
Genetic Influences on the Periodontal Microbial-Host Crosstalk

7

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7.1 Introduction

Previous chapters of this book have discussed the importance of subgingival microbial colonization and of the inflammatory-immune response as triggers of periodontal tissue breakdown. In fact, it appears clear that periodontal health or pathology is the result of the interaction between the human host and its invading microbes. Or perhaps we should not define them as ‘invading’, since it is well known that microbes not only coexist with their human host, but also provide multiple vital functions for the survival of the host itself [1]. Compelling evidence has now emerged to suggest that host genetic variants have a fundamental effect in regulating the host’s relationships with the microbial ‘guests’ and a better knowledge of how these effects are implemented is crucial in the understanding of disease processes. This chapter will review the evidence on the effect of host genetic factors on periodontal microbial colonization and will provide examples of how this could have an impact in clinical practice.

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7.2 Infectogenomics

In the fourteenth century, one-third to half of the population living in Europe was exterminated by a mysterious disease which was called ‘the Black Death’. It is now known that this disease, later named ‘plague’, was most likely a rodent-associated, flea-borne zoonosis caused by Gram-negative bacterium *Yersinia pestis* [2]. Interestingly, among all people who came into contact with the bacterium, a large proportion became infected and died, some were ill but managed to survive and some had no clinical signs of infection. It appears plausible that each subject responded to infection with *Yersinia pestis* as well as to other potentially fatal infections in a way that was largely determined by his/her genetic make-up [2, 3]. A more up-to-date example is given by the HIV, the virus responsible for AIDS. Following transmission, HIV enters the bloodstream and infects helper T cells, macrophages and dendritic cells, causing killing of T cells (especially CD4 T cells) by CD8 cytotoxic lymphocytes, with reduction in CD4 T cell numbers and loss of cell-mediated immunity [4]. The HIV most commonly uses chemokine receptors CCR5 and/or CXCR4 co-receptor to enter its target cells. The CCR5 receptor is coded for by the CCR5 gene on chromosome 3. A deletion of a 32-bp segment in this gene (named CCR5-Δ32) has been discovered to result in a non-functional receptor which prevents this way of HIV R5 entry

[4]. This genetic variant is rare in Africans/Asians and more common in North European, possibly due to selective pressure by previous epidemics [3, 5, 6]. Homozygosity to this gene variant is characterized by resistance to infection by the most common strain of HIV, while heterozygosity seems to confer partial resistance with slower progression after onset of AIDS [7]. Based on this principle, CCR5 receptor-antagonist drugs have been experimented for the treatment of AIDS [8].

The evidence described above is in line with a concept defined ‘infectogenomics’, suggesting that host genetic factors play a major role in determining the response to bacterial colonization [9, 10]. This concept can be extended also to the presence of common ‘symbiotic’ bacteria and not just pathogens. In other words, host genotypes may influence the composition of human biofilms, including oral biofilms [11]. Therefore, the composition of microbial biofilms in the human body will be dictated by a combination of genetic variants, coupled with environmental factors. As a result of this, a group of human diseases originate from a genetically determined failure to properly recognize or respond to members of the normal human microbiota [11]. This disease-predisposing effect can potentially extend not just to microbial diseases, in the traditional meaning of the term, such as, for example, bacterial vaginosis and periodontitis, but also to diseases not traditionally considered of microbial origin. Among them, rheumatoid arthritis, reactive arthritis and even cancer, which could be influenced by microbial shifts (dysbiosis) even at distant sites. Hence the concept of genetic dysbiosis, which suggests that host genetic variants could be responsible for a range of chronic human diseases through an effect on dysbiosis of microbial biofilms [11].

7.3 Evidence for Genetic Variants Influencing the Response to Microbial Challenge

Where is the evidence for the Infectogenomics principles outlined above? Circumstantial evidence can be derived from studies showing that monozy-

gotic twins frequently have more similar gut microbiomes than non-twin siblings [12]. Furthermore, microbial profiles of faecal samples collected at various times from a given individual are more similar to each other than to the intestinal microbial communities in a different individual [13]. A little dip into the human genome can allow a better understanding of the host-microbial axis. More than 60 million common genetic variants in 19,000–25,000 genes located in 23 pairs of chromosomes are listed in the Single Nucleotide Polymorphism Database (dbSNP) by the National Center for Biotechnology in collaboration with National Human Genome Research Institute [14]. Different individuals are thought to be 99.4% identical in chromosomal structure and 99.9% identical at sequence level [15]. Functional SNPs are located in the gene promoter (affecting gene activity) or in the coding region of the gene (affecting the protein produced). The disease-predisposing effects may be determinant such as for haemophilia A, caused by a specific mutation (single gene defect) in the F8 gene leading to defects in coagulation factor VIII [16]. However, most diseases are characterized by a complex susceptibility profile, where a variety of SNPs contribute to the disease risk. Such SNPs may be involved, for example, in microbial recognition (determining aberrant responses to the normal microbiota), in the inflammatory cascade or in DNA repair (associated with a reduction in the ability to repair damaged DNA).

7.3.1 Microbial Recognition Genes

Following the earlier example of the HIV, it seems reasonable to believe that genetic variants affecting microbial recognition will have a major role in determining the composition of microbial biofilms. The search for the possible ‘microbial recognition’ gene affecting microbial colonization could focus on pattern-recognition receptors (PRRs), which recognize evolutionarily conserved constituents of microbes called pathogen-associated molecular patterns (PAMPs). PRRs include normally cell-bound proteins such as toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type-lectin like receptors (CLRs), scavenger receptors

(SCs), innate DNA receptor proteins termed AIM2-like receptors (ALRs), members of the complement pathways and peptidoglycan-recognition proteins (PRPs) and soluble PRRs (including collectins, ficolins, pentraxins, galectins, sCD14 and natural IgM). Upon microbial interaction, PRRs activate a series of downstream mechanisms through selective cells signalling, leading to the generation of pro- or anti-inflammatory proteins. Mutations in the coding or promoter regions of PRR genes could result in an altered ability to recognize microbial patterns, affecting the 'binding/recognition' process and its downstream pathways, leading to aberrant response to microbial challenge and shifts in the normal biofilm composition [17].

The circumstantial evidence for a role of host genetic variants in determining microbial colonization is strengthened by observations on inflammatory bowel disease, encompassing Crohn's disease (CD) and ulcerative colitis (UC). Genetic variants in the NOD2 gene, coding for an intracellular **pattern recognition receptor** able to recognize molecules containing bacterial **muramyl dipeptide** [18], are now recognized as increasing the risk of CD [19]. In an experimental ileal inflammation model in mice [20], when NOD2-CD-susceptible animals were subjected to *Toxoplasma gondii*-induced ileitis, an increase in inflammation and dysbiosis was noticed (shift from mainly Gram-positive to Gram-negative bacteria, associated with invasive *E. coli*) compared to non-genetically susceptible animals. Furthermore, genetic variants in the NOD2 and autophagy-related 16-like 1 protein (ATG16L1) have been associated with gut microbiota structure alterations, including decreased *Faecalibacterium* levels and increased *Escherichia* levels [21]. These results are likely due to an alteration of the inflammatory cascade resulting from an aberrant response upon microbial recognition through the NOD2 receptor.

Some evidence exists also for the effect of microbial recognition genes on microbial presence in vaginal biofilms. In a study on the vaginal microbiota of 144 pregnant women, detection of *A. vaginae* and *G. vaginalis* by PCR was studied in relation to 34 single nucleotide polymorphisms pertaining to 9 genes involved with Toll-like

receptor-mediated pathogen recognition and/or regulation. While no association between these SNPs and presence of bacterial vaginosis was detected, some of the studied SNPs were associated with carriage of *A. vaginae* and *G. vaginalis* during early pregnancy. The authors suggested that some degree of genetic susceptibility involving pathogen recognition may occur, which influences vaginal presence of potential pathogenic microorganism [22]. In a separate study on 238 pregnant women, TLR4 genotypes were associated with increases in vaginal pH and in vaginal detection of *Gardnerella vaginalis*, *Prevotella*, *Bacteroides* and *Porphyromonas*, suggesting that genetic variants may drive a change in the vaginal environment which favours the growth of pathogenic bacteria [23].

7.3.2 Genes Involved in Inflammatory Pathways

It is now becoming clear that inflammation can have profound effects on microbial communities, causing a progressive decrease in the microbial diversity through an increased availability of substrates for growth of Gram-negative bacteria (e.g. iron and serum, dead or dying cells) and loss of niche and substrates for Gram-positive flora (e.g. mucus, goblet cells) [24]. Therefore, it is conceivable that variants in genes directly involved in inflammatory pathways, could impact the threshold for dysbiosis and the ability to resolve the dysbiosis-inflammation cycle generated by an acute trigger [20]. Based on this concept, genetic variants affecting inflammatory responses may be major candidates for an effect on microbial biofilm composition. The evidence for this comes mainly from studies on periodontal disease which will be discussed in the next section.

7.4 Genetic Effects on Microbial Colonization: Studies in Periodontal Disease

Previous chapters of this book described how pathogenic pathways leading to periodontal breakdown involve the role of subgingival

microbes, host response and environmental factors and how important the crosstalk between host and bacteria is. This chapter introduced the concept of ‘Infectogenomics’ to mean the effect of host genetic background on the colonizing microbes, and examples relative to inflammatory bowel disease and bacterial vaginosis have been provided. In the last 10–15 years, evidence for periodontal infectogenomics [25] has also emerged. In particular, it is striking how the JP2 leukotoxic strain of *A. actinomycetemcomitans* has a strong tropism of for subjects of mainly North African and West African descent, increasing the risk of development of Localised Aggressive Periodontitis (LAgP) [26]. Since carriage of this strain does not seem to depend on geographic location but rather on ancestry, it is likely to be linked with heritability and with the host genetic make-up. Figures 7.1 and 7.2 show a typical clinical and radiographic presentation



Fig. 7.1 Clinical photograph of 15-year-old non-smoker LAgP patient of Afro-Caribbean origin, showing buccal migration of the upper right central incisor and generally good oral hygiene

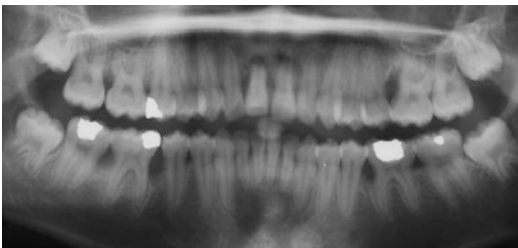


Fig. 7.2 Panoramic radiograph of patient shown in Fig. 7.1. Please note localized alveolar bone loss affecting mainly upper right central incisor and first molars

of a case of Localized Aggressive Periodontitis (LAgP), characterized by a molar-incisor pattern of bone and periodontal attachment loss.

The understanding that heritability accounts for about half of the risk of developing periodontitis [27] has led to a flourish of studies trying to identify inflammatory, metabolic or structural gene polymorphisms which could predispose to periodontal diseases [28]. Most studies focused on selected candidate SNPs, starting from the case-control association study suggesting an effect of the Interleukin-1 (IL-1) ‘composite genotype’ on disease predisposition [29]. More recent studies are often using an explorative genome-wide approach [30, 31]. However, studies so far failed to reach a consensus after analyses in different populations and settings, with promising studies pointing towards the role of SNPs in ANRIL (antisense non-coding RNA in the INK4 locus), COX2 (cyclooxygenase 2), IL-10 (Interleukin-10) and DEFDB1 (β -defensin-1) and possibly others in disease predisposition [31, 32]. These genes are involved, respectively, in glucose and fatty acid metabolism regulation (ANRIL gene) [33], coding for antimicrobial peptides involved in the epithelial response to microbial invasion (DEFDB1 gene) [34] and in the periodontal inflammatory response (COX2 and IL-10 genes) [35, 36].

Sigmund Socransky and Anne Haffajee were probably the first to investigate the relationships between SNPs supposed to affect the periodontitis trait and presence of subgingival microbes. In their 2000 paper, they observed an association between IL-1 genotypes and presence of subgingival microbes [37]. In particular, more IL-1 ‘genotype positive’ subjects [29] exhibited high mean counts of ‘red’ and ‘orange’ subgingival species than ‘genotype negative’ subjects. Bacteria found at higher levels in IL-1 genotype positive subjects were *Bacteroides forsythus*, *Treponema denticola*, the *Fusobacterium nucleatum* subspecies, *Fusobacterium periodonticum*, *Campylobacter gracilis*, *Campylobacter showae*, *Streptococcus constellatus*, *Streptococcus intermedius*, *Streptococcus gordonii* and 3 *Capnocytophaga* species. The differences in bacterial colonization by genotype were mainly visible in deep peri-

odontal pockets (>6 mm). Based on these results, the authors postulated that genetic variants might either directly affect bacterial growth and virulence or alter the inflammatory milieu, favouring the growth of specific bacteria. In contrast with these findings, no associations between IL-1 composite genotypes and subgingival bacteria analysed by PCR were detected in a similar study published shortly afterwards [38].

Our group has extensively investigated the associations between candidate genetic variants affecting the inflammatory response (e.g. interleukin-1 and interleukin-6 genes) and subgingival detection of periodontopathogenic bacteria by culture and polymerase chain reaction (PCR). In 45 untreated aggressive periodontitis (AgP) patients from London, IL6 and Fc- γ polymorphisms were both associated with increased odds of detecting *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythensis* after adjustment for age, ethnicity, smoking and disease severity [39]. In particular, subjects with supposedly pro-inflammatory IL6 genotypes [40, 41] had increased detection of *A. actinomycetemcomitans* and *P. gingivalis*. The study was repeated in a rural population living in Andhra Pradesh, India [42]. Subjects had subgingival plaque samples taken and analysed by checkerboard DNA-DNA analysis for 40 periodontal taxa and had their DNA extracted for IL6 SNP analyses. In this population not exposed to regular dental care and to use of antibiotics, most subjects harboured *A. actinomycetemcomitans* and *P. gingivalis* subgingivally, which did not allow any analysis on bacterial detection by genotype. However, associations between IL6 genotypes and elevated counts of *A. actinomycetemcomitans* and *Campylobacter sputigena* were observed, strengthening the previous report. This was further confirmed when a population of 267 chronic and aggressive periodontitis patients was studied. Host DNA samples were extracted from blood samples and analysed for five IL6 SNPs, while subgingival plaque samples were analysed by PCR for the presence of *A. actinomycetemcomitans* and *P. gingivalis*. The study confirmed again the association between IL6 supposedly ‘pro-inflammatory’

genetic variants and presence of *A. actinomycetemcomitans* and of both bacteria concomitantly [43]. To explore this concept further, we conducted a pilot treatment study on 12 AgP patients selected based on their IL6 genotypes (‘pro-inflammatory IL6 haplotype positive’ vs. ‘IL6 haplotype negative’). In this population, higher *A. actinomycetemcomitans* counts were detected subgingivally in IL6 ‘haplotype positive’ subjects before treatment. Despite a reduction after non-surgical and surgical treatment, these subjects showed a sharp increase in counts of *A. actinomycetemcomitans* again 3 months after periodontal treatment, suggesting a strong genetic influence on gingival pocket re-colonization, which was not observed in IL6 ‘haplotype negative’ subjects [44].

A larger study used a genome-wide approach in 1020 subjects participating in the Atherosclerosis Risk In Communities (ARIC) study to investigate the relationship between host genotypes and eight periodontal pathogens analysed by checkerboard DNA-DNA hybridization [45]. They detected no genome-wide significant signals, but suggestive evidence ($p < 5 \times 10^{-6}$) of association for 13 genetic loci and ‘red’ and ‘orange’ complex microbiota. The same effect direction was detected in a second sample of 123 African-American participants. Interestingly, these authors confirmed the moderate association our group previously reported between IL6 SNPs and high ‘red complex’ colonization. No association was detected between any of the identified SNPs with CP diagnosis, suggesting once more the examination of bacterial colonization as a distinct trait to ‘presence of disease’ [45]. Recently, in a case-control study analysing polymorphism TBX21-1993T/C (rs4794067) in healthy ($n = 218$), chronic periodontitis ($n = 197$) and gingivitis patients ($n = 193$), no associations were detected between genotypes and presence of ‘red complex’ bacteria [46]. A summary of genetic variants shown to be associated to detection of subgingival periodontal bacteria is provided in Table 7.1. A more systematic and comprehensive review of the literature on periodontal infectogenomics has been recently published [47].

Table 7.1 Summary of genetic variants shown to be associated with detection of subgingival periodontal bacteria in some of the studies reviewed in this chapter

Study	Population	Study design	Genetic variant-microbial association
[37]	U.S. University-based	CP case-control candidate gene association study with checkerboard DNA-DNA microbial analysis	IL-1 genotypes: counts of <i>B. forsythus</i> , <i>T. denticola</i> , <i>F. nucleatum</i> , <i>F. periodonticum</i> , <i>C. gracilis</i> , <i>C. showae</i> , <i>S. constellatus</i> , <i>S. intermedius</i> , <i>S. gordonii</i> , 3 <i>Capnocytophaga</i> species
[39]	UK University-based	AgP case-control candidate gene association study with microbial culture analysis	IL6 and Fc- γ R genotypes: <i>A. actinomycetemcomitans</i> , <i>P. gingivalis</i> detection
[42]	Indian rural village	Cross-sectional candidate gene association study with checkerboard DNA-DNA microbial analysis	IL6 genotypes: <i>A. actinomycetemcomitans</i> , <i>C. sputigena</i> counts
[43]	UK University-based	Mixed CP and AgP case-control candidate gene association study with microbial PCR analysis	IL6 genotypes: <i>A. actinomycetemcomitans</i> , <i>P. gingivalis</i> detection
[45]	U.S. University-based	GWAS with checkerboard DNA-DNA microbial analysis	13 loci (including KCNK1, FBXO38, UHRF2, IL33, RUNX2, TRPS1, CAMTA1 and VAMP3): suggestive evidence of associations with 'red' and 'orange' complex/ <i>A. actinomycetemcomitans</i>

CP chronic periodontitis, AgP aggressive periodontitis

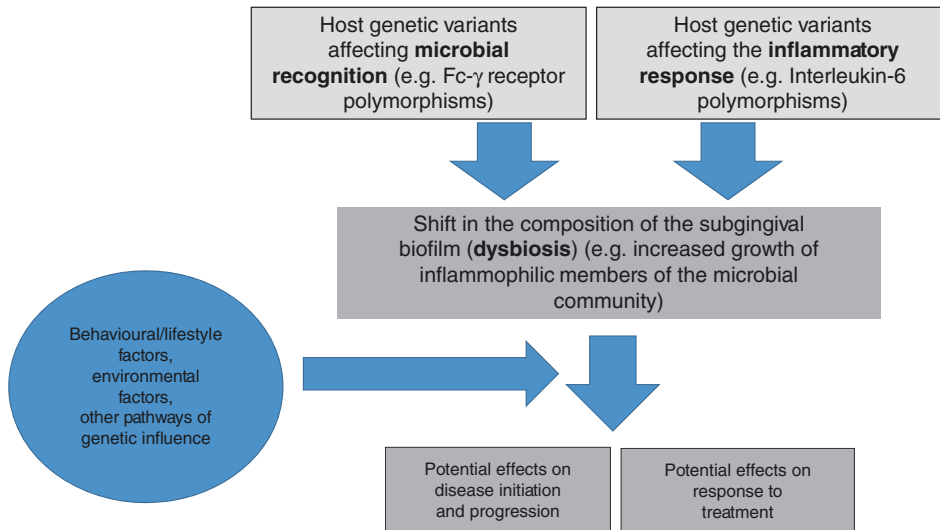


Fig. 7.3 Schematic representation of periodontal infectogenomics

Summarizing the findings above, there is now increasing evidence that host genetic variants can have an effect on periodontal pathology by influencing the subgingival bacteria composition. The shifts in *A. actinomycetemcomitans*, *P. gingivalis*

and more in general 'red complex' bacteria may be a small representation of changes in the subgingival biofilm ('dysbiosis') (see Fig. 7.3). There is still a lack of studies investigating whether health-associated bacteria may be affected by specific

genetic variants. The supposed shift towards a more pathogenic microbiota may occur through the effects on microbial recognition and inflammation discussed above. It is conceivable that a more inflamed milieu, characteristic of patients with ‘pro-inflammatory’ genetic profiles, may favour the growth of bacteria which grow well in inflamed environments, then shifting the whole microbiota towards a disease-predisposing one.

7.5 Challenges and Future Directions

Periodontal genetic research still has a long way to go before it identifies clear predisposing host gene variants in different populations. This is complicated by issues such as sample size, definition of health and disease, genetic methodology, difficulty at controlling for other predisposing factors and epigenetic influences. Recently, gene variants in the IRF5 gene have been associated with IBD [48], while SNPs in the DEFDB1 gene and PRDM1 gene have been associated with chronic and aggressive periodontitis, respectively ([31, 49]). These gene polymorphisms appear to be able to contribute to a disturbance of the immunological barrier, thus promoting dysbiosis of the local microflora, potentially predisposing to disease. Hence, it would be interesting to focus periodontal infectogenomics research on a variety of genes with an effect on microbial recognition and host response. The availability of new metagenomics techniques gives the possibility to explore the associations with health-associated as well as with pathogenic bacteria. Furthermore, there is a lack of studies in periodontally healthy populations, which could give some insights on the genetic influence on the host-microbe crosstalk in health-associated biofilms. Among subjects with healthy periodontia living in a rural population in India, the association between IL6 genetic variants and *A. actinomycetemcomitans* was confirmed (as in periodontitis patients) [42]. However, a paucity of data exists on healthy subjects in other settings. A better knowledge of how gene variants affect the composition of the subgingival biofilm could shed light into potential

pathogenic pathways and could open new management avenues. The above-mentioned pilot study in AgP patients selected based on their IL6 haplotypes [44] could, for example, suggest that ‘IL6 positive’ subjects may benefit from adjunctive antimicrobial therapy, as they might be more likely to have a tendency to re-developing dysbiotic, disease-associated biofilms also after treatment. Studying mechanisms of association between the subgingival biofilm and other biofilms elsewhere in the body, such as in gastrointestinal tract, vagina and skin, could shed light into mechanisms of host-bacteria crosstalk.

Clinical Relevance

- Host genetic variants seem to play an important role in determining the composition of microbial biofilms in the human body, including the dental biofilm.
- Some subjects may be more predisposed to periodontal disease onset and progression through the activity of host genetic variants in the response to the microbial challenge.
- Knowing which subjects are more predisposed to colonization by specific microbes could affect the clinical management of periodontitis cases.

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