Journal of
PERIODONTAL RESEARCH

J Periodont Res 2012; 47: 273–285

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2011.01437.x

Mini Review

Genetic polymorphism studies in periodontitis and Fcγ receptors

L. Chai[1], Y.-Q. Song[2], W. K. Leung[3]
[1]School of Dentistry, University of Queensland, Brisbane, Qld, Australia, [2]Department of Biochemistry, Li Ka Shing Faculty of Medicine and [3]Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China

Chai L, Song Y-Q, Leung WK. Genetic polymorphism studies in periodontitis and Fcγ receptors. J Periodont Res 2012; 47: 273–285. © 2011 John Wiley & Sons A/S

Periodontitis is a complex chronic subgingival plaque-induced inflammatory disease influenced by multiple factors, including genetics, behavior and the environment. Many genetic association studies have been conducted in periodontology. One of the most extensively investigated gene families is the Fcγ receptor gene family, which plays a key role in regulating host immune responses to bacteria. Unlike other genetic polymorphisms reported in periodontology, most Fcγ receptor polymorphisms reported not only have established biological functions but are reported to associate with other autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. There are, however, few recent reviews summarizing the association of this gene family with periodontitis. This article critically reviews the current understanding of genetic polymorphism studies in periodontitis, then summarizes the research status of Fcγ receptor polymorphisms and periodontitis and also of other genes involved in the regulatory network of Fcγ receptors, with special reference to their anticipated biological roles. Moreover, some possible future research directions in the related area are discussed.

Lei Chai, DDS, PhD, School of Dentistry, University of Queensland, 200 Turbot Street, Brisbane, QLD 4000, Australia
Tel: +61 7 33658033
Fax: +61 7 33658199
e-mail: l.chai@uq.edu.au

Key words: Fcγ receptor; genetics; periodontitis; polymorphism; receptor

Accepted for publication October 10, 2011

Human periodontitis is a chronic infectious disease that is characterized by plaque-induced destruction of periodontal soft tissue and alveolar bone. The etiology of the disease is unclear, but it is commonly believed that bacterial infection interacts with host defense, which is modified by a multitude of agents, such as genetic, behavioral and environmental factors (1). In the past decade, many studies have been carried out to investigate genetic susceptibility to periodontal diseases. In this respect, one of the most extensively studied gene families is that of the Fcγ receptor (FcγR), which has been proved to be essential in the pathogenesis of periodontal disease. In this review, we summarize current genetic association studies on the FcγR gene family and other genes in its immune network. We hope the review will give readers a general idea of the association between FcγR and periodontitis, as well as the current status of genetic study in periodontology and its future directions.

Periodontitis is a complex disease

A classification system based on genetic involvement puts diseases into three categories: chromosomal, Mendelian and complex (2). Chromosomal disorders are characterized by gross abnormalities in chromosome number or structure, and often result in preterm death related to developmental abnormalities. Mendelian disorders are caused by a few rare mutations of a single gene or, exceptionally, of more than one gene (3,4). Mendelian disorders usually display familial patterns of inheritance, including autosomal recessive, autosomal dominant or X-linked transmission of the disease-related alleles, and there is a direct correlation between genotype and phenotype. It is generally accepted that complex diseases have a multifactorial pathogenesis and develop as a result of the interplay between several genes or genetic variants and environmental factors (including bacterial infection and smoking), somatic mutations and epigenetic modifications (5). Thus, inherited genetic variation is not the

direct cause of a complex disease but instead mediates the risk of disease development in response to exposure to one or more environmental factors; therefore, the clinical and genetic heterogeneity of such disorders makes the analysis of their exact causes extremely difficult (6; Fig. 1).

Genetic factors can influence the intensity and severity of host responses to bacterial challenge, which may result in various levels of periodontal tissue destruction. As a consequence, different patients might exhibit different levels of immune responses to the same level of infection (7–9). Specifically, different allelic variants can lead to variations in different aspects of host immune responses, such as innate immunity, adaptive immunity and autoimmune reaction (10). Genetic variations may also serve as either protective or risk factors for diseases such as periodontitis (11). For these reasons, periodontitis is considered a complex disease whose phenotype is determined by both genetic make up and environmental influences on the host bacterial interaction within an individual. Therefore, genetic polymorphism studies of periodontitis need careful design and cautious interpretation (9).

Genetic polymorphism study of complex human diseases

Until the availability of detailed genetic maps thanks to the Human Genome Project, the identification of DNA mutations that caused rare disorders, such as cystic fibrosis and Huntington's disease, depended on genetic linkage and positional cloning studies (12–15). However, such approaches were unsuccessful in identifying loci that contribute to complex diseases. In 1996, Risch and Merikangas (16) suggested that association studies could be more powerful than linkage studies in identifying susceptibility loci. Furthermore, some researchers postulated the hypothesis that common variants are the base of common diseases, suggesting that common DNA variation, as opposed to rare mutations, could be responsible for a proportion of common diseases (17–19). Although that hypothesis remains controversial, resources for association studies, such as dense genetic maps of single nucleotide polymorphisms (SNPs) across the human genome, enable investigators to more rapidly identify disease-associated loci that could have a major impact on public health (20). Association studies are currently the focus of most study designs for identifying loci involved in complex diseases, such as cardiovascular diseases, diabetes, cancer and periodontal diseases.

There are two approaches for studying candidate SNPs: direct and indirect. In the direct association study, the proposed causative SNP is genotyped directly. Despite the proven success of the direct approach using nonsynonymous SNPs (nonsynonymous change may either be missense or nonsense, where a missense change in the coding sequence results in a different amino acid, while a nonsense change in the coding sequence results in a premature stop codon; 21), a major challenge is predicting or determining *a priori* which SNPs are likely to be causative or predictive of the phenotype of interest, in particular, because our current knowledge about the pathogenesis of most complex diseases and SNP functions is limited. Hence, the selection of the candidate SNPs is usually difficult. The indirect approach, on the other hand, is much like a linkage study in that it assays multiple markers while assuming them to be neutral, without assuming the location of the causative gene or locus (22). It is most often a case–control study on subjects drawn from the general population and uses a measure of allelic association or site correlation (known as linkage disequilibrium) to detect historical recombination. This strategy, however, also has some problems: sample selection reduces statistical power, particularly for rare alleles; haplotypes at multiple loci cannot be resolved, thereby precluding some powerful mapping strategies; and clinical samples are less readily analysable using stratification by phenotypic differences and environmental factors, which may be critical to understanding disease susceptibility (23).

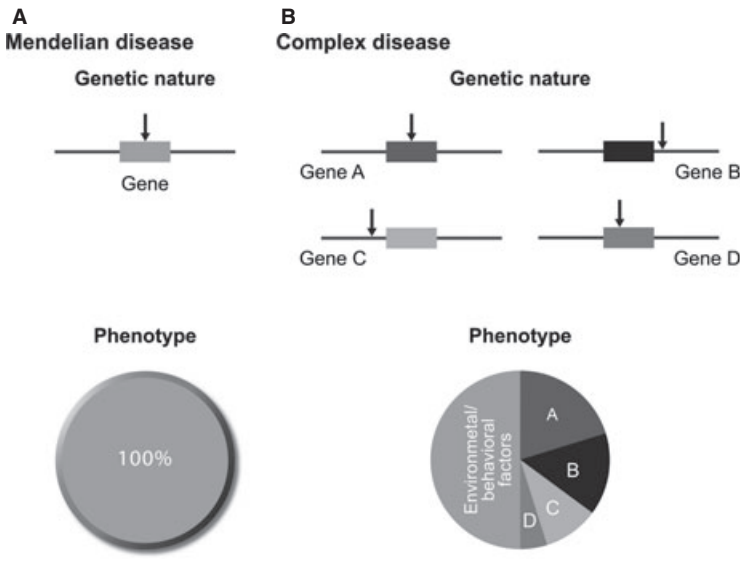

Fig. 1. Impact of mutation or variations on disease phenotype for Mendelian or complex diseases. (A) In Mendelian diseases, mutation in a single dominant gene is necessary and sufficient to produce the clinical phenotype and cause disease. (B) In complex disorders with multiple causes, variations or mutations in a number of genes encoding different proteins result in a genetic predisposition to a clinical phenotype. Pedigrees reveal no Mendelian inheritance pattern, and gene mutations are often neither sufficient nor necessary to explain the disease phenotype. Environment and behavioral factors are major contributors to the pathogenesis of complex diseases (157). Box(es) indicate gene(s) involved; arrow indicates genetic mutation or variations.

Among recent developments in genomic research is the genome-wide association study (GWAS), which seems more promising than traditional association studies in identifying molecular pathways of diseases and, to a lesser extent, risk variants of complex diseases, because it scans the whole genome for association without any prior assumptions about the biological process, hence it can possibly find out those variants that would not usually be suspected to be associated based on our current limited knowledge of the biological functions of the genes (24). However, researchers are still debating the usefulness of the GWAS in helping to predict individual genetic risk of complex diseases, because most GWASs carried out so far have not identified variants by which we can accurately predict genetic risks, because the associated variants found out are common and typically have very small effects on the variability of the traits, hence they can explain only a small portion of the heritability (25–27). The majority of effect sizes of risk alleles that have been found so far in GWASs are small, typically with an odds ratio of < 1.5, and with many around 1.1 and 1.2, which represent the limit of detection given the experimental sample sizes employed to date. Alternatively, an individual identified gene variant results in only 10–20% greater susceptibility to a certain disease. Those findings suggested that many GWASs so far may not have sufficient power to discover associations with such small effects (Fig. 2; 28). Larger-scale GWASs (sample size of more than 10,000) are thus required (27). Moreover, for most diseases, GWAS results usually indicate a substantial number of variants that generate small increases in disease risk; such variants cannot individually explain much of the genetic variance. Therefore, a combined strategy such as one using rare and low-frequency variants and structure variants may be required (26,27).

Genetic polymorphism study of periodontitis

In the past decade, many association studies on periodontitis have been reported. However, owing to the complicated nature of the disease and the limitations of the study approaches used, our knowledge of the genetic background of periodontitis is still scant (29). Most of the published research into genetic polymorphisms in periodontitis focuses on genes that play roles in immunoregulation or metabolism, such as genes for cytokines, cell-surface receptors, chemokines and enzymes, as well as genes related to antigen recognition. The direct association approach is most commonly used, but ethnic heterogeneity, different clinical classification systems and other factors, such as variations in sample size and often nonstandardized control criteria, mean that the diverse results remain difficult to comprehend (9). Among the studied genes, FcγRs are one of the gene families that has gained much attention because they link the cellular and humoral immunity and play a pivotal role in the host vs. bacteria response (30). Unlike the interleukin (IL)-1 cluster, however, there are few reviews summarizing the FcγR gene family polymorphisms and periodontitis, although genetic studies of FcγR polymorphisms and

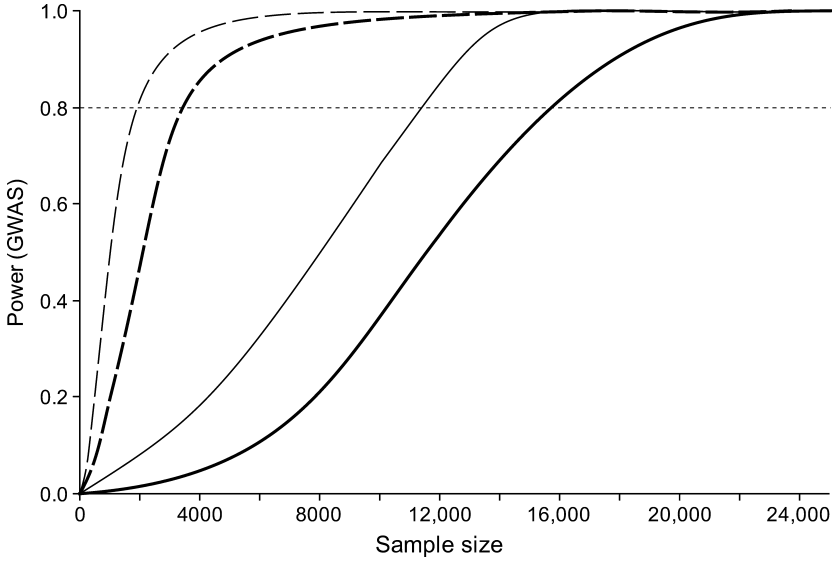

Fig. 2. The power of a genome-wide association study (GWAS) is determined by study sample size and minor allele frequency (MAF), as well as the odds ratio of the risk variant. This figure demonstrates, for a disease with 0.5–1% prevalence (continuous lines, e.g. aggressive periodontitis) and risk variant odds ratio 1.2, the relationship between the expected power and sample size for different MAFs. It shows that when MAF is 0.2 (thin continuous line), sample size over 11,000 cases and 11,000 controls could reach an accepted power of 0.8. However, when MAF is dropped to 0.1 (thick continuous line), the required sample size is increased to 16,000 for both case and control subjects (28). When a disease has 15–20% prevalence (dashed lines, e.g. chronic periodontitis) and risk variant odds ratio is 1.2, a MAF of 0.1–0.2 (between thin and thick dashed lines) means that a sample size of 1500–3000 cases and controls could reach an acceptable power of 0.8. Therefore, most of the GWASs today, particularly those for periodontitis, may not have sufficient power to detect genetic association of complex diseases with small effects.

periodontitis are numerous. The following sections will focus on research that has been undertaken to study the role of FcγRs and other factors, such as the IL-1 family and other cytokines, that are relevant to FcγRs within and beyond their regulatory network in relation to periodontitis, in the context of genetic polymorphisms.

Fcγ receptor polymorphisms

Biology of Fcγ receptors

The Fc receptors for immunoglobulin G (IgG), or FcγRs, were identified more than 40 years ago with the observation that IgG antibodies could be directly cytophilic for macrophages when presented on opsonized red blood cells (31). This binding property of IgG antibodies was found to be independent of the antibody's hypervariable (Fab) region and required only the constant (Fc) portion of the IgG. Subsequent *in vitro* studies established the role of FcγRs in triggering effector responses, such as macrophage phagocytosis, natural killer (NK) cell antibody-dependent cell-mediated cytotoxicity, neutrophil activation and the paradoxical inhibition of B cell activation by IgG immune complexes (32–36). Currently, three different classes of the human FcγR family are recognized, encompassing nine genes (CD64: *FcγRIa*, *Ib* and *Ic*; CD32: *FcγRIIa*, *IIb* and *IIc*; CD16: *FcγRIIIa* and *IIIb* and *FcγRIV*), which have been mapped to the long arm of chromosome 1 (1q21 and 1q23–24; 37–40). While FcγRI has a high affinity for the antibody-Fc region, FcγRII and FcγRIII have a low affinity for the Fc region of IgG (41,42). A new member of the FcγR family, FcγRIV, was recently identified in mice. It is considered to be conserved in all mammalian species and to have intermediate affinity (43–45). Although only single copies of the low-affinity Fc-receptor genes are present in most species, duplications and diversification processes have led to the presence of multiple genes in the human genome (46). The copy number variation of FcγRs is becoming recognized as one of the important genetic polymorphisms for this gene family, as we will discuss later in this review. Unfortunately, most probably owing to their highly homologous sequences, many genome databases list these low-affinity FcγRs not as separate genes but, incorrectly, as allelic versions of one gene (38).

Most FcγR subclasses consist of a separate ligand-binding chain, whose extracellular domain contains the IgG-binding region, and signaling chains essential for the initiation of signal transduction. The exception is neutrophil FcγRIIIb, which is attached to the outer layer of the cell membrane via a glycosyl-phosphatidylinositol anchor. Functionally, there are two different FcγR classes: activating and inhibitory receptors, which transmit their signals via immunoreceptor tyrosine-based activation (ITAM) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs), respectively (47). The general characteristics of human FcγRs are summarized in Table 1. The paired expression of activating and inhibitory molecules on the same cell is the key for the generation of a balanced immune response (47).

Fcγ receptors and periodontitis

The FcγRs are found on a wide variety of immune cells, such as polymorphonuclear granulocytes, lymphocytes and dentritic cells, in both gingival epithelium and pocket epithelium of periodontal tissues (48). Indeed, strong, specific IgG responses against periodontopathic bacteria have been observed in gingival tissue and gingival crevicular fluid (49). Furthermore, micro-organisms and bacterial antigens that have been opsonized with antibody can be either phagocytosed via FcγRs on neutrophils or internalized via FcγRs by antigen-presenting cells (dendritic cells, monocytes, macrophages and B cells). As a consequence, T cells and NK cells may become activated; a variety of cytokines and chemokines may also be released (50). As FcγRs on leukocytes in effect link cellular and humoral branches of the immune system, they can be considered to be an essential component of the host-defense mechanism against bacteria (30). Therefore, any alteration in FcγR expression and function would alter host immune responses against periodontal pathogens, hence susceptibility to periodontal diseases.

Since the recent realization that FcγRIV is a highly conserved member of the FcγR family, researchers have begun refocusing on the affinity of individual Fc receptors for different antibody isotypes (51). One hypothesis is that the low-affinity inhibitory FcγRIIb differentially regulates each activating Fc receptor type, depending on the antibody isotype by which it is regulated (38). The *in vivo* activity of an IgG antibody, therefore, can be predicted on the basis of its activation/inhibition ratio, which in turn is influenced by several factors. Inflammatory mediators, including interferon-γ (IFN-γ), complement component 5a (C5a) and T helper 1 cytokines, such as interleukin-1β and tumor necrosis factor-α (TNF-α), can upregulate activating Fc receptors, while simultaneously decreasing the level of FcγRIIb expression (51–53). The expression of FcγRI can be upregulated by IFN-γ, resulting in elevated

Table 1. General characteristics of human Fcγ receptors (FcγRs)

Receptor class	kDa	Chromosome	Genes	Signaling motif	Affinity for IgG (K_a)
FcγRI (CD64)	72	1q21.1	*FCGR1A*	—	High (10^8–10^9/M)
			FCGR1B	—	
			FCGR1C	—	
FcγRII (CD32)	40	1q23–24	*FCGR2A*	ITAM	Low ($< 10^7$/M)
			FCGR2B	ITIM	
			FCGR2C	ITAM	
FcγRIII (CD16)	50–80	1q23–24	*FCGR3A*	—	Medium ($\pm 3 \times 10^7$/M)
			FCGR3B	—	Low ($< 10^7$/M)

Abbreviations: ITAM, immunoreceptor tyrosine-based activation motif; and ITIM, immunoreceptor tyrosine-based inhibitory motif.

mRNA expression of TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), IL-3 and IL-13 (54). In contrast, T helper 2 cytokines, such as IL-4, IL-5, IL-10, IL-13 or transforming growth factor-β (TGF-β), upregulate expression of inhibitory Fc receptors and downregulate that of activating Fc receptors on innate immune effector cells (52,54–57). It should be noted, however, that cytokine-mediated regulation of FcγR expression is cell type specific. For example, IL-4 upregulates FcγRIIb expression on myeloid cells, but downregulates FcγRIIb expression on activated cells (58). Table 2 lists members the FcγR regulatory network and their relation with FcγRs (except FcγRIV).

Fcγ receptor polymorphisms

Functional bi-allelic polymorphisms have been identified for four FcγR subclasses: FcγRIIa, FcγRIIc, FcγRIIIa and FcγRIIIb (59–63). FcγRIIa contains either an arginine (-R131) or a histidine (-H131) at amino acid position 131 in the second extracellular immnunoglobulin-like domain (RefSNP: rs1801274; 64,65). Depending on the amino acid, the receptor affinity for IgG2 is strongly affected (66). For example, FcγRIIa-H/H131 neutrophils internalize human IgG2-opsonized bacteria more efficiently than FcγRIIa-R/R131 neutrophils (67). Several studies have shown that allelic polymorphisms in the first extracellular domain (EC1) of FcγRIIc corresponding to amino acid position 13 (EC1-13) with either a CAG or a TAG can possibly determine the expression and function of FcγRIIc on normal human NK cells because CAG is a codon for Gln while TAG is a stop codon, hence resulting in either a functional open reading frame or a null allele (59,60). Furthermore, receptor affinity for both monomeric and immune-complexed IgG1and IgG3 is higher for the FcγRIIIa-158V allotype than the FcγRIIIa-158F allotype (rs396991; 68). Neutrophil-specific FcγRIIIb polymorphisms are characterized by their reactivity to anti-FcRIII monoclonal antibodies and alloantisera that recognize determinants of the biallelic neutrophil antigen (NA) system. Receptors that react with only monocolonal antibody Gran 11 and anti-NA1 alloantibodies are regarded as NA1NA1, while receptors that react with only anti-NA2 alloantibodies are NA2NA2 and the remainder, which can react with both anti-NA1 and anti-NA2 alloantibodies, are NA1NA2 (61,69). The NA1–NA2 polymorphisms caused by five bases changes, in codons 36, 38, 65, 82 and 106, lead to four predicted amino acid substitutions within the first extracellular immunoglobulin-like domain (69). As a result, NA1 has only four potential N-linked glycosylation sites, compared with six in NA2 FcγRIIIb (69). Neutrophils from FcγRIIIb-NA2 individuals bind IgG1 or IgG3 less efficiently than neutrophils from individuals with FcγRIIIb-NA1 (67). *In vitro* findings also have suggested differential protein expression profiles in neutrophils between FcγRIIIb genotypes (70). Figure 3 illustrates the function of FcγRs and summarizes most of their related polymorphism studies.

Apart from SNPs, Fcγ receptor genes also exhibit variation in their copy numbers. Copy number variation has been demonstrated for *FCGR3B*, *FCGR2C* and *FCGR3A* genes, but not for *FCGR2A* or *FCGR2B* (71). Copy number variation in *FCGR3B* has been shown to be associated with surface expression of FcγIIIb in neutrophils. In addition, neutrophils isolated from donors with more than or equal to two gene copies displayed enhanced IgG-induced effector responses, as well as increased cell adherence in IgG-coated surfaces compared with those from donors with fewer than two gene copies (72). The copy number variation of *FCGR3B* has been reported to be associated with several chronic inflammatory diseases, such as systemic lupus erythematosus (72), rheumatoid arthritis (73) and immune-mediated glomerulonephritis (74). The *FCGR2C* copy number variation is found to be associated with idiopathic thrombocytopenic purpura (75). The same study also reported that NK cells from individuals with two or three copies of *FCGR3A* seem to express higher levels of receptor and exhibit greater antibody-dependent killing capacity than those from individuals with one copy of the gene (75). It should be noticed that although a number of studies have made use of well-validated complementary techniques for the assessment of copy number variation, there is controversy concerning the accuracy and sensitivity of some of these techniques, because they are still at an early stage of technical development.

Table 2. Factors that regulate human FcγRs and their actions

Regulatory function of FcγRs	Factors	References
Increase expression of FcγRI via upregulation of mRNA expression	Granulocyte macrophage colony-stimulating factor	Okayama *et al.* (54) Pricop *et al.* (56)
	Interleukin-3	Radeke *et al.* (52)
	Tumor necrosis factor-α	
Upregulate activating FcγRs and reduce FcγRIIb expression	Complement component 5a	Guyre *et al.* (51)
	Interferon-γ	Okayama *et al.* (54)
	Interleukin-1β	Pricop *et al.* (56)
	Tumor necrosis factor-α	Radeke *et al.* (52) Shushakova *et al.* (53)
Upregulate FcγRIIb expression on myeloid cells and downregulate FcγRIIb expression on activated B cells	Interleukin-4	Rudge *et al.* (58)
Upregulate inhibitory FcγRIIb and reduce expression of activating FcγRs on innate effector cells	Interleukin-5	Okayama *et al.* (54)
	Interleukin-10	Pricop *et al.* (56)
	Interleukin-13	Radeke *et al.* (52)
	Transforming growth factor-β	Tridandapani *et al.* (57) Nimmerjahn *et al.* (55)

Fcγ receptor polymorphisms and periodontitis

Most studies on the association between genetic polymorphisms of FcγRs and

periodontitis are based on the bi-allelic polymorphisms mentioned above. Studied groups have come from Caucasian, African-American, Japanese and Chinese populations. Different definitions of periodontitis that have been used include early onset periodontitis, adult periodontitis, aggressive periodontitis, chronic periodontitis and recurrent periodontitis (30,76–88; Table 3). Unsurprisingly, the differing populations, periodontitis types and study designs have led to mixed conclusions (89). Apart from most of the association studies trying to establish confirmed association between those single FcγR polymorphisms mentioned above and periodontitis, some researchers tried to use different strategies to look for associated variations, e.g. Chai *et al*. (76) has screened 103 SNPs in FcγRs and reported a novel SNP (rs445509) in FcγRIIIa that may associated with chronic periodontitis in Chinese. Some researchers focus on FcγR polymorphism biofunction in pathogenesis of periodontitis, but the results seem controversial as described in the association studies mentioned above.

Nicu *et al*. (90) have investigated the function of FcγR genetic variants on host against periodontopathogenic bacteria. It has been reported that periodontitis patients with FcγRIIa H/H-131 genotype seemed to suffer more bone loss comparing with periodontitis patients having the H/R or R/R genotype, and their polymorphonuclear leukocytes showed higher reactivity in response to periodontopathogenic bacteria than those of patients with other genotypes (90). Neutrophils with FcγRIIa-R/R131 genotype have been reported to be associated with lower phagocytic index of *E. coli* in aggressive periodontitis patients (91). In contrast, subjects with FcγRIIa-H/H131 gentoype seem to exhibit a higher percentage of IL-1β-producing cells than -R/H131 and -R/R131 subjects, indicating inter-individual differences in periodontitis risk (92). Wolf *et al*. (87), however, failed to demonstrate a clinically relevant relationship between FcγRIIa polymorphism and periodontal status in a prospective follow-up study. Shimomura-Kuroki *et al*. (93) failed to detect any association between Fcγ RIIa-131 polymorphisms and localized aggressive periodontitis in Japanese adolescent subjects. It has been reported that FcγRIIIb-NA2-carrying polymorphonuclear leukocytes seem less efficient in phagocytosis and induction of an oxidative burst upon interaction with IgG1- and IgG3-opsonized *Porphyromonas gingivalis* (94). However, in the same prospective study mentioned above, Wolf *et al*. (87) failed to demonstrate any clinically relevant relationship between FcγRIIIb (NA1–NA2) polymorphism and periodontal status. Moreover, it should be noticed that FcγRIIb polymorphisms may also play an important role in the pathogenesis of periodontitis, because there are large numbers of FcγRII-bearing B lymphocytes in periodontal lesions, and so far FcγRIIb is the only known inhibitory receptor in the FcγR family that is pivotal in the regulation of B cell activation. Indeed, an association between FcγRIIb-232T and aggressive periodontitis has been shown in Japanese subjects (89). The FcγRIIb-232T allele might be related to the lower levels of antibody response to *P. gingivalis* in Japanese chronic periodontitis patients (95). So far, there are no studies about copy number variation of FcγRs and periodontitis.

A recent meta-analysis about FcγR polymorphisms and their association with periodontal disease included a total of 17 studies reporting association of FcγRIIIb NA1–NA2 polymorphism with both aggressive and chronic periodontitis, weak evidence for association between FcγRIIa H131R polymorphism and aggressive periodontitis in Asians, and no relationship between FcγRIIIa F158V and periodontal disease (96). However, it should be noticed that the inclusion criteria for this meta-analysis did not consider the sample size of each individual study, which as discussed in this review is a key issue in any association studies.

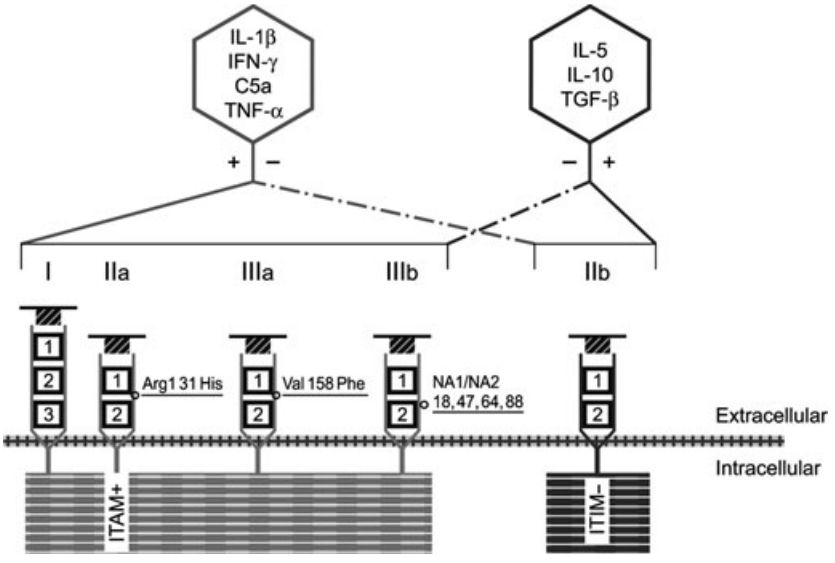

Fig. 3. Fcγ receptors and their regulatory factors. FcγI, FcγIIa, IIIa and IIIb are immune activation receptors. FcγIIa activates an immune response through an immunoreceptor tyrosine-based activation motif (ITAM). FcγIIb is the inhibitory receptor and mediates an immune response via an immunoreceptor tyrosine-based inhibition motif (ITIM). The most studied polymorphisms are labeled on corresponding receptors. The left hexagon indicates factors that activate those activating receptors but inhibit FcγIIb. In the right hexagon are factors inhibit activating receptors but enhance inhibition of FcγIIb. The continuous line means upregulation, while the dash-dotted line means downregulation. Influences of other minor regulatory factors, such as granulocyte macrophage colony-stimulating factor, interleukin-3, interleukin-4 and interleukin-13 were not included. IL-1β, Interleukin-1β; IFN-γ, interferon-γ; C5a, complement component 5a; TNF-α, tumor necrosis factor-α; IL-5, interleukin-5; IL-10, interleukin-10; TGF-β, transforming growth factor-β.

Table 3. Summary of findings from studies of association between human FcγR genes and periodontitis[a]

Periodontitis	Population	*Fcγ RIIa*	*Fcγ RIIb*	*Fcγ RIIIa*	*Fcγ RIIIb*	References
Aggressive (early onset)	Caucasian	+	ND	+	–	Loos *et al.* (30)
		–	ND	–	+	Nibali *et al.* (84)
	African-American	–	ND	–	+	Fu *et al.* (80)
	Japanese	–	ND	–	+	Kobayashi *et al.* (81)
		–	+	–	–	Yasuda *et al.* (89)
	Chinese	+	ND	ND	–	Chung *et al.* (77)
	Brazilian	+	ND	ND	+	de Souza *et al.* (79)
Chronic (adult)	Caucasian	+	ND	–	–	Loos *et al.* (30)
		+	ND	ND	ND	Yamamoto *et al.* (88)
		–	ND	ND	–	Wolf *et al.* (87)
	Japanese	–	ND	–	–	Kobayashi *et al.* (82)
		ND	ND	+	ND	Sugita *et al.* (86)
		–	+	–	–	Yasuda *et al.* (89)
		ND	+	ND	ND	Honma *et al.* (95)
	Chinese	–	ND	ND	–	Chung *et al.* (77)
Severe chronic (adult)	Caucasian	+	ND	+	–	Meisel *et al.* (83)
		+	ND	–	–	Loos *et al.* (30)
		+	ND	ND	ND	Yamamoto *et al.* (88)
	Japanese	–	ND	+	–	Kobayashi *et al.* (81)
	Chinese	–	–	+	–	Chai *et al.* (76)
Recurrent chronic (adult)	Caucasian	–	ND	–	–	Colombo *et al.* (78)
	Japanese	–	ND	+	+	Kobayashi *et al.* (81)
		ND	ND	ND	+	Sugita *et al.* (85)

Abbreviations: +, positive association reported; –, negative association reported; ND, not determined.

[a]No data available regarding *FcγRIa*, *FcγRIb*, *FcγRIc*, *FcγRIIc* and *FcγRIV* polymorphism and periodontitis.

In general, although researchers have shown some evidence that FcγRIIa and FcγRIIIb polymorphisms, as well as FcγRIIb polymorphisms, may be associated with periodontitis, more studies on various populations are needed to confirm whether these conclusions can be extrapolated to the general population.

Genetic polymorphisms within the Fcγ receptor regulatory network

As shown in Table 2, the members of the FcγR regulatory network can be categorized as follows: activation factors, such as IL-1β, TNF-α, IFN-γ, IL-13, C5, IL-3 and GM-CSF; inhibitory factors, such as IL-10, TGF-β and IL4; and cytokines that have both functions (Fig. 3). Compared with FcγRs, cytokines of the regulatory network, such as IL-1 and TNF-α, have received more attention in terms of the number of studies on polymorphisms and susceptibility to periodontitis. Nevertheless, some members of the regulatory network have been neglected, especially in periodontal susceptibility studies. The following sections will discuss genetic polymorphisms within the FcγR regulatory network in detail.

Activation factors

Interleukin-1 family— The biological activity, molecular biology and clinical relevance of the IL-1 family have been studied extensively. Interleukin-1 is a potent proinflammatory cytokine that is released by macrophages, platelets and endothelial cells. The gene encoding this cytokine lies on chromosome 2q13–21 (97–99). In 1997, Kornman *et al.* (100) reported an association between polymorphisms in the genes encoding IL-1α (–889; rs1800587) and IL-1β (Y3953; rs1143634; termed the 'composite genotype') and an increased severity of periodontitis. This initial study has been highly influential in arousing interest in gene polymorphisms and periodontitis. The IL-1 family has become the most studied in the search for genetic associations with periodontitis and can serve as a useful example for considering the strengths and limitations of using gene polymorphisms in disease association studies in periodontitis.

On the basis of numerous studies of IL-1 composite genotypes and periodontitis, Kinane *et al.* (10) summarized current understanding of the association between IL-1 family genotypes and periodontitis. The overall findings are as follows: (i) the IL-1 composite genotype appears irrelevant in aggressive periodontitis; (ii) such composite genotype may be in linkage disequilibrium with the gene contributing to susceptibility to chronic periodontitis; (iii) the composite polymorphisms may be part of several involved in the genetic risk for chronic periodontitis; (iv) the polymorphism is only a useful marker in a defined population (101,102); (v) confirmation of the functional significance of this gene polymorphism remains to be established; and (vi) clinical utilization of the composite polymorphisms for risk assessment and prognostic determination is premature. A recent meta-analysis supports these opinions by showing a statistically significant association between IL-1 cluster polymorphisms and chronic periodontitis (103). The same meta-analysis also found a weak positive association with IL-1β (–511; rs16944; 103).

Tumor necrosis factor— Tumor necrosis factor is a proinflammatory cytokine that possesses a wide range of immunoregulatory functions. It has the potential to stimulate the production of secondary mediators, including chemokines or cyclo-oxygenase products, which consequently amplify the degree of inflammation (104,105). The *TNF* gene is located on chromosome 6 within the major histocompatibility complex, in the 6p21.3 Class III human leukocyte antigen zone (106). Research on some SNPs, such as –1031T/C (rs1799964), –863C/A (rs1800630), –857C/T (rs1799724) and –308G/A (rs1800629), in the promoter region of this gene has revealed conflicting findings for their association with periodontitis (107–115). Meta-analysis of studies done so far on –308G/A could

not establish an association between the polymorphism and susceptibility to chronic periodontitis (103).

Miscellaneous factors— Studies on IFN-γ –874T/A (rs2430561) and chronic periodontitis have shown mostly negative results (116–118). A study on the IL-13 promoter polymorphisms –1112C/T (rs1800925) and –1512A/C (rs1881457) in aggressive periodontitis also did not show significant results (119). Other inflammatory mediators, such as C5 rs17611, have been found to be associated with severe chronic periodontitis in the Chinese population (120). Additionally, C5 1632C/T (rs25681) and 2404A/G (rs17611) have been found to be associated with bronchial asthma (121), and rs17611 and rs2300929 with liver fibrogenesis (122).

Inhibitory factors

Interleukin-10— Interleukin-10 stimulates the production of protective antibodies and downregulates pro-inflammatory cytokines produced by monocytes (123–125). The gene encoding IL-10 has been mapped to chromosome 1q31–32 (126). Three promoter SNPs have been described: -–1087G/A (rs1800896), –819C/T (rs1800871) and –592C/A (rs1800872; 127,128). These three loci exhibit strong linkage disequilibrium (129). There is some evidence of association of such polymorphism with periodontitis, but only in particular populations (130–133). Microsatellite polymorphisms have been identified in the 5′-flanking region of the gene, but no association with periodontitis has been established (113,134).

Transforming growth factor-β1— Transforming growth factor-β1 is released during tissue injury and by inflammatory cells exposed to bacteria and their products (135). It has both therapeutic and pathologic potential (136). The gene is located on chromosome 19q13.1 (137), and SNP –509C/T (rs1800469) has been reported to be associated with periodontitis in Brazilian Caucasians but not Czech Caucasians (138,139).

Interleukin-4— Interleukin-4 can rescue B lymphocytes from apoptosis and enhance their survival, thus playing a role in promoting B-cell-mediated autoimmunity (116). It is also a potent downregulator of macrophage function (140,141). The gene has been mapped to chromosome 5q31.1 (142), with a promoter SNP at position –590 (rs2243250) and a 70 bp variable-number tandem repeat polymorphism at intron 2 (11). Case–control reports relating to aggressive periodontitis and chronic periodontitis susceptibility and severity across several populations did not find a connection between these polymorphisms and periodontitis (143–147).

Other regulatory members

So far, no study on IL-3 and IL-5 polymorphisms in periodontal diseases has been reported. However, reports on IL-3 + 79T/C (rs40401) in association with asthma and atopy (148), IL-3 –16T/C and –131T/C in association with rheumatoid arthritis (149), and IL-5 rs2522411 and –703C/T in association with atopic dermatitis (150,151) have been published. Granulocyte macrophage colony-stimulating factor 545G/A (rs2069616), 3606T/C (rs25881) and 3928C/T (rs25882) have also been found to be associated with atopic diseases (152). Whether any of these genetic polymorphisms are related to periodontitis still needs further investigation.

Limitations and future directions

Other than genetic polymorphism studies, large-scale genomic screening and large-scale population investigations in periodontal research, such as multicommunity screening, are rare. The paucity of research may be due to the complex natural course of periodontitis, lack of a robust classification system, difficulties in searching matched controls, or other factors (29). Most of the studies about FcγR polymorphisms and periodontitis have focused on single or several variations of the candidate genes in a certain population (e.g. studies listed in Table 3), and have provided vast quantities of diverse data that are difficult to interpret and lead to general conclusions. Even for the most extensively studied variations in the IL-1 cluster, meta-analysis can only give a positive conclusion in Caucasians (10,103). Moreover, the number of studies providing thorough data (e.g. allele type, genotype and haplotype) together with Hardy–Weinberg equilibrium and minor allele frequencies, is small.

Although the FcγR genetic polymorphism studies related to periodontal diseases in the past decade have given us some evidence that FcγR genetic variants can modify host immune responses and lead to different phenotypes of periodontal disease, it is too early to draw any conclusions. With the completion of the Human Genome Project and the availability of cutting-edge technology, the application of genetic information and technology to the diagnosis and treatment of periodontitis is conceptually compelling. Nonetheless, it is important to maintain a realistic perspective of the clinical utility of genetic information (153,154). In the future, researchers should also be cautious of numerous weak associations that may turn out to be spurious at repeated testing (155). It is not enough that only the racial and ethnic backgrounds of the subjects are taken into account; studies must have sufficient numbers of cases and controls, with the controls carefully chosen to make the association between polymorphisms and periodontitis much clearer. The choice of candidate genes must also be justifiable and the data clearly presented to show the range of effect and risk attributable to the gene variation. In many currently published genetic association studies, the reported associated SNPs show no obvious function, thus providing few clues on pathogenesis. Recent developments in high-throughput target resequencing can overcome this limitation by searching for variants in targeted gene regions, such as exons or other regions with known function (156). Combination strategies can also be utilized, such as combination of genome-wide scanning and candidate

gene strategy, to improve both the efficiency and the efficacy of studies, especially periodontal genetic studies, for which it is usually difficult to screen a large population. It should be kept in mind that our knowledge of FcγR genetics is expanding and that new technology for detecting different kinds of variations is continually being developed. The most important task for us before we dig in is to understand this new knowledge and technology thoroughly and find a way to incorporate this knowledge and technology, given the unique nature of periodontitis. Only that can help us to establish a reasonable and practical strategy for association study in periodontitis.

Acknowledgements

The work described in this paper was fully supported by University Research Committee Grants 10206094 and 10207348, as well as partially supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (HKU772110M). A draft of the manuscript was edited by Trevor Lane, Faculty of Dentistry, The University of Hong Kong.

References

1. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontol 2000* 1997;**14:**216–248.
2. Lazaridis KN, Juran BD. American Gastroenterological Association future trends committee report: the application of genomic and proteomic technologies to digestive disease diagnosis and treatment and their likely impact on gastroenterology clinical practice. *Gastroenterology* 2005;**129:**1720–1752.
3. Shelling AN, Ferguson LR. Genetic variation in human disease and a new role for copy number variants. *Mutat Res* 2007;**622:**33–41.
4. Sidransky E. Heterozygosity for a Mendelian disorder as a risk factor for complex disease. *Clin Genet* 2006;**70:** 275–282.
5. Chakravarti A, Little P. Nature, nurture and human disease. *Nature* 2003;**421:** 412–414.
6. Juran BD, Lazaridis KN. Applying genomics to the study of complex disease. *Semin Liver Dis* 2007;**27:**3–12.
7. Loos BG, John RP, Laine ML. Identification of genetic risk factors for periodontitis and possible mechanisms of action. *J Clin Periodontol* 2005;**32**(suppl 6)**:**159–179.
8. Nibali L, Donos N, Henderson B. Periodontal infectogenomics. *J Med Microbiol* 2009;**58:**1269–1274.
9. Yoshie H, Kobayashi T, Tai H, Galicia JC. The role of genetic polymorphisms in periodontitis. *Periodontol 2000* 2007;**43:**102–132.
10. Kinane DF, Shiba H, Hart TC. The genetic basis of periodontitis. *Periodontol 2000* 2005;**39:**91–117.
11. Mout R, Willemze R, Landegent JE. Repeat polymorphisms in the interleukin-4 gene (IL4). *Nucleic Acids Res* 1991;**19:** 3763.
12. Group T. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993;**72:**971–983.
13. Gusella JF, Wexler NS, Conneally PM *et al*. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 1983;**306:**234–238.
14. Riordan JR, Rommens JM, Kerem B *et al*. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;**245:** 1066–1073.
15. Rommens JM, Iannuzzi MC, Kerem B *et al*. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989;**245:**1059–1065.
16. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996;**273:**1516–1517.
17. Chakravarti A. Population genetics – making sense out of sequence. *Nat Genet* 1999;**21:**56–60.
18. Collins FS, Guyer MS, Charkravarti A. Variations on a theme: cataloging human DNA sequence variation. *Science* 1997; **278:**1580–1581.
19. Lander ES. The new genomics: global views of biology. *Science* 1996;**274:**536–539.
20. Collins FS, Green ED, Guttmacher AE, Guyer MS. A vision for the future of genomics research. *Nature* 2003;**422:**835–847.
21. Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* 2004;**305:**869–872.
22. Crawford DC, Akey DT, Nickerson DA. The patterns of natural variation in human genes. *Annu Rev Genomics Hum Genet* 2005;**6:**287–312.
23. Kruglyak L, Nickerson DA. Variation is the spice of life. *Nat Genet* 2001;**27:**234–236.
24. Khor CC, Goh DL. Strategies for identifying the genetic basis of dyslipidemia: genome-wide association studies vs. the resequencing of extremes. *Curr Opin Lipidol* 2010;**21:**123–127.
25. Janssens AC, van Duijn CM. Genome-based prediction of common diseases: advances and prospects. *Hum Mol Genet* 2008;**17:**R166–R173.
26. Manolio TA, Collins FS, Cox NJ *et al*. Finding the missing heritability of complex diseases. *Nature* 2009;**461:**747–753.
27. Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk of complex disease. *Curr Opin Genet Dev* 2008;**18:**257–263.
28. Cichon S, Craddock N, Daly M *et al*. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. *Am J Psychiatry* 2009;**166:**540–556.
29. Chai L, Corbet EF, Leung WK. Genetic polymorphisms and periodontitis. In: Walchuck RE, ed. *Periodontitis: Symptoms, Treatment and Prevention. Public Health in the 21st Century Series*. Hauppauge, New York: Nova Science Publishers, Inc., 2010:209–219.
30. Loos BG, Leppers-Van de Straat FG, Van de Winkel JG, Van der Velden U. Fcgamma receptor polymorphisms in relation to periodontitis. *J Clin Periodontol* 2003;**30:**595–602.
31. Berken A, Benacerraf B. Properties of antibodies cytophilic for macrophages. *J Exp Med* 1966;**123:**119–144.
32. Anderson CL, Shen L, Eicher DM, Wewers MD, Gill JK. Phagocytosis mediated by three distinct Fc gamma receptor classes on human leukocytes. *J Exp Med* 1990;**171:**1333–1345.
33. Chan PL, Sinclair NR. Regulation of the immune response. V. An analysis of the function of the Fc portion of antibody in suppression of an immune response with respect to interaction with components of the lymphoid system. *Immunology* 1971;**21:**967–981.
34. Phillips NE, Parker DC. Fc-dependent inhibition of mouse B cell activation by whole anti-mu antibodies. *J Immunol* 1983;**130:**602–606.
35. Titus JA, Perez P, Kaubisch A, Garrido MA, Segal DM. Human K/natural killer cells targeted with hetero-cross-linked antibodies specifically lyse tumor cells in vitro and prevent tumor growth in vivo. *J Immunol* 1987;**139:**3153–3158.
36. Young JD, Ko SS, Cohn ZA. The increase in intracellular free calcium associated with IgG gamma 2b/gamma 1 Fc receptor-ligand interactions: role in

phagocytosis. *Proc Natl Acad Sci USA* 1984;**81:**5430–5434.
37. Le Coniat M, Kinet JP, Berger R. The human genes for the alpha and gamma subunits of the mast cell receptor for immunoglobulin E are located on human chromosome band 1q23. *Immunogenetics* 1990;**32:**183–186.
38. Nimmerjahn F, Ravetch JV. Fcgamma receptors: old friends and new family members. *Immunity* 2006;**24:**19–28.
39. Sammartino L, Webber LM, Hogarth PM, McKenzie IF, Garson OM. Assignment of the gene coding for human FcRII (CD32) to bands q23q24 on chromosome 1. *Immunogenetics* 1988;**28:** 380–381.
40. Takai S, Kasama M, Yamada K *et al*. Human high-affinity Fc gamma RI (CD64) gene mapped to chromosome 1q21.2-q21.3 by fluorescence in situ hybridization. *Hum Genet* 1994;**93:**13–15.
41. Hulett MD, Hogarth PM. Molecular basis of Fc receptor function. *Adv Immunol* 1994;**57:**1–127.
42. Ravetch JV, Kinet JP. Fc receptors. *Annu Rev Immunol* 1991;**9:**457–492.
43. Davis RS, Dennis G Jr, Odom MR *et al*. Fc receptor homologs: newest members of a remarkably diverse Fc receptor gene family. *Immunol Rev* 2002;**190:**123–136.
44. Mechetina LV, Najakshin AM, Alabyev BY, Chikaev NA, Taranin AV. Identification of CD16-2, a novel mouse receptor homologous to CD16/Fc gamma RIII. *Immunogenetics* 2002;**54:**463–468.
45. Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. FcgammaRIV: a novel FcR with distinct IgG subclass specificity. *Immunity* 2005;**23:**41–51.
46. Qiu WQ, de Bruin D, Brownstein BH, Pearse R, Ravetch JV. Organization of the human and mouse low-affinity Fc gamma R genes: duplication and recombination. *Science* 1990;**248:**732–735.
47. Ravetch JV, Lanier LL. Immune inhibitory receptors. *Science* 2000;**290:**84–89.
48. Yuan ZN, Schreurs O, Gjermo P, Helgeland K, Schenck K. Topical distribution of Fc gammaRI, Fc gammaRII and Fc gammaRIII in inflamed human gingiva. *J Clin Periodontol* 1999;**26:**441–447.
49. Ogawa T, McGhee ML, Moldoveanu Z *et al*. Bacteroides-specific IgG and IgA subclass antibody-secreting cells isolated from chronically inflamed gingival tissues. *Clin Exp Immunol* 1989;**76:**103–110.
50. van Sorge NM, van der Pol WL, van de Winkel JG. FcgammaR polymorphisms: implications for function, disease susceptibility and immunotherapy. *Tissue Antigens* 2003;**61:**189–202.
51. Guyre PM, Morganelli PM, Miller R. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. *J Clin Invest* 1983;**72:**393–397.
52. Radeke HH, Janssen-Graalfs I, Sowa EN *et al*. Opposite regulation of type II and III receptors for immunoglobulin G in mouse glomerular mesangial cells and in the induction of anti-glomerular basement membrane (GBM) nephritis. *J Biol Chem* 2002;**277:**27535–27544.
53. Shushakova N, Skokowa J, Schulman J *et al*. C5a anaphylatoxin is a major regulator of activating versus inhibitory FcgammaRs in immune complex-induced lung disease. *J Clin Invest* 2002;**110:**1823–1830.
54. Okayama Y, Kirshenbaum AS, Metcalfe DD. Expression of a functional high-affinity IgG receptor, Fc gamma RI, on human mast cells: up-regulation by IFN-gamma. *J Immunol* 2000;**164:**4332–4339.
55. Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. *Science* 2005;**310:**1510–1512.
56. Pricop L, Redecha P, Teillaud JL *et al*. Differential modulation of stimulatory and inhibitory Fc gamma receptors on human monocytes by Th1 and Th2 cytokines. *J Immunol* 2001;**166:**531–537.
57. Tridandapani S, Wardrop R, Baran CP *et al*. TGF-beta 1 suppresses [correction of supresses] myeloid Fc gamma receptor function by regulating the expression and function of the common gamma-subunit. *J Immunol* 2003;**170:**4572–4577.
58. Rudge EU, Cutler AJ, Pritchard NR, Smith KG. Interleukin 4 reduces expression of inhibitory receptors on B cells and abolishes CD22 and Fc gamma RII-mediated B cell suppression. *J Exp Med* 2002;**195:**1079–1085.
59. Ernst LK, Metes D, Herberman RB, Morel PA. Allelic polymorphisms in the FcgammaRIIC gene can influence its function on normal human natural killer cells. *J Mol Med* 2002;**80:**248–257.
60. Metes D, Ernst LK, Chambers WH, Sulica A, Herberman RB, Morel PA. Expression of functional CD32 molecules on human NK cells is determined by an allelic polymorphism of the FcgammaRIIC gene. *Blood* 1998;**91:**2369–2380.
61. Ory PA, Goldstein IM, Kwoh EE, Clarkson SB. Characterization of polymorphic forms of Fc receptor III on human neutrophils. *J Clin Invest* 1989;**83:**1676–1681.
62. Salmon JE, Millard S, Schachter LA *et al*. Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. *J Clin Invest* 1996; **97:**1348–1354.
63. van de Winkel JG, Capel PJ. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol Today* 1993;**14:**215–221.
64. Warmerdam PA, van de Winkel JG, Gosselin EJ, Capel PJ. Molecular basis for a polymorphism of human Fc gamma receptor II (CD32). *J Exp Med* 1990;**172:**19–25.
65. Warmerdam PA, van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. *J Immunol* 1991;**147:**1338–1343.
66. Parren PW, Warmerdam PA, Boeije LC *et al*. On the interaction of IgG subclasses with the low affinity Fc gamma RIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. *J Clin Invest* 1992;**90:**1537–1546.
67. Bredius RG, Fijen CA, De Haas M *et al*. Role of neutrophil Fc gamma RIIa (CD32) and Fc gamma RIIIb (CD16) polymorphic forms in phagocytosis of human IgG1- and IgG3-opsonized bacteria and erythrocytes. *Immunology* 1994;**83:**624–630.
68. Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. *Blood* 1997;**90:**1109–1114.
69. Ory PA, Clark MR, Kwoh EE, Clarkson SB, Goldstein IM. Sequences of complementary DNAs that encode the NA1 and NA2 forms of Fc receptor III on human neutrophils. *J Clin Invest* 1989;**84:**1688–1691.
70. Yokoyama T, Kobayashi T, Yamamoto K, Yamagata A, Oofusa K, Yoshie H. Proteomic profiling of human neutrophils in relation to immunoglobulin G Fc receptor IIIb polymorphism. *J Periodontal Res* 2010;**45:**780–787.
71. Breunis WB, van Mirre E, Geissler J *et al*. Copy number variation at the FCGR locus includes FCGR3A, FCGR2C and FCGR3B but not FCGR2A and FCGR2B. *Hum Mutat* 2009;**30:**E640–E650.
72. Willcocks LC, Lyons PA, Clatworthy MR *et al*. Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. *J Exp Med* 2008;**205:**1573–1582.
73. McKinney C, Fanciulli M, Merriman ME *et al*. Association of variation in Fcgamma receptor 3B gene copy number with rheumatoid arthritis in Caucasian samples. *Ann Rheum Dis* 2010;**69:**1711–1716.
74. Aitman TJ, Dong R, Vyse TJ *et al*. Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. *Nature* 2006;**439:**851–855.

75. Breunis WB, van Mirre E, Bruin M *et al*. Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. *Blood* 2008; **111:**1029–1038.
76. Chai L, Song YQ, Zee KY, Leung WK. SNPs of Fc-gamma receptor genes and chronic periodontitis. *J Dent Res* 2010;**89:**705–710.
77. Chung HY, Lu HC, Chen WL, Lu CT, Yang YH, Tsai CC. Gm (23) allotypes and Fcgamma receptor genotypes as risk factors for various forms of periodontitis. *J Clin Periodontol* 2003;**30:**954–960.
78. Colombo AP, Eftimiadi C, Haffajee AD, Cugini MA, Socransky SS. Serum IgG2 level, Gm(23) allotype and FcgammaRIIa and FcgammaRIIIb receptors in refractory periodontal disease. *J Clin Periodontol* 1998;**25:**465–474.
79. de Souza RC, Colombo AP. Distribution of FcgammaRIIa and FcgammaRIIIb genotypes in patients with generalized aggressive periodontitis. *J Periodontol* 2006;**77:**1120–1128.
80. Fu Y, Korostoff JM, Fine DH, Wilson ME. Fc gamma receptor genes as risk markers for localized aggressive periodontitis in African-Americans. *J Periodontol* 2002;**73:**517–523.
81. Kobayashi T, Sugita N, van der Pol WL *et al*. The Fcgamma receptor genotype as a risk factor for generalized early-onset periodontitis in Japanese patients. *J Periodontol* 2000;**71:**1425–1432.
82. Kobayashi T, Westerdaal NA, Miyazaki A *et al*. Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. *Infect Immun* 1997;**65:**3556–3560.
83. Meisel P, Carlsson LE, Sawaf H, Fanghaenel J, Greinacher A, Kocher T. Polymorphisms of Fc gamma-receptors RIIa, RIIIa, and RIIIb in patients with adult periodontal diseases. *Genes Immun* 2001;**2:**258–262.
84. Nibali L, Parkar M, Brett P, Knight J, Tonetti MS, Griffiths GS. NADPH oxidase (CYBA) and FcgammaR polymorphisms as risk factors for aggressive periodontitis: a case–control association study. *J Clin Periodontol* 2006;**33:**529–539.
85. Sugita N, Kobayashi T, Ando Y *et al*. Increased frequency of FcgammaRIIIb-NA1 allele in periodontitis-resistant subjects in an elderly Japanese population. *J Dent Res* 2001;**80:**914–918.
86. Sugita N, Yamamoto K, Kobayashi T *et al*. Relevance of Fc gamma RIIIa-158V-F polymorphism to recurrence of adult periodontitis in Japanese patients. *Clin Exp Immunol* 1999;**117:**350–354.
87. Wolf DL, Neiderud AM, Hinckley K, Dahlen G, van de Winkel JG, Papapanou PN. Fcgamma receptor polymorphisms and periodontal status: a prospective follow-up study. *J Clin Periodontol* 2006;**33:**691–698.
88. Yamamoto K, Kobayashi T, Grossi S *et al*. Association of Fcgamma receptor IIa genotype with chronic periodontitis in Caucasians. *J Periodontol* 2004;**75:**517–522.
89. Yasuda K, Sugita N, Kobayashi T, Yamamoto K, Yoshie H. FcgammaRIIB gene polymorphisms in Japanese periodontitis patients. *Genes Immun* 2003; **4:**541–546.
90. Nicu EA, Van der Velden U, Everts V, Van Winkelhoff AJ, Roos D, Loos BG. Hyper-reactive PMNs in FcgammaRIIa 131 H/H genotype periodontitis patients. *J Clin Periodontol* 2007;**34:**938–945.
91. Nibali L, O'Dea M, Bouma G *et al*. Genetic variants associated with neutrophil function in aggressive periodontitis and healthy controls. *J Periodontol* 2010;**81:**527–534.
92. Yamamoto K, Kobayashi T, Sugita N, Tai H, Yoshie H. The FcgammaRIIa polymorphism influences production of interleukin-1 by mononuclear cells. *Int J Immunogenet* 2007;**34:**369–372.
93. Shimomura-Kuroki J, Yamashita K, Shimooka S. Tannerella forsythia and the HLA-DQB1 allele are associated with susceptibility to periodontal disease in Japanese adolescents. *Odontology* 2009; **97:**32–37.
94. Kobayashi T, van der Pol WL, van de Winkel JG *et al*. Relevance of IgG receptor IIIb (CD16) polymorphism to handling of Porphyromonas gingivalis: implications for the pathogenesis of adult periodontitis. *J Periodontal Res* 2000;**35:**65–73.
95. Honma Y, Sugita N, Kobayashi T, Abiko Y, Yoshie H. Lower antibody response to Porphyromonas gingivalis associated with immunoglobulin G Fcgamma receptor IIB polymorphism. *J Periodontal Res* 2008;**43:**706–711.
96. Dimou NL, Nikolopoulos GK, Hamodrakas SJ, Bagos PG. Fcgamma receptor polymorphisms and their association with periodontal disease: a meta-analysis. *J Clin Periodontol* 2010;**37:**255–265.
97. Auron PE, Webb AC, Rosenwasser LJ *et al*. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc Natl Acad Sci U S A* 1984;**81:**7907–7911.
98. Cameron P, Limjuco G, Rodkey J, Bennett C, Schmidt JA. Amino acid sequence analysis of human interleukin 1 (IL-1). Evidence for biochemically distinct forms of IL-1. *J Exp Med* 1985; **162:**790–801.
99. March CJ, Mosley B, Larsen A *et al*. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* 1985;**315:**641–647.
100. Kornman KS, Crane A, Wang HY *et al*. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;**24:**72–77.
101. Armitage GC, Wu Y, Wang HY, Sorrell J, di Giovine FS, Duff GW. Low prevalence of a periodontitis-associated interleukin-1 composite genotype in individuals of Chinese heritage. *J Periodontol* 2000;**71:**164–171.
102. Walker SJ, Van Dyke TE, Rich S, Kornman KS, di Giovine FS, Hart TC. Genetic polymorphisms of the IL-1alpha and IL-1beta genes in African-American LJP patients and an African-American control population. *J Periodontol* 2000; **71:**723–728.
103. Nikolopoulos GK, Dimou NL, Hamodrakas SJ, Bagos PG. Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls. *J Clin Periodontol* 2008;**35:**754–767.
104. Jiang Y, Magli L, Russo M. Bacterium-dependent induction of cytokines in mononuclear cells and their pathologic consequences in vivo. *Infect Immun* 1999;**67:**2125–2130.
105. Offenbacher S, Odle BM, Braswell LD *et al*. Changes in cyclooxygenase metabolites in experimental periodontitis in Macaca mulatta. *J Periodontal Res* 1989;**24:**63–74.
106. Ragoussis J, Bloemer K, Weiss EH, Ziegler A. Localization of the genes for tumor necrosis factor and lymphotoxin between the HLA class I and III regions by field inversion gel electrophoresis. *Immunogenetics* 1988;**27:**66–69.
107. Chiu YF, Chuang LM, Hsiao CF *et al*. An autosomal genome-wide scan for loci linked to pre-diabetic phenotypes in nondiabetic Chinese subjects from the Stanford Asia-Pacific Program of Hypertension and Insulin Resistance Family Study. *Diabetes* 2005;**54:**1200–1206.
108. Craandijk J, van Krugten MV, Verweij CL, van der Velden U, Loos BG. Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. *J Clin Periodontol* 2002;**29:**28–34.
109. Donati M, Berglundh T, Hytonen AM, Hahn-Zoric M, Hanson LA, Padyukov L. Association of the -159 CD14 gene polymorphism and lack of association of the -308 TNFA and Q551R IL-4RA polymorphisms with severe chronic periodontitis in Swedish Caucasians. *J Clin Periodontol* 2005;**32:**474–479.
110. Endo M, Tai H, Tabeta K, Kobayashi T, Yamazaki K, Yoshie H. Analysis of single nucleotide polymorphisms in the 5′-flanking region of tumor necrosis

factor-alpha gene in Japanese patients with early-onset periodontitis. *J Periodontol* 2001;**72:**1554–1559.
111. Folwaczny M, Glas J, Torok HP, Mende M, Folwaczny C. Lack of association between the TNF alpha G -308 A promoter polymorphism and periodontal disease. *J Clin Periodontol* 2004;**31:** 449–453.
112. Galbraith GM, Hendley TM, Sanders JJ, Palesch Y, Pandey JP. Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. *J Clin Periodontol* 1999;**26:**705–709.
113. Kinane DF, Hodge P, Eskdale J, Ellis R, Gallagher G. Analysis of genetic polymorphisms at the interleukin-10 and tumour necrosis factor loci in early-onset periodontitis. *J Periodontal Res* 1999;**34:** 379–386.
114. Shapira L, Stabholz A, Rieckmann P, Kruse N. Genetic polymorphism of the tumor necrosis factor (TNF)-alpha promoter region in families with localized early-onset periodontitis. *J Periodontal Res* 2001;**36:**183–186.
115. Soga Y, Nishimura F, Ohyama H, Maeda H, Takashiba S, Murayama Y. Tumor necrosis factor-alpha gene (TNF-alpha) -1031/-863, -857 single-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese. *J Clin Periodontol* 2003;**30:** 524–531.
116. Babel N, Cherepnev G, Babel D *et al*. Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. *J Periodontol* 2006; **77:**1978–1983.
117. Fraser DA, Loos BG, Boman U *et al*. Polymorphisms in an interferon-gamma receptor-1 gene marker and susceptibility to periodontitis. *Acta Odontol Scand* 2003;**61:**297–302.
118. Reichert S, Machulla HK, Klapproth J *et al*. Interferon-gamma and interleukin-12 gene polymorphisms and their relation to aggressive and chronic periodontitis and key periodontal pathogens. *J Periodontol* 2008;**79:**1434–1443.
119. Gonzales JR, Mann M, Stelzig J, Bodeker RH, Meyle J. Single-nucleotide polymorphisms in the IL-4 and IL-13 promoter region in aggressive periodontitis. *J Clin Periodontol* 2007;**34:**473–479.
120. Chai L, Song YQ, Zee KY, Leung WK. Single nucleotide polymorphisms of complement component 5 and periodontitis. *J Periodontal Res* 2010;**45:**301–308.
121. Hasegawa K, Tamari M, Shao C *et al*. Variations in the C3, C3a receptor, and C5 genes affect susceptibility to bronchial asthma. *Hum Genet* 2004;**115:** 295–301.
122. Hillebrandt S, Wasmuth HE, Weiskirchen R *et al*. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. *Nat Genet* 2005;**37:**835–843.
123. Illera VA, Perandones CE, Stunz LL, Mower DA Jr, Ashman RF. Apoptosis in splenic B lymphocytes. Regulation by protein kinase C and IL-4. *J Immunol* 1993;**151:**2965–2973.
124. Mori M, Morris SC, Orekhova T, Marinaro M, Giannini E, Finkelman FD. IL-4 promotes the migration of circulating B cells to the spleen and increases splenic B cell survival. *J Immunol* 2000;**164:**5704–5712.
125. Singh RR. IL-4 and many roads to lupuslike autoimmunity. *Clin Immunol* 2003;**108:**73–79.
126. Kim JM, Brannan CI, Copeland NG, Jenkins NA, Khan TA, Moore KW. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. *J Immunol* 1992;**148:** 3618–3623.
127. Kube D, Platzer C, von Knethen A *et al*. Isolation of the human interleukin 10 promoter. Characterization of the promoter activity in Burkitt's lymphoma cell lines. *Cytokine* 1995;**7:**1–7.
128. Lazarus M, Hajeer AH, Turner D *et al*. Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. *J Rheumatol* 1997;**24:**2314–2317.
129. Wang WY, Barratt BJ, Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet* 2005;**6:**109–118.
130. Berglundh T, Donati M, Hahn-Zoric M, Hanson LA, Padyukov L. Association of the -1087 IL 10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians. *J Clin Periodontol* 2003;**30:** 249–254.
131. Gonzales JR, Michel J, Diete A, Herrmann JM, Bodeker RH, Meyle J. Analysis of genetic polymorphisms at the interleukin-10 loci in aggressive and chronic periodontitis. *J Clin Periodontol* 2002;**29:**816–822.
132. Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB, Camargo LE, Line SR. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis. *J Clin Periodontol* 2004;**31:** 443–448.
133. Yamazaki K, Tabeta K, Nakajima T *et al*. Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. *J Clin Periodontol* 2001;**28:**828–832.
134. Eskdale J, Kube D, Tesch H, Gallagher G. Mapping of the human IL10 gene and further characterization of the 5′ flanking sequence. *Immunogenetics* 1997;**46:**120–128.
135. Wahl SM, Costa GL, Mizel DE, Allen JB, Skaleric U, Mangan DF. Role of transforming growth factor beta in the pathophysiology of chronic inflammation. *J Periodontol* 1993;**64:**450–455.
136. Border WA, Noble NA. Fibrosis linked to TGF-beta in yet another disease. *J Clin Invest* 1995;**96:**655–656.
137. Fujii D, Brissenden JE, Derynck R, Francke U. Transforming growth factor beta gene maps to human chromosome 19 long arm and to mouse chromosome 7. *Somat Cell Mol Genet* 1986;**12:**281–288.
138. de Souza AP, Trevilatto PC, Scarel-Caminaga RM, de Brito RB Jr, Barros SP, Line SR. Analysis of the MMP-9 (C-1562 T) and TIMP-2 (G-418C) gene promoter polymorphisms in patients with chronic periodontitis. *J Clin Periodontol* 2005;**32:**207–211.
139. Holla LI, Buckova D, Fassmann A, Halabala T, Vasku A, Vacha J. Promoter polymorphisms in the CD14 receptor gene and their potential association with the severity of chronic periodontitis. *J Med Genet* 2002;**39:**844–848.
140. Essner R, Rhoades K, McBride WH, Morton DL, Economou JS. IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J Immunol* 1989;**142:**3857–3861.
141. Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA. Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. *Proc Natl Acad Sci USA* 1989;**86:**3803–3807.
142. Sutherland GR, Baker E, Callen DF *et al*. Interleukin 4 is at 5q31 and interleukin 6 is at 7p15. *Hum Genet* 1988;**79:** 335–337.
143. Gonzales JR, Kobayashi T, Michel J, Mann M, Yoshie H, Meyle J. Interleukin-4 gene polymorphisms in Japanese and Caucasian patients with aggressive periodontitis. *J Clin Periodontol* 2004; **31:**384–389.
144. Kang BY, Choi YK, Choi WH *et al*. Two polymorphisms of interleukin-4 gene in Korean adult periodontitis. *Arch Pharm Res* 2003;**26:**482–486.
145. Michel J, Gonzales JR, Wunderlich D, Diete A, Herrmann JM, Meyle J. Interleukin-4 polymorphisms in early onset periodontitis. *J Clin Periodontol* 2001;**28:**483–488.
146. Pontes CC, Gonzales JR, Novaes AB Jr *et al*. Interleukin-4 gene polymorphism and its relation to periodontal disease in a Brazilian population of African heritage. *J Dent* 2004;**32:**241–246.
147. Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB Jr, Line SR. Investigation of IL4 gene polymorphism in

individuals with different levels of chronic periodontitis in a Brazilian population. *J Clin Periodontol* 2003;**30:**341–345.

148. Park BL, Kim LH, Choi YH *et al*. Interleukin 3 (IL3) polymorphisms associated with decreased risk of asthma and atopy. *J Hum Genet* 2004;**49:**517–527.
149. Yamada R, Tanaka T, Unoki M *et al*. Association between a single-nucleotide polymorphism in the promoter of the human interleukin-3 gene and rheumatoid arthritis in Japanese patients, and maximum-likelihood estimation of combinatorial effect that two genetic loci have on susceptibility to the disease. *Am J Hum Genet* 2001;**68:**674–685.
150. Namkung JH, Lee JE, Kim E *et al*. IL-5 and IL-5 receptor alpha polymorphisms are associated with atopic dermatitis in Koreans. *Allergy* 2007;**62:**934–942.
151. Yamamoto N, Sugiura H, Tanaka K, Uehara M. Heterogeneity of interleukin 5 genetic background in atopic dermatitis patients: significant difference between those with blood eosinophilia and normal eosinophil levels. *J Dermatol Sci* 2003; **33:**121–126.
152. He JQ, Ruan J, Chan-Yeung M *et al*. Polymorphisms of the GM-CSF genes and the development of atopic diseases in at-risk children. *Chest* 2003;**123:**438S.
153. Cooper DN, Nussbaum RL, Krawczak M. Proposed guidelines for papers describing DNA polymorphism-disease associations. *Hum Genet* 2002;**110:**207–208.
154. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001;**29:**306–309.
155. Ioannidis JP, Ntzani EE, Trikalinos TA. 'Racial' differences in genetic effects for complex diseases. *Nat Genet* 2004;**36:** 1312–1318.
156. Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* 2009;**324:**387–389.
157. Peltonen L, McKusick VA. Genomics and medicine. Dissecting human disease in the postgenomic era. *Science* 2001; **291:**1224–1229.