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Genetic study of families affected with aggressive periodontitis

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Periodontitis is a multifactorial disease associated with several risk and susceptibility factors (66). Risk factors are part of the causal chain, or expose the host to the causal chain. The presence of a risk factor directly increases the probability of a disease occurring, and the absence of a risk factor reduces this possibility. Risk factors are modifiable. Contrary to modifiable risk factors, susceptibility factors often refer to nonmodifiable determinants or background factors, such as age, gender and genetic make-up.

It is now evident that genetics form an important aspect in most diseases, including periodontitis. Elucidation of the genetic basis of periodontitis should permit a better understanding of disease etiology, allowing improved classification, diagnosis and treatment of periodontal diseases.

Aggressive periodontitis (formerly termed early-onset periodontitis, subdivided into prepubertal periodontitis, juvenile periodontitis and rapidly progressive periodontitis) is a group of infrequent types of periodontitis, characterized by early age of onset and rapid destruction of the tooth-supporting tissues in otherwise healthy individuals (5, 51). In patients with aggressive periodontitis, exposure to local irritants usually cannot account for the marked alveolar destruction, suggesting that host factors are involved in determining susceptibility to the disease. Although a variety of factors, such as microbial, environmental and behavioral factors, and systemic disease, are suggested to influence the risk of aggressive periodontitis, an individual's genetic make-up is a crucial factor influencing their systemic or host response-related risk (42, 55).

It is commonly believed that family studies remain the best approach for studying possible genetic causes of aggressive periodontitis. Therefore, this review focuses on family studies of aggressive periodontitis and the utility of genetic analytical methods in periodontology.

Familial aggregation

Many studies have indicated that the prevalence of aggressive periodontitis is disproportionately high among certain families (5, 7, 11, 12). In many families, the percentage of affected siblings may reach 40–50% (6, 8, 49, 52, 75, 76, 92), or even higher (50, 80, 95, 96). Recently, our group analyzed 93 Chinese nuclear families with aggressive periodontitis, including 93 probands and 155 relatives (22), and determined the heritability of aggressive periodontitis in Chinese people to be 0.64 (unpublished information, Y. Tian, L. Xu, H. Meng et al.). A previous study by our group indicated that root abnormality could be a local contributing factor for aggressive periodontitis (55, 100). Inherent anatomic and morphologic features of teeth might not only have a significant impact on the management and prognosis of the involved teeth, but may also affect the severity of periodontal destruction (55, 100).

The notable familial aggregation of cases indicates that genetic factors may be important in susceptibility to aggressive periodontitis. Familial aggregation of periodontal disease may also reflect exposure to common environmental factors, including oral hygiene, diet and nutrition, exposure to pollutants and behaviors such as smoking (active and passive). Certain infectious agents may also cluster in families. To determine the evidence for genetic factors in familial aggregation of a trait, more formal genetic studies are required, but until now, the research tools to pursue this have been unavailable (9, 30).

Genetic analysis of inheritance

Three genetic analysis methods can be used to study modes of inheritance: pedigree analysis; segregation analysis; and linkage analysis.

Pedigree analysis

Pedigree analysis is an important method in medical genetics. Most early family studies used a pedigree-analysis method. The increased prevalence of aggressive periodontitis among female family members, and transmission of the disorders through generations in some pedigrees, has been used to support the hypothesis of X-linked dominant inheritance of this form of periodontitis (7, 26, 54, 68, 80, 95). Autosomal-recessive inheritance of aggressive periodontitis has been proposed on the basis of pedigree structural features in which no parents are affected (24, 40). However, autosomal-dominant inheritance based on family studies has been proposed for aggressive periodontitis. Of note here is that these studies were of one or a few families, and even though there were many affected individuals in one family (77, 84), it is difficult to assess the mode of transmission in one family (Table 1).

Pedigree analysis is direct and simple, but it has its shortcomings in that only the pedigree structure and the disease phenotype are considered. Thus, different inclusion criteria among probands and their family members, or incorrect diagnosis, will directly result in inaccurate conclusions. For example, in Sussman & Baer's report (84), a 17-year-old female proband, and her mother and her grandmother, were stated to have juvenile periodontitis. However, the disease of a 50-year-old individual (the mother), with almost all natural teeth remaining and only some 2–3 mm bone loss in the maxilla, cannot be diagnosed as juvenile periodontitis (84).

Segregation analysis

Segregation analysis is a formal method of studying families with a disease to assess the likelihood that the condition is inherited as a genetic trait. Segregation analysis includes simple and complex segregation analysis. Simple segregation analysis is a classical method that compares the observed proportion of affected siblings and offspring with the expected proportion, under a particular genetic hypothesis. Its genetic principle requires that genes are passed from parents to children in a predictable manner, and usually segregate in families as predicted by Mendel's laws (61). Most importantly, segregation ratios are 1:2 in autosomal-dominant mode and 1:4 in autosomal-recessive inheritance.

Melnick et al. (54) described a family in which five of six siblings, three of whom were female, had aggressive periodontitis with a history of early tooth loss in both maternal and paternal forebears. In a second family, four of eight siblings, all of whom were female, had aggressive periodontitis. A segregation analysis was performed on these two families and on another 19 families with aggressive periodontitis reported from six studies found in the dental literature (7, 11, 12, 24, 25, 40). Analysis of these data led Melnick et al. (54) to conclude that aggressive periodontitis was most probably inherited as an X-linked dominant trait with a decreased penetrance of 78%, and the female:male ratio of affected persons was approximately 2:1. Numerous other case reports of families and individuals have supported this conclusion (65, 67, 94).

On the basis of an analysis of 129 first-degree relatives of 31 probands with aggressive periodontitis, and a segregation ratio of approximately 0.25, Saxén (74) proposed an autosomal-recessive model of inheritance for aggressive periodontitis. Another study reported on 30 families in which there were no affected parents and nine affected siblings (of 52 examined), and concluded that aggressive periodontitis may be an autosomal-recessive disease (75). By contrast, approximately 50% of the children in the family suffered from aggressive periodontitis (77), suggesting the possibility of autosomal-dominant inheritance. Recently, using segregation analyses, our group analyzed 73 Chinese nuclear families (a total of 233 individuals) with aggressive periodontitis. The genetic ratio was calculated using an *a priori* method and a theoretical total offspring correction method (48). The result was 0.2495, which was close to the theoretical ratio for autosomal-recessive inheritance. This study indicated that in general the genetic mode for aggressive periodontitis fits an autosomal-recessive inheritance in individuals of Chinese Han heritage. Furthermore, autosomal-dominant inheritance could not be excluded in those families whose parent(s) suffered from severe chronic periodontitis (unpublished information, X.Y. Ren, L. Xu, H.X. Meng, H.S. Zhao, R.F. Lu, Z.B. Chen, X.H. Feng, D. Shi, L. Zhang, Y. Tian).

Complex segregation analysis is a method that compares various genetic and nongenetic models, with support for the best-fitting model being assessed

Table 1. Pedigree analysis in the study of aggressive periodontitis

Author, year of publication (ref. no.)	Subjects	Principal findings
Benjamin et al., 1967 (7)	Twelve families (52 individuals)	XD mode. The female to male ratio was 3:1
Butler 1969 (11)	A family The proband and his sister had JP. His mother and maternal aunt and grandfather had become edentulous at an early age	XD mode
Fourel, 1972 (24)	A family (more than 20 individuals)	AR mode
Jorgenson et al., 1975 (40)	A family	AR mode
Melnick et al., 1976 (54)	Two families Family 1: five of six siblings, three of them female, had JP Family 2: four of eight siblings, all females, had JP	XD mode with decreased penetrance. The female to male ratio was 2:1
Sussman et al., 1978(84)	Proband, 17, female Mother and grandmother were affected	AD mode
Vandesteen et al., 1984 (95)	A family (29 individuals in the pedigree trees) The proband and six siblings all had JP; both parents were edentulous since early adulthood; both maternal grandparents and at least two siblings of the mother had been affected Paternal side: two of the father's siblings were affected, but the paternal grandparents were not affected	XD mode
Spektor et al., 1985 (80)	One large family (40 individuals in the pedigree trees) The proband had JP Father (healthy) mother (affected) Twelve siblings (six affected, six unaffected) Both maternal grandparents of the proband had become edentulous at an early age. Four of 10 siblings of the proband's mother had EOP. The paternal grandparents did not have EOP and periodontitis was not unusually prevalent in the siblings of the proband's father	XD mode
Page et al., 1985 (68)	A family (six individuals) The parents had JP in their teens, and had two affected chidren and two unaffected children	XD mode

AD mode, autosomal-dominant mode; AR mode, autosomal-recessive mode; EOP, early onset periodontitis; JP, juvenile periodontitis; XD mode, X-linked dominant mode.

using maximum-likelihood statistical tests. Using this approach, support for earlier claims of an X-linked dominant transmission for aggressive periodontitis has been refuted (32, 33, 49) and the autosomal-recessive model is preferred (6, 9, 49).

Two large, complex, segregation-analysis studies have been carried out (6, 52). In one study, 227 probands with aggressive periodontitis were included, and a complex segregation analysis on 100 families (comprising 631 individuals in total) was carried out.

The results confirmed that the often-reported female preponderance of aggressive periodontitis appears to be an ascertainment bias. The segregation analysis results were consistent with an autosomal major locus being sufficient to explain the family patterns of aggressive periodontitis in the entire data set, and also in both the Black and non-Black subsets. It was determined that a dominant mode of transmission was most likely, with a penetrance of about 70%. On the other hand, the results of another study, of 28 families with 372 individuals, suggested that the best-fitting model is an autosomal-recessive model (6).

Complex segregation analysis does not necessarily provide the best model. Because it is a comparison between two models, segregation analyses are only as good as the models tested. When comparing genetic models of transmission, genetic characteristics, including mode of transmission (e.g. autosomal, X-linked, dominant, recessive, complex, multilocus, or random environmental), penetrance, phenocopy rates and frequencies for disease and nondisease alleles, are some of the characteristics included in the different models evaluated. If important assumptions of the model being tested are incorrect, this will limit the results.

Segregation analyses of families with aggressive periodontitis appear to support a major locus hypothesis. However, this approach has two major weaknesses. First, it cannot distinguish the nongenetic factors in familial aggregation of a trait which might mimic genetic effects (e.g. transmission of virulent biotypes of bacteria among close relatives); and, second, it has virtually no power to detect heterogeneity and complexity in genetic etiology among different families, such as dominant major genes in one subset of families, recessive major genes in other families and multiple genes of less effect in others (Table 2).

Thus, both pedigree analysis and segregation analysis have shortcomings in that only the pedigree structure and the disease phenotype are considered and not genetic marker polymorphisms.

Linkage analysis

The problems of segregation analysis are overcome by gene-mapping approaches of linkage analysis. Linkage analysis is a technique used to localize the gene for a trait to a specific chromosomal location. This approach is based on the fact that alleles at syntenic gene loci in close proximity on the same chromosome tend to be passed together as a unit (i.e. co-segregate) from parents to their offspring. Such genes are said to be 'linked'. Genetic linkage studies are usually accomplished by collecting pedigrees with affected individuals, and excess allele sharing among affected individuals is sought for some markers. This approach measures the co-transmission of disease and alleles of a marker locus within a family (with different marker alleles 'traveling' with the disease in different families).

Because the precise chromosomal location of the genetic marker is known, when linkage is detected, the gene responsible for the trait can be placed in the vicinity of the linked genetic polymorphism. In this manner, the alternative hypothesis of transmission of shared environmental factors, such as diet, hygiene, or infectious agents, is ruled out, because these factors cannot influence the meiotic segregation of chromosomal regions detected by the marker loci. Linkage can therefore be used to prove the genetic basis of a disease.

For aggressive periodontitis, only a very limited amount of gene-mapping data has been reported. An investigation utilizing this methodology reported linkage of localized aggressive periodontitis to the vitamin D-binding locus on region q of chromosome 4 in a large family from southern Maryland (10). These results, however, were not confirmed in a subsequent study of 19 unrelated families (15 African–American and four Caucasian families) (31). Such data are currently considered to support the existence of genetic heterogeneity in localized aggressive periodontitis forms, and of distinct forms of aggressive periodontitis. Li et al. (46) reported evidence of a gene responsible for localized aggressive periodontitis to be located on chromosome 1q25. To date, a gene of major effect for aggressive periodontitis has not been identified (Table 3).

It is notable that linkage analysis is likely to succeed for simple Mendelian diseases, but not for complex genetic traits, for a variety of reasons (29, 88). A limiting factor in the traditional application of linkage to complex diseases is that complex diseases arise as a result of the combined effect of multiple genes of minor effect. When multiple genes each contribute a small amount to the disease phenotype, traditional parametric linkage studies are much less powerful. Consequently, attention has shifted away from model-dependent parametric linkage analysis to model-free, nonparametric 'association' analysis as an alternative means of locating disease-susceptibility genes, especially because association studies can sometimes detect weaker effects than linkage analysis (35).

Table 2. Segregation analysis in the study of aggressive periodontitis

Author, year of publication (ref. no.)	Genetic analysis	Subjects	Principal findings
Melnick et al., 1976 (54)	Segregation analysis and pedigree analysis	Two families Family 1: five of six siblings, three of them female, had JP Family 2: four of eight siblings, all females, had JP	XD mode
Saxén, 1980 (74)	Simple segregation analysis	Thirty-one families (158 subjects: 60 parents, 64 siblings and 3 children) Neither parents nor the children were affected with JP. 11/64 siblings were affected	AR mode
Saxén et al., 1984 (75)	Simple segregation analysis	Thirty families (60 parents and 52 siblings) No parents were affected; 9/52 siblings were affected	AR mode
Beaty et al., 1987(6)	Complex segregation analysis	Twenty-eight families (372 individuals: 62 had JP, 95 were unaffected and 215 unknown)	AR mode
Long et al., 1987 (49)	Complex segregation analysis	33 families (199 individuals) Compared the likelihoods of 33 kindreds	AR mode
Boughman et al., 1988 (9)	Complex segregation analysis	28 families (372 individuals: 62 had JP,95 were unaffected and 215 unknown)	AR mode
Marazita et al., 1994 (52)	Complex segregation analysis	One-hundred families (631 individuals in total) African–Americans	Dominant mode with penetrance of about 70%
Hodge et al., 2000 (37)	Segregation analysis and linkage study	A large family (40 individuals in the pedigree trees) North European Caucasians	Dominant mode

AR mode, autosomal-recessive mode; EOP, early onset periodontitis; JP, juvenile periodontitis; XD mode, X-linked dominant mode.

Association analysis

Population-based association studies

Association analysis is another gene-mapping approach. It has two subtypes: population-based association tests; and family-based association tests (34).

Traditionally, disease-marker associations have been detected using population-based association tests, which compare the frequency of the marker allele in affected cases vs. those in unrelated controls (with patients and controls chosen at random from the population). Accordingly, this population-based association analysis is traditionally called case–control association design.

In contrast to linkage studies, which look for co-inheritance of chromosomal regions with disease in families, association studies look for differences in the frequency of genetic variants between unrelated affected individuals and controls, with the expectation that a risk-conferring variant and/or alleles on the disease haplotype will be more common in affected individuals (15, 70). Thus, a positive association can occur for three different reasons: first, the allele itself is a cause of the disease; second, the allele is in linkage disequilibrium with a susceptible allele at the disease gene; and, third, population subdivision and admixture (sampling, or statistical artifact) may lead to disease association, even in the absence of association. The most important principle of associate analysis is linkage disequilibrium, meaning that a specific allele at the linked locus is more commonly found in individuals affected by the disease. It is now generally understood that, as a consequence of selection, random genetic drift, co-ancestry or gene flow, alleles at

Table 3. Linkage and family-based association study of aggressive periodontitis

Author, year of publication (ref. no.)	Genetic analysis	Subjects	Principal findings
Boughman et al., 1986 (10)	Linkage study	A large, five-generation family from southern Maryland (more than 70 individuals)	An AD mode of JP: its localization to chromosome 4 and linkage to dentinogenesis imperfecta and Gc
Hart et al., 1993 (31)	Linkage study	Fifteen African–American and four Caucasian families (228 individuals)	Exclusion of the 4q candidate region for EOP
Li et al., 2004 (46)	Linkage study	Four African–American families (28 subjects)	L-AgP is linked to human chromosome 1q25
Diehl et al.,1999 (16)	Family-based association study	Twenty-eight African–American and seven Caucasian–American families (285 individuals with DNA available for 246)	Allele '1' of both interleukin-1A-889 and interleukin-1B +3953 was transmitted more frequently with the EOP phenotype
Reproduced with permission from Ren et al., 2009 (71)	Family-based association study	Seventy-three nuclear families (DNA available for 204 subjects) from China	Allele A of S100A8 gene SNP rs3795391 (A / G) showed significantly preferential transmission to the AgP-affected individuals in the additive genetic model

AD mode, autosomal-dominant mode; EOP, early onset periodontitis; JP, juvenile periodontitis; L-AgP, localized aggressive periodontitis; SNP, single nucleotide polymorphism.

different loci may not be randomly associated with each other in a population.

Because case–control studies have the advantage of easy sample collection of unrelated affected individuals and controls, this approach has been used extensively in periodontology. Kornman et al. (44) first reported an association between polymorphisms in the genes encoding interleukin-1 and an increased severity of chronic periodontitis. Subsequently, many gene polymorphisms, such as interleukin-6, interleukin-4, interleukin-10, tumor necrosis factor-alpha, Fc-γ receptors, human leukocyte antigen, vitamin D receptors, *N*-formylpeptide receptors and S100A8, have been investigated as possible markers of increased susceptibility to aggressive periodontitis (27, 36, 41, 45, 47, 60, 78, 83). Conflicting results have been presented regarding the relationship between the genotype of these genes and susceptibility to aggressive periodontitis in different populations. These results may reflect the genetic heterogeneity of aggressive periodontitis, of distinct forms of aggressive periodontitis, but these studies may not exclude the possibility of false positives as a result of population stratification, which is a major limitation of the case–control design owing to the unrelated control individuals not being well matched to cases. Nonetheless, there are many methods available which have the ability to overcome the shortcomings of case–control designs, and these are discussed in more detail under the next two headings.

Family-based association studies

The transmission disequilibrium test is a new method of genetic epidemiology (81). This method is a family-based experimental design, selecting pedigree members as controls and using disease and marker data within families. Thus, this approach avoids potential pitfalls caused by the mismatching of case and control groups or by the admixture of subpopulations consisting of different racial or ethnic groups.

The main principle of the transmission disequilibrium test requires counting the number of transmissions of allele 1 vs. allele 2 from heterozygous parents to affected offspring. Under the null hypothesis that the polymorphic marker has no effect on disease risk, the two alleles are expected to be transmitted with equal frequency. Deviation from the 1:1 expected ratio of transmission indicates that the marker is in linkage disequilibrium with the disease. This means that the marker polymorphism itself, or another DNA site in very close proximity to the marker, influences the risk of developing the disease. In addition, theoretical analyses indicate that this approach may be much more powerful than tradi-

tional linkage-based approaches for detecting alleles of relatively small effect, such as a two fourfold increase in disease risk (72).

To date, only very limited family-based association studies of aggressive periodontitis have been reported. Diehl et al. (16) studied the association between genetic polymorphisms at interleukin-1alpha and interleukin-1beta and aggressive periodontitis in 28 African–American families and seven Caucasian–American families. They obtained highly significant evidence of linkage disequilibrium for both African–American and Caucasian–American subjects with generalized aggressive periodontitis. A similar trend was noted for localized aggressive periodontitis. Disequilibrium with generalized aggressive periodontitis was equally strong for smoking and nonsmoking subjects. Interleukin-1alpha and interleukin-1beta polymorphisms were found to be in strong disequilibrium with each other in Caucasian–Americans, but not in African–Americans. These findings indicated that aggressive periodontitis is a complex, oligogenic disorder, with interleukin-1 genetic variation contributing an important, but not exclusive, influence on disease risk. While this is an important family-based association study, a limitation is that the authors applied three different transmission disequilibrium tests to the analyses of their family data using three different computer programs, deleting all families with a missing parent, and therefore suffers a considerable loss of power for data sets. Furthermore, because of the low heterozygosity level of the interleukin-1 markers studied, only a limited number of their subjects were informative for transmission disequilibrium analysis. Thus, most of their tests were performed using SIB-PAIR, a program for elementary genetical analyses, in which only the data of the sibships can be used (16).

Our group first reported an association between S100A8 genetic polymorphisms and aggressive periodontitis in 73 Chinese nuclear families, including 204 individuals (71). The study indicated that there was a statistically significant association of the single nucleotide polymorphism of S100A8 rs3795391 with aggressive periodontitis in the additive genetic model. Allele A (a major allele) showed significantly preferential transmission to the aggressive periodontitis-affected individuals. This study was the first to use the novel genetic analysis method, Family-Based Association test software (http://www.biostat.harvard.edu/~fbat/default.html), in periodontology.

The Family-Based Association test is an extension of transmission disequilibrium analysis and can deal with any type of pedigree structure, including incomplete nuclear-families (i.e. families without parents or families with only one parent). The statistical results are based on the number of informative families in which there should be at least one heterozygous parent (Fig. 1). Of importance is that the Family-Based Association test statistic is based on the distribution of the offspring genotypes being conditional on any trait information and on the parental genotypes, not on the parental trait information. Therefore, problems of diagnosis of aggressive periodontitis in older individuals can be better managed. This is helpful because it is well recognized that genetic studies of aggressive periodontitis are hampered by many factors, including a variable onset of the trait, lack of phenotypic information for edentulous family members or the problems of diagnosis in older individuals (9, 42). Many genetic studies, especially segregation analysis and linkage studies of multigenerational pedigrees, have been flawed by having to use different inclusion criteria among probands and their family members (16, 37, 52).

Twin models

Twin studies have been invaluable in studying the genetic basis of simple and complex traits. Monozygous twins, who arise from a single fertilized ovum, are therefore genetically identical. Dizygous (20) twins arise from two separate fertilized ovum, and share, on average, 50% of their descendent genes in the same way as siblings do. The terms concordance and disconcordance are often used in twin studies. Any discordance in disease between monozygous twins must be caused by environmental factors, while discordance between dizygous twins could arise from environmental and/or genetic variance. One assumption of the classic twin study-design is that monozygous and dizygous twins have similar environment variances. Thus, for a trait predominantly environmental in origin, the concordance rates of monozygous and dizygous twins would be expected to be similar. However, if a disease has a genetic background, concordance rates would be greater in monozygous twins than in dizygous twins. Therefore, the difference in concordance between monozygous and dizygous twins for a particular phenotype can be used to estimate the relative contribution of genes (heredity) and environmental factors to a disease.

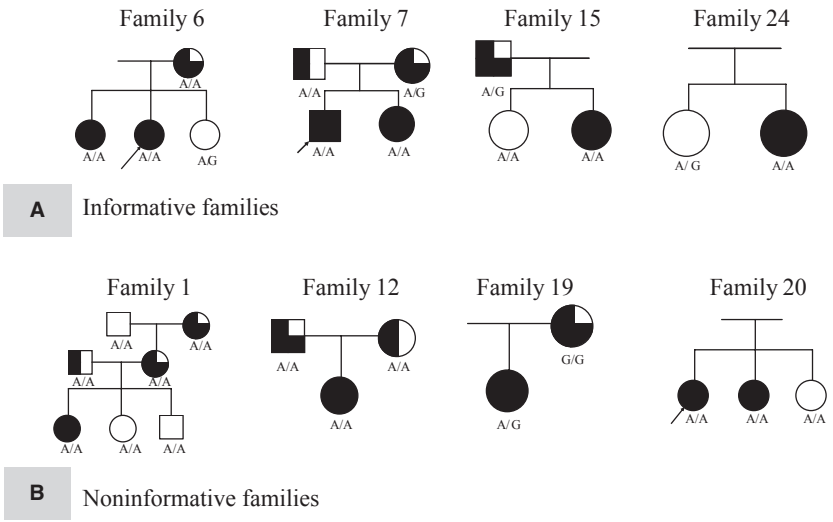

Fig. 1. Typical family trees with genotype of the single nucleotide polymorphism rs3795391 of S100A8 being marked for each individual. Squares designate males and circles designate females. The proband is indicated with an arrow in families that have more than one member with aggressive periodontitis. Parents with gingivitis and mild chronic periodontitis were designated as periodontally healthy. (A) Informative families. (B) Noninformative families. Symbols: □○, healthy periodontium; ■●, affected with aggressive periodontitis; ◧◔, adult affected with severe periodontitis; ◧◐, adult affected with moderate periodontitis. From Ren et al.(71).

For periodontal diseases, all twin studies have studied the more prevalent forms (chronic periodontitis and chronic gingivitis) (13, 56–59). Heritability estimates indicated that 38–82% of the population variance for measures of periodontal disease may be attributed to genetic factors. Chronic periodontitis was estimated to have approximately 50% heritability. However, to date, no twin study on aggressive periodontitis has been reported.

Family studies of periodontal pathogens

Bacteria play an essential role in the etiology of periodontitis. The intrafamilial transmission of periodontal pathogens may explain, in part, a familial aggregation of aggressive periodontitis and may have important prophylactic and treatment implications. Most bacterial species isolated from subgingival plaque are indigenous to the oral cavity: *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are detected infrequently in periodontal health, which makes these species prime candidates for using to study person-to-person transmission.

It has been shown that periodontal pathogens are not restricted to subgingival areas, also being found in the saliva, supragingival plaque and various mucous membranes in patients with periodontitis (14, 21, 62, 99). It seems likely that saliva and direct mucosal contact are the main transmission routes of periodontal pathogens. In a large study on the transmission of *P. gingivalis* within families, this microorganism was found, using the polymerase chain reaction (PCR), in 564 members of 104 multigeneration families. It was noted that a *P. gingivalis*-colonized mother resulted in a risk for colonization of their child that was 4.7 times greater than for a child with a mother who was not colonized with *P. gingivalis*. This risk was 2.98 for colonized fathers. The risk for colonization was highest for a child when both parents were colonized with *P. gingivalis* (90). Umeda et al. (91) observed that *Tannerella forsythia*, *Prevotella intermedia* and *Prevotella nigrescens* were detected more frequently in children whose parents were positive for these pathogens than in children whose parents were negative. In a study on 113 pairs of children and their mothers, it was reported that the detection rate of the red-complex species (*P. gingivalis*, *T. forsythia*, *Treponema denticola*) in children whose mothers possessed the same species was significantly higher than in those whose mothers did not possess them (86). Thus, if a child harbors one of the periodontopathogens then at least one of the parents is also likely to be positive for the same bacterium (64). Recently, in a study of 78 Japanese children (3–9 years of age, including 10 siblings) and their mothers for the occurrence of 11 species of periodontal bacteria, a high consistency of colonization

with *P. gingivalis*, *T. denticola*, *P. intermedia* and *P. nigrescens* in nine of the 10 siblings was found. Kappa statistical analysis revealed that the detection of *Capnocytophaga gingivalis*, *Capnocytophaga ochracea*, *Campylobacter rectus* and *T. denticola* in children was consistent with that in their mothers (43). Studies from our group have found that the prevalence of *A. actinomycetemcomitans* is low in Chinese populations. However, if *A. actinomycetemcomitans* is detected in probands with aggressive periodontitis, its prevalence is also high (75%) in family members of these probands (unpublished information, X. Feng, L. Zhu, L. Xu, H. Meng et al.).

Genotyping of *A. actinomycetemcomitans* within families

Detection of the same bacterial species in family members does not prove transmission. For further evidence, typing of the bacterial isolates is necessary. Therefore, the transmission pathway may become clearer if serotyping or genotyping methods (arbitrarily-primed PCR, amplified fragment length polymorphism, pulse-field gel electrophoresis, PCR-single strand conformation polymorphism (SSCP), etc.) were employed to distinguish the serotype or genotype of the same bacterial species (3, 4, 19). For genotyping, usually the isolation of target bacteria is necessary. Using the immunodiffusion technique it has been reported that the child always harbored the same serotype of *A. actinomycetemcomitans* as the parent (1). Furthermore, different individuals within the same family sometimes show only one type of *A. actinomycetemcomitans* restriction fragment length polymorphism. When members among the same family showed two types of restriction fragment length polymorphism, children were always infected with the *A. actinomycetemcomitans* strains found in at least one of the parents (18). Using arbitrarily primed-PCR to genotype *A. actinomycetemcomitans* isolates from family members, 11 of 12 families had identical *A. actinomycetemcomitans* genotypes among family members (4, 69). This suggests transmission of this microorganism among family members (4). Recently, it has been observed that in the families of patients with aggressive periodontitis, the likelihood of intrafamilial transmission of *A. actinomycetemcomitans* was statistically significant (the child proband shared the *A. actinomycetemcomitans* clonal types with their parents in five of six families and with their siblings in three of six families). Thus, parents and siblings of an individual with *A. actinomycetemcomitans*-positive aggressive periodontitis may have increased susceptibility to periodontitis (19). In contrast to these findings, several studies found no evidence for the vertical transmission of periodontal pathogens between parents and children. For example, a comparison of the PCR-generated amplitypes of *A. actinomycetemcomitans* has shown that there is a wide distribution of amplitypes among the probands and immediate relatives (87). No clear transmission paths were observed in this specific population.

Genotyping of *P. gingivalis* within families

Although *A. actinomycetemcomitans* is considered as a major etiologic agent of aggressive periodontitis (79), several studies of Asian populations have observed that *P. gingivalis* is detected more frequently than *A. actinomycetemcomitans* in patients with aggressive periodontitis (23, 38). This suggests that *P. gingivalis*, rather than *A. actinomycetemcomitans*, is a predominant periodontal pathogen in these populations. Vertical transmission of *P. gingivalis*, based on genotyping isolates from parents and children, has rarely been observed. However, some studies have shown that horizontal transmission of *P. gingivalis* between spouses exists (3, 73, 82). It has also been reported that the clonal stability of *P. gingivalis*, under natural conditions, is high and that the transmission of *P. gingivalis* occurs frequently among siblings but not among spouses (98).

Data from our study on Chinese populations showed that 10 of 22 (45.5%) probands with aggressive periodontitis shared the same *P. gingivalis* fimA genotype with their families. Of these 10 families, nine harbored the fimA genotype, implying that the fimA genotype may play an important role in the transmission of *P. gingivalis* between family members (unpublished information, L. Zhu, X. Feng, L. Xu, H. Meng et al.).

Periodontitis is a multifactorial disease associated with a complex microflora, so the issue of bacterial transmission is very complicated. Identical genotypes in family members are not 100% proof of transmission, as there is no definitive number of genotypes and finding identical genotypes may occur by chance. Accordingly, the intrafamilial transmission of periodontal pathogens may play a less important role than host genetic factors in the familial aggregation of aggressive periodontitis.

Clinical, microbiological and immunological studies of families with aggressive periodontitis

It has been widely reported that neutrophil dysfunctions are associated with the pathogenesis of aggressive periodontitis (28, 39, 63, 92–94, 97). The role of the specific host immune response to bacteria in the pathogenesis of aggressive periodontitis has also been investigated extensively during the last two decades. The main finding has been a significant elevation in the serum immunoglobulin G (IgG) levels to certain periodontal pathogens, particularly *A. actinomycetemcomitans* and *P. gingivalis*, in subjects with aggressive periodontitis compared with healthy controls.

Several comprehensive family studies of aggressive periodontitis have been reported. Page et al. (68) studied a family with a high prevalence of aggressive periodontitis and found depressed neutrophil chemotaxis in all affected subjects (including both parents and two affected children), but not in the unaffected family member. The results showed that the presence of disease correlated strongly with abnormal neutrophil chemotaxis and the presence of serum antibodies reacting with *A. actinomycetemcomitans*. Numerous other case reports on families and individuals support this conclusion (28, 63, 76). By contrast, Vandesteen et al. (95) showed variability of clinical and laboratory test phenotypes among relatives in one family with aggressive periodontitis. None of the members of the studied family had *A. actinomycetemcomitans* in their periodontal pockets, or serum antibodies to *A. actinomycetemcomitans*. The pocket flora were predominantly gram-negative, anaerobic rods with a high prevalence of *Bacteroides* species, and serum antibodies specific to *Bacteroides* species were detected in the sera of five of the seven patients studied. There was a relatively good correlation between the bacterial species isolated from the periodontal pockets and the antibodies found in the serum. They suggested that a pocket flora in which *A. actinomycetemcomitans* is predominant, and the presence of serum antibodies to *A. actinomycetemcomitans*, may not be universal features of aggressive periodontitis. Although abnormal leukocyte chemotaxis was generally common in patients with aggressive periodontitis, in this family no correlation between this defect and the presence of these diseases was found. The observed variability may result from a different mechanism than the more commonly reported neutrophil dysfunction.

In later studies, Lopez (50) examined a consanguineous family with localized aggressive periodontitis and generalized aggressive periodontitis. The author found that all patients with localized aggressive periodontitis had depressed polymorphonuclear neutrophil chemotaxis, while patients with generalized aggressive periodontitis had normal polymorphonuclear neutrophil chemotaxis. This study indicated that the underlying cause of generalized aggressive periodontitis was not always related to leukocyte dysfunction. In this family, because *A. actinomycetemcomitans* was the pathogen most commonly found in the subgingival microflora of the patients with generalized aggressive periodontitis, it was assumed that *A. actinomycetemcomitans* may play a key role in the etiology of aggressive periodontitis.

Neutrophil defects in aggressive periodontitis have been well studied. In a comprehensive study of 22 families of probands with localized aggressive periodontitis, Van Dyke et al. (92) reported that all siblings affected with localized aggressive periodontitis had a neutrophil abnormality, indicating that a high proportion of the localized aggressive periodontitis was attributable to neutrophil defects. Boughman et al. (8) studied neutrophil chemotaxis and serum antibodies to *A. actinomycetemcomitans* in 39 sibship families with 116 individuals. In the study, all individuals of 14 sibships had localized aggressive periodontitis; all individuals of 14 other sibships had generalized aggressive periodontitis; and 11 sibships had at least one sibship with each form of periodontitis. The associations of disease with these risk factors (neutrophil chemotaxis and serum antibodies to *A. actinomycetemcomitans*) were stronger in the localized aggressive periodontitis-only sibship families. These results may reflect the genetic heterogeneity of aggressive periodontitis, in particular, of distinct forms of aggressive periodontitis (localized aggressive periodontitis and generalized aggressive periodontitis). To date, it is generally regarded that the underlying cause of localized aggressive periodontitis is related to leukocyte dysfunction in certain races, and there is a relatively good correlation between depressed neutrophil chemotaxis and the presence of serum antibodies reacting with *A. actinomycetemcomitans*.

Marazita et al. (53) examined the IgG2 levels (the predominant antibody to *A. actinomycetemcomitans*) in 123 families with aggressive periodontitis and in 508 unrelated control individuals with nonaggressive periodontitis. They found that the IgG2 levels were elevated in individuals with localized aggressive

periodontitis but not in individuals with generalized aggressive periodontitis; and that the IgG2 levels in African–Americans were higher than in Caucasians, regardless of the aggressive periodontitis status. Segregation analysis under the regressive-model approach of Bonney was applied to analyze IgG2 levels for evidence of major locus segregation. After adjusting for localized aggressive periodontitis status, race, gender and age, the best-fitting model was an autosomal co-dominant major-locus model (accounting for approximately 62% of the variance in IgG2). In a study of 17 Caucasian and 43 African–American families with two or more members affected with localized or generalized aggressive periodontitis (274 subjects with IgG2 measurements), only 16% of the variance in IgG2 was attributable to age, race and smoking (17). Even with the addition of localized aggressive periodontitis, the model explained only 19% of the IgG2 variance. By contrast, heritability of IgG2 levels was estimated to be 38% and highly significant, demonstrating a substantial genetic basis. Furthermore, bi-trait variance-component analyses of IgG2, and quantitative measures of aggressive periodontitis, indicate that different genes appear to control IgG2 levels and disease susceptibility.

There are several other interesting reports on microbiological agents and lymphocyte dysfunctions that should be considered in relation to the progression of periodontal disease (2, 85). Takahashi et al. (85) found high antibody titers to *A. actinomycetemcomitans*, *P. gingivalis* and *C. rectus* in the serum of the proband. High serum antibody titers to *P. gingivalis* were found in the mother, and to *C. rectus* in the unaffected sister, compared with the healthy control patients. Interestingly, increased serum antibody titers to *P. gingivalis* were found in the mother, although her teeth had been extracted 6 years previously. This result was similar to that reported by Vandesteen et al. (95), in which the edentulous parents of the proband showed high antibody titers to *Eikenella corrodens* in the mother, and serum antibody to *Bacteriodes intermedius* and *Capnocytophaga spullgena* in the father, although they had lost their teeth 15 or 22 years previously. These findings were unexpected because one would not expect to find serum antibodies to bacteria that had previously inhabited the periodontal pockets of extracted teeth. Various possibilities have been considered to explain these findings. Perhaps these individuals were being exposed to these bacteria other than via the periodontal pockets. Alternatively, serum antibody to certain periodontal pathogens may persist for much longer than previously thought, antigenic determinants of these bacteria may cross-react with those of other bacteria to which these individuals were exposed, these individuals may have had an abnormal hyper-reaction to specific antigens. Another possibility may be that some unknown factors may be involved in the host response which contributes to the maintenance of high antibody titers. Arai et al. (2) examined a family consisting of a mother with aggressive periodontitis, an elder daughter with localized aggressive periodontitis and a younger daughter with simple gingivitis. All subjects exhibited high T4/T8 ratios and high IgG titers to *A. actinomycetemcomitans*. These results indicate that lymphocyte dysfunctions may be considered in relation to the progression of periodontal disease.

Trevilatto et al. (89) reported the clinical, microbiological and genetic profile of a 14-member Brazilian family with aggressive periodontitis. The proband had detectable levels of *A. actinomycetemcomitans*, *P. gingivalis*, *Bacteroides forsythus* and *Treponema denticola*. Allele 2 of the interleukin-1alpha (−889) polymorphism was found in all individuals, as was allele 1 of the interleukin-1beta (+3953) gene. Alleles 1 and 2 (50% each) of interleukin-1beta (−511), allele 1 of tumor necrosis factor-alpha (−308) and allele 2 (in homozygosity or heterozygosity) of the interleukin-RN (intron 2) gene were present. No evidence was found to suggest that the present microbiological and genetic parameters were related to the prediction of periodontitis susceptibility in the family. Although there were many affected individuals within this family, it must be recognized that this study, in only one family, has very low power to explore the association between genetic profile and susceptibility of periodontitis.

Summary and conclusions

There are numerous reports in the literature on familial aggregation of aggressive periodontitis. The prevalence of aggressive periodontitis is very high among certain families: the percentage of affected siblings and affected pedigree members may reach 40–50%, or even more, indicating that genetic factors may be important in the susceptibility to aggressive periodontitis.

Some types of aggressive periodontitis seem to be inherited in a Mendelian manner. Views as to whether the transmission of the trait is X-linked dominant, autosomal-recessive or autosomal-dominant have been the subject of much debate in the literature. In most early reports, the increased prevalence

among females, and transmission of the disorders through generations in pedigrees, has been used to support the hypothesis of X-linked dominant inheritance. By segregation analysis (especially complex segregation analysis), earlier support for X-linked dominant transmission has been refuted, and an autosomal-recessive model was preferred. However, some instances of intergenerational transmission suggest the possibility of a dominant inheritance. These studies may reflect the heterogeneity and complexity in genetic etiology among different families, which is consistent with the fact that human diseases and syndromes with a similar clinical appearance are known to result from different mechanisms. Of interest is that in both pedigree analysis and segregation analysis, only the pedigree structure and the disease phenotype are considered and no consideration is given to genetic marker polymorphisms.

The problems of segregation analysis are overcome by gene-mapping approaches of linkage and association analyses. To date, considerable effort has been expended to identify gene polymorphisms associated with the risk for periodontal diseases. Several localized aggressive periodontitis loci on chromosomes 1, 4, 6 and 9 have been identified by linkage analysis. Reports of population-based genetic polymorphisms associated with periodontal disease are increasing, and only very limited family-based association studies of aggressive periodontitis have been reported. Family-based experimental designs, selecting pedigree members as controls and using disease and marker data within families, avoids potential false-positive results caused by the mismatching of case and control groups or admixture of subpopulations.

To date, it has been commonly reported that the underlying cause of localized aggressive periodontitis is related to leukocyte dysfunction in certain races, and there is a relatively good correlation between neutrophil abnormality and the presence of serum antibodies reacting with *A. actinomycetemcomitans*.

In conclusion, aggressive periodontitis is a multifactorial, genetically complex disease. Complex diseases are the result of many accumulative susceptibility factors (including genetic make-up, innate immunologic deficiency and serum antibodies reacting with specific microflora). Environmental factors (such as oral hygiene and smoking) are usually etiologically important. Although many factors, including a variable onset of the trait, lack of phenotypic information for edentulous family members or the problems of diagnosis in older individuals, may influence genetic studies of aggressive periodontitis, the assessment of within- and among-family variability remains the best approach for identifying possible causal mechanisms and sources of heterogeneity. Identification of susceptibility factors for aggressive periodontitis will shed light on the underlying genetic mechanisms. Such information is important for the design of new treatment procedures to prevent or slow down the development of aggressive periodontitis.

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