21, have a high prevalence of periodontal disease (Dow, 1951; Loevy, 1979). In a population-based study of periodontal disease and oral hygiene among individuals aged 10-24 years in New Zealand, Cutress (1971) found that individuals with Down syndrome had a higher prevalence of

severe periodontal disease than other mentally retarded individuals and individuals with normal intelligence at all ages; furthermore, persons with Down syndrome who were institutionalized had a higher prevalence than those who lived at home. These differences in prevalence could not be explained by differences in oral hygiene or other environmental factors. Other studies suggested that the host response to oral bacteria in individuals with Down syndrome may differ from that in other individuals. In an experimental gingivitis study, gingivitis started earlier in the children with Down syndrome than in controls, and the pattern of leukocyte locomotion differed between the two groups (Reuland-Bosma *et al.*, 1986). In a more recent study, Izumi and colleagues (1989) found decreased neutrophil chemotaxis among individuals with Down syndrome. Interestingly, persons with Down syndrome are also at increased risk for infections in the upper respiratory tract, indicating some sort of altered immune response to bacterial infections in general.

Genetically Inherited Conditions

Early-onset periodontal disease is associated with genetically inherited conditions that are characterized by defects in collagen biosynthesis (*e.g.*, Ehlers-Danlos syndrome, type IV and type VIII), neutrophil function (*e.g.*, Chediak-Higashi syndrome and chronic neutropenia), calcium metabolism (familial hypophosphatemic vitamin-D-resistant rickets), and genetically inherited conditions of unknown etiology (reviewed by Genco and Löe, 1993). Although the abnormalities causing periodontal disease or predisposing to it are not clear in all of these cases, all are believed to be related to genetically determined defects in connective tissue structure, or to neutrophil number or function. On the other hand, Papillon-Lefèvre Syndrome is transmitted as an autosomal recessive trait, but there is very little evidence that the basic gene disorder results in defective connective tissue structure or neutrophil dysfunction (reviewed by Hart and Shapira, 1994). The possibility of an epithelial cell defect has received some support.

Multifactorial Conditions

Periodontal disease also has a statistically high association with more common, familial conditions: non-insulin-dependent diabetes (NIDDM), insulin-dependent diabetes (IDDM), and osteoporosis (reviewed by Oliver and Tervonen, 1994, and Genco and Löe, 1993). In a series of studies in Pima Indians, who have a very high prevalence of NIDDM, the prevalence and incidence of periodontal disease were found to be higher among those with NIDDM; the higher prevalence could not be explained by the common potential environmental risk factors for periodontal disease, such as smoking or oral hygiene (Nelson *et al.*, 1990; Shlossman *et al.*, 1990; Emrich *et al.*, 1991). Periodontitis is also more prevalent among teenagers and adults with IDDM, and may be associated with some of the often-encountered complications of diabetes, such as retinopathy and nephropathy (Glavind *et al.*, 1968; Cianciola *et al.*, 1982; Rylander *et al.*, 1986). The reasons for the increased periodontal disease risk among patients with diabetes remain unclear; some but not all investigators have found differences in pathologic structure within the gingiva (*e.g.*, capillary basement membranes; Frantzis *et al.*, 1971) or impaired neutrophil function (Fikrig *et al.*, 1977; Manouchehr-Pour *et al.*, 1981).

The relationship between osteoporosis and periodontal disease is even less clear. Most studies addressing this issue have been plagued by important deficiencies: crude measurements of periodontitis (*e.g.*, tooth loss), or lack of comprehensive information on important risk factors for periodontal disease (*e.g.*, cigarette smoking, oral hygiene).

In summary, studies of the association between periodontal disease and genetic or familial conditions provide evidence that immune function (*e.g.*, neutrophil chemotaxis) and tissue structure (*e.g.*, altered collagen synthesis) play an important role in the development of periodontal disease, and that some of the inter-individual variations in these factors may have a genetic basis.

Early-onset Periodontitis

Family Studies

Evidence for a genetic contribution to individual differences in risk of periodontal disease is clearest for early-onset periodontitis. Some of the pioneering initial studies of the mode of inheritance of susceptibility to early-onset periodontitis concluded that the increased prevalence in women as well as the lack of father-to-son transmission in families indicated that susceptibility is inherited as an X-linked dominant trait (Melnick *et al.*, 1976). More extensive analysis of these data has shown that these two indications of X-linked inheritance are due to the differential ascertainment of women or girls with periodontal disease in families (Hart *et al.*, 1992, 1991). When the original pedigrees were analyzed redressing for ascertainment bias, they were found to be supportive of autosomal inheritance of EOP (Hart *et al.*, 1992). Both autosomal-recessive inheritance (Saxén and Nevanlinna, 1984; Beaty *et al.*, 1987; Boughman *et al.*, 1988, 1992) and autosomal-dominant inheritance (Boughman *et al.*, 1986; Marazitta *et al.*, 1991) of early-onset periodontitis are supported by existing data. In the largest study to date (100 families), Marazitta and colleagues (1994) found the strongest evidence for an autosomal-dominant susceptibility gene, with 70% penetrance. This was the best-fitting model for both African-American and Caucasian-American families; the gene frequency was much higher

in Blacks, reflecting the higher population prevalence of periodontal disease (EOP) in that group. It is important to understand the basis for the incomplete penetrance estimated in this model. It may be due to an inaccurate assessment of (or the definition of) affected status (*e.g.*, all edentulous individuals being classified as unaffected), or to other susceptibility genes or risk factors for periodontal disease (*e.g.*, tobacco use, personal oral hygiene habits, or exposure to disease-causing microorganisms). If innovative research can provide new knowledge about the basis for incomplete penetrance of a susceptibility gene, more effective preventive interventions for those at high risk of periodontal disease could be devised.

Genetic linkage studies have been routinely used to locate disease susceptibility genes in the genome; such studies typically involve detailed genetic and phenotypic studies in families that appear to manifest a genetically inherited disease predisposition. In a large, five-generation family, an autosomal-dominant form of localized juvenile periodontitis was ascertained to be linked to Gc (group-specific component, a vitamin-D-binding protein locus) on the long arm of chromosome 4 (4q) (Boughman *et al.*, 1986). A linkage study of 19 unrelated families by Hart *et al.* (1993) could not, however, confirm this linkage to 4q, suggesting rather that the form of LJP linked to 4q was unlikely to be a gene of major effect in most African-American EOP families. Genetic heterogeneity for early-onset periodontitis could not be ruled out; furthermore, important differences existed among these various study populations. Because of the likely genetic heterogeneity for inherited susceptibility to early-onset periodontal disease, it will be important to design studies of sufficient size to detect linkage in subsets of families. Fortunately, the data from linkage studies can be combined, so that adding families with well-characterized and clearly defined periodontal disease to the literature will better enable researchers to understand the genetic basis of early-onset periodontal disease. Better delineation of etiologically homogeneous groups will certainly facilitate genetic linkage studies, as well as other genetic studies. For example, some families with early-onset periodontal disease exhibit decreased neutrophil function, while others do not (Van Dyke *et al.*, 1985); the use of such criteria should help to delineate more homogeneous groups for genetic studies in the immediate/near future.

Population association studies

Associations between specific genetic markers and diseases are often used to indicate a specific genetic effect. A higher prevalence of a specific genetic marker among individuals with periodontal disease indicates that the marker itself confers increased susceptibility to periodontal disease or that a periodontal disease susceptibility gene is closely linked to that genetic locus. In such studies, it is necessary to guard against a type of confounding called "population stratification", which occurs when gene frequencies differ in subgroups of a population and these subgroups are differentially represented in the case and comparison groups. A pertinent example for periodontal disease is race: Since the prevalence of periodontal disease is higher in African-Americans than in Caucasian-Americans, and since gene frequencies may differ between the two groups, the association between specific genetic markers and periodontal disease should be analyzed separately for Blacks and Caucasians in the US.

The association between specific HLA antigens and early-onset periodontal disease is of particular interest because of the role of interplay between bacterial infection and host immune response in the development of periodontal disease. Most recently, Shapira and colleagues (1994) found that, among non-Ashkenazi Jews, HLA antigens A9 and B15 are associated with the generalized form of early-onset periodontal disease but not with the localized form. Earlier studies in other populations also reported an association between HLA-A9 and early-onset periodontal disease (Reinholdt *et al.*, 1977; Marggraf *et al.*, 1983; Klouda *et al.*, 1986; Amer *et al.*, 1988); the criteria for case selection suggest that primarily generalized early-onset periodontal disease cases were included in these studies (Shapira *et al.*, 1994). Shapira and colleagues (1994) conclude that generalized early-onset periodontal disease and localized early-onset disease are different diseases "under different genetic control". However, family studies of early-onset periodontal disease show that localized and generalized disease can occur in the same family (Boughman *et al.*, 1990), and that localized disease can progress to the generalized form (Shapira *et al.*, 1994). Some confusion has arisen from two small-family studies of HLA antigens and periodontal disease (Cullinan *et al.*, 1980; Saxén and Koskimies, 1984). These studies have been mistakenly construed as being genetic linkage studies, which they were *not*. It has nevertheless been suggested that HLA associations are not due to an inherited periodontal disease susceptibility locus close to HLA in the genome, and that any family clustering of periodontal disease cannot be explained solely by segregation of HLA antigens in families. Rather, specific HLA antigens (A9 and B15) may specifically confer increased risk of periodontal disease because of a difference in their biologic activity (expression) when compared with other HLA antigens. Another way to interpret the observed difference in HLA associations for generalized and localized disease is to consider HLA-A9 or -B15 as *cofactors* for periodontal disease. There may be susceptibility genes that are unlinked to HLA, and their expression in terms of periodontal phenotype (localized or generalized, degree of severity)

may depend on HLA antigens or other risk factors for periodontal disease (such as poor dental hygiene or cigarette smoking). For example, individuals with benign chronic neutropenia often manifest severe periodontal disease, but the severity of periodontal disease appears also to be related to oral hygiene (Genco and Löe, 1993).

Adult Periodontitis

TWIN STUDIES

Twin studies are typically used to detect genetic variance for traits or conditions that are multifactorial. The comparison of the similarity of identical twins with that of fraternal twins is based on the difference in shared genes and the similarity of shared environments, so that a greater degree of similarity for identical twins is evidence for genetic variance (see above). For the simple model on which estimates of heritability are based, for Mendelian traits one expects the correlation for identical twins to be no more than twice that for fraternal twins; however, this does not always hold for multigenic traits. An even greater difference between the similarity of identical and fraternal twins suggests that a more complex genetic model, gene-environment interaction, or greater shared environment for identical twins, is a better explanation of the observed correlations. A near-equal correlation for identical and fraternal twins suggests that shared environmental factors, and not genes, account for any observed similarities. Researchers in Minnesota (Bouchard, 1984) have also studied twins reared apart. Presumably, such twins have no more shared environment than any two unrelated individuals, and certainly share less of their environment than do twins reared together. Thus, including twins reared apart in a study design facilitates stronger conclusions about the importance of genetic variation and shared environment in familial clustering.

An early study of periodontal health in 26 twin pairs aged 12-17 years found no evidence of a genetic contribution to variation in gingival recession, sulcus depth, or indices of gingivitis, calculus, and plaque (Ciancio *et al.*, 1969); however, more recent and well-controlled studies of more appropriately aged twins support the idea that genetic variation may contribute to individual differences in risk for the more common adult periodontitis. For example, in a large, population-based twin study of questionnaire-assessed periodontal disease, identical twins were found to be more often concordant for periodontal disease than were fraternal twins (Corey *et al.*, 1993). Thus, if one is an MZ twin, the risk of developing periodontal disease if one's sibling has had periodontal disease is 21-38%, *vs.* 8-16% for fraternal twins; both of these concordance rates are higher than the prevalence of periodontal disease among twins (5%) and among non-twin spouses (4%). However, age and gender differences in risk for periodontal disease, and other risk factors for periodontal disease (*e.g.*, cigarette smoking), were not specifically taken into account in these analyses.

Twin similarity for clinical measures of periodontal disease and for potential host risk factors for periodontal disease has recently been assessed (Michalowicz *et al.*, 1991a,b; Rudney *et al.*, 1994). After age and gender effects on each measure were taken into account, the similarities of identical twins reared together, fraternal twins reared together, and identical twins reared apart were compared for attachment loss, pocket probing depth, gingival index, and plaque index (Michalowicz *et al.*, 1991b). The greater similarity of identical twins, whether reared together or apart, suggested that there is a *genetic contribution* to variation in levels of supragingival plaque and clinical measures of periodontal health. However, the low degree of similarity in fraternal twins for most measures suggested that the genetic model may not be a straightforward additive model, but may instead be an interaction between genes at one locus (dominance), among genes at more than one locus (epistasis), or between genes and other risk factors.

The difference in similarity of alveolar bone height for identical twins reared together and fraternal twins suggests that genetic variation contributes to individual differences in alveolar bone height; the difference in similarity between identical twins reared apart and those reared together suggests that shared environmental factors also contribute to variation in alveolar bone height (Michalowicz *et al.*, 1991a). Regarding salivary protein levels, the similarities for total protein, lactoferrin, total peroxidase, and myeloperoxidase were greater for identical twins than for fraternal twins (Rudney *et al.*, 1994). For most of these measurements, the pattern of correlations for identical and fraternal twins was consistent with a simple genetic and shared environment model. However, for total peroxidase, identical twins were quite similar while fraternal twins were not at all similar, suggesting a more complex genetic model or a more complex relationship between genes and environment.

FAMILY STUDIES

Family studies of traits (such as measurements of periodontal health) typically examine the correlations among family members to assess familial clustering, but often do not have the sensitivity to "tease out" the effects of shared genes *vs.* shared environment because of limited knowledge of family relationships (*i.e.*, when only parents and their offspring are studied). More extensive family studies of periodontal health have examined the correlations of periodontal measurements and risk factors for periodontal disease among parents and their children to assess whether shared genes and/or shared environmental factors are more likely to explain any observed simi-

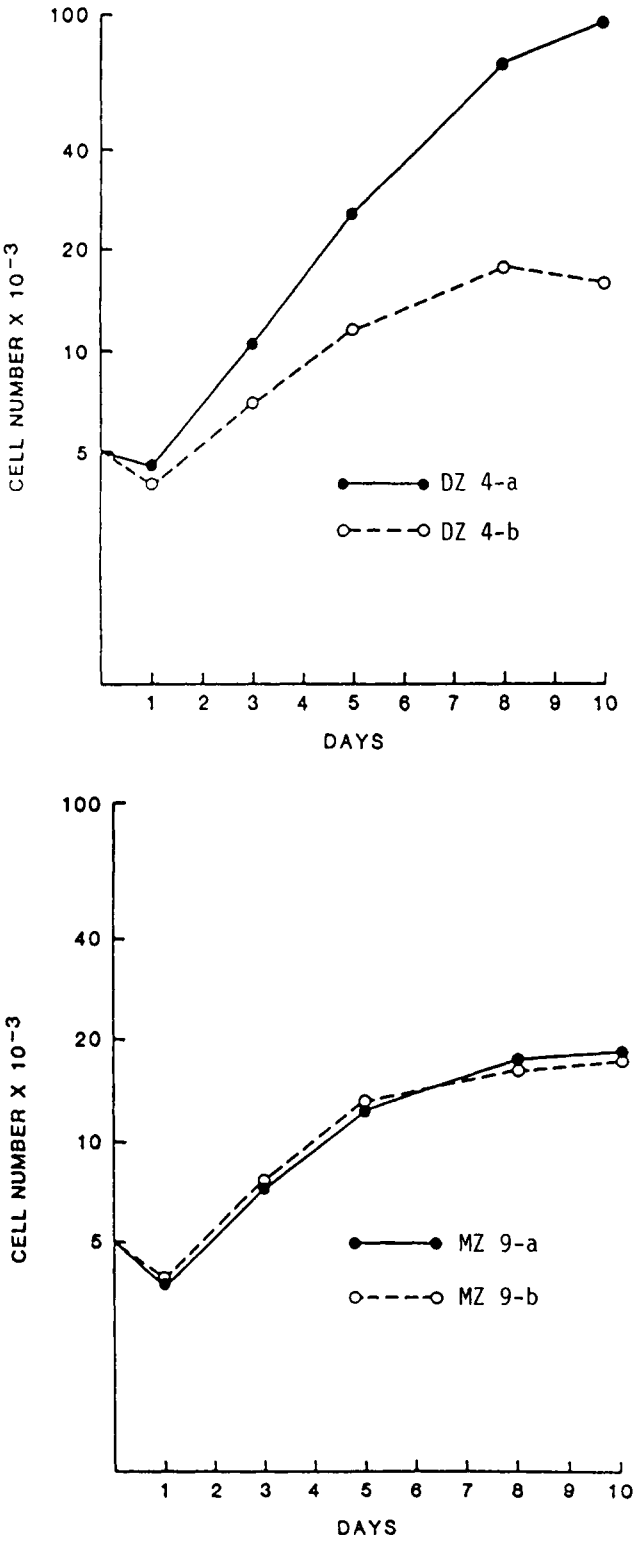

Figure 4. (A) Ten-day cell proliferation graph of cultured gingival fibroblasts from a set of young adult, healthy fraternal (DZ) twins with GI and PI of zero. (B) Identical experiment, but with fibroblasts from a set of identical (MZ) twins. See text for discussion [previously unpublished data, adapted from Cockey *et al.*, 1989].

larity of family members. A study of families in which the biological parents and at least one child 14 years or older were available for dental examinations concluded that, after adjustment for age, gender, tobacco use, and toothbrushing frequency, shared *environmental* factors could explain the observed correlations among parents and their children (Rao *et al.*, 1979). However, the children were relatively young (20 years or younger) (Chung *et al.*, 1977a,b), a time in life when susceptibility genes for adult periodontal disease may not be fully expressed. The children in this study are now approaching their parents' ages at the time of first evaluation (31-50 years); it would be interesting to re-assess their periodontal status and the correlations among family members.

In an Indonesian population lacking any regular dental care, Van der Velden and colleagues (1993) studied sibship clustering of periodontal measurements for individuals aged 15-25 years. Sibship clustering was observed for measurements of plaque, calculus, and attachment loss. Using analysis of covariance, the authors concluded that the clustering of data on attachment loss could be explained by differences in the amounts of dental plaque among sibships. Because of the age restriction, age effects would have been minimal, but other risk factors for periodontal disease (such as tobacco use; Bergström and Floderus-Myrhed, 1983) were not considered.

In a study of periodontal health in 75 families not selected for any dental condition, gingival and plaque indices and attachment loss were measured in 175 individuals (Beaty *et al.*, 1993). The participants were predominantly African-American, women, and age 40 years or younger. After adjustment for race, gender, age, and reported oral hygiene practices, the authors found significant familial clustering only for the plaque index, with a family effect accounting for 34% of inter-individual variation. The family effect was not statistically significant for the gingival index or attachment loss, although it accounted for about 30% of variation for each variable. To examine further the patterns of correlations among relatives while simultaneously adjusting for the effects of periodontal disease risk factors, Beaty *et al.* (1993) used the regressive models (described below). For the plaque index, the data were consistent with a multifactorial inheritance model, with a correlation of about 0.26 for parents and offspring and for siblings. The patterns of correlations for the gingival index and attachment loss were not so simple: The correlations for mother-child were positive, while the correlations for father-child were negative or near zero; sibling correlations were lower than those for mother-child. Specific genetic models of inheritance (*e.g.*, autosomal dominant) were not examined. Although these researchers accounted for several important risk factors for periodontal disease, they did not include tobacco smoking; it would be interesting to

know how inclusion of smoking affects the correlations, specifically if the gender differences are attenuated. Beaty *et al.* (1993) emphasized the importance of considering gender-specific transmission and including periodontal disease risk factors when analyzing family data.

A further extension of the family model is formally to analyze the segregation of periodontal measurements or periodontal disease in nuclear families (parents and their children) or in extended pedigrees to determine the mode of inheritance of periodontal disease susceptibility. There are several potential difficulties in periodontal disease research: First, genetic heterogeneity in the etiology of adult periodontal disease may make detection of a specific mode of inheritance difficult. This does not pose a great problem if the mode of inheritance for the various genetic susceptibilities is the same (*e.g.*, autosomal-dominant or autosomal-recessive), but it can be a significant problem if the modes of inheritance differ across families (*e.g.*, autosomal-dominant and autosomal-recessive traits). Second, it may be difficult to determine affected status or to measure periodontal health in some individuals (edentulous family members, adults who may have had early-onset periodontitis rather than adult periodontal disease). Third, there are other factors that affect periodontal disease susceptibility, some of which may be heritable themselves, such as a propensity for tobacco use, or which may cluster in families, such as at-home oral hygiene habits. Although traditional genetic segregation analysis techniques cannot readily take into account specific covariates, extensions of regression techniques that account for the dependency among relatives (regressive models) can simultaneously assess different modes of genetic inheritance and take into account the effects of covariates (Bonney, 1984, 1986). Measured genetic factors (*e.g.*, "high-risk" HLA marker) can also be included as risk factors or to assess the interaction between the genetic marker and other periodontal disease risk factors. Aside from Beaty *et al.* (1993), these types of analyses of family data have, to date, not been performed for adult periodontal disease or for periodontal health in adults.

Population Association Studies

The association between HLA haplotypes and adult periodontal disease is also of interest because of the role of immune response to infections in the expression of periodontal health. Studies of adult periodontal disease have primarily included teenagers and young adults, and in some instances it has been unclear if the individuals with periodontal disease had early-onset, rather than adult, periodontal disease. These studies have for the most part shown a lower frequency of HLA-A2 among those subjects with periodontal disease (Kaslick *et al.*, 1975, 1980; Terasaki *et al.*, 1975), and a higher frequency of HLA-A9 (Klouda *et al.*, 1986; Amer *et al.*, 1988).

TABLE 5

Intraclass Correlation Coefficients for Phenytoin-altered Protein and Collagen Production by MZ and DZ Twin Pair Gingival Fibroblasts [from Hassell *et al.*, 1990]

Twin Type	Intraclass Correlation Coefficient (r_i)	
	Protein	Collagen
MZ	0.908*	0.452*
DZ	0.627*	0.047

*$p < 0.05$, rejecting the hypothesis that $r_i = 0$.

Association studies of clear cases of adult periodontal disease, as well as family-based genetic linkage studies, may prove helpful in clarifying the relationship between HLA antigens and the genetics of periodontal disease susceptibility.

The Human Twin *Paradigm*: In vitro, *This Time*

Since the first paper by Galton in 1876, the human twin paradigm has been a powerful model for studying "nature *vs.* nurture" in medical research. Except for the abominable aberration that was the Third Reich, however, moral and ethical strictures have fortunately precluded the exploitation of this and other human research paradigms. Some recent studies (Cockey *et al.*, 1987, 1989; Hassell, 1992; Hassell and Harris, 1995; Hassell *et al.*, 1996), however, have demonstrated that it is possible to pursue the "twin paradigm" *in vitro*, using cells cultured from twins. This permits "exposure" of cells from MZ and DZ twins to putative pathogens and other agents known to be associated with periodontal pathology (*e.g.*, drugs [Vesell, 1984] such as phenytoin, nifedipine, etc.). The *in vitro* proliferation rates of fibroblasts derived from gingival biopsies of MZ and DZ twins, for example, have been shown to be under strong genetic control (Fig. 4). The protein and collagen production by cultured twin gingival fibroblasts in response to phenytoin has also been shown to be under genetic control (Table 5). The exposure of such cells to extracts from putatively pathogenic micro-organisms is being used to evaluate levels of genetic susceptibility of fibroblasts to bacterial challenge, at the level of the cytoskeleton (Hassell *et al.*, 1995, 1996). Therefore, the *in vitro* twin method may serve in the future to supplement the classic *in vivo* human twin method for assessing heritability of human disease and cellular function—the first step in therapy for genetic disorders is their ascertainment. From a tiny biopsy (of gingiva, skin, etc.), somatic cells can be challenged *in vitro* and evaluated for their functions, as a reflection of the phenotype (Thompson and Holliday, 1983). In addition,

direct, well-controlled studies can be designed, investigated, and replicated. The use of somatic cells, which need to be derived only once, can be serially propagated in culture and stored indefinitely; additionally, they permit multiple, diverse investigations to be conducted with the same cells. The proposed *in vitro* paradigm offers a new and original approach for investigating genetic control of human pathology. The knowledge derived from such studies is equally relevant to both clinical and basic scientists, since the scope of this new method is unlimited in terms of diversity of application or research design. Any type of exogenous agent (carcinogenic, mutagenic, immunologic, chemical, biologic, metabolic, physical, or nutritional) could be evaluated for its putative pharmacogenetic effect in inducing disease.

Future of Genetic Research in Caries and Periodontology

As noted by Boraas and co-workers (1988), several genetically controlled factors have previously been identified or implicated to be involved in the susceptibility to dental caries—for example, salivary factors and the oral microflora (Mandel, 1974), tooth eruption patterns (Gedda and Brenci, 1966), tooth morphology (Wood and Green, 1969; König, 1963), dental arch shape (Kolmakow and Puranen, 1985), and interdental space dimension (Corruccini and Potter, 1980), as well as nutritional factors (Forrai and Bánkövi, 1984). All of these factors warrant detailed study in the future, for it is one thing to know *that* dental caries experience is a heritable characteristic, but quite another to know *how/why* this is so, *i.e.*, to understand the biologic mechanisms. Along this line, Rudney and co-workers (1994) recently reported significant genetic contributions to observed variations in salivary protein concentrations (*e.g.*, lactoferrin and peroxidase) in adult human twins. Similarly, Malamud *et al.* (1988) observed significant genetic influences on parotid fluid flow rate, protein level, and the ability of parotid saliva from human MZ and DZ twins to agglutinate *Strep. sanguis* and *Strep. mutans*. Rudney *et al.* (1994) deduced that genetic regulation of hormones or neurotransmitters (see, *e.g.*, Propping *et al.*, 1986) could be responsible for heritable patterns of saliva secretion rates or composition (Johnson *et al.*, 1987).

It is rather well-accepted today that tooth morphology (*e.g.*, occlusal fissure shape and depth) can play a significant role in caries susceptibility. That tooth shape and size are genetically determined is likewise well-established. Future research will be targeted toward elucidation of the genetic mechanisms of such control.

During his presentation of the annual Seymour J. Kreshover lecture at NIDR in 1987, Slavkin described work on-going in his laboratory and elsewhere, in which recombinant DNA and other technology is making it possible to identify and describe dental structural genes (Slavkin, 1988). The complete nucleic acid sequence for (mouse) amelogenin has been defined, and is located on both X and Y human chromosomes. Slavkin elucidated what he referred to as the "new genetics", which is a composite of traditional genetics, molecular biology, and recombinant DNA technology, which is providing novel strategies for understanding gene regulation of oral tissues. For example, it is known that amelogenesis imperfecta and dentinogenesis imperfecta are inherited disorders (Ball *et al.*, 1982; Boughman *et al.*, 1986), which likely reflect alterations in regulatory and/or structural genes (Slavkin, 1988). Techniques currently available should, in the future, make it possible to understand not only these gross dental disease states, but also the more subtle aspects of genetic control of dental morphology and composition that lead to elevated susceptibility to dental caries attack.

With regard to periodontal diseases, at least three lines of research will be important to pursue if we are to achieve a better understanding of the genetic basis of inter-individual risk variance for this family of diseases: (1) delineation of more etiologically homogeneous categories of periodontal disease; (2) integration of known risk factors for periodontal disease into genetic analyses of familial clustering; and (3) identification of candidate genes that can be tested as susceptibility genes for periodontal disease. As in other etiologically heterogeneous conditions (such as diabetes mellitus), it will be difficult to make progress without subcategorization into more homogeneous groups. *But we will have to be extremely careful about the criteria.* For example, early-onset periodontitis can be clinically categorized into localized and generalized forms, but these clinical entities may *not* be etiologically distinct. Subcategorization may require measurement of other host characteristics (*e.g.*, neutrophil chemotaxis).

Because the nature of periodontal disease may be multifactorial, it will be quite important to consider known risk factors for periodontal disease when studying familial clustering or possible genetic mechanisms, because many risk factors for periodontal disease tend to cluster in families, through genetic or cultural mechanisms. Not taking into account such factors as age, race, oral hygiene, or tobacco use could lead to false conclusions about genetic contributions to familial clustering of the disease. In addition, most of the recent twin data for adult periodontal disease suggest that the genetic mechanisms of inheritance are not simple—they may involve gene-gene or gene-environment interactions. However, not all of the recent twin studies considered some of the important risk factors for periodontal disease (*e.g.*, oral hygiene, smoking).

It will be important to identify *candidate genes* that may be the basis for genetic susceptibility to periodontal disease. Genes that may affect immune response to oral

bacteria are the most obvious. Learning more about traits that predispose to periodontal disease, such as tissue response characteristics, may provide additional clues about possible candidate genes. Identification of such genes could enable clinicians better to identify high-risk individuals for targeted prevention and treatment (Johnson *et al.*, 1988).

Finally, at least until additional new knowledge emerges, one must accept the likelihood that no specific "periodontal disease susceptibility" gene exists, and that genetic control of susceptibility resides in the genetic control of one or more aspects of the host response (reviewed by Ebersole and Taubman, 1994). One candidate in this regard is genetic control of antimicrobial proteins (*e.g.*, lysozyme, lactoferrin, peroxidases, and secretory IgA), which can influence the colonization and growth of periodontal pathogens (Schenk *et al.*, 1993). Rudney *et al.* (1994) suggest that genetic variation in whole saliva levels of such proteins could act as a genetic influence on susceptibility to periodontitis. All protective proteins and all destructive proteins are gene products of human cells. As such, they are "genetically" regulable and stand as potential players in determining resistance or susceptibility to periodontal diseases.

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