Gene polymorphisms in periodontal health and disease

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Gene polymorphisms are locations within the genome that vary in sequence between individuals and are very prevalent, affecting at least 1% of the population (104). Polymorphisms of human genes occur at one or more of the following sites (Fig. 1):

1. the promoter or 5'-flanking region;
2. the exon(s) or the gene coding regions;
3. the intron(s) or the gene intervening regions;
4. the 3'-untranslated region (69).

The most common form of polymorphisms is the single nucleotide polymorphism, which is a change in a single base pair (bp) in the genomic DNA (104). Single nucleotide polymorphisms can affect gene function. For example, a single nucleotide polymorphism located in a promoter region may influence the amount of mRNA produced. Another class of polymorphism is the simple sequence repeats, of which the common forms are the dinucleotide and trinucleotide repeats (70). The variable number of tandem repeats can also influence the function of a gene, but the repeats are more likely to be linked with a functional polymorphism elsewhere in the gene. A third category of gene polymorphism involves insertions or deletions (116). Insertions and deletions can be as small as 1 base, in which case they may also be classified in the category of single nucleotide polymorphisms, but can also consist of a few bases, one or more exons, or even a whole gene.

Single nucleotide polymorphisms in disease

When the human genome project was completed and published, a new era of human genetics was born (65). This resource now permits the analysis of genes and their variants in any number of human conditions. Indeed, the potential to associate gene variations with disease and disease susceptibility is highly attractive. Initial studies indicate that the most common source of human genetic variation is found in single nucleotide polymorphisms. Thus, it may make considerable sense to use single nucleotide polymorphisms for more accurate diagnosis of diseases as well as improving prognostic processes and even developing novel therapies (119).

Although traditional linkage analyses have been used to identify gene loci in classical single gene inherited disorders, such approaches have generally failed to be of much use for the more complex multifactorial diseases such as diabetes, cardiovascular disease, rheumatoid arthritis and, of course, periodontitis. Often these complex diseases have multiple gene associations which individually have weak effects but which collectively combine with other influences, such as environment factors, result in variable disease manifestation (118).

Single nucleotide polymorphisms within candidate genes may be causally related to changes in protein expression, structure and function. These, in turn, may lead to variations in phenotypic expression. This may be a useful development, but large scale screening for single nucleotide polymorphisms is still a very complex undertaking (11). Alternative approaches include selective targeting of candidate genes coding for particular features of a disease. One such example is the targeting of genes considered to be specifically related to inflammatory cell function in inflammatory diseases. Known as the candidate pathway approach, this involves the study of genes based on prior knowledge of the disease in relation to its phenotype (141). Even using this approach one can envisage probing hundreds, if not thousands, of genes participating in the plethora of inflammatory pathways. Clearly, there will be a heavy reliance on genomic informatics to make this
work and to establish this as a viable mode of investigation (102).

The candidate pathway approach to identifying single nucleotide polymorphisms associated with disease can be via indirect or direct candidate association. Although the indirect method, such as tagging single nucleotide polymorphisms to study potential genetic etiologies of complex diseases, is used, considerable problems are still encountered with this approach. The main criticism of this method is the assumption that allelic heterogeneity within target loci is very small and thus disease susceptibility is due to a small number of well-conserved single nucleotide polymorphisms occurring at a high frequency in all populations (116). Unfortunately, this clearly is not always the case and this common disease/common variant paradigm is flawed if the disease is complex and results from multiple rare variants at numerous loci (114). Although improvements in single nucleotide identification methods and discovery are improving, it is not yet possible to evaluate all of the possible nucleotide variations in gene coding and regulatory regions in all individuals to allow direct association studies to be carried out. At present, direct association studies are limited to studying specific single nucleotide polymorphisms whereby a relationship is tested between proposed functional variants and known disease risk.

The field of single nucleotide polymorphism discovery is moving at a very fast pace; however, current methods are still unable to cope with large scale population-based genetic screening. At present, prescreening methods can distinguish the alleles of single nucleotide polymorphisms, but without always identifying their precise position or the base pair which is affected. Once a novel single nucleotide polymorphism is identified, the fragment containing the variant sequence must be sequenced. Such prescreening methods have been quite useful in identifying rare single nucleotide polymorphisms, as well as finding polymorphisms or mutations in human populations suffering from defined diseases. Sequencing is still considered the gold standard for prescreening purposes, but other methods based on conformation (strand–strand conformation polymorphism, cleavage fragment length polymorphism, conformation-sensitive gel electrophoresis) or melting (denaturing high-performance liquid chromatography, denaturing gradient gel electrophoresis two-dimensional gel scanning) have been used, with accuracy levels ranging from quite low to very high.

Differences of more than 3 million nucleotides can be seen when comparing the genomes of two individuals as a result of single nucleotide polymorphisms (130). Thus, there is considerable work to be done not only to unravel single nucleotide differences but also to correlate them with disease.

**Single nucleotide polymorphisms in inflammatory diseases**

Inflammation is a complex process that develops following initial tissue trauma and is completed with induction of tissue repair. The innate immune system is of central importance to the initiation, progression, and containment of the inflammatory response. Hence, genetic variations that disrupt innate immune sensing of tissue injury could explain individual differences in the ability of the immune system to respond to tissue injury, the diversity of the clinical presentation of inflammation, and the response to current medical treatment (6). Such genetic variations may identify patients at high risk for the development of abnormal inflammatory responses. The single base variations, known as single nucleotide polymorphisms, are the most commonly used variants. There has been great interest in exploring single nucleotide polymorphisms in those genes involved in the control of the inflammatory cascades. The rationale for studying gene single nucleotide polymorphisms in such conditions is that it can be used to identify potential markers of susceptibility, severity, and clinical outcome (6, 151). Accordingly, such an approach might be useful in identifying potential markers for responders and nonresponders to various medications and therapies as well as help identify targets for therapeutic intervention.
To date, many studies have focused on genes and polymorphisms in the regulation of the immune and inflammatory systems. All of these studies have been based on the direct candidate association approach.

**Single nucleotide polymorphisms in periodontal disease**

In general a person’s genetic background influences their susceptibility to many kinds of diseases and conditions. Periodontitis, a chronic inflammatory disease, caused by gram-negative microorganisms in the periodontal pockets, is no exception. Many reviews have been published in recent years supporting the evidence that genes influence an individual’s predisposition for the initiation and progression of periodontal disease (75). In this review we describe studies conducted to reveal polymorphic genes related to the severity or progression of periodontitis, and suggests future directions and strategies for genetic research in periodontal disease.

For this concise review, 140 original articles were selected from those retrieved in a PubMed search using key words such as periodontitis, genetic analysis, genetic study, human leukocyte antigen (HLA), and other related words. These reports were divided into six groups:

- HLA (1, 2, 4, 8, 15, 28, 33, 55, 56, 68, 77, 92, 103, 105, 106, 108, 117, 135, 145, 147);
- immuno-receptors (14, 16, 34–36, 47, 57, 72, 78–82, 91, 95, 136, 137, 153, 155–157);
- proteases (24, 26, 60, 62, 66, 67);
- structural molecules (19, 21, 22, 48, 49, 51, 53, 64, 138, 142, 143, 148);
- others (9, 37, 50, 63, 74, 83, 90, 96, 100, 121, 128, 139, 140, 158, 159).

Many investigators predict that information concerning polymorphism will be useful in the prevention and therapy of periodontitis, as well as in the recognition of patients in need of more comprehensive therapy. The early identification of risk factors for the development of periodontitis may also form the basis for more focused and cost-effective preventive approaches. This review also discusses the potential of comprehensive genetic analyses to decipher the relationship between periodontal disease and environmental factors. Table 1 summarizes the overall findings of these studies and depicts the number of studies which have demonstrated a correlation between a particular polymorphism and periodontal disease. Clearly, there is considerable variation in results and this highlights the need for further uniformly planned case control studies with adequately numbers of matched subjects as well as appropriate follow-up times. Information from such studies would be expected to help with the identification of individuals with increased susceptibility to periodontitis. Ultimately, such research could lead to the rational use of cytokine modulating therapies.

### Table 1. Results of literature search

<table>
<thead>
<tr>
<th>Category (n)</th>
<th>Gene (n)</th>
<th>Correlation (n)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
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<tr>
<td>Cytokine (66) and its receptor</td>
<td>IL-1 (36)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>TNF (14)</td>
<td>6</td>
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<td>HLA allele (20)</td>
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<td>Immuno-receptor (21)</td>
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</tr>
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<td></td>
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<tr>
<td>Others (15)</td>
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<td>9</td>
</tr>
<tr>
<td>Total (140)</td>
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<td>76</td>
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To summarize findings on published gene polymorphisms and periodontitis, 140 original articles were selected from those retrieved from a PubMed search using appropriate keywords such as ‘periodontitis’ and ‘gene analysis’. The articles selected were divided into six categories: Cytokine, HLA allele, Immuno-receptor, Protease, Structural molecule, and Others.

IL-1, interleukin 1. MMP, matrix metalloproteases. Positive, polymorphisms of target genes correlate with periodontal outcome. Negative, polymorphisms of target genes do not correlate with periodontal outcome. n, number of studies found in literature.

### Cytokines

#### Interleukin-1

Polymorphisms of the interleukin (IL)-1 gene have been proposed as potential genetic markers for periodontal diseases (Table 1). Many investigators have reported a positive association between periodontitis and the presence of specific polymorphism of the IL-1 gene. However, an unveiled interaction exists between the IL-1 genetic polymorphism and environmental factors such as smoking (97–99).
Smokers bearing the genotype-positive IL-1 allele combination may be at an increased risk of developing periodontitis. This suggests that genetic–environmental interaction is more important than genetic factors alone for determination of susceptibility to periodontitis.

Tumor necrosis factor-α

Tumor necrosis factor-α (TNF-α) is one of the most widely studied cytokines in periodontitis (30, 32). Polymorphisms in the promoter region of the TNF-α gene at positions −238 (G to A) and −308 (G to A) have been reported (32). The −308 A-allele has been associated with high promoter activity and enhanced TNF-α production (32, 134). However, in general a lack of association between TNF-α polymorphism and periodontitis has been consistently reported. For example, in a study of TNF-α genotypes for three bi-allelic polymorphisms (−238, −308 or +252 gene polymorphisms) no differences were observed between patients and controls or between patients with different disease severity (40). A subsequent study of four bi-allelic polymorphisms in the TNF-α gene (at positions −376, −308, −238 and +489) also revealed no differences between test and control subjects (17).

Human leukocyte antigens

The human leukocyte antigen (HLA) complex plays an important role in immune responsiveness (68, 103) and may be involved in antigen recognition of periodontal pathogens. HLA class II molecules have been identified on immune cells (108). These molecules are involved in the interaction between T and B lymphocytes and in the production of high-affinity IgG antibodies (28). Several investigators have studied populations of patients with different forms of periodontitis to investigate the expression of various HLA antigens (1, 2, 8). We have studied polymorphisms of HLA-DR molecules in patients with periodontitis and found a significant association between several DRB1 alleles and the disease (145). In contrast, Hodge et al. (55) found no association between the presence of HLA-DQB1 in European Caucasians and the occurrence of early onset periodontitis. The difficulty in finding convincing associations between HLA alleles and periodontitis may be due to racial differences in HLA allele distribution and to small numbers of study subjects.

Immuno–receptors

The association of immuno-receptors to periodontitis has been well studied (Table 1). In particular, receptors for the Fc domain of IgG (Fc gamma R, FcRγR) provide a critical link between specific humoral responses and the cellular branch of the immune system (79, 95). FcRγRs are categorized as a family of receptors, expressed on the cell surface of leukocytes, which bind IgG antibodies and immune complexes (91). In humans, FcRγRs are expressed on natural killer cells, macrophages, T lymphocytes, monocytes, and mast cells (42). The interaction between FcRγRs and IgG triggers a variety of biological responses, including phagocytosis, endocytosis, antibody-dependent cellular cytotoxicity, release of inflammatory mediators, and enhancement of antigen presentation (75). As shown in Table 1, the majority of reports indicate that polymorphisms of FcRγ gene tend to be associated with both aggressive and chronic forms of periodontitis. These alleles may be in linkage disequilibrium with a gene causing periodontitis, although it still remains unclear whether the outcome of periodontitis is associated with the functional defect of FcRγRs.

Protease and structure molecules

Matrix metalloproteinase

Matrix metalloproteinases are one of the most important groups of enzymes involved in periodontal connective tissue destruction (24, 26). Despite this, there are very few reports concerning polymorphisms of genes for matrix metalloproteinases and periodontitis (Table 1). Itagaki et al. (66) reported that matrix metalloproteinase-1 and/or matrix metalloproteinase-3 single nucleotide polymorphisms were not associated with susceptibility to periodontitis in a Japanese population. More recently, polymorphisms in the gene for matrix metalloproteinase-2 were studied and no definitive correlation with periodontitis could be found (60). Due to the limited number of studies carried out to date, it is difficult to relate single nucleotide polymorphisms of matrix metalloproteinase genes with periodontitis.

Cathepsin C

Aggressive periodontitis in prepubertal children is often associated with genetic disorders such as
Papillon–Lefèvre syndrome (48). This syndrome is associated with mutations in the cathepsin C gene. Cathepsin C is a lysosomal protease that plays an essential role in immune and inflammatory processes (19) and may also play a role in the development of periodontitis. Most mutations in the cathepsin C gene have been shown to result in a loss of enzyme function (22). Whether the pathogenetic role of cathepsin C gene variants also relates to types of periodontitis other than syndrome-associated periodontitis remains to be confirmed. Interestingly, Hewitt et al. (53) have recently reported a decreased cathepsin C activity associated with the development of chronic periodontitis in patients who do not suffer from any syndrome such as Papillon–Lefèvre syndrome.

**Trends in gene polymorphism studies in periodontitis**

The number of reports describing genetic analysis using gene and HLA markers for periodontitis has dramatically increased since 1999 (Fig. 2). Of these studies, the polymorphism studies of genes coding for cytokines have received the most attention. Sixty-four reports describing cytokine genes, including IL-1 gene (36 reports) and TNF gene (14 reports) have been published. Since Kornman et al. (84) reported the correlation between IL-1 genotype and severity of periodontitis in 1997, studies investigating cytokine genes have included other cytokines such as immune-regulating cytokines (IL-4, IL-10, and interferon-γ), growth factors (transforming growth factor), and related receptors. In the past, studies concerning the correlation between HLA and periodontitis have used HLA serotypes. However, since 1994 studies have used HLA genotypes (145). Unfortunately, to date, the number of HLA genotype studies in relation to periodontitis remains small. An increasing number of genetic studies in the field of immuno-receptors, structural molecules and periodontitis are emerging. More recently, multigene analysis has been performed using sophisticated molecular biological devices (104, 123, 139, 140) and such approaches have been performed for other common diseases (69, 70, 85). Large-scale analyses, i.e. 100,000 single nucleotide polymorphisms (single nucleotide polymorphisms) on 940 subjects (124), 6000 microsatellite markers in DNA samples of 198 subjects (41), and wide-range genomic regions (107), have been performed for many genetic markers simultaneously, enabling the identification of genetic markers in disease. Furthermore, the ease of large-scale analysis may facilitate multifactorial analysis of the correlation using environmental or other factors.

Although positive correlations have been reported for many kinds of genes such as IL-1 and HLA, conflicting results have also been reported (Table 1). The cause of these discrepancies appears to be environmental factors and racial disequilibrium of targeted genes. In addition, the number of research groups specializing in studies of the genetic aspects of periodontitis is limited, with most groups focusing on only one field (cytokine, immune receptors, proteinases, structural molecules, etc.). Clearly, in order to adequately cover the unbalanced distribution of genes to be searched in relation to periodontitis, multicenter, large-scale single nucleotide polymorphism analyses will be needed.

**Genetic analyses for diagnosis of periodontitis**

Various stages in the progression of periodontitis may be under genetic control, predisposing an individual not only to the initiation and progression of periodontitis, but also to the outcomes of treatment. Recognizing this role for genetic control means that risk assessment could well be served and targeted through genetic analyses (Fig. 3). The candidate genes may be involved in regulating host reactions pertaining to the microbial infection, regulation of inflammation and immune reactions, or regulation of tissue regeneration.

 Significant variations in genes (polymorphisms, deletions, insertions) have the potential to result in very serious cellular malfunction. These malfunctions can affect systemic conditions and, in some cases, can be lethal. However, if the variation is minor or moderate, the disease may occur only under appropriate conditions. Indeed, predisposition towards common diseases, such as type 2 diabetes mellitus and hypertension, can be determined genetically, but the occurrence of the disease requires specific ongoing conditions. Thus, genetic analysis cannot provide a diagnosis without additional analysis of environmental factors. Hence, for a multifactorial disease such as periodontitis, genetic diagnosis (#1 in Fig. 4) must be combined with an assessment of environmental factors (#2 in Fig. 4). This concept is summarized in Fig. 5A. Although genetic predisposition to periodontitis is weak (dotted line), repeated assaults by environmental factors (vertical solid line
with arrow) may increase the susceptibility to periodontitis or the rate of disease progression (elbow-shaped line).

Furthermore, an increased predisposition towards periodontal disease may appear as a function of the amplified steepness of the basal predisposition (dotted line in Fig. 5B). Hence, the higher positioning of ‘Pre-disposition’ along the Y-axis, as shown in Fig. 5B, leads to increased periodontal disease activity. As a result of these effects, the rate of disease progression (Fig. 5B) strongly rises at each environmental assault. In the future, diagnosis for susceptibility to periodontitis should be performed with these processes in mind.

**Conclusion**

Future strategies for the utilization of genetic polymorphisms in periodontics will need to consider at least two factors. The first is to perform large-scale genetic analyses using as many target genes and subjects as reasonably possible. Secondly, there needs to be a development of new statistical analytical methods to combine both genetic and environmental factors. To achieve these goals, multicenter research studies will be needed to maximize cost- and time-effectiveness. As periodontitis is a multifactorial disease, studies have used nonparametric linkage analysis, case–control association analysis, and transmission disequilibrium analysis to determine susceptible or resistant genes for periodontitis. Recently, genome-wide association studies using single nucleotide polymorphisms or microsatellite polymorphisms have become realistic due to the development of promising high-throughput and cost-effective single nucleotide polymorphism typing. As suggested by Tamiya et al. (146), microsatellite-based genome-wide association analysis complemented by
Fig. 4. Relationship of environmental factors on genetic variation causing functional abnormality. Genetic variations affect functions of the host tissues at severe to low levels. Environmental factors such as smoking could exacerbate a slight functional abnormality caused by genetic variation and result in manifestation of disease.

Fig. 5. Effects of multiple assaults of environmental factors on disease progression and the effect of increased predisposition caused by genetic factors. A) Periodontitis susceptibility may be regulated by a gene polymorphism (dotted line) without ever resulting in development of overt disease. However, with multiple effects of environmental factor assaults, the disease may become apparent (solid line from A–D factors). Hence, periodontitis symptoms might be unaffected by a gene polymorphism. B) Increased predisposition towards periodontal disease may also result as a function of the basal predisposition (dotted line). Hence higher ‘Predisposition’ leads to increased periodontal disease activity. As a result of these effects, rate of disease progression (the elbow-shaped line) (Fig. 4B) rises significantly following each environmental assault.
end-stage single nucleotide polymorphism typing will be an interesting strategy for genetic dissection of multifactorial pathologies such as common diseases, including periodontitis.

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