Association of Fc gamma-receptors IIa, IIIa, and IIIb genetic polymorphism with susceptibility to chronic periodontitis in South Indian population

Veenu Madaan Hans, Dhoom Singh Mehta[1], Mayank Hans[2]

Abstract

Background and Objective: Fc gamma receptors (FcγRs) are the members of the immunoglobulin superfamily and may play a role in the pathogenesis of periodontitis. Genetic variation in these receptors and its link with various forms of periodontitis is being studied in different populations. The aim of the present study is to determine whether specific FcγRIIa, FcγRIIIa, and FcγRIIIb alleles and/or genotypes are associated with risk for susceptibility to generalized chronic periodontitis (GCP) in South Indian population. **Materials and Methods:** The study population consisted of 120 South Indian subjects; 60 with GCP and 60 periodontally healthy. Deoxyribonucleic acid (DNA) was extracted from samples collected by scrapping buccal epithelium. FcγRIIa and FcγRIIIa genotyping were performed by polymerase chain reaction (PCR) amplification of DNA with allele-specific primers followed by allele-specific restriction digestion of the products. However, FcγRIIIb genotyping was done by allele-specific PCR. **Results:** No significant difference in the distribution of FcγRIIa H/R and FcγRIIIa NA1/NA2 genotypes or their respective alleles was observed in GCP patients and healthy subjects. For FcγRIIIa F/V genetic polymorphism, the homozygous V/V genotype and V allele were significantly overrepresented in GCP patients while F/F genotype and F allele in controls. **Conclusion:** The present study demonstrates that FcγRIIIa V/V genotype, as well as V allele, could be a possible risk factor for chronic periodontitis in South Indian population.

Keywords: Fc gamma receptor, generalized chronic periodontitis, genetic polymorphism, South Indian

Introduction

Chronic periodontitis, as defined by 1999 World Workshop for Classification of Periodontal Disease and Conditions, is an infectious disease resulting in inflammation within the supporting tissue of the teeth, progressive attachment, and bone loss.[1] It is now known that specific bacteria are essential for the initiation and progression of periodontal disease, although host inflammatory response also plays a role in the amount of destruction to the supporting tissues.[2] Even though this disease affects a significant percentage of the population, some people are more susceptible to periodontopathic organisms than others.[3] Both genetic and environmental factors may play a role in this inter-individual variation.[4]

Genetic polymorphism of various receptors participating actively in immunity toward infections can lead to changed affinities of the receptors. Fc gamma receptors (FcγRs) are vital receptor found on the surfaces of leukocytes acting in defense against the infectious diseases.[5] In humans, there are three families of the FcγRs present on leukocytes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). These receptor families are defined and based on the molecular weights, ligand binding properties, and certain monoclonal antibodies. These receptors are FcγRIa, Ib, Ic, FcγRIIa, IIb, IIc, and FcγRIIIa and IIIb.[6] Among these receptors, functional polymorphism has been identified for four FcγR subclasses, that is, FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb.[7-9]

FcγRIIa expresses two alleles with a single base change guanine (G) to alanine (A) at nucleotide 494. The proteins encoded by these two alleles differ by two amino acids, arginine (R) and histidine (H) at residue 131 that appears

Department of Periodontology, Faculty of Dental Sciences, SGT University, Gurgaon, [2]Department of Dentistry, ESIC Medical College and Hospital, Faridabad, Haryana, [1]Department of Periodontology and Implantology, Bapuji Dental College and Hospital, Davangere, Karnataka, India

Correspondence: Dr. Veenu Madaan Hans,
404, Double Storey, New Rajender Nagar,
New Delhi - 110 060, India.
E-mail: veenudoc@yahoo.co.in

Access this article online	
Quick Response Code:	Website: www.contempclindent.org
	DOI: 10.4103/0976-237X.166831

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Hans VM, Mehta DS, Hans M. Association of Fc gamma-receptors IIa, IIIa, and IIIb genetic polymorphism with susceptibility to chronic periodontitis in South Indian population. Contemp Clin Dent 2015;6:S141-6.

© 2015 Contemporary Clinical Dentistry | Published by Wolters Kluwer - Medknow

to be determinant to IgG2 binding.[10] FcγRIIIa illustrates a single nucleotide polymorphism at nucleotide 559 which involves a guanine to thiamine substitution that corresponds to change of phenylalanine (F) to valine (V) at position 158 in the Ig-like domain 2.[11] The natural killer (NK) cells and monocytes expressing the FcγRIIIa 158 V/V genotype bind IgG1, IgG3, and IgG4 more effectively than cells with the FcγRIIIa 158F/F genotype.[12,13] FcγRIIIb is the most abundantly expressed receptor on polymorphonuclear leukocytes. It abides two allotype forms, neutrophil antigens (NAs) 1 and 2. These alleles divulge minor amino acid differences at position 65 and 82 in two extraglycolation sites in NA2.[14] Various studies account a much lower IgG mediated phagocytosis of bacteria in NA2/NA2 than in NA1/NA1.[15]

Studies well document the association/linkage of FcγR polymorphism with various forms of periodontitis. It has been shown that the FcγRIIa 131 H/H may be associated with a risk for chronic periodontitis in Caucasian smokers.[16] Similarly, a significant over-representation of FcγRIIIa-158F has been found in Japanese patients with recurrence of chronic periodontitis when compared with patients without recurrence.[17]

In a similar study on genetic polymorphism of FcγRIIa, IIIa, and IIIb genes in South Indian population with generalized aggressive periodontitis (GAgP), it was concluded that FcγRIIIa V/V genotypes and/or V allele as well as FcγRIIIb NA2/NA2 and/or NA2 allele along with FcγRIIa R allele may be a risk factor for GAgP.[18]

Considering the frequency of many allele variations between ethnic groups and geographically distinct populations, and that studies concerning FcγR gene polymorphism and generalized chronic periodontitis (GCP) are not many; particularly from India, this study was premeditated. Hence, the purpose of the present study was to evaluate the association among the FcγRIIa, IIIa, and IIIb gene polymorphism with GCP in South Indian population.

Materials and Methods

Study population

The design and methods of the study were reviewed and approved by the Ethical Committee for the use of human subjects in Research of Bapuji Dental College and Hospital, Davangere, Karnataka, India. Sixty South Indian patients with GCP (29 males and 31 females; mean age was 37.54 years) referred to Department of Periodontics were recruited for the study. Sixty race matched healthy controls (32 males and 28 females; mean age was 32.23 years) were also included in this study. Participants were explained about the nature of the study and an informed consent was obtained with a signed form. All participants were interviewed for systemic health history and current medications as well as smoking status. Subjects with history or current sign of systemic disease affecting periodontal status were excluded from this study. Likewise, only nonsmokers were engaged for this study.

The inclusion criteria for GCP groups were following: Attachment loss around more than 30% of sites with clinical attachment level (CAL) ≥5mm around the involved teeth. Furthermore, the amount of destruction was consistent with the amount of local factors. Subjects who showed neither attachment loss nor pockets >3mm at more than one site were classified as healthy controls. Patients with <15 teeth, use of antibiotics in last 6 months before entered into the study, pregnant and lactating women were excluded from this study.

Clinical assessments

The following clinical parameters were assessed in all patients during their first visit by two calibrated examiners: (a) Plaque index (PI),[19] (b) gingival index (GI),[20] (c) gingival bleeding index (GBI),[21] (d) probing pocket depth (PPD) which was determined by distance from free gingival margin to base of the pocket, and (e) CAL expressed as the mean distance from cemento-enamel junction to the base of the pocket. The PPD and CAL were assessed at six sites per tooth: Mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual by using William's periodontal probe (Hu Friedy®, USA). The measurements were rounded to the nearest millimeter. Full mouth intraoral periapical radiographs and orthopantomogram were also examined for radiographic evidence of alveolar bone loss. Clinical characteristics of both the study groups are summarized in Table 1.

Isolation of genomic deoxyribonucleic acid

To obtain genomic deoxyribonucleic acid (DNA) from participants in both groups buccal scrapping was obtained with a custom made sterile wooden spatula. The collected sample was immediately immersed in the sterile tubes containing 1000μl of Krebs buffer solution (118 mM of NaCl - 20%; 4.7mM of KCl - 2%; 1.2 mM of KH_2PO_4; and $MgSO_4$ each; 4.2 mM of sodium hydrogen carbonate; 2.0 mM of $CaCl_2$ 2%; 10 mM of glucose; H_2O - 2%). Krebs buffer maintains the homeostasis and the viability of cells so that the DNA is not lost during transit. Each sample was labeled

Table 1: Intergroup comparisons of full mouth clinical parameters in study subjects

Clinical parameters	GCP (*n*=60)	Healthy control (*n*=60)
PI	1.880±0.402*	0.709±0.290
GI	1.874±0.308*	0.078±0.137
GBI	75.30±16.156*	2.201±6.065
Mean PD	4.079±0.837*	1.441±0.278
Mean CAL	5.697±1.194*	0.000

Intergroup comparison with one-way ANOVA.*P<0.001 HS. HS: Highly significant; PI: Plaque index; GI: Gingival index; GBI: Gingival bleeding index; CAL: Clinical attachment level; PD: Probing depth; GCP: Generalized chronic periodontitis

with a code number by an assistant so that laboratory is unaware, which sample belongs to which group. The tubes were sealed tightly and sent to the laboratory within 24 h of taking sample. DNA was isolated from each sample and stored at 4°C before processing for the genomic analysis. The genotyping was performed twice for each sample and a sample in which discrepancy was found between the two results, genotyping was performed for a third time. The third result was considered as final, although such samples were only two, one in periodontally healthy controls and another in GCP patients.

Determination of Fc gamma receptor genotypes

Fc gamma receptor IIa-R-H131

The polymerase chain reaction (PCR) amplification for FcγRIIa was carried out according to the methodology described by Jiang *et al*.[22] The genomic DNA (100 ng) was added to the reaction mixture containing 200 μM dNTPs, 2.0 mM $MgCl_2$, 0.25 μM of sense and antisense primers (Sigma Aldrich®, Bengaluru, Karnataka, India) (5' GGA AAA TCC CAG AAA TTC TCG C 3' [forward primer], 5' CAA CAG CCT GAC TAC CTA TTA CGC GGG 3' [reverse primer]), and 1 unit of Taq polymerase (Chromus Biotech®, Bengaluru, Karnataka, India). The amplification protocol included 1 cycle of 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The DNA obtained by PCR was digested by restriction enzyme FnuDII (Fermentas®, Genetics Biotech Asia Pvt. Limited, New Delhi, India) as per manufacturer's recommendations to obtain the allele distribution. The digested PCR product was resolved at 16 amp on 3% agarose gel stained with ethidium bromide. The PCR product was digested by FnuDII enzyme giving rise to 343 bp and 322 bp fragments for H and R allele, respectively, which was then resolved on 3% of agarose gel and viewed under ultraviolet transilluminator.

Fc gamma receptor IIIa-158V-F

The amplification for FcγRIIIa was carried out with a nested PCR and allele-specific restriction analysis assay according to a method described by Koene *et al*.[12] The DNA was amplified with sense and antisense primers (Sigma Aldrich®, Bengaluru, Karnataka, India) 5' ATA TTT ACA GAA TGG CAC AGG 3' (forward primer) and 5' GAC TTG GTA CCC AGG TTG AA 3' (reverse primer). Amplification protocol of 5 min at 95°C, 35 cycles of 30 s at 95°C, 1-min at 50°C, and 1.5 min at 72°C. 0.5 μl of the first PCR product was used as a template for a nested PCR reaction with the primer pair, 5' ATC AGA TTC GAT CCT ACT TCT GCA GGG GGC AT 3' and 5' ACG TGC TGA GCT TGA GTG ATG GTG ATG TTC AC 3', using 30 cycles of 30 s at 95°C, 30 s at 62°C, and 40 s at 72°C. The PCR product was digested with Nla III (Fermentas®, Genetics Biotech Asia Pvt. Limited, New Delhi, India) at 37°C followed by electrophoresis. The homozygous F/F158 fragments were not digested whereas the heterozygous F/V158 fragments were partially digested yielding 3 bands of equal intensity (94 bp, 61 bp, and 33 bp). On the other hand, the homozygous V/V158 fragments were maximally digested yielding 3 bands (a very low intensity 94 bp band and equal intensity 61 bp and 33 bp bands).

Fc gamma receptor IIIb-NA1-NA2

FcγRIIIb genotyping was performed by PCR employing allele specific sense and antisense oligonucleotide as described by van Schie and Wilson.[23] Two different PCRs were carried out for detection of FcγIIIb alleles; NA1 and NA2. For NA1 gene amplification, 5' CAG TGG TTT CAC AAT GTG AA 3' (forward primer) 5' CAT GGA CTT CTA GCT GCA CCG 3' (reverse primer) (Sigma Aldrich®, Bengaluru, Karnataka, India) was used. The PCR reaction-conditions to amplify this 142 bp product included 1 cycle of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The NA2-specific sense primer 5'-CTC AAT GGT ACA GCG TGC TT-3' and antisense primer 5'-CTG TAC TCT CCA CTG TCG TT-3' (Sigma Aldrich®, Bengaluru, Karnataka, India) was employed to amplify a 169 bp product. Rest of the composition of reaction mixture was similar to that of NA1 PCR. This reaction was carried out at 1 cycle of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s. 5μl of NA1 PCR and 5μl of NA2 PCR products mixed with ethidium bromide dye were loaded into the same well in the electrophoresis unit. The 142bp and 169bp products were resolved on 2% of agarose gel at 16 amp.

Statistical analysis

The FcγRIIa, FcγRIIIa, and FcγRIIIb genotype distribution was compared between patients with GCP and healthy controls by Chi-square test (3 × 2 contingency table). The same test (Chi-square) was also used for comparing allelic frequency in both groups (2 × 2 contingency table). *A P* value below 0.05 was considered as statistically significant. For any two-locus test with $P < 0.05$, the odds ratio (OR) and 95% confidence interval (CI) were calculated. Calculations were performed using Primer of Biostatics software. Deviation of observed genotype distribution from expected distribution according to Hardy–Weinberg Law[24] was assessed by Chi-square test. Mean values for all clinical parameters in both groups were calculated and inter-group comparison was made using one-way ANOVA.

Results

Clinical parameters

Table 1 provides a summary of the baseline clinical characteristics of cases and controls. GCP group had consistently higher mean PD and CAL values followed than the control group. Furthermore, the PI, GI, and GBI scores of GCP group were significantly higher than the control group.

Distribution of genotypes

Genotype frequencies of FcγRIIa, FcγRIIIa, and FcγRIIIb of both groups did not deviate from Hardy–Weinberg equilibrium [Table 2]. The distribution of FcγRIIa, FcγRIIIa,

Table 2: Single locus test for Hardy-Weinberg equilibrium

Group	FcγRIIa				FcγRIIIa				FcγRIIIb			
	H	*R*	χ^2	*P**	*V*	*F*	χ^2	*P**	NA1	NA2	χ^2	*P**
GCP (*n*=60)	0.36	0.64	5.576	0.062	0.62	0.38	3.751	0.153	0.60	0.40	2.852	0.240
Healthy control (*n*=60)	0.48	0.52	5.006	0.082	0.32	0.68	2.721	0.257	0.70	0.30	0.989	0.610

**P*>0.05 NS. NS: Nonsignificant

and FcγRIIIb genotypes and their allelic frequency is shown in Table 3. On the comparison between GCP and control groups for FcγRIIa (χ^2 = 2.40), the difference in the distribution of genotypes was statistically insignificant. Comparing the FcγRIIIa genotype distribution in both groups, F/F homozygous genotype was the most frequent in control group (53.33%) whereas homozygous V/V was the most frequently present in GCP group (46.66%). On comparison between the GCP and control (χ^2 = 15.57) the distribution of genotypes was highly significant ($P < 0.001$). On intergroup comparison, the distribution of F and V allele was highly significant between GCP and control groups ($P < 0.001$, OR = 3.47, 95% CI [2.03–5.91]). On the comparison between GCP and control group, the difference in genotype distribution for FcγRIIIb was statistically insignificant (χ^2 = 2.26, $P > 0.05$). The differences in the allelic distribution of NA1 and NA2 were also statistically insignificant between GCP and control group ($P > 0.05$).

Table 3: Distribution of FcγR genotypes and alleles in the study subjects

	GCP *n*=60 (%)	Healthy controls *n*=60 (%)
FcγRIIa		
Genotype		
R/R131	30 (50)	22 (36.66)
R/H131	16 (26.66)	18 (30)
H/H131	14 (23.33)	20 (33.33)
Allelic frequency		
R131	76	62
H131	44	58
FcγRIIIa		
Genotype		
158V/V	28* (46.66)	10 (16.66)
158V/F	18 (30)	18 (30)
158F/F	14 (23.33)	32 (53.33)
Allelic frequency		
158V	74#	38
158F	46	82
FcγRIIIb		
Genotype		
NA1/NA1	26 (43.33)	32 (53.33)
NA1/NA2	20 (33.33)	20 (33.33)
NA2/NA2	14 (23.33)	8 (13.33)
Allelic frequency		
NA1	72	84
NA2	48	36

**P*<0.001 (HS) χ^2=15.570; #*P*<0.001 (HS) χ^2=21.696; OR=3.47, 95% CI (2.03-5.91). HS: Highly significant; OR: Odds ratio; CI: Confidence interval; FcγR: Fc gamma receptor; GCP: Generalized chronic periodontitis

Discussion

The inflammatory response implicated for the progression of periodontitis is influenced by many genetic factors.[25] These genes determine a normal, diminished, or an exaggerated response of the host to microbial insults.[26] Many candidate genes have been studied that influence development of inflammatory response in a periodontal pocket. Our study focuses on the influence of FcγR genetic polymorphism on development of GCP. Many studies have been carried out on the relation of this genetic polymorphism with periodontitis in various populations; however, a similar study on South Indian population has not been reported yet to the best of our knowledge.

Chronic periodontitis is an immune-inflammatory disease requiring bacteria for initiation, as well as a progression of the disease. Although micro-organisms associated with periodontal disease have components and by products capable of doing direct damage to the tooth supporting tissues, the majority of destruction is caused by the activation of the immune-inflammatory process.[27] Human phagocytic cells exhibit surface receptors that bind to immunoglobulins, for instance, FcγRs. Polymorphism of FcγRs is implicated as determinants of susceptibility to infection.[7] The results of our study showed that FcγRIIIa V/V genotype, as well as V allele, could be a possible risk factor for GCP although we could not establish a correlation between FcγRIIa and FcγRIIIb polymorphism and GCP.

The inclusion criteria for the study groups were strictly adhered to remove the effect of confounding factors. Subjects with factors that affect the initiation and progression of chronic periodontitis were excluded from the study such as smoking, systemic diseases, and physiological variations such as pregnancy. Subjects into the disease group were selected according to the classification criteria of 1999 World Workshop on Periodontics[1] to eliminate the variability in subject selection.

FcγRIIa mediates phagocytic function on monocytes, macrophages, and neutrophils. Its polymorphism FcγRIIa R131/H131 influences binding with IgG2 and IgG3.[28] FcγRIIa R allele is associated with deficient handling of IgG2 leading

to deficient clearance of immune complexes or pathogens by phagocytes.[29] Data from our study shows an insignificant but over-representation of R/R genotype in GCP group than controls. These results are in accordance with studies by other authors who found no association between FcγRIIa and GCP.[30,31] Although, contrary results have also been reported indicating that subjects with 131H/H genotype are more likely to suffer from the disease.[16]

FcγRIIIa has been implicated in the regulation of phagocytosis by macrophages as well as cytokine production by NK cells and lymphocytes. FcγRIIIa is considered to be crucial for IgG binding on NK cells, monocytes, and macrophages.[32] Research has shown that V/V homozygous NK cells result in more active cellular response to IgG stimulus than F/F homozygous and binds IgG1, IgG3, and IgG4 more effectively.[13] We found a more efficient FcγRIIIa V/V genotype to be more common in GCP patients indicating the subjects with V/V genotype are more likely to suffer from the disease. Although, immune cells are considered to play a protective role in periodontal pathogenesis, it has been established that upon interaction with bacterial products, immune cells release substances injurious to periodontal tissues.[27] The binding of IgG to FcγRIIIa induce stimulation of NK cells leading to pro-inflammatory cytokine release including interleukin-1β, interferon-γ, and tumor necrosis factor-α.[33] These pro-inflammatory mediators are known to play a role in periodontal connective tissue destruction as well as an alveolar bone loss. Thus, a more aggressive polymorphism of FcγRIIIa V/V may be responsible for more obvious expression of periodontal disease. Comparable results in GCP patients were obtained in a similar study in Caucasians,[30] whereas, that in Japanese subjects no correlation of this genotype was found with the severity of disease in GCP patients.[34]

We also observed an association of V allele with GCP with OR of 3.47, which is in accordance with various other studies on same genotype where V allele was associated with severe bone destruction.[30] Another study in Japanese subjects associated V allele with the severity of GCP, although the results for homozygous V/V were nonsignificant.[34] Furthermore, V allele was found to be a putative susceptibility factor for periodontitis in North European Caucasians.[26]

The NA1-NA2 polymorphism of FcγRIIIb is a determinant of IgG1 and IgG3 mediated effect in neutrophils. It was found that NA2 bearing neutrophils phagocytosed IgG1 and IgG3 opsonized periodontopathic bacteria lesser effectively than NA1 neutrophils.[15] Thus, phagocytosis of periopathogens in gingival tissues through neutrophils carrying NA1 or NA2 polymorphism may determine the course of the periodontal disease. Although, NA1/NA2 polymorphism has been linked with GAgP, the studies on its association with GCP are limited.[18] Except for one study where FcγRIIIbNA2/NA2 was more prevalent in GCP patients,[30] none of the studies have found a relationship between this genotype and chronic periodontitis.[35,36] Our results also did not show any significant difference in the distribution of FcγRIIIb genotypes between GCP and controls.

The results of various studies on FcγR polymorphism in a different population tend to demonstrate different results. This can possibly be explained on the basis of difference in population group studied as well as the criteria for patient selection. Furthermore, periodontitis being a complex disease may have several etiological agents which may act as a confounding factor for the results. Furthermore, IgG mediated leukocyte function is regulated by the interaction of multiple FcγR classes. Thus, the complex interaction of various FcγR classes may explain the leukocyte function and disease expression more than single FcγR genotypes.

Conclusion

Our results predict that FcγRIIIa V/V genotype and V allele could be associated with GCP in South Indian population. Although, more extensive studies covering larger populations are required to establish the relevance of the relationship between FcγR polymorphism and GCP.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1-6.
2. Lamster IB, Smith QT, Celenti RS, Singer RE, Grbic JT. Development of a risk profile for periodontal disease: Microbial and host response factors. J Periodontol 1994;65 5 Suppl: 511-20.
3. Genco RJ. Current view of risk factors for periodontal diseases. J Periodontol 1996;67 10 Suppl: 1041-9.
4. Beck JD, Slade GD. Epidemiology of periodontal diseases. Curr Opin Periodontol 1996;3:3-9.
5. Ogawa T, McGhee ML, Moldoveanu Z, Hamada S, Mestecky J, McGhee JR, *et al.* Bacteroides-specific IgG and IgA subclass antibody-secreting cells isolated from chronically inflamed gingival tissues. Clin Exp Immunol 1989;76:103-10.
6. Su K, Wu J, Edberg JC, McKenzie SE, Kimberly RP. Genomic organization of classical human low-affinity Fc gamma receptor genes. Genes Immun 2002;3 Suppl 1:S51-6.
7. van de Winkel JG, Capel PJ. Human IgG Fc receptor heterogeneity: Molecular aspects and clinical implications. Immunol Today 1993;14:215-21.
8. van der Pol W, van de Winkel JG. IgG receptor polymorphisms: Risk factors for disease. Immunogenetics 1998;48:222-32.
9. Budde P, Bewarder N, Weinrich V, Schulzeck O, Frey J. Tyrosine-containing sequence motifs of the human immunoglobulin G receptors FcgRIIb1 and FcgRIIb2 essential for endocytosis and regulation of calcium flux in B cells. J Biol Chem 1994;269:30636-44.
10. Parren PW, Warmerdam PA, Boeije LC, Arts J, Westerdaal NA, Vlug A, *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;90:1537-46.

11. Ravetch JV, Perussia B. Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. J Exp Med 1989;170:481-97.
12. Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M. Fc gamma RIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gamma RIIIa, independently of the Fc gamma RIIIa-48L/R/H phenotype. Blood 1997;90:1109-14.
13. Wu J, Edberg JC, Redecha PB, Bansal V, Guyre PM, Coleman K, *et al.* A novel polymorphism of Fc gamma RIIIa (CD16) alters receptor function and predisposes to autoimmune disease. J Clin Invest 1997;100:1059-70.
14. Ory PA, Goldstein IM, Kwoh EE, Clarkson SB. Characterization of polymorphic forms of Fc receptor III on human neutrophils. J Clin Invest 1989;83:1676-81.
15. Bredius RG, Fijen CA, De Haas M, Kuijper EJ, Weening RS, Van de Winkel JG, *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;83:624-30.
16. Yamamoto K, Kobayashi T, Grossi S, Ho AW, Genco RJ, Yoshie H, *et al.* Association of Fc gamma receptor IIa genotype with chronic periodontitis in Caucasians. J Periodontol 2004;75:517-22.
17. Sugita N, Yamamoto K, Kobayashi T, Van Der Pol W, Horigome T, Yoshie H, *et al*. Relevance of Fc gamma RIIIa-158V-F polymorphism to recurrence of adult periodontitis in Japanese patients. Clin Exp Immunol 1999;117:350-4.
18. Hans VM, Mehta DS. Genetic polymorphism of Fcγ-receptors IIa, IIIa and IIIb in South Indian patients with generalized aggressive periodontitis. J Oral Sci 2011;53:467-74.
19. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condtion. Acta Odontol Scand 1964;22:121-35.
20. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
21. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229-35.
22. Jiang XM, Arepally G, Poncz M, McKenzie SE. Rapid detection of the Fc gamma RIIA-H/R 131 ligand-binding polymorphism using an allele-specific restriction enzyme digestion (ASRED). J Immunol Methods 1996;199:55-9.
23. van Schie RC, Wilson ME. Evaluation of human Fc gamma RIIA (CD32) and Fc gamma RIIIB (CD16) polymorphisms in Caucasians and African-Americans using salivary DNA. Clin Diagn Lab Immunol 2000;7:676-81.
24. Smith CA. A note on testing the hardy Weinberg law. Ann Hum Genet 1970;33:377-83.
25. Michalowicz BS, Diehl SR, Gunsolley JC, Sparks BS, Brooks CN, Koertge TE, *et al*. Evidence of a substantial genetic basis for risk of adult periodontitis. J Periodontol 2000;71:1699-707.
26. Loos BG, John RP, Laine ML. Identification of genetic risk factors for periodontitis and possible mechanisms of action. J Clin Periodontol 2005;32 Suppl 6:159-79.
27. Kornman KS, Page RC, Tonetti MS. The host response to the microbial challenge in periodontitis: Assembling the players. Periodontol 2000 1997;14:33-53.
28. 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.
29. 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;147:1338-43.
30. 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;2:258-62.
31. Sugita N, Kobayashi T, Ando Y, Yoshihara A, Yamamoto K, van de Winkel JG, *et al*. Increased frequency of Fc gamma RIIIb-NA1 allele in periodontitis-resistant subjects in an elderly Japanese population. J Dent Res 2001;80:914-8.
32. Vance BA, Huizinga TW, Wardwell K, Guyre PM. Binding of monomeric human IgG defines an expression polymorphism of Fc gamma RIII on large granular lymphocyte/natural killer cells. J Immunol 1993;151:6429-39.
33. Sulica A, Metes D, Gherman M, Whiteside TL, Herberman RB. Divergent effects of Fc gamma RIIIA ligands on the functional activities of human natural killer cells *in vitro*. Eur J Immunol 1996;26:1199-203.
34. Kobayashi T, Yamamoto K, Sugita N, van der Pol WL, Yasuda K, Kaneko S, *et al.* The Fc gamma receptor genotype as a severity factor for chronic periodontitis in Japanese patients. J Periodontol 2001;72:1324-31.
35. Chung HY, Lu HC, Chen WL, Lu CT, Yang YH, Tsai CC. Gm (23) allotypes and Fc gamma receptor genotypes as risk factors for various forms of periodontitis. J Clin Periodontol 2003;30:954-60.
36. Wolf DL, Neiderud AM, Hinckley K, Dahlén G, van de Winkel JG, Papapanou PN. Fc gamma receptor polymorphisms and periodontal status: A prospective follow-up study. J Clin Periodontol 2006;33:691-8.