Review

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Periodontal infectogenomics

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Multicellular creatures consist of a symbiosis between the host and its colonizing bacteria. The oral cavity may contain as many as 19 000 bacterial phylotypes, while each individual presents a proportion of these microbes. Infectogenomics studies the interaction between host genetic variations and composition of the microbiota. This review introduces the concept of periodontal infectogenomics, defined as the relationship between host genetic factors and the composition of the subgingival microbiota. In particular, the evidence for the effect of genetic variants in neutrophil and cytokine genes and the presence of periodontopathogenic bacteria will be discussed. The influence of genetic factors may affect clearance or persistence of pathogenic bacteria subgingivally, therefore increasing the risk for the development of common pathogenic conditions such as gingivitis and periodontitis, leading to early tooth loss. Mechanisms of interaction between genetic and microbiological factors and prospects for future studies will be discussed.

Introduction

Multicellular creatures are supra-organisms consisting of a symbiosis between the host and one or more colonizing bacteria – the microbiota (McFall-Ngai *et al.*, 2005). Recent estimates of the numbers of bacterial species using massively parallel DNA sequencing have suggested that the oral cavity may contain as many as 19 000 bacterial phylotypes (Keijser *et al.*, 2008). This is an almost unimaginable number. It is assumed that each individual will only have a proportion of the total numbers of bacteria found in the general populations measured. Genetic factors in the host seem to play a major role in deciding which bacteria (commensal and pathogenic) are able to colonize the host. This has led to the concept of infectogenomics, a term which was initially introduced to define the study of the interaction between host genetic variations and colonization by pathogenic microbes (Kellam & Weiss, 2006). In this review, the term will be broadened to include the growing numbers of members of the human bacterial microbiota. Periodontal diseases are the most common chronic inflammatory diseases in humans. This paper will review the evidence for the association between host genetic and microbiological factors in patients affected by periodontitis.

The concept of infectogenomics

The response to infections varies enormously between individuals and the interplay between the fitness of the host and of the pathogen determines sickness (Bellamy, 2004; Wang, 2005; van Opijnen & Berkhout, 2005). Exposure of the host to a pathogen can result in resistance, subclinical

infection or overt infection. This concept has led to a growing interest in the genetic predisposing factors for life-threatening infectious diseases such as malaria, tuberculosis, hepatitis and AIDS (reviewed by Bellamy, 2004). Understanding the host genetic basis for these different responses may improve our understanding of infectious disease pathogenesis and help in the treatment and control of infection (Cooke & Hill, 2001). Host genetic factors may affect a pathogen's infectivity in at least two different ways: pathogen invasion and pathogen proliferation. However, in reality, this is a simplistic view, as many functions and mechanisms between invasion and proliferation may actually overlap. Some examples are provided below.

Genetic factors predisposing to pathogen invasion

The *CCR5* gene (chromosome 3) codes for the CCR5 receptor, which binds chemokines. However, this protein is also a co-receptor for HIV, aiding its entry into target cells (T cells and macrophages). A genetic variant in this gene (*CCR5-Δ32* – deletion of a 32 bp segment) results in a nonfunctional receptor, thus preventing HIV entry. This confers complete resistance to the majority of HIV strains which enter through this receptor in the homozygous state and partial resistance with slower progression in the heterozygous state (Galvani & Slatkin, 2003).

Genetic factors predisposing to pathogen clearance/proliferation

Factors that are related to pathogen proliferation may also affect the interaction between microbes and host genome.

For instance, the β -globin gene (chromosome 11) codes for haemoglobin (Hb). A genetic variant in this gene (HbS, A→T single nucleotide mutation resulting in a glutamate→valine substitution) causes the formation of fibrous precipitates in cellular haemoglobin, with no major consequences in the heterozygous state. However, in the case of HbS homozygosity, the presence of long-chain polymers of HbS distorts the shape of the red blood cells, making them fragile and susceptible to breaking within capillaries (sickle cell anaemia). *Plasmodium falciparum* is the causative agent of malaria and it spends part of its life in red blood cells. In HbS homozygous individuals, the presence of *P. falciparum* causes the red blood cell to rupture, and therefore, although it can enter its target cells, it cannot proliferate. As a consequence, HbS heterozygous subjects are more resistant to malaria than subjects homozygous for the normal gene (Cooke & Hill, 2001).

Other factors

A classic monogenic disease which results in unexpected bacterial infection is cystic fibrosis. Here the genetic mutation, normally the loss of the amino acid phenylalanine located at position 508 in the cystic fibrosis transmembrane conductance regulator, results in this protein failing to function properly with changes in lung fluid composition (e.g. mucus) and an increased colonization with bacteria, particularly *Pseudomonas aeruginosa* (Campodónico *et al.*, 2008).

Crohn's disease and the gut microbiota

Crohn's disease is a chronic inflammatory bowel disease, possibly characterized by an aberrant response to members of the intestinal microbiota. It has been linked with *NOD2/CARD15* genetic variants (nucleotide-binding oligomerization domain 2/caspase activation recruitment domain 15) and with two separate autophagy genes, *ATG16L1* and *IRGM* (Chapman *et al.*, 2009). These genes have different pathogenic pathways, as the *NOD2/CARD15* genes affect intracellular receptors which recognize the peptidoglycan of bacteria and thus are focused on bacteria entering gut epithelial cells, while *ATG16L1* and *IRGM* genes affect bacterial clearance and antigen processing via autophagy (pathogen clearance/proliferation). Therefore, genetic factors linked with both pathogen invasion and proliferation may be associated with the presence and/or persistence of specific intestinal bacteria, which might trigger the host response typical of Crohn's disease. In fact, it is thought that the florid inflammation seen in Crohn's might be advantageous in case of a particular infection (Cooke & Hill, 2001), such as dairy-associated bacterial infections caused by *Listeria*, *Brucella* or mycobacteria (Gasche *et al.*, 2008). There is growing interest in applying the techniques of bacterial metagenomics to human diseases and in particular diseases of the gut (Frank & Pace, 2008).

Thus, there are a growing number of human diseases where there is a clear relationship between infectious agents,

genetic susceptibility and tissue pathology. There is also the slow emergence of evidence that micro-organisms, particularly bacteria, are responsible for what have long been regarded as idiopathic diseases. The most striking examples of this are *Helicobacter pylori* and gastric ulceration and gastric cancer (Wessler & Backert, 2008), and other bacteria and viruses and nasopharyngeal carcinoma and colorectal cancer (Niller *et al.*, 2009; Duncan *et al.*, 2009).

Periodontitis

With the realization of the enormity of the colonization of *Homo sapiens* with bacteria has come the perception that most of the bacteria that we live with, from cradle to grave, cause no adverse events in our lives. We seem to have evolved to live in perfect harmony with these bacteria, and, by-and-large, our skin, guts and urogenital tracts show no sign that they are colonized by bacteria that could cause tissue pathology. However, there is one body site where this cooperativity between our prokaryotic and eukaryotic selves breaks down – the oral cavity. Unless daily care is taken to remove plaque bacteria, the mouth is subject to the incredibly common diseases caries and periodontitis. The pathogenesis of caries is reasonably well worked out and will not be discussed. Periodontitis is a destructive inflammatory disease of the periodontium (*peri* around; *odontos* the teeth) (Page *et al.*, 1997) affecting an extremely large percentage of the human population (Papapanou, 1999; Henderson *et al.*, 2009) and leading to tooth loss. The two most common forms of periodontitis are chronic periodontitis and aggressive periodontitis (Armitage, 1999). Aggressive periodontitis usually affects younger individuals and has a faster rate of progression, while chronic periodontitis has a slower progression rate and is often associated with local plaque retentive factors, such as difficult-to-brush molar areas, overhanging restorations and overcrowded teeth. Periodontitis is a bacterially driven disease (Socransky & Haffajee, 1991). However, it is still the subject of speculation as to whether this disease is a generic response to all plaque bacteria or is caused by a selected group of oral bacteria. These two arguments have been termed the non-specific and specific plaque hypotheses. Currently, at least three bacteria have been confirmed as periodontopathogenic: *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* (American Academy of Periodontology, 2005). Several other bacteria are currently considered putative periodontopathogens (Table 1). The pathogenic damage characteristic of periodontitis is thought to result from an immunopathological reaction triggered by the presence of bacteria in the crevice between the teeth and the gums. In this context, several studies have clearly shown that a lack of oral hygiene may rapidly cause inflammation of the gums (gingivitis) but that this condition does not necessarily mature into periodontitis (Löe *et al.*, 1965; Neely *et al.*, 2001). This epidemiological evidence suggests a genetic, in addition to an environmental, disease-modifying effect for periodontitis. Host

Table 1. Recognized and putative periodontopathogenic bacteria according to criteria defined by Haffajee & Socransky (1994) (reviewed by Socransky & Haffajee, 2008)

Recognized periodontopathogenic bacteria	Putative periodontopathogenic bacteria
<i>Aggregatibacter actinomycetemcomitans</i>	<i>Treponema denticola</i>
<i>Porphyromonas gingivalis</i>	<i>Campylobacter rectus</i>
<i>Tannerella forsythia</i>	<i>Prevotella intermedia</i>
	<i>Prevotella nigrescens</i>
	<i>Eikenella corrodens</i>
	<i>Peptostreptococcus micros</i>
	<i>Eubacterium nodatum</i>

susceptibility has been associated with severity of plaque-induced gingivitis (Trombelli *et al.*, 2004). Genetic factors have been strongly associated with periodontitis (Michalowicz *et al.*, 1991), and efforts in periodontal research have recently focused on the possible effect of genetic variants in periodontal pathogenesis. A few single gene disorders have been detected, which are able to determine the onset of periodontitis in the presence of subgingival plaque, usually associated with syndromes (Kinane *et al.*, 2005). However, most cases of periodontitis seem to show a polygenic predisposition, determined by the cumulative effect of subtle gene variants (Kinane *et al.*, 2005). Among these, cytokine [such as interleukin-1 (IL-1), IL-6] and neutrophil (such as Fc γ receptor) gene polymorphisms with an effect on inflammatory responses have emerged as reasonable candidates for single nucleotide polymorphism analysis, from association studies (Table 2). However, to date no consensus has been reached on any of

these factors as predisposing to periodontitis (Kinane *et al.*, 2005).

Periodontal infectogenomics

One end of the Gaussian distribution of the periodontitis hypothesis is that particular bacteria are the causative agents of periodontitis and these bacteria only (or preferably) colonize individuals with specific genetic predisposition. Surprisingly, only a few studies have focused on the possible influence of genetic factors on the presence of subgingival bacteria. Clear evidence for the role of genetic factors in the colonization by specific subgingival pathogens has emerged from studies on the *A. actinomycetemcomitans* JP2 clone. This clone has a 530 bp deletion from the promoter of the leukotoxin gene missing with the consequence that it produces more of this leukocyte-killing toxin (Henderson *et al.*, 2003). This

Table 2. Recognized and putative genetic risk factors predisposing to periodontitis

The genes indicated in the first column are involved in the pathogenesis of syndromic forms of periodontitis (reviewed by Kinane *et al.*, 2005).

Genes with recognized defects able to cause periodontitis	Genes with putative polymorphisms predisposing to periodontitis
Elastase 2 (ELA-2)	Interleukin-1
Growth factor independence-1 (GFI-1)	Interleukin-2
Haematopoietic cell-specific Lyn substrate 1 associated (HAX-1)	Interleukin-4
Granulocyte colony-stimulating factor (GCSF)	Interleukin-10
Lysosomal-trafficking regulator (LYST)	Tumour necrosis factor
Integrin beta 2 (ITGB2)	Transforming growth factor-beta 1
Solute carrier family 35 (SLC35C1)	Fc receptors
KINDLIN 1	CD14 receptor
Cathepsin C	Vitamin D receptor
Type III collagen	Matrix metalloproteases
Alkaline phosphatase (ALPL)	Human leukocyte antigen (HLA)
	FMLP receptor
	Oestrogen receptor α
	Fibrinogen
	Plasminogen
	N-Acetyltransferase (NAT2)
	CYBA p22phox

variant has been consistently associated with people of North African/Mediterranean origin (Rylev & Kilian, 2008) and a recent longitudinal study of Moroccan adolescents found that individuals carrying only the JP2 clone had a considerably increased risk of developing periodontitis compared with individuals who did not harbour this clone subgingivally (Haubek *et al.*, 2008). This shows the tropism of this bacterial clone towards people within specific ethnic groups and leads to speculation that the predominant periodontal pathogenic microbiota preferably develop in subjects with a specific genetic susceptibility. In a study of patients with chronic periodontitis (Socransky *et al.*, 2000), subjects positive for the composite IL-1 genotype (Kornman *et al.*, 1997) had increased counts of 14/40 bacteria (especially red and orange complexes, such as *Tannerella forsythia* and *Treponema denticola*). The authors concluded that differences in host genotype influence the composition of the subgingival microbiota. More recently, epidemiological and *in vitro* functional studies are bringing forward further evidence on this topic. Studies from our group have shown an association between IL-6 gene and Fc γ gene receptor variants and subgingival detection of *A. actinomycetemcomitans* and *P. gingivalis* in periodontitis patients (Nibali *et al.*, 2007, 2008). Following a pattern similar to the above-described mechanisms of infectogenomics, we can distinguish two pathways for periodontal infectogenomics.

Genetic factors predisposing to periodontal pathogen invasion

The following requisites are indispensable for microbes to invade subgingival sites: (i) the ability to attach to the tissue surface; (ii) the ability to multiply; (iii) the ability to compete against other microbial species and; (iv) the ability to defend against host responses (Socransky & Haffajee, 1991). The innate bacterial characteristics dictate the first three features. Where the host genotype becomes important is mainly in relation to (iv), where the huge machinery of inflammation and immunity comes into play in terms of recognizing bacteria and attempting to kill and remove them. The major cells involved in killing extracellular bacteria are the polymorphonuclear leukocytes (PMNs or neutrophils) and, to a lesser extent, the monocyte and macrophage. A key requirement is that these cells can recognize pathogenic bacteria and can ingest them for intracellular killing. Genetic factors coding for surface receptors of neutrophils, macrophages or monocytes [Toll-like receptors (TLRs), formyl peptide receptors, Fc γ receptors] involved in recognizing and killing bacteria may affect bacterial clearance. Evidence for this comes from *in vitro* studies. For example, TLR4 polymorphisms have been shown to affect responsiveness to *P. gingivalis* from gingival epithelial cells (Kinane *et al.*, 2006). Neutrophils can recognize Ig-opsonized bacteria through specific Fc γ receptors (van Sorge *et al.*, 2003). The Fc γ RIIa 131 H allele exhibits higher affinity for IgG2-opsonized particles and has been suspected to be involved in

susceptibility to periodontitis (Loos *et al.*, 2005). Subjects with Fc γ RIIa H allele exhibited increased phagocytosis of *A. actinomycetemcomitans* and *P. gingivalis* *in vitro* (Nicu *et al.*, 2007) and, in agreement with this, less frequency of detection of *A. actinomycetemcomitans* and *P. gingivalis* subgingivally (Nibali *et al.*, 2007). Furthermore, we observed increased phagocytosis of pre-opsonized *Escherichia coli* in subjects with Fc γ RIIa H genotypes with and without periodontitis (L. Nibali and others, unpublished).

Genetic factors predisposing to periodontal pathogen clearance/proliferation

Once bacteria have colonized the periodontal tissues, in susceptible individuals they can proliferate and trigger the immunopathological reactions that determine tissue destruction. Increased inflammatory response to plaque accumulation in subjects carrying specific gene polymorphisms may increase the chance of overgrowth of particular components of the opportunistic microbiota (such as, for example, *A. actinomycetemcomitans*) that grow well in inflamed areas. For example, IL-6 is a multi-functional cytokine crucial in the inflammatory response to infectious agents (especially Gram-negative bacteria) (Dalrymple *et al.*, 1996). Homozygosity for the G allele at position -174 in the promoter region of the IL-6 gene has been linked to increased promoter activity and increased serum concentrations of IL-6 (Fishman *et al.*, 1998) and is suspected as a susceptibility factor for periodontitis (Trevilatto *et al.*, 2003; Nibali *et al.*, 2009). We demonstrated an association between IL-6 genetic variants and subgingival detection of periodontopathogenic bacteria (*A. actinomycetemcomitans* and *P. gingivalis*) in two independent periodontitis patients cohorts (Nibali *et al.*, 2007, 2008). We concluded that IL-6 hyperproducers (based on their IL-6 genotypes) may be predisposed to colonization by specific bacteria and in turn to rapidly progressive forms of periodontitis upon chronic stimulation with these bacteria. Therefore, IL-6 genetic factors may affect microbial proliferation, and the transformation of bacterial subgingival colonization into a chronic inflammatory process.

Selective pressure

Microbiological factors can potentially modify the genetic profile of a population. In other words, microbial infections (especially if life-threatening) may have shaped the host genotypes throughout generations (Cooke & Hill, 2001). For example, selective pressure owing to malaria has selected HbS homozygous individuals who, because they were not killed by malaria, survived beyond reproductive age and produced offspring. Therefore, in areas where malaria was endemic, the HbS trait is much more common than in other areas. Furthermore, certain infectious diseases (such as smallpox) may have determined the geographical differences in the prevalence of the CCR5

genetic mutation that we observe today (Galvani & Slatkin, 2003). Selective pressure through dairy-associated bacterial infection within Neolithic cattle farming populations might have led to regional diversity in *NOD2* mutations and therefore to Crohn's disease predisposition (Gasche *et al.*, 2008). Similarly, we could speculate that other infections may have selected certain hyperinflammatory genetic variants (IL-6 variants being one of the possible examples) that facilitated the survival of individuals, but at the same time caused an excessive inflammatory reaction at the periodontal level, with consequent rapid alveolar bone loss (periodontitis). The considerable racial variation in IL-6 genotype frequency, with the supposedly hyperinflammatory -174 GG genotype (Fishman *et al.*, 1998; Bennermo *et al.*, 2004) much more common in blacks than in whites, may reflect different selective pressures from different infectious diseases throughout history.

Future developments

Periodontal diseases are the most common chronic inflammatory diseases of humans. Depending on different definitions and different populations, periodontitis alone (excluding gingivitis) is estimated to have a prevalence of 13–57% (Rylev & Kilian, 2008). Therefore, it may serve as a useful model to study the relationship between host genome and microbial challenge. However, several questions on periodontal infectogenomics still remain unanswered. In particular, periodontal infectogenomics is complicated by the biofilm nature of subgingival bacteria (Socransky & Haffajee, 1991), where bacteria – some of which are considered exogenous (Haubek *et al.*, 1996; Kaplan *et al.*, 2002) – are organized in a biofilm at least 50–100 cells thick and may not behave independently but as part of a complex. Genome-wide association studies and pyrosequencing analysis of the periodontal microflora may help uncover more genetic variants that can have an effect on subgingival bacterial invasion and proliferation. In conclusion, the field of periodontal infectogenomics can prospectively bring evidence of associations between genetic and microbial factors, which can clarify different pathogenic pathways in different forms of periodontitis, and possibly assist in prevention and management. As for other diseases, we should not only seek microbial virulence markers, but we should try to identify the human genetic factors that predispose to invasion by pathogens and to their proliferation (Kellam & Weiss, 2006). Advances in gene expression profiling may shed more light into pathogenic processes and possibly provide the chance for adjunctive pharmacological treatment (Kellam, 2006).

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