Genetic aspects of dental disorders

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Abstract

This paper reviews past and present applications of quantitative and molecular genetics to dental disorders. Examples are given relating to craniofacial development (including malocclusion), oral supporting tissues (including periodontal diseases) and dental hard tissues (including defects of enamel and dentine as well as dental caries). Future developments and applications to clinical dentistry are discussed. Early investigations confirmed genetic bases to dental caries, periodontal diseases and malocclusion, but research findings have had little impact on clinical practice. The complex multifactorial aetiologies of these conditions, together with methodological problems, have limited progress until recently. Present studies are clarifying previously unrecognized genetic and phenotypic heterogeneities and attempting to unravel the complex interactions between genes and environment by applying new statistical modelling approaches to twin and family data. Linkage studies using highly polymorphic DNA markers are providing a means of locating candidate genes, including quantitative trait loci (QTL). In future, as knowledge increases; it should be possible to implement preventive strategies for those genetically-predisposed individuals who are identified to be at risk.

Key words: Heredity, caries, periodontal diseases, malocclusion, gene mapping, enamel defects, dentine defects.

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Introduction

The three most common problems in dentistry today remain dental caries, periodontal diseases and malocclusion. While there has always been anecdotal evidence of a genetic basis to each of these problems, for example, 'My mother had chalky teeth too' or 'He's inherited his father's teeth and his mother's jaws' or 'Bad gums run in my family', it is true to say that well-planned and executed scientific studies aimed at clarifying the genetic basis of these conditions are few and far between. This is partly because their aetiologies are multifactorial, making it difficult to design well-controlled studies. Furthermore, most researchers of dental caries and periodontal diseases have concentrated on environmental aetiological factors, for example, dental plaque, dietary factors, and oral hygiene, presumably because these have been thought to be most important.

A multifactorial aetiology for all three conditions has generally been assumed, with both genetic and environmental contributions to observed variability. The paucity of evidence of any clear-cut single gene effects has meant that genetic research in these areas has had little impact up to now on clinical dental practice.

As Neale and Cardon[1] have pointed out, several questions need to be answered before a complete understanding can be gained about how genetic factors influence a feature or disorder. These include:

- How important are genetic effects on human differences?
- What kinds of action and interaction occur between gene products in the pathways between genotype and phenotype?
- Are the genetic effects on a trait consistent across sexes?
- Are there some genes that have particularly outstanding effects when compared with others?
- Whereabouts on the human gene map are these genes located?

Modern methods of quantitative genetic analysis allow the first three questions to be addressed and

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Table 1. Abbreviations used in the text

AD	Autosomal dominant
AR	Autosomal recessive
CIPD	Chronic inflammatory periodontal disease
cDNA	Complementary DNA
CpG	Coding part of gene
DI	Dentinogenesis imperfecta
DNA	Deoxyribonucleic acid
DNP1	Dentine matrix protein 1
DPP	Dentine phosphoprotein
DZ	Dizygous
EOP	Early onset periodontitis
h^2	Heritability estimate
HLA	Human leukocyte antigen
LOD	Logarithm of odds
LJP	Localized juvenile periodontics
mRNA	Messenger RNA
MSTRA	Minnesota study of twins reared apart
Mx	Genetic modelling program
MZ	Monozygous
p	Short arm of chromosome
PCR	Polymerase chain reaction
PMN	Polymorphonuclear leukocyte
PRP	Proline-rich proteins
q	Long arm of chromosome
QTL	Quantitative trait locus
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SPP1	Osteopontin
SSCP	Single strand chain polymorphism
Va	Additive genetic variance
Vd	Dominance variance
Ve	Environmental variance
Vec	Common environmental variance
Vew	Specific environmental variance
Vg	Genetic variance
Vi	Epistasis variance
VNTR	Variable number of tandem repeat
Vp	Phenotypic variance
XAI	X-linked amelogenesis imperfecta
YAC	Yeast artificial chromsome
Θ	Theta – recombination fraction

provide some insight into the fourth. Resolution of the fifth question requires application of the methods of molecular genetics.

Analysis of multifactorial traits

Rigorous statistical analysis of multifactorial traits began with R. A. Fisher's seminal paper[2] in which he showed how the correlations between relatives of different degree could be explained using the principles of Mendelian inheritance. Although the impact of quantitative genetics has been considerable in agriculture where selective breeding is possible, until recently most studies in human populations have been confined to partitioning observed variation into genetic and environmental components based on comparisons between relatives, for example, parents and offspring, siblings, half-sibs, twins.

The variability between individuals in a trait's phenotype (Vp) can be considered to result from a combination of the genetic variance (Vg) and the environmental variance (Ve). Assuming that there is no interaction between these two sources of variation, Vp=Vg+Ve, genetic variance can be partitioned further into an additive component (Va) representing the sum of the effects of all the genes influencing the feature under study, a dominance component (Vd) resulting from the interaction of alleles at a single gene locus, and an epistatic component (Vi) due to the interaction of genes at different loci: that is, Vg=Va+Vd+Vi. The environmental variance can be partitioned into a common environmental component (Vec) shared by family members and a specific environmental component (Vew).

Heritability estimates, h^2, that can range in value from 0 to 1.0 (or 0-100 per cent) indicate how much of the observed variation of a character can be attributed to genetic effects. The ratio Vg/Vp is referred to as broad-sense heritability whereas the ratio Va/Vp is termed narrow-sense heritability. A list of abbreviations used in this paper is provided in Table 1.

It is important to realize that heritability estimates need to be interpreted with caution as they relate only to the population under study at a particular time, including the prevailing environmental influences. Furthermore, as Smith and Bailit[3] have noted, 'contrary to popular opinion, the extent to which genes determine a trait has no relationship whatsoever with the success of environmental intervention'. Traditional quantitative genetic analyses in human populations deal with variation between individuals not with mean values. For this reason it is inappropriate to say for example that 'tooth size is strongly genetic' – rather one should say that 'variation in tooth size between individuals has a strong genetic component'.

Genetic and environmental factors have often been assumed to be independent for the purposes of analysis, but in practice this is unlikely to be the case. Three factors that should be considered are: assortive mating whereby there is non-random pairing between mates for the trait under investigation; genotype-environmental correlation when different genotypes are not distributed at random in all possible environments; and genotype-environment interaction in which environmental effects on phenotype differ according to genotype. Unfortunately, most previous genetic studies of dental disorders have been based on assumptions that have never been tested.

Twin studies

The classical twin approach for separating the effects of nature and nurture involves comparing identical (monozygous) twins and non-identical (dizygous) twins. Differences between monozygous (MZ) twin pairs reflect environmental factors, whereas differences between dizygous (DZ) twin pairs are due to both genetic and environmental factors. Therefore, greater similarities between MZ

twin pairs compared with DZ twin pairs can be interpreted as reflecting genetic influences on the feature(s) being studied.

The traditional twin analysis method based on correlations is limited, but the development of sophisticated genetic modelling methods made possible with improved computing power has now provided an opportunity to fit complex multivariate models to human data, test their goodness of fit, make estimates of genetic and environmental parameters, and specify interactions between them. Apart from comparisons of monozygous and dizygous twins, there are other twin models that provide insights into the contributions of genetic and environmental factors to observed variability. The monozygous co-twin model involves comparisons of monozygous twins where each member of a pair has been exposed to different environmental effects. For example, identical twins might be treated with different appliances to correct similar malocclusions and the outcomes compared.[4]

Studies of twins reared apart overcome the problem of twins displaying similarities because of their common environment. Since 1978, Bouchard and his colleagues[5] in the USA have been studying monozygous and dizygous twins who were separated at birth and reunited in adulthood. The twins travel from all over the world to Minnesota where they undergo an intensive week of psychological and medical evaluations.

Another approach involving twins, the monozygous twin half-sib model, offers a powerful way of resolving shared genetic and environmental disease risks in families, as well as clarifying maternal effects and the importance of assortative mating.[6] Monozygous twins are assumed to have identical genotypes, so their offspring are genetically related as half-sibs but are socially first cousins. A nested analysis of variance similar to that used in analysing data from half and full-sibling litters in animal studies can be applied to provide estimates of genetic and environmental effects.

Segregation and linkage analysis

Segregation analysis is a statistical method for determining the mode of inheritance of a particular phenotype from family data, particularly with the aim of elucidating single gene effects or so-called major genes. With increasing computer power, models have been developed to detect the contribution of individual genetic loci that have large effects against a background of polygenic and environmental effects.[7,8] Once evidence of major genes has been detected, linkage analysis provides a means of determining where individual genes are located within the genome. Until recently, however, application of these methods to clarify the genetic basis of dental disorders has been limited by the difficulties of obtaining data from large family pedigrees and also in identifying appropriate polymorphic marker loci.[9,10]

Molecular approaches

With marked advances in molecular genetic technology in recent years, gene mapping techniques are now providing powerful approaches for locating genes associated with various diseases and disorders. Functional cloning uses the protein sequence and thereby the putative corresponding DNA sequence to clone the relevant gene, or by extracting the messenger RNA (mRNA) from the tissue to produce a complementary DNA (cDNA). This cDNA corresponds to the DNA sequence of the coding regions (exons) of a gene.

Positional cloning, also known as reverse genetics, is used to identify the location of the mutant gene on a particular chromosome by virtue of its cosegregation with polymorphic DNA markers. The first generation of these markers were termed restriction fragment length polymorphisms (RFLPs). RFLPs arise as a result of minor alterations in the DNA sequence on pairs of chromosomes. The DNA, usually obtained from peripheral blood leucocytes, is digested with a restriction enzyme which recognizes particular DNA sequences and cuts at a certain point in the sequence. The resulting DNA fragments are then separated in an agarose gel where the distance they migrate depends upon their size, shorter fragments migrating further than larger fragments over a given period of time. The DNA is then transferred from the gel to a nylon membrane (Southern blotting) where it can be probed by markers.

The markers are DNA fragments which have been mapped to parts of chromosomes. Because of the variation in cutting sites, in an ideal situation the probe will bind to two different sized fragments of DNA. The probe is labelled using a radioisotope and appears as one or more bands on an autoradiograph. The different bands are referred to as alleles, and by following the segregation of these alleles with the disease, the position of the gene is established. The limitation of RFLPs is that individuals are frequently homozygous at a given marker, that is, they have two alleles of the same size.

To establish linkage (the position of the diseased gene in relation to the RFLPs) affected individuals need to be heterozygous, that is, have two alleles of different sizes. Linkage analysis depends upon having a sufficient number of meioses, either in one or more large pedigrees or multiple smaller pedigrees. It is difficult to establish linkage without a number of three-generation (or more) pedigrees. Linkage also relies upon the fact that, at meiosis, recombination events occur on the chromosomes. Thus, some

individuals will inherit exact copies of their parents' chromosomes while others will inherit chromosomes which represent rearrangements of the original chromosomes. These recombination events are the key to mapping of a gene.

More recently, a new generation of polymorphic markers has been employed. These variable number of tandem repeat (VNTR) markers rely upon variations in the number of repeat sequences in non-coding regions of chromosomes. The VNTRs may be either dinucleotide repeats (repeats of two DNA bases, usually cytosine and adenine) or tri-, tetra-, or penta-nucleotide repeats. VNTRs have an advantage over RFLPs in that the number of repeats is (in theory) infinitely variable and these markers are more likely to be heterozygous. VNTRs obviate the need for Southern blotting. They are identified using the polymerase chain reaction (PCR) which uses primer sequences flanking the variable segment to amplify the DNA using a thermal cycler. The resulting amplified DNA fragments are then separated by electrophoresis in a polyacrylamide gel and revealed by autoradiography. Detection systems other than radioactive systems are now available and some of these processes can be automated.

Cosegregation of a disease with one or more DNA markers can be confirmed by statistical analysis. The measures of cosegregation are the LOD score (logarithm of the odds for linkage as opposed to no linkage) with a value of three being regarded as significant, this indicating a one-thousand-fold likelihood of linkage. The other measure is the recombination fraction which is an indication of the distance from the marker to the gene. With a high LOD score and a low recombination fraction the researcher can be fairly certain that the gene responsible for the disease has been localized.

The next stage is to clone the gene which can be a long and frustrating process. Numerous techniques are available to accomplish this. If the disease has been localized to a small area of a chromosome, the current strategy would be to use the markers either side of the disease (flanking markers) to probe a yeast artificial chromosome (YAC) library and this, in turn, can be used to screen other libraries containing smaller fragments of DNA such as cosmid libraries. Typical YACs are considerably larger than cosmids so this approach enables the relevant section of DNA to be analysed on a smaller scale. Other techniques that can be used include identification of the coding parts at the beginning of genes (CpG islands) and exon trapping. Once the gene has been isolated it can then be sequenced and the coding regions (exons) and non-coding regions (introns) identified. Following this, mutations in affected individuals can be identified using techniques such as single strand chain polymorphisms (SSCP) or direct sequencing.

Craniofacial development, including malocclusion

Dental occlusion reflects the interplay between a number of factors including tooth size, arch size and shape, the number and arrangement of teeth, size and relationships of the jaws, and also the influences of the soft tissues including lips, cheeks and tongue. The term 'malocclusion' is generally used to refer to variations from normal occlusal development, and although in some instances it is possible to specify the cause of a particular malocclusion, for example, genetic syndromes, embryological defects, or trauma, most malocclusions represent variations from normal development for which there is no apparent cause.

Indeed, the term 'occlusal variation' seems a more appropriate term than 'malocclusion' as it emphasizes the continuous range of dental occlusal relationships that are observed, with the more severe cases being located at the extremes of the distribution. With so many factors involved in the development of occlusion it is little wonder that most examples of malocclusion display multifactorial inheritance, with both genetic and environmental influences contributing to phenotypic variability.

Most previous studies have found a relatively strong genetic contribution to variation in craniofacial morphology, but standard errors of heritability estimates have rarely been provided.[11] Indeed, Hunter[12] has questioned the value of continuing to pursue traditional family studies to estimate heritabilities for dento-facial variables, given the lack of any real clinical application to the findings. However, as Proffit[13] has pointed out, history indicates that prevailing views about the causes of malocclusion (that is, heredity versus environment) have affected the type of treatment offered to patients to a surprising degree. Hopefully, a better understanding of the relative effects of genetic and environmental influences on different occlusal features should ultimately lead to improved preventive and treatment planning rationales in clinical dentistry.

There have been several excellent reviews of genetic studies of craniofacial development and morphology.[11,14-18] In their comprehensive review of the problems and methods in studies of the genetics of dental occlusion, Smith and Bailit[3] listed five main research objectives:

1) Elucidating modes of inheritance

2) Detecting the effects of admixture and inbreeding

3) Performing linkage analyses

4) Estimating heritabilities

5) Comparing population differences

Modes of inheritance

As already mentioned, occlusal variation appears (or is assumed) to conform to a multifactorial mode of inheritance, although strong familial similarities may be due to single major genes. For example, the famous 'Hapsburg jaw' seen in consecutive generations of an Austrian royal family may have been caused by a small number of segregating major genes. It is also possible that epistatic factors, that is, the interaction between genes at different loci, may play a more important role than most researchers have thought.

Admixture and breeding effects

Although many workers have suggested that racial admixture increases the occurrence of malocclusion, Smith and Bailit[3] have pointed out that the only detailed study aimed at testing this hypothesis by Chung and colleagues[19] failed to show any increased risk associated with admixture. The notion that admixture might lead to an increased frequency of malocclusion in humans appears to have originated from the work of Stockard and Johnson[20] in which gross deformities of the jaws of dogs were produced by cross-breeding different inbred strains. It has subsequently been noted that Stockard's results reflected the segregation of a gene for achondroplasia which is present in many breeds of small dogs but rare in humans, and therefore the results of Stockard's animal experiments cannot be extrapolated directly to humans with any validity.[13]

The X and Y chromosomes

Linkage studies in humans have been mainly restricted to studies of the sex chromosomes and the work of Gorlin and his colleagues,[21] together with the on-going research of Professor Lassi Alvesalo in Finland, have clarified the roles of the X and Y chromosomes on craniofacial morphology. Pattern profiles of dental crown size show the dosage effect of the sex chromosomes,[22] with both the X and Y chromosomes appearing to exert growth-promoting effects on human tooth crown size.

The X chromosome appears to mainly regulate enamel thickness. On the other hand, the Y chromosome seems to affect both enamel and dentine. The X and Y chromosomes also seem to influence craniofacial growth and development. Cephalometric analysis of a sample of 47,XXY males indicates pronounced facial prognathism in the Klinefelter males, especially in the mandible. Mandibular corpus length is significantly increased and there is a tendency for reduction of the cranial base angle.[23] Studies of 45,X females indicate a retrognathic face, with short mandible and flattened cranial base angle.[24] There is an increased prevalence of cross-bite, large maxillary overjet, distal molar occlusion and tendency to open bite in 45,X females reflecting imbalanced growth of the craniofacial skeleton.[25] It is suggested that the X chromosome may alter morphology of the cranial base by affecting growth at the synchondroses, that is, cartilaginous joints, and it also appears to have a direct effect on mandibular shape.

Of particular interest is the finding that the human dental enamel amelogenin gene is located on both the X and Y chromosomes,[26] although the gene on the X chromosome is predominant. Using molecular genetic techniques, the amelogenin gene has been localized to the distal portion of the short arm of the X chromosome and to the peri-centromeric region of the Y. Amelogenin is one of the major matrix proteins secreted by ameloblasts and it is thought to direct the growth of hydroxyapatite crystals. This finding fits in with studies at a phenotypic level. It also appears that mutation of the human amelogenin gene is associated with some X-linked types of amelogenesis imperfecta, a finding that will be discussed in more detail subsequently. This provides an excellent example of how studies at both molecular and phenotypic levels can complement each other and also have important clinical implications.

Heritability

Early traditional twin studies[27] and intrafamilial comparisons[28] indicated that occlusal traits were under reasonably strong genetic control. However, more recent reports in twins[29] and in first-degree relatives[30,31] have emphasized the importance of environmental factors.

Studies of tooth size in Australian twins have indicated a relatively strong genetic influence on observed variability, and a model incorporating additive genetic and individual environmental influences provided a good fit to the data. In addition, significant or near significant contributions of non-additive genetic variance were noted for the mesiodistal crown dimension of canines and first premolars, and a significant shared environmental component of variation to maxillary first molar dimensions. The findings for the canine and first premolar mesiodistal dimensions are consistent with expectations derived from evolutionary theory concerning the presence of dominance variation in morphological features that have been subjected to strong selective pressures in the past.[32]

The best model for Carabelli trait includes additive genetic effects, together with general and specific environmental components. An estimate of heritability around 90 per cent indicates a very strong genetic contribution to observed variation. It also seems possible that features like Carabelli trait may be influenced by genes interacting at the same

or different loci.[33] Dental arch shape also appears to be under relatively strong genetic influence, although there is evidence of some independence between the maxilla and mandible.[34]

Studies of occlusal variation in Australian twins have shown that heritability estimates for various occlusal features are generally low in magnitude, emphasizing the importance of environmental influences.[35] The trend noted by other workers for genetic components of variance to be greatest for overjet, less for overbite and least for molar relationships has also been confirmed by these studies. There is also an apparent lack of genetic determination for crossbite, a relationship in the coronal plane.

Although most genetic studies of craniofacial growth and morphology have concentrated on osseous structures, the functional matrix concept proposes that the morphology of the craniofacial skeleton is determined by the surrounding soft tissues.[36] Although a relationship has been demonstrated between the morphology of craniofacial features and the amount of muscular activity,[37,38] little is known about the genetic basis to variation in soft tissue structure or function. In this regard the finding of significant genetic variance in masseter muscle electrical activity and morphology in recent twin studies is of considerable interest.[39,40]

Comparisons of MZ twins who show different expressions of various dental or facial features provide a useful means of unravelling the influence of genes and environment to observed variability. For example, several pairs of MZ twins who display different expressions of missing, peg-shaped and diminutive upper lateral incisors have been noted.[41] These findings support the view of a relationship between tooth size and agenesis. A multifactorial threshold model for tooth development is proposed that links tooth size and tooth number. Presumably, developmental influences may modify phenotypic expression of lateral incisor crown form in those MZ twins whose genetic make-up places them near the threshold for agenesis.

The study of Australian twins and their families has been extended recently to include young children with deciduous dentitions. It is planned to follow these children through the mixed dentition stage until all permanent teeth are present. A longitudinal approach should overcome many of the problems associated with previous cross-sectional studies and provide information that can be applied clinically for predictive purposes. There is now increasing interest in early intervention to correct incipient malocclusions and based on the preliminary findings of clinical trials in Finland, Varrela and Alanen[42] claim that 'orthodontics may be on the verge of a fundamental change in its clinical practice which could be of benefit to dental health as a whole'.

Population differences

Variations in dental occlusion between different human populations have been described and interpreted in genetic terms.

Growth records of Aborigines living at Yuendumu in the Northern Territory of Australia have provided, and continue to provide, a unique resource for clinicians, anthropologists and geneticists. The special value of this material for clinicians is that it provides the essential perspective upon which to base preventive and treatment decisions. From a genetic viewpoint it has been shown that a fairly large percentage of the variation observed in tooth size is due to genetic factors. For example, heritability estimates are around 60 per cent.[43] Certain teeth are more variable than others in their size, shape, timing of emergence, etc, and this pattern follows Butler's field theory.

Midfacial growth, alveolar bone development and tooth migration associated with vigorous masticatory function tends to provide space for unimpeded emergence and alignment of permanent teeth in Aboriginals. This is in contrast to commonly observed crowded dental arches in industrialized populations. A feature of dental arch growth in many Aboriginal children, especially males, is the tendency for a greater increase in maxillary arch breadth than mandibular arch breadth. This growth pattern leads to a variation of dental occlusion termed alternate intercuspation which resembles the dental relationship found in many species of herbivores. It is suggested that alternate intercuspation, which would be regarded as a malocclusion and termed 'scissors bite' according to the modern clinical concepts, is in fact an efficient adaptation to vigorous masticatory function.

Studies of genetic contributions to arch size variability indicate that the heritability for maxillary arch breadth in Aborigines is low, that is, that variation is largely due to environmental factors.[44] Also, recent studies have shown that occlusal variation increased significantly in the Yuendumu people within one generation after adoption of a more westernized diet.[45]

There seems to have been an increase in the frequency of malocclusion during the period of human evolution. Although examples of crowding and malalignment of teeth have been found in prehistoric specimens, the prevalence appears to be lower than in modern day societies.[46] Whether this increase in malocclusion is a reflection of genetic or non-genetic effects is still not completely resolved but there is apparently an association between increased occlusal variation and the adoption of modern industrialized lifestyles.[46]

Future developments and applications to clinical dentistry

One of the major deficiencies in most genetic studies of common dental disorders to date has been the use of inappropriate systems of classification for the conditions or traits being studied. It is little wonder that genetic analyses of malocclusion based on Angle's classification, dental caries expressed as DMF scores, or periodontitis measured by pocket depth have been largely uninformative, given the complex aetiologies and continuous distributions of these conditions. Potter[47] has emphasized the importance of clarifying unrecognized heterogeneities in the aetiology of diseases such as periodontitis before attempting genetic analyses. She believes that a combined biologic-genetic approach is most likely to prove fruitful in discovering major susceptibility genes that might then be mapped.

An important basis for investigations of craniofacial growth is to establish the appropriate developmental units for study. Atchley and Hall[48] have emphasized that before any comprehensive theory for developmental change can be formulated, fundamental developmental units need to be identified along with their underlying controlling factors. These authors have provided a quantitative genetic model for the evolution of mandibular development in the mouse that might eventually be applied to the craniofacial structures of humans. They have drawn on the work of Moss[49] and Cheverud *et al.*[50] who used finite element scaling analysis to compare mandibular morphology in inbred strains of mice. Rather than computing linear and angular variables as usually occurs in conventional craniometric or cephalometric analyses, finite element analysis uses data derived from sets of interconnected landmarks or nodes to provide triangles or quadrangles that are termed 'finite elements'. These elements then become the basic units for analysis.

Application of 3D methods for visualizing hard and soft tissues in the craniofacial region (for example, magnetic resonance imaging), together with morphometric techniques such as finite element analysis that focus on particular regions of the skull, promise to provide a more realistic assessment of craniofacial morphology and growth than the conventional 2D methods used up until now.[11,51] These data could then be analysed using multivariate genetic modelling methods such as Mx to investigate their covariance structure.

Another problem to overcome in human studies of dental disorders relates to sampling. Firstly, there is a difficulty in obtaining data from large numbers of related individuals to provide sufficient statistical power for genetic analyses. Secondly, individuals representing the entire range of variation of the trait or disorder under investigation need to be included in studies to avoid introducing bias. For example, most studies of craniofacial growth and morphology, with the exception of a recent investigation by King, Harris and Tolley[52] have excluded individuals who have received orthodontic treatment. Thirdly, longitudinal studies are more likely to be informative for predictive purposes than cross-sectional approaches, but the cost of the former is often prohibitive.

Table 2. Classification of the periodontal diseases

I	Gingivitis
II	Early onset periodontitis
	Prepubertal – localized or generalized
	Juvenile – localized or generalized
III	Adult onset periodontitis
	Adult-type periodontitis
	Rapidly progressive periodontitis
	Refractory periodontitis
IV	Periodontal abscess
V	Periodontitis associated with systemic or inherited diseases
	HIV – gingivitis
	HIV periodontitis
	Neutropenias
	Diabetes, etc

The critical questions that need to be answered before real progress can be made are: 'How many genes are involved?' and 'Where are the genes located?'.

A gene that influences a continuously variable or so-called quantitative feature is termed a quantitative trait locus or QTL. For traits that display a multifactorial mode of inheritance, it would be expected that several QTLs will be involved together with various environmental effects. Although traditional approaches have usually been unable to identify these genes because of the superimposed effects of other genes and environmental variation, it is theoretically possible to locate them if they are linked with genetic markers, for example, RFLPs or VNTRs. Considerable progress has been made in mapping QTLs in rodents using either segregating populations or recombinant inbred strains together with genetic markers.[53] VNTRs are ideally suited for linkage studies of quantitative traits in humans as they are very common, often have multiple codominant alleles, and are distributed throughout the human genome.[54] Although recent genetic mapping of hypodontia in Finnish families has excluded a number of candidate genes that are known to be important in dental development, for example, epidermal and fibroblast growth factors,[55] the search is on in earnest!

The periodontal diseases

Currently, the periodontal diseases are considered as a group of related, but vastly different, inflammatory diseases affecting the support structures of the

periodontium. While many different classifications have been proposed, the American Academy of Periodontology has adopted a relatively simple classification related to age of onset and severity of disease (Table 2). These diseases range in severity from gingivitis which is largely a reversible condition if the causative agents are removed and controlled, to the very aggressive forms of early onset periodontitis, which manifest in several forms all of which demonstrate early and rapid destruction of the periodontium and can be extremely difficult to manage. In the middle ground lies the relatively benign form of adult-type periodontitis which affects many individuals but is generally regarded as being of less long-term threat to the well-being of the periodontium and is relatively easily treated.

Without question, all of the above conditions are associated with bacterial plaque accrual adjacent to the gingival tissues. However, although disease will not develop in the absence of plaque it is apparent that plaque alone is not sufficient to lead to disease. For example, there are many instances where individuals may have significant plaque and calculus deposits yet manifest little overt evidence of disease. The converse holds true also in that some individuals appear to have very minimal plaque and calculus deposits yet manifest significant periodontal destruction.

The most likely explanation for the above dilemma lies in the multifactorial nature of the disease in which host and environmental factors play a significant role in the development of disease. If this paradigm is accepted, then it is likely that some individuals will be at higher risk of developing disease. Thus, within this framework the issue of a genetic component to the disease experience of different individuals must be taken into account. In recent years there have been several very good published reviews on the subject of genetics and periodontitis to which the reader is referred for a more detailed discussion of this topic.[56-59]

Host genome considerations

Epidemiology

Population studies can be used to study groups of individuals with differing levels of inbreeding or outbreeding to determine the influence of genetics on the manifestation of disease in known populations. This method has been used with some success in the study of periodontal disease. An effect of racial mixing as well as inbreeding has been noted for gingivitis.[60,61] This has led to the proposal that recessive genes might be associated with increased risk for development of periodontitis. Nonetheless, these early studies mistakenly assumed that gingivitis progressed to periodontitis and that these two diseases were of similar origin. Today it is recognized that gingivitis and periodontitis are quite separate diseases with separate aetiologies and thus any extrapolation between gingivitis and periodontitis is of little value.

Differences in periodontal disease experience between different populations have also been studied. Chung *et al.*[60,61] found that different racial groups residing in Hawaii demonstrated different levels of disease, with Caucasians and Japanese showing a high level of oral health while the Hawaiians had a poor level of health. In a study of two Pacific Island populations who have relatively separate gene pools yet are ethnically similar, have similar diets, lifestyle and oral hygiene practices, Cutress, Powell and Ball[62] found that these two populations varied significantly in their experience of progressive disease. This observation has been suggested to implicate a role for genetic factors in determining disease susceptibility.[63]

Family studies have also provided insights into determining the genetic contribution to various diseases. While these are useful for diseases that manifest with a single discrete trait (or set of traits) as Mendelian segregation, in multifactorial or multi-presentation diseases such as the periodontal diseases, such analyses cannot distinguish between environmental and genetic influences on disease trait manifestation.

Nevertheless, for some forms of periodontal disease, localized juvenile periodontitis (LJP) in particular, pedigree patterns have been determined that seem to indicate a genetic component to this specific disease. However, this concept has been complicated by reports that have variously indicated localized juvenile periodontitis to be X-linked dominant,[64] autosomal recessive,[65] or autosomal dominant.[66] These findings indicate that even this clinically well-defined condition may have significant genetic heterogeneity. One study has even gone so far as to suggest that the putative localized juvenile periodontitis gene is located on chromosome 4q12-q13.[67] However, more recently, this finding has been questioned as not being representative of all localized juvenile periodontitis cases and further serves to highlight the genetic heterogeneity of this disease.

Since MZ twins have an identical genetic composition and DZ twins share approximately 50 per cent of their genome, twin studies utilizing both MZ and DZ twins enable determination of both hereditary variability and the influence of environment on genetic expression. As with all hereditary studies, those involving twins have limitations that must be recognized. Firstly, for twins reared together, this method of study presupposes that the environment during development and maturation is very similar and thus any differences noted in

Table 3. Monogenetic and chromosomal defects associated with periodontal defects

Condition	Tissue/cell/ biochemical defect	Periodontal condition	Mode of inheritance
Connective tissue disorders			
Ehlers-Danlos syndrome			
Type IV	Collagen type III	Fragile tissues and EOP	AR or AD
Type VII	Procollagen peptidase	Fragile tissues and EOP	AR or AD
Type IX	Collagen	Fragile tissues and EOP	X-linked
Mucopolysaccharidoses	Proteoglycans	Gingival overgrowth	
Mannosidosis	Mannose	Gingival overgrowth	
Familial fibromatoses	Collagen	Gingival overgrowth	Variable
Metabolic disorders			
Acatalasia	Catalase	Gingival necrosis and EOP	AR
Hypophosphatasia	Alkaline phosphatase	Poorly mineralized bone and cementum and EOP	AR (?AD)
Leukocyte defects			
Chediak Higashi syndrome	Neutrophil	EOP	AR
Chronic neutropenia	Neutrophil	EOP	AD
Cyclic neutropenia	Neutrophil	EOP	AD
Leukocyte adhesion defect	Neutrophil	EOP	AD
Dermatological defects			
Papillon Lefevre syndrome	Keratin/epithelium	EOP	AR
Chromosomal disorders			
Trisomy 21	Multiple biochemical	CIPD, EOP	

Abbreviations: EOP=Early onset periodontitis. AR=Autosomal recessive. AD=Autosomal dominant. CIPD=Chronic inflammatory periodontal disease.

phenotype between MZ and DZ twins must be due to genetic differences. However, subtle environmental differences between MZ and DZ twins do exist during both prenatal development (for example, birth weight) and postnatal development (for example, parenting differences) that may have a significant bearing on phenotype which is extremely difficult to control in such studies.

Although environmental factors are considered to be important in the establishment of periodontitis, individuals reared or living in similar environments may manifest significantly different disease patterns. However, MZ twins reared either together or apart have been found to have a more similar periodontal disease experience than DZ twins reared in the same manner with respect to both disease severity and distribution.[57] These findings have possibly provided the most convincing data to date that some, as yet to be identified, genetic factors may influence the manifestation of periodontal disease.

From these various epidemiological studies it may be concluded that the early onset forms of periodontal disease such as LJP probably have a distinct host genomic component to their manifestation. However, there are little convincing data to support a similar conclusion for adult-type periodontitis.

Genetic markers for periodontal diseases

Genetic risk factors may be studied by establishing an association between the disease and inherited tissue markers. In an infectious disease such as periodontal disease, the association between the HLA antigens and various forms of the disease has been of interest with several studies reporting the incidence of various class I and II HLA antigens in patients with early onset periodontal disease. In particular, the HLA antigens A9, A28, BW15 and DR4 have been found to be associated with early onset forms of periodontitis.[68-70]

Of interest is the observation that the HLA-A9 and HLA-BW15 antigens have been associated with the generalized but not localized forms of juvenile periodontitis implying differing genetic factors may be responsible for these two conditions.[71] Furthermore, unique intronic gene variations have been noted in the gene for HLA-DQb in patients (and some other normal family members) with early onset periodontitis.[71] However, other studies have indicated that there are no HLA associations with manifestations of various types of periodontal disease.[72,73] Thus, it is unclear whether there is an association between HLA antigens and periodontal disease due to an inherited periodontal disease susceptibility factor which is close to the gene for HLA, or segregation of HLA antigens in families who have a high risk for developing early onset forms of periodontitis.

Genetic segregation analyses of serum IgG2 levels have indicated that some forms of early onset periodontitis have a clear, albeit variable, genetic linkage.[74] The results from this study indicated the possibility of a two locus model explaining the manifestation of early onset forms of periodontal disease in which there may be an autosomal dominant major locus for early onset periodontitis conferring disease susceptibility.[74] Additionally, other alleles which control IgG2 responses may regulate some features of the disease. Thus, an interesting model has been proposed whereby an individual with the

'at risk' genotype for early onset periodontitis but who has a strong IgG2 response may manifest the localized form of early onset periodontitis. On the other hand, an individual with the 'at risk' genotype for early onset periodontitis who also fails to mount a strong IgG2 response may manifest a more generalized form of the disease.[75]

Other genetic markers which have been studied include the markers associated with the ABO blood group. While several reports have indicated that some blood groups may be associated with an increased incidence of periodontal disease,[76,77] others have not been able to confirm these findings.[78]

Inherited disorders with associated periodontal diseases

There are many inherited diseases and syndromes that have periodontal disease in one form or another as one of their distinct clinical features (Table 3). Whether these conditions are monogenetic, chromosomal or multifactorial, they all serve to demonstrate that genetic mutations affecting a broad range of tissues, cells, biochemical processes and host defence processes are affected to varying degrees, leading to increased susceptibility to developing periodontal disease. Significantly, the genes responsible for all of these conditions do not appear to cluster on one particular chromosome and this further highlights the multifactorial and polydisperse nature of the periodontal diseases. Although many of these conditions are relatively rare, they have provided some important insights into various components of host susceptibility to periodontal infections.

Connective tissue disorders

Conditions affecting connective tissue metabolism include various forms of Ehlers Danlos syndrome,[79] the mucopolysaccharidoses,[80] mannosidosis[81] and familial fibromatoses.[82] Of these, all but the fibromatoses are monogenetic syndromes. Ehlers Danlos syndrome types IV, VII and IX have all been associated with an early onset form of periodontitis. The mucopolysaccharidoses and mannosidosis are generally associated only with gingival overgrowth and little evidence of significant periodontal destruction. Similarly, the familial fibromatoses do not tend to demonstrate any association with destructive periodontal disease.

Metabolic disorders

Acatalasia is a monogenetic condition affecting the production of catalase which is important in removing hydrogen peroxide generated during normal cell metabolism. Since hydrogen peroxide can be toxic, and also leads to generation of superoxide radicals, tissue destruction is a common sequela to its accumulation. In patients manifesting acatalasia both gingival necrosis and severe alveolar bone destruction have been noted.[83]

Hypophosphatasia is a monogenetic condition characterized by deficient production of alkaline phosphatase leading to significant skeletal abnormalities. Along with diminished bone mineralization, defects in cementum formation have been noted. Hypophosphatasia and its associated cementum defect have been implicated in forms of early onset periodontitis.[84]

Leukocyte defects

In most forms of periodontal disease there is a significant rise in the circulating antibodies to various periodontal pathogens. However, individual variation is very high in these responses and this may be related to differences in levels of individual gene expression for various immunoglobulins. High IgG2 levels to antigens of various periodontal pathogens have been noted in localized juvenile periodontitis and rapidly progressive periodontitis,[85] indicating that the humoral response to periodontal pathogens is not very efficient in eliminating these organisms.

Apart from lymphocytes, polymorphonuclear leukocytes (PMN) are also critical in providing host defence against bacterial infections. Indeed, polymorphonuclear leukocytes are considered to be the primary line of host defence and if defective in their function can lead to significant and severe problems with controlling infections. The most striking link between neutrophils and onset of aggressive forms of periodontal disease is seen in patients suffering from the various forms of neutropenia. In these conditions there is a significant reduction in neutrophil numbers such that these individuals are very susceptible to recurrent bacterial infections of which periodontal infections are very common.[86] While some forms of neutropenia occur spontaneously, others appear to be of a familial nature and may be transmitted via an autosomal dominant mode. Apart from the problems with adequate numbers of neutrophils in neutropenia, qualitative defects in neutrophil function have also been noted to contribute to severe periodontal destruction. For example, Chediak-Higashi syndrome is a genetically transmitted disease characterized by reduced neutrophil function and extreme susceptibility to recurrent bacterial infections of which rapidly progressive early onset periodontitis is a characteristic feature.[87]

Apart from these systemic conditions which manifest an identifiable neutrophil deficiency, defects in PMN function such as chemotaxis have also been noted in approximately 75 per cent of patients with early onset forms of periodontitis.[88] A genetic component to this defect is considered likely in these conditions since the PMNs in these

Table 4. Common modifying and predisposing factors in periodontal diseases

Modifying	Predisposing
Systemic disease	Tooth shape
Smoking	Tooth composition
Disorders of cellular immunity	Calculus
Medications and hormones	Iatrogenic features
Stress	

individuals have a reduced expression of the cell surface adhesion protein GP-100 which appears to be inherited in an X-linked fashion.[89] Further evidence that this defect is inherent in the cells rather than acquired from an interaction with the environment relates to the persistence of the defect despite successful treatment of the periodontal condition.

Dermatological disorders

Papillon Lefevre syndrome is a monogenetic condition that is characterized by hyperkeratosis of the palmar and plantar surfaces together with a very destructive form of early onset periodontitis. The periodontal destruction can be so rapid as to lead to complete loss (exfoliation) of all teeth by age 3-4 years.[90] The underlying mechanism responsible for the rapid periodontal breakdown is unclear although some reports have implied that there may be inherent immunologic and neutrophil deficiencies.

Chromosomal disorders

The best recognized chromosome defect which has a periodontal component is trisomy 21 or Down syndrome. Both early onset periodontitis as well as advanced adult type chronic inflammatory periodontitis have been noted in these individuals. A genetic component to this condition has been confirmed by comparing Down syndrome individuals with cerebral palsy children who had a similar problem with maintaining adequate oral hygiene.[91] In the cerebral palsy individuals there was little evidence of the advanced periodontal destruction noted in those diagnosed with Down syndrome. Nonetheless, since a number of other 'environmental' factors such as whether the individual lives at home or in an institution, tongue abnormalities, tooth morphology, malocclusion and chewing patterns may impact on periodontal disease experience in individuals with Down syndrome, the severity of the problem may be a combination of both genetic and environmental influences.

Other intrinsic and extrinsic environmental factors of possible genetic association

Apart from the above considerations with respect to genetics and periodontal disease, numerous other modifying and predisposing factors influence the manifestation of the various periodontal diseases. Modifying factors are defined as any condition that alters the way in which the host might respond to bacterial challenge, while predisposing factors are those conditions that enhance the accumulation of dental plaque or hinder its efficient removal (Table 4). Of these factors, several have a 'genetic component'.

Tooth shape

Both normal and abnormal variations in tooth crown and root form can have a significant impact on the accumulation of dental plaque and thus on the manifestation of periodontal disease. For example, buccal and lingual crown contours, position of contact points, number of roots, root shape, development grooves and other developmental anomalies not only provide an appropriate niche for plaque accumulation but often make adequate removal by either the patient or dentist extremely difficult (if not impossible). Apart from these examples which relate to plaque accumulation or retention, tooth anatomy has also been linked to some forms of early onset periodontitis in which the roots appear to have a long and spindly appearance. Whether such root shape is under genetic control has never been addressed.

Tooth structure

The genetic bases of abnormalities of enamel and dentine are considered in more detail later in this paper. In the presence of enamel anomalies, surface roughness may lead to inadequate plaque removal. In addition, the poor appearance of the hard and soft tissues may lead to further problems of poor motivation for maintenance of adequate oral hygiene. In the case of cementum anomalies, such as cementopathia or cemental tears, there is the potential for inadequate attachment of the periodontal ligament fibres and subsequent compromised biological function.[92,93]

Systemic disease

The manifestation of many systemic diseases which may lead to compromised host function should also be considered. For example, diabetes and rheumatoid arthritis are examples of diseases which may have a genetic component and may have enhanced periodontal breakdown as a secondary feature.[94] In these cases the role of genetics is one of modifying the host responses such that upon significant bacterial plaque challenge the appropriate humoral and cellular responses cannot function adequately. These examples of genetic influences on the manifestation of periodontal diseases further serve to highlight the multifactorial nature of the

disease and confirm the unlikely scenario that a single gene defect (or indeed cluster of defects) will be associated with the development of many of the more common forms of periodontal disease.

Health beliefs

The role of genetics in development of health beliefs is complex. There is some evidence to suggest that oral health beliefs are passed from generation to generation but whether this is truly related to genetic or environmental influences is difficult to answer.[95] Twin and family studies have indicated that maternal influences, learned behaviour and cultural differences all contribute to an individual's health beliefs and thus will impact on variables such as oral hygiene.[95,96]

Other aspects of general behaviour such as propensity to smoke or suffer from stress-related problems may also have a heritable component to them. The complexities of determining the genetic influence of these parameters on periodontal disease are, at present, only beginning to be recognized.

Microbial genetics

There is no question that bacterial plaque is a necessary component for the development of all of the periodontal diseases. However, it is also clear that neither severity nor type of disease always correlates with the amount or type of bacteria present. Thus, not all individuals manifest similar disease to a given plaque mass or plaque type. Such observations account for the observed individual variation in disease experience but do not explain such variability.

To date it is not clear whether bacterial colonization is influenced by host genotype, but this cannot be discounted since differences in both the types of bacteria and antibody responses have been noted between various racial groups.[97] However, as discussed above, host response to plaque bacteria may be under some form of genetic control. In addition to host response in considering the question of genetic aspects of periodontal disease, it is worthy to note the role of genetic variability in the inhabitants of the microbial flora.

Currently, there are some 300 different species known to be capable of inhabiting the subgingival microenvironment which may be associated with both periodontal health and disease.[98] At present, understanding of all of these bacteria is limited to only a very small number. Nonetheless, studies have indicated clearly a great deal of genetic variability within various bacterial species. For example, the major pathogen associated with early onset forms of periodontal disease *Actinobacillus actinomycetem - comitans* may be present in at least three different serotypes (a, b and c) of which serotype b appears to be associated with early onset periodontitis.[99] With respect to genetic influences of bacteria such as *Actinobacillus actinomycetemcomitans*, it is interesting to note that while transmission from one individual to another is possible, the infection of an individual is usually clonal.[100]

Other aspects of bacterial genetics that should be taken into account in periodontics are the appearance of strains which are resistant to antibiotics. This is particularly relevant with the recent emergence of strains of periodontal pathogens showing resistance to tetracycline, an antibiotic considered to be particularly useful in managing Gram negative anaerobic infections.

Clinical implications

The major clinical implications of studying the role of the host genome in the various periodontal diseases lie in leading to a better understanding of the variability in disease manifestation as well as being of some diagnostic value. By recognizing that some forms of periodontal disease may have a strong genetic component it has become necessary to identify those individuals and subsequently screen their immediate relatives for signs of developing problems. In addition, by recognizing that some forms of periodontal disease may form part of a syndromic condition, early recognition of these signs can aid in the identification of such individuals.

Apart from studies on the host genome, considerations of the microbial genome have also impacted significantly on clinical practice and will continue to do so. For example, genetic mapping studies on bacteria isolated from family members have clearly determined that many periodontal pathogens can be transmitted to family members over a prolonged period of time.[101] The significance of this is greatest for severe periodontal infections where particular pathogens are identified and thus care should be taken to minimize transmission. In addition the issue of spontaneous mutations occurring within the bacterial genome is becoming a major concern. The ramifications of spontaneous mutations will impact on both the development of sensitive diagnostic probes as well as the development of antibiotic resistant strains of bacteria.

Although somewhat removed from immediate clinical applicability, gene replacement therapy for both the host and parasite genome is a rapidly growing research area.[102] The implications for this kind of research are exciting and warrant close attention in the years to come. For example, through the identification of certain antigenic components (fimbrillin) on the surface of the periodontal pathogen *Porphyromonas gingivalis*, work is underway to explore means of constructing a recombinant

adenovirus containing the fimbrillin gene which could be transferred into salivary gland cells. In doing so, the salivary glands would secrete fimbrillin locally and result in the local production of secretory IgA against the fimbrillin protein which in turn may be capable of neutralizing the colonization of dental plaque by *P. gingivalis*. Of course, there are numerous technical and clinical obstacles which will need to be overcome before such an approach becomes accepted.

Other areas where genetic replacement therapy may prove valuable could be in the introduction into the oral environment of genetically manipulated bacteria which no longer express their pathogenic determinants but will still inhabit, in a nonpathogenic manner, a periodontal pocket.

Conclusions

There are many confounding parameters in establishing a genetic link for manifestations of the periodontal diseases as far as the human genome is concerned. Of the many factors which act as risk, modifying or predisposing factors in periodontal disease, some may be heritable, such as diabetes and propensity for tobacco use, whereas others may simply cluster within families or cultures, such as oral hygiene practices and other health beliefs, which will impact on the disease process. Furthermore, classic Mendelian heritability has never been shown for these diseases. Nonetheless, some evidence does exist to implicate the genome in manifestation of adult type periodontal disease, albeit in a secondary manner.

There is more compelling evidence to suggest that the early onset forms of periodontal disease such as juvenile periodontitis and rapidly progressing periodontitis do have a significant genetic component. However, due to the significant genetic and aetiologic variability within these diseases the determination of specific heritable factors has been difficult. In the future, these problems may be overcome by the use of linkage studies which can distinguish identifiable DNA changes without the need to resorting to phenotype expression.

Apart from the above conditions there are numerous examples of severe forms of periodontitis manifesting in several inherited monogenetic or chromosomal disorders. These provide good evidence for a genetic component to some periodontal diseases and allow an insight into the role of the host genome in the manifestation of a multifactorial disease such as periodontal disease.

The role of the microbial genome should not be overlooked in a discussion of genetics and the manifestation of the periodontal diseases. While the host response is necessary for development of disease, the bacteria must still be considered primary aetiologic agents in the development of periodontitis.

The issue of genetics in a common human disease such as the periodontal diseases is very significant and involves aspects of disease aetiology, susceptibility, manifestation and management. In the future, dentists will be challenged to embrace these developments and to implement the discoveries which arise from 'genetic' research.

Dental hard tissues

Amelogenesis imperfecta

Amelogenesis imperfecta can be inherited as an autosomal dominant trait, or in autosomal recessive or X-linked forms. X-linked disorders are characterized by an absence of male-to-male transmission by virtue of the fact that males who are affected must pass on their Y chromosome to their sons. The corollary to this is that any X-linked disorder must be passed on from father to daughter. The degree to which females manifest the trait is variable and much depends upon the detail of the examination and extent of the investigations. Thus, some X-linked disorders, such as haemophilia, are not identifiable in females at the clinical level but are at the biochemical and molecular levels. Conversely, some X-linked disorders, such as X-linked hypohydrotic ectodermal dysplasia, are manifest in females to variable degrees such as reduction in size or absence of upper lateral incisors.

In X-linked amelogenesis imperfecta (XAI) males are fully affected (because of the absence of a compensating normal X chromosome) whereas females are often good examples of lyonization, a phenomenon caused by the inactivation of one X chromosome in each somatic cell during development. A female heterozygous for amelogenesis imperfecta will have vertical markings of the teeth, either vertical ridging in contour or in colour/translucency. The molecular basis of X-linked amelogenesis imperfecta has been reviewed elsewhere.[103]

Briefly, the amelogenin gene was localized to the terminal portion of the short arm of the human X chromosome (as well as to the pericentromeric region of the Y chromosome).[26] Clearly, then, the amelogenin gene was a candidate gene for XAI so that mutations in this gene might cause XAI. Lagerstrom *et al.*[104] established linkage of XAI to the Xp22.1-p22.3 region of the X chromosome, the same region to which the amelogenin gene had been localized. Studies in two unrelated families comfirmed and refined this localization,[105] but also identified a second locus on the long arm of the X chromosome which was responsible for XAI in another family. It was clear, therefore, that there was genetic heterogeneity in XAI with (at least) two

genes on the X chromosome involved in enamel formation.

Nakahori *et al*.[106] identified human genomic DNA sequences with homology between the X and Y chromosomes and established that open reading frames (potential exons) corresponded to the mouse amelogenin cDNA sequence. Using primers based on these sequences, Lagerstrom *et al*.[107] and Aldred *et al*.[108] identified mutations in the amelogenin gene. Since then, an increasing number of mutations in the amelogenin gene have been identified in affected individuals from families with XAI.[103] The gene responsible for the disease resulting from a mutation in the second locus on the X chromosome has not yet been cloned.

Autosomal dominant amelogenesis imperfecta

Autosomal dominant amelogenesis imperfecta (ADAI) has been mapped to the long arm of chromosome 4 in three Swedish families.[109] The gene is located in the same region as that involved in dentinogenesis imperfecta (see later) as well as genes thought to be involved in enamel development. These genes are the albumin gene and the ameloblastin gene. It is not yet clear whether mutations in these genes are responsible for the ADAI in these families. Other families have shown no evidence for linkage to this region indicating genetic heterogeneity in ADAI as well as XAI. Tuftelin is another enamel protein whose gene has been mapped to chromosome 1q (the long arm),[110] but it is not yet certain whether mutations in this gene cause ADAI.

Dentinogenesis imperfecta

Dentinogenesis imperfecta was first identified as a disorder distinct from amelogenesis imperfecta by Finn.[111] Shields *et al.*[112] classified DI into type I (with osteogenesis imperfecta), type II (without osteogenesis imperfecta) and type III (the Brandywine type). DI was mapped to chromosome 4q by linkage analysis with tight linkage to the Gc locus (the Group-Specific Component, now known to be the vitamin D-binding protein) blood group.[113]

Subsequent clinical studies suggested that DI-II and DI-III could occur in the same family and these were regarded as being allelic, that is, DI-III is a variant of DI-II (II).[114] Boughman *et al.*[115] demonstrated that DI-III and juvenile periodontitis mapped to 4q11-q21 and discussed whether DI types II and III might be due to closely linked genes or be allelic. They also questioned whether DI-II and DI-III might represent variable expression of the disease in different families. The observation that dentine phosphoprotein (DPP) levels were increased in DI led MacDougall *et al.*[116] to investigate the possibility that DPP might be a candidate gene for DI. Using a degenerative oligonucleotype probe and a somatic cell hybrid panel they found no evidence that the gene was associated with DI. Crall *et al.*[117] found that DI-II was closely linked to the interferon-induced protein 10 (INP10) on chromosome 4q. The dentine matrix protein 1 (DNP1) gene is expressed in odontoblasts but not in pulp cells or preodontoblasts. The DNP1 gene is localized to mouse chromosome 5q21 which corresponds to human chromosome 4q21, making DNP1 a candidate gene for DI-II.[118]

Other proteins and their corresponding genes are involved in dentine formation. Osteopontin (SPP1) is the principal phosphorylated glycoprotein in bone. A highly polymorphic tandem repeat polymorphism was used by Crosby *et al*.[119,120] to investigate families with DI-II but no mutations in the exons were found, hence it was felt that the mutated SPP1 gene was unlikely as a cause of DI. Aplin *et al*.[121] used a DNP1 repeat sequence to map the locus to 4q21. DI was linked to this locus in two families with a LOD scores of 11.01 (Θ=0.001). DNP1 therefore is a strong candidate gene for DI. At the time of writing, however, the gene involved in dentinogenesis imperfecta has not yet been isolated and characterized.

Dentinal dysplasia is another autosomal dominant form of inherited defects of dentine. There is some question regarding the validity of the type 2 coronal dentinal dysplasia. Genetic studies using molecular biological techniques should enable the elucidation of the classification of these entities.

Dental caries

The genetic basis for dental caries was reviewed by Sofaer.[122] Evidence from experimental caries in rats suggests that there is an approximately 50 per cent genetic contribution to the development of caries.[123-125] Advances in the understanding of dental caries in humans have been limited. Inbreeding studies and investigations of inter-racial breeding have suggested no genetic effect on the DMFT index,[60,126,127] suggesting that recessive genes are unlikely to play a major role in susceptibility to caries.

Twin studies have yielded some evidence for a genetic basis for susceptibility to dental caries, although it must be acknowledged that fluoride (and other environmental factors presumably) can override this genetic influence.[128] MZ twins are more similar than DZ twins in relation to caries experience,[129-131] and this seems to apply particularly to the lower anterior teeth[132] and smooth surface approximal lesions.[133]

The Minnesota Study of Twins Reared Apart (MSTRA) has produced the most convincing

evidence for heritability of caries susceptibility. The MSTRA studies have supported the association between dental caries and genetic background and extended it. The number of teeth present, number of teeth and surfaces restored, number of teeth and surfaces restored or carious on two sides correlated better in MZ than DZ twins.[134] These observations have been supported by further work based on an extended sample of these twins.[135] The susceptibility to caries and other dental hard tissue factors was similar to the assessment of heritability in periodontal disease, IQ, religion, values and attitudes, personality and interests. Yu *et al.*[136] found an association between caries experience and the proline-rich proteins (PRP) in saliva. These PRPs are a complex of eight proteins coded by a region on chromosome 12p. The inheritance of PRPs follows an autosomal dominant mode but no linkage analysis studies have yet been carried out to investigate this further. Various other factors have been invoked as being involved in the susceptibility or otherwise to dental caries,[122] but the evidence for these is questionable. Lehner *et al.*[137] found a differing response to streptococcal antigens depending upon the individual's HLA status. This might indicate a role for the HLA system in the susceptibility or otherwise to caries as well as other dental and medical conditions, although this has not been supported by a clinical study.[138]

Summary

The investigations reviewed in this paper have provided a solid foundation of knowledge about the influence of genetic factors on disorders of craniofacial growth, the oral supporting tissues and the dental hard tissues. Research findings related to craniofacial variation are already influencing approaches to orthodontic management. Advances in the understanding of the genetic basis of amelogenesis imperfecta have been impressive, but progress in understanding dentinogenesis imperfecta has been slow. Although there seems to be increasing recognition of the importance of genetic influences on periodontal diseases and dental caries, application to clinical practice remains limited.

However, as Slavkin[139] and Barnett[140] have emphasized recently, the significant advances in human genetics that are now taking place, should soon enable screening of those individuals at risk and the implementation of targetted preventive measures to provide protection from disease onset.

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