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Fcγ receptor polymorphisms and periodontal status: a prospective follow-up study

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Abstract

Aims: The aims of this study were to assess: (i) the distribution of Fcγ receptor polymorphisms among patients with chronic periodontitis (''cases'') and control subjects with no/minimal loss of periodontal tissue support in a Caucasian population; (ii) whether these polymorphisms can serve as severity markers for periodontitis; and (iii) whether they have any bearing on the response to periodontal therapy.

Methods: The study sample consisted of 132 cases and 73 controls of comparable age and gender. Full-mouth periodontal status was assessed. Subgingival plaque (PL) samples and blood samples were obtained and analysed with respect to 19 bacterial species and homologous serum immunoglobulin G titres. Polymorphisms in the Fcγ receptor IIa (131R/H) and IIIb (NA1/NA2) were assessed by polymerase chain reaction. Patients underwent periodontal therapy and were followed up at 4 and 30 months.

Results: Neither polymorphism showed a skewed distribution among cases and controls. At baseline, periodontitis patients with Fcγ RIIa-H/H131 genotype had more PL and deeper pockets than patients in other genotype groups ($p < 0.05$). Both bacterial levels and antibody titres were unrelated to genotype. The longitudinal analysis failed to detect an association between genotype and response to periodontal therapy.

Conclusions: The present data failed to demonstrate a clinically relevant relationship between the Fcγ receptor IIa (131R/H) or IIIb (NA1/NA2) polymorphism and periodontal status.

Key words: bacterial antibody; Fcγ receptor polymorphism; genetics; periodontal infection

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It is currently accepted that susceptibility to periodontal disease is not conferred by the presence of periodontal pathogens alone (Page & Beck 1997). In fact, the risk for development and/or progression of periodontitis is thought to be determined in part by the host's genotype (Michalowicz 1994). In search of candidate genes that may confer susceptibility to periodontitis, researchers have focused primarily on genes whose protein products play a role in inflammation and the immune response such as interleukin-1 (IL-1) (Kornman et al. 1997), tumour necroses factor-α (TNF-α) (Craandijk et al. 2002), and Fcγ receptors (FcγR) (Kobayashi et al. 1997, 2001). Specific genetic polymorphisms in Fcγ R, for example, are associated with impaired immune function and with increased risk for meningococcal disease (Domingo et al. 2002), cerebral malaria (Omi et al. 2002), and recurrent respiratory track infections (Sanders et al. 1994, van Sorge et al. 2003).

FcγR are molecules found on the surfaces of leucocytes such as macrophages and neutrophils. These receptors serve as an important link between the humoral and cellular immune system. The binding of antibody–antigen complexes to FcγR can trigger phagocytosis, antibody-dependent cytotoxicity, release of inflammatory mediators, and enhancement of antigen presentation (van de Winkel & Capel 1993). There are three classes of leucocyte FcγR: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). Functional polymorphisms have been identified in two of the three classes; FcγRII and FcγRIII. A single nucleotide substitution in the gene for FcγRIIa results in either a histidine (H) or arginine (R) at position 131 within the second immunoglobulin (Ig)-like domain of the receptor. This variation has been shown to impact the affinity of the receptor for IgG, with the arginine allele having a lower affinity (Rascu et al. 1997, van der Pol & van de Winkel 1998). Similarly, there are two allotypes of FcγRIIIb, termed neutrophil antigens 1 and 2 (NA1, NA2). Cells bearing the NA2 receptor display less phagocytic

ability than those with the NA1 receptor (Rascu et al. 1997, van der Pol & van de Winkel 1998).

Studies in the periodontal literature have found associations between polymorphisms in FcγR and periodontal status (Kobayashi et al. 1997, 2001, Sugita et al. 1999, Loos et al. 2003). However, the data are conflicting and difficult to interpret due to variations in the ethnic backgrounds of the study populations, the reported allele frequencies, and the periodontal disease definitions used. Additionally, the documented associations may have been race related. To date, no prospective longitudinal study has assessed the relationship between FγR and periodontal status using concomitantly clinical, microbiologic, and serologic data.

The aims of the present study were threefold: first, to compare the distribution of polymorphisms in FcγR IIa and IIIb among northern European subjects with chronic periodontitis and controls i.e., subjects with no, or only minimal loss of periodontal tissue support; second, to determine whether these polymorphisms can serve as severity markers for periodontitis as assessed by clinical, microbiologic, and serologic means; and lastly, to assess whether these polymorphisms have any bearing on the response to conventional periodontal therapy.

Materials and Methods

Study sample and inclusion criteria

The design and methods of the study were approved by the Committee of Research Ethics, Göteborg University, Sweden, and have been described in an earlier report (Papapanou et al. 2000). In brief, northern European Caucasians with destructive periodontitis (''cases'') were recruited from first time referrals for periodontal specialty care. Inclusion criteria included: (i) >20 years of age; (ii) moderate to advanced periodontal disease, with several inflamed periodontal pockets, loss of attachment, and radiographic evidence of bone loss; (iii) no previous history of periodontal therapy; and (iv) no use of antibiotics within the preceding 6-month period. Periodontally ''intact'' subjects qualified as controls if they displayed: (i) no sites with probing depths (PDs) exceeding 5 mm with concomitant loss of clinical attachment >1 mm, (ii) in ages below 45 years, no inter-proximal or lingual sites with clinical attachment loss of >3 mm, and (iii) in ages over 45 years, no more than four inter-proximal sites with maximum clinical attachment loss of 5 mm. There were no restrictions applied regarding levels of plaque (PL) or gingivitis. All patients signed an informed consent form before enrolment into the study.

Table 1. Age and gender distribution among periodontitis cases and controls

Age (years)	Cases		Controls		Total
	male	female	male	female	
$\leqslant 35$	2	4	2	5	13
36–55	44	43	23	24	134
$\geqslant 56$	18	21	8	11	58
Total	64	68	33	40	205

Table 1 provides a description of the study sample that included 205 subjects, 132 cases and 73 controls. The age and gender distribution was reasonably well balanced, with cases being on average 51.7 years old (SD 8.8) and 48.5% male, and controls 49.6 years old (SD 10.7) and 45.2% male. Cases were examined at baseline, 4 months (approximately, 2 months after completion of periodontal therapy), and 30 months at which time clinical, microbiologic, and serologic data were collected. One hundred and thirty-seven subjects (89 cases and 42 controls) were available at the 30-month follow-up examination.

Interview and clinical examination

All participants were interviewed with respect to systemic health history and current medications. Smoking habits were recorded according to a simplified protocol and coded as follows: 0 = never smokers; 1 = former smokers who did not exceed 15 cigarettes/day (former light/moderate smokers); 2 = former smokers exceeding 15 cigarettes/day (former heavy smokers); 3 = current smokers not exceeding 15 cigarettes/day (current light/moderate smokers); and 4 = current smokers exceeding 15 cigarettes/day (current heavy smokers).

All subjects underwent a full-mouth clinical examination of the periodontal tissues at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) at all present teeth excluding third molars. A manual, calibrated periodontal probe with a probe tip diameter of 0.45 mm was used to assess the following parameters:

PD: defined as the distance between the gingival margin and the bottom of the probeable pocket to the nearest whole mm.
Location of the gingival margin: the distance between the cemento-enamel junction (CEJ) and the gingival margin recorded to the nearest whole millimetres. This measure was given a positive sign in case of gingival recession and a negative sign when the gingival margin was located coronal to the CEJ. The distance was deemed non-readable whenever the CEJ was obscured by dental restorations or was impossible to identify.

The algebraic sum of the above two parameters was used to compute the *clinical attachment level (CAL)*.
PL was recorded dichotomously without the use of any disclosing agent.
Bleeding on probing (BOP) was also recorded dichotomously and was deemed positive if it occurred within 15 s after the assessment of PD.

All clinical assessments were performed by two calibrated examiners (authors A. M. N. and P. N. P.) as previously described (Papapanou et al. 2000).

Bacterial plaque samples

Subgingival PL samples were obtained before the clinical examination from the same tooth sites at each of the three time points (baseline, 4, and 30 months), as described earlier (Papapanou et al. 2000). A maximum of 14 sites per subject, at one randomly selected upper quadrant and the diagonally lower quadrant, were sampled.

Processing of the bacterial plaque samples

Digoxigenin-labelled, whole genomic DNA probes were prepared from the following 19 bacterial strains by random priming using the High-Prime labelling kit (Boehringer-Mannheim, Mannheim Germany):

Porphyromonas gingivalis (strain FDC 381), *Porphyromonas endodontalis* (OMGS 1205), *Prevotella intermedia* (ATCC 25611), *Prevotella nigrescens* (ATCC 33563), *Prevotella melaninogenica* (OMGS 1503), *Tannerella forsythia* (ATCC 43037), *Actinobacillus actinomycetemcomitans* (FDC Y4), *Fuso-*

bacterium nucleatum (ATCC 10953), *Treponema denticola* (OMGS 3271), *Micromonas micros* (ATCC 33270), *Campylobacter rectus* (ATCC 33238), *Eikenella corrodens* (ATCC 23834), *Eubacterium nodatum* (OMGS 3356), *Selenomonas noxia* (OMGS 3118), *Streptococcus intermedius* (OMGS 3177), *Streptococcus oralis* (ATCC 35037), *Veillonella parvula* (ATCC 10790), *Capnocytophaga ochracea* (ATCC 33624), and *Actinomyces naeslundii* genospecies 2 (ATCC 15987).

Analysis of PL samples was performed according to the ''checkerboard'' DNA–DNA hybridization technique (Socransky et al. 1994) as earlier described (Papapanou et al. 2000). Bacterial counts were quantified in a scale from 0 to 5 where 0, a level below detection: 1, a level lower than 10^5 bacteria; 2, a level equal to 10^5 bacteria; 3, a level between 10^5 and 10^6 bacteria; 4, a level equal to 10^6 bacteria; and 5, a level higher than $>10^6$ bacteria.

Blood samples

Ten millilitres of venous blood was obtained from each person by means of untreated Vacutainer™ blood collection tubes (Beckton-Dickinson, Franklin Lakes, NJ, USA). Cases were sampled at baseline, 4 and 30 months, and controls, at baseline and 30 months. The blood samples were stored at +4°C overnight and thereafter centrifuged at 1400 g for 10 min. Both serum and the cellular component were collected, aliquoted, and stored at minus 70°C until further analysis.

Assessment of serum IgG antibodies to periodontal bacteria

The ''checkerboard'' immunoassay (Sakellari et al. 1997) was used to detect levels of serum antibody as previously described (Papapanou et al. 2000). Titres were quantified in ng/ml.

Determination of FcγRIIa and FcγRIIIb genotypes

Genomic DNA was isolated from ethylene diamine tetraacetic acid (EDTA)-anticoagulated peripheral blood using QIAAmp blood kits (Qiagen, Hilden, Germany). Determination of FcγRIIa and FcγRIIIb genotypes was performed using polymerase chain reaction at the University Medical Centre Utrecht, Department of Immunology, Immunotherapy Laboratory. FcγRIIa and FcγRIIIb genotyping was carried out using allele specific primers as described earlier (de Haas et al. 1995, Carlsson et al. 1998, respectively).

Periodontal therapy

All patients were treated by a single periodontist (author A. M. N.) as described earlier (Papapanou et al. 2000), according to their individual needs. Periodontal therapy included instructions in oral hygiene, extractions of non-salvageable teeth, and full-mouth scaling and root planing. Access periodontal surgery was performed in 67 patients (75.3%), at one or several quadrants. Adjunctive systemic antibiotics (tetracycline, metronidazole, or combination of amoxicillin and metronidazole, at a standard dosage for a week) were prescribed to a total of 49 patients (55.1%), all of whom were treated surgically.

Data analysis

Statistical analysis was performed using SAS 9.1 for windows (SAS Institute Inc., Cary, NC, USA). In all analyses, the individual subject was the computational unit. The distribution of polymorphisms in cases and controls was analysed by means of the chi-square test. The mean values for all clinical parameters (PL and gingivitis scores, PDs, and attachment levels) were calculated for each subject. Bacterial scores from individual PL samples were averaged to describe each subject's bacterial burden for a particular species. Associations between polymorphism status (FcγRIIa-H/H, H/R, or R/R; FcγRIIIb NA1/NA1, NA1/NA2, NA2/NA2) and clinical parameters were assessed by ANOVA or the Kruskall–Wallis test, as appropriate. To adjust for the multiple comparisons performed, the level of significance required to be reached when comparing a single species or titre was 0.0026, to maintain an overall level of α error of 0.05 (Bonferroni's correction for 19 tested species).

Generalized linear equations were used to adjust for age and smoking status categorically. Three age categories (<35, 35–55, and >55 years), and three smoking status groups (never smokers, former smokers, and current smokers) were used in these adjustments. In the event of a positive association, pair-wise comparisons were performed with the Bonferroni's correction.

The longitudinal analyses examining the effect of the investigated polymorphisms on treatment outcomes were performed in cases only. For each FcγR polymorphism, differences in the three groups with respect to changes in clinical, microbiologic, and serologic status after treatment were assessed. Longitudinal multivariate analyses were adjusted for age, smoking, and baseline status using generalized linear modelling.

Results

Table 2 provides a summary of the baseline clinical characteristics of cases and controls. Cases had consistently fewer teeth, more sites with PL and gingivitis, and higher mean PDs and attachment levels compared with controls. When comparing the relative distributions of FcγRIIa genotypes among cases and controls, no skewed distribution was observed (χ^2 p-value 0.416). The distribution of individual alleles approximated 50% in both cases and controls (Table 3). Twenty-eight per cent of cases and 26% of controls were homozygous for the histidine allele, while 30% of cases and 23% of controls were homozygous for the arginine

Table 2. Baseline clinical characteristics of cases ($n = 132$) and controls ($n = 73$)

	# Teeth	% sites with BOP	% sites with PL	% sites with PD $\geqslant$6 mm	% sites with CAL $\geqslant$6
Cases					
<35	25.5 (2.0)	65.6 (16.5)	41.5 (23.1)	13.0 (15.7)	6.7 (10.0)
36–55	24.0 (4.1)	65.0 (19.4)	51.9 (24.3)	15.4 (21.8)	14.0 (13.2)
>56	20.4 (5.8)	65.2 (23.1)	51.1 (25.6)	16.8 (16.2)	20.8 (17.6)
Controls					
<35	28.0 (0.0)	19.0 (10.1)	21.7 (19.5)	0.1 (0.2)	0.1 (0.2)
36–55	27.2 (1.2)	23.5 (13.7)	35.7 (25.3)	0.1 (0.2)	0.6 (2.5)
>56	26.6 (1.7)	21.2 (11.4)	38.4 (28.2)	0.0 (0.0)	0.3 (0.8)

Mean values (standard deviations).
CAL, clinical attachment level; PL, plaque; PD, probing depth; BOP, bleeding on probing.

Table 3. Distribution of FcγRIIa genotypes and allelic frequencies among cases and controls; *n* (%)

	Cases	Controls	Total
Genotype			
R/R	40 (30)	17 (23)	57 (28)
R/H	55 (42)	37 (51)	92 (45)
H/H	37 (28)	19 (26)	56 (27)
Allelic frequency			
R131 (%)	51	49	50
H131 (%)	49	51	50

Table 4. Distribution of FcγRIIIb genotypes and allelic frequencies among cases and controls; *n* (%)

	Cases	Controls	Total
Genotype			
NA1/NA1	21 (16)	8 (11)	29 (14)
NA1/NA2	62 (47)	40 (55)	102 (50)
NA2/NA2	49 (37)	25 (34)	74 (36)
Allelic frequency			
NA1 (%)	39	38	39
NA2 (%)	61	62	61

allele. A majority of both cases and controls were heterozygous (42% and 51%, respectively). Similarly, the distribution of FcγRIIIb genotypes was not skewed among cases and controls (χ^2 *p*-value = 0.472; Table 4). Sixteen per cent of cases and 11% of controls were homozygous for the NA1 allotype, while 37% of cases and 34% of controls were homozygous for the NA2 allotype. Individual allele frequencies were almost identical in cases and controls, with the NA2 allele being more prevalent (overall frequency 61% for NA2 and 39% for NA1).

Next, we examined whether the analysed polymorphisms served as severity markers of periodontitis. The clinical status of the periodontitis patients by genotype group is presented in Table 5. A statistically significant association was found between the variable ''percent of sites with plaque'' and FcγRIIa ($p = 0.0174$). Indeed, pair-wise comparisons revealed that subjects of the FcγRIIa-H/H genotype had significantly higher per cent of sites with PL than subjects of the FcγRIIa-R/R genotypes (60.8% *versus* 46.1%). The variable ''per cent of sites with BOP'' was also significantly associated with FcγRIIa (R/R, 61.8%; R/H, 62.6%; H/H, 72.4%; $p = 0.0414$); however, pair-wise comparison with the Bonferroni's correction did not reveal any statistically significant differences among the groups. A statistically significant association was found between FcγRIIa and mean PD ($p = 0.0144$). Pair-wise comparisons revealed that subjects of the FcγRIIa-H/H genotype had significantly higher mean PD than subjects of the FcγRIIa-R/H or FcγRIIa-R/R genotypes; however, the difference in mean PD between the genotypes was a mere 0.5 mm. FcγRIIa was significantly associated with the variable ''per cent of sites with PD $\geqslant$ 6 mm'' as well ($p = 0.0086$). Pair-wise comparisons revealed that subjects of the FcγRIIa-H/H genotype had a higher percentage of sites with PD $\geqslant$ 6 mm than those of the FcγRIIa-R/H orFcγRIIa-R/R genotypes (21.6% *versus* 13.9% and 12.8%, respectively). There were no statistically significant associations detected between FcγRIIa and attachment levels. No associations were detected between any of the tested clinical parameters and FcγRIIIb.

Table 5. Baseline clinical characteristics of ''Cases'' by polymorphism (mean values, standard deviations in parenthesis; *p*-values are adjusted for age and smoking)

FcγRIIa-R/H131	R/R ($n = 40$)	H/R ($n = 55$)	H/H ($n = 37$)	*p*-value
% sites with PL*	46.1 (22.1)	48.4 (24.3)	60.8 (25.2)	**0.0174**
% sites with BOP	61.8 (19.6)	62.6 (19.7)	72.4 (20.6)	**0.0414**
Mean probing depth†	3.6 (0.7)	3.6 (0.8)	4.1 (0.9)	**0.0144**
% sites with PD $\geqslant$6† 6 mm*	12.8 (10.0)	13.9 (13.2)	21.6 (16.8)	**0.0086**
Mean attachment loss	2.8 (0.9)	3.2 (1.4)	3.4 (1.6)	0.0764
% sites with CAL $\geqslant$6 mm	11.7 (9.6)	16.0 (15.8)	19.1 (17.5)	0.0697

FcγRIIIb-NA1/NA2	NA1/NA1 ($n = 21$)	NA1/NA2 ($n = 62$)	NA2/NA2 ($n = 49$)	*p*-value
% sites with PL	50.5 (19.8)	48.9 (25.0)	54.3 (25.8)	0.5663
% sites with BOP	62.3 (19.3)	63.3 (20.2)	68.7 (20.7)	0.3164
Mean probing depth	3.6 (0.9)	3.8 (0.8)	3.8 (0.7)	0.5536
% sites with PD $\geqslant$6	12.3 (15.1)	16.6 (14.4)	16.0 (12.7)	0.5325
Mean attachment loss	2.7 (1.2)	3.2 (1.4)	3.3 (1.4)	0.1518
% sites with CAL $\geqslant$6	10.9 (12.6)	15.8 (14.1)	17.2 (16.7)	0.2078

Bold values are statistically significant.
*Statistically significant pair-wise comparison between R/R–H/H genotypes.
†Statistically significant pair-wise comparison between R/R–H/H and R/H–H/H genotypes.
CAL, clinical attachment level; PL, plaque; PD, probing depth; BOP, bleeding on probing.

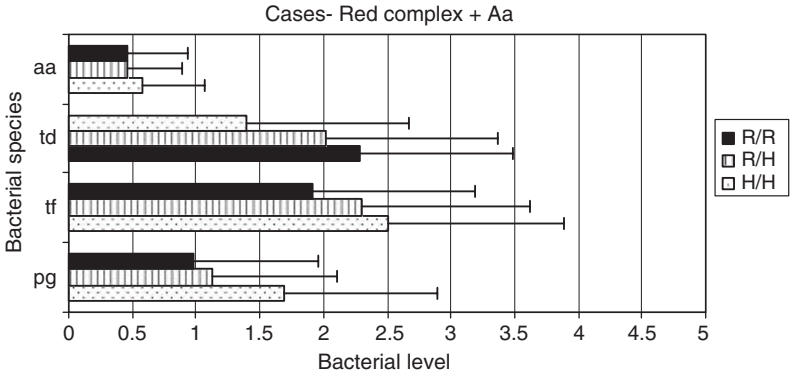

Fig. 1. Colonization (log microbial counts) of periodontitis patients by red complex species (*Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola*), and *Actinobacillus actinomycetemcomitans* by FcγRIIa polymorphism. Units on the *x*-axis are means based on a scale from 0 to 5, where: 0, a level below detection; 1, a level lower than 10^5 bacteria; 2, a level equal to 10^5 bacteria; 3, a level between 10^5 and 10^6 bacteria; 4, a level equal to 10^6 bacteria; and 5, a level higher than $>10^6$ bacteria.

Figure 1 depicts the levels of red complex bacteria (*P. gingivalis*, *T. forsythia*, and *T. denticola)* and *A. actinomycetemcomitans* by the FcγRIIa polymorphism group. No conspicuous differences in bacterial levels were observed. The ANOVA test revealed that both *P. gingivalis* and *T. denticola* levels were associated with FcγRIIa at the $p < 0.05$ level; however, the association did not hold after adjusting for multiple comparisons. Figure 2 illustrates the comparable levels of red complex bacteria and *A. actinomycetem-*

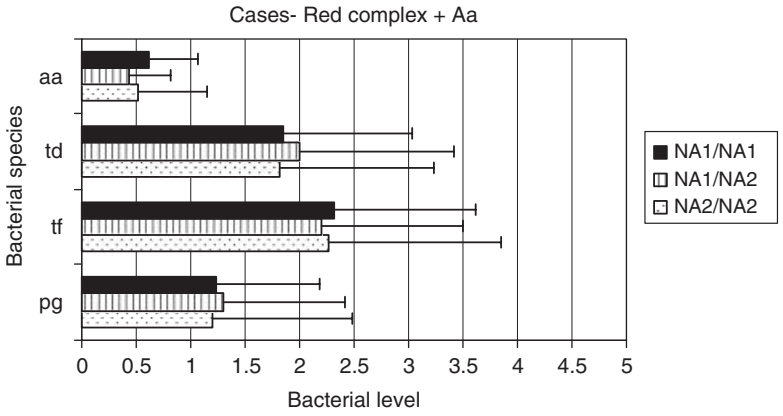

Fig. 2. Colonization (log microbial counts) of periodontitis patients by red complex species (*Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola*) and *Actinobacillus actinomycetemcomitans* by FcγRIIIb polymorphism. Units on the *x*-axis are means based on a scale from 0 to 5, as described in Fig. 1.

Table 6. Clinical parameters in cases at baseline, 4 and 30 months by FcγRIIa polymorphism (mean values, standard deviations in parentheses)

	R/R	H/R	H/H
% PL			
Baseline	46.1 (22.1)	48.4 (24.3)	60.8 (25.2)
4 months	38.5 (19.5)	39.1 (20.3)	39.9 (19.0)
30 months	22.3 (18.4)	19.8 (13.4)	24.9 (15.0)
% BOP			
Baseline	61.8 (19.6)	62.6 (19.7)	72.4 (20.6)
4 months	23.6 (16.4)	24.8 (16.7)	26.8 (16.2)
30 months	30.8 (17.3)	28.5 (14.3)	32.4 (17.3)
Mean PD			
Baseline	3.6 (0.7)	3.6 (0.8)	4.1 (0.9)
4 months	2.9 (0.5)	3.1 (0.6)	3.1 (0.6)
30 months	2.5 (0.3)	2.5 (0.2)	2.5 (0.4)
% PD			
⩾6 mm baseline	12.8 (10.0)	13.9 (13.2)	21.6 (16.8)
⩾6 mm 4 months	4.7 (5.0)	6.3 (8.5)	6.1 (5.9)
⩾6 mm 30 months	1.1 (1.7)	0.6 (1.2)	1.2 (2.4)
Mean CAL			
Baseline	2.8 (0.9)	3.2 (1.4)	3.4 (1.6)
4 months	2.7 (0.9)	3.2 (1.3)	3.3 (1.3)
30 months	2.7 (0.8)	3.1 (1.1)	3.5 (1.4)
% CAL			
⩾6 mm baseline	11.7 (9.6)	16.0 (15.8)	19.1 (17.5)
⩾6 mm 4 months	9.9 (8.9)	13.9 (15.3)	16.6 (13.7)
⩾6 mm 30 months	7.3 (6.8)	9.3 (10.6)	17.4 (16.1)

CAL, clinical attachment level; PL, plaque; PD, probing depth; BOP, bleeding on probing.

comitans by FcγRIIIb genotype. No significant findings with respect to either polymorphism were observed for the examined orange complex species, (*C. rectus*, *M. micros, F. nucleatum*, *P. nigrescens*, *P. intermedia*, and *E. nodatum*) or for the tested health-associated species (*A. naeslundii*, *C. ochracea*, *V. parvula*, *and E. corrodens*; data not shown).

Analyses of relationships between antibody titres and FcγR genotype failed to demonstrate any statistically significant associations between IgG levels and polymorphism genotypes (data not shown).

To address the third aim of the study, we examined the response to periodontal therapy in 89 cases as reflected by longitudinal changes in clinical, microbiologic, or serologic parameters. Variations in these therapeutic outcomes were assessed among the polymorphism groups. Tables 6 and 7 present the clinical data of the ''cases'' at baseline, 4 and 30 months by FcγRIIa, and FcγRIIIb polymorphism, respectively. A reduction in PL, BOP, and PD as well as a gain in clinical attachment after therapy was observed for all polymorphism groups. There were no statistically significant differences in the change of any of the clinical parameters from baseline to 4 months or from baseline to 30 months with respect to FcγRIIa or FcγRIIIb genotypes.

No differences among genotype groups were detected in the longitudinal microbial analysis for both FcγRIIa and FcγRIIIb. Similarly, neither the FcγRIIa, nor the FcγRIIIb polymorphism had any significant impact on the longitudinal change in IgG titres from baseline to 4 months or baseline to 30 months (data not shown).

Discussion

To the best of our knowledge, this is the first study to assess prospectively the impact of FcγR polymorphisms on the clinical, microbiological, and serologic features of periodontitis in subjects with and without destructive disease. The main findings can be summarized as follows: in a subject sample of northern European Caucasians, the distribution of FcγRIIa and FcγRIIIb polymorphisms was similar among patients with periodontitis and periodontitis-free controls. Patients with periodontitis harbouring the FcγRIIa-H/H131 genotype had more PL and deeper pockets than patients of either the FcγRIIa-R/H131 or FcγRIIa-R/R131 genotypes. However, there were no significant differences in CALs, bacterial loads, or antibody titres among the different genotypes. Finally, the longitudinal analysis failed to demonstrate any bearing of the investigated polymorphisms on the response to conventional periodontal therapy.

The distribution of FcγR genotypes in the present study is in accord with earlier findings in Caucasian populations. Loos et al. (2003) examined 68 periodontitis patients and 61 periodontally intact controls genotyped for FcγRIIa, FcγRIIIb, and a more recently described FcγRIIIa polymorphism. The latter is the result of a single nucleotide substitution that yields either a valine or phenylalanine at position 158 within the receptor. The valine form has a higher affinity for both monomeric and immune-complexed IgG1, IgG3, and IgG4 (Koene et al. 1997). While the authors did not report any differences in FcγRIIa, FcγRIIIa, and FcγRIIIb genotype distribution among periodontitis

Table 7. Clinical parameters in cases at baseline, 4 and 30 months by FcγRIIIb polymorphism (mean values, standard deviations in parentheses)

	NA1/NA1	NA1/NA2	NA2/NA2
% PL			
Baseline	50.5 (19.8)	48.9 (25.0)	54.3 (25.8)
4 months	34.7 (15.7)	38.8 (18.4)	41.4 (22.0)
30 months	19.5 (14.5)	20.8 (14.5)	24.4 (17.8)
% BOP			
Baseline	62.3 (19.3)	63.3 (20.2)	68.7 (20.7)
4 months	20.8 (12.1)	24.1 (15.2)	27.6 (18.8)
30 months	29.8 (12.5)	29.9 (16.4)	31.1 (17.6)
Mean PD			
Baseline	3.6 (0.9)	3.8 (0.8)	3.8 (0.7)
4 months	2.9 (0.6)	3.0 (0.6)	3.1 (0.6)
30 months	2.5 (0.4)	2.5 (0.3)	2.5 (0.3)
% PD			
⩾6 mm baseline	12.3(15.1)	16.6 (14.4)	16.0 (12.7)
⩾6 mm 4 months	4.3 (4.9)	5.4 (5.4)	6.7 (8.6)
⩾6 mm 30 months	1.0 (2.2)	0.7 (1.7)	1.1 (1.7)
Mean CAL			
Baseline	2.7 (1.2)	3.2 (1.4)	3.3 (1.4)
4 months	2.9 (1.1)	3.0 (1.2)	3.3 (1.2)
30 months	2.8 (1.1)	2.9 (1.2)	3.2 (1.0)
% CAL			
⩾6 mm baseline	10.9 (12.6)	15.8 (14.1)	17.2 (16.7)
⩾6 mm 4 months	11.1 (9.9)	12.7 (13.2)	14.9 (14.5)
⩾6 mm 30 months	8.8 (10.4)	10.3 (12.9)	11.8 (11.2)

CAL, clinical attachment level; PL, plaque; PD, probing depth; BOP, Bleeding on probing.

cases and controls, they did observe an overrepresentation of the FcγRIIIa-V158 allele in cases, and an over-representation of the FcγRIIa-H/H131 genotype in subjects classified as having aggressive periodontitis.

A series of studies addressing the relationship between FcγR polymorphisms and periodontal disease was conducted by a group of investigators from Japan. Typically, the results of these studies found associations between polymorphisms in FcγRIIIb and periodontal status (Kobayashi et al. 1997, 2000). In their earliest paper, Kobayashi et al. (1997) compared genotype distributions in FcγR IIa and IIIb between adult periodontitis patients and race-matched periodontally healthy controls, as well as in patients with and without disease recurrence (defined as ⩾1 site with ⩾2 mm loss of attachment mm during a 1-year period following periodontal treatment). Although no skewed distribution of either polymorphism was detected among cases and controls and no significant differences were observed in clinical parameters between genotypes, an over-representation of the FcγRIIIb-NA2/NA2 and NA1/NA2 genotypes was noted among subjects with recurrence of periodontitis. The authors concluded that FcγRIIIb-NA2 allotype represents a risk factor for recurrent disease, but the lenient definition of recurrence (resulting in 85% of the examined cohort classified as progressing) may have conceivably influenced the reported findings. In general, the data from the literature are difficult to interpret, as the definitions of periodontal disease and health have varied significantly between studies.

An additional inconsistency in the literature relates to whether the high- or low-affinity alleles (and the resulting presumably ''good'' or ''bad'' polymorphisms) are associated with periodontitis. In the case of FcγRIIIb, most studies find the ''unfavourable'' NA2 allele to be associated with periodontal disease (Kobayashi et al. 1997, Sugita et al. 2001). Studies of FcγRIIa, however, have found the high-affinity alleles to be associated with periodontal disease (Loos et al. 2003, Yamamoto et al. 2004). With respect to FcγRIIIa, Sugita et al. (1999) reported an overrepresentation of the FcγRIIIa-F158 allele in periodontitis patients with disease recurrence. Similarly, Kobayashi et al. (2000) showed higher occurrence of the FcγRIIIa-F158 allele in patients with generalized, early-onset periodontitis, compared with periodontally healthy controls. In contrast, Kobayashi et al. (2001) found the FcγRIIIa-V158 allele to be over-represented in patients with severe chronic periodontitis *versus* subjects with moderate disease. Again, these conflicting findings are difficult to reconcile as both the histidine and valine alleles are the high-affinity alleles that conceivably facilitate efficient phagocytosis and effective clearance of infectious agents. In an attempt to explain why the potentially favourable allele was associated with higher levels of disease, Yamamoto et al. (2004), who reported an over-representation of the FcγRIIa-H/H131 genotype in North American patients with periodontitis *versus* ''periodontally healthy'' controls, hypothesized that, in the presence of bacterial antigens, possession of the ''efficient'' FcγRIIa on the neutrophil surface may result in ''hyper-function'' and enhanced release of cellular products that may cause injury to the periodontal tissues.

Among the clinical, microbiologic, and serologic variables that were concomitntly used in this study to assess the periodontal status of subjects with different FcγR genotypes, only PL levels and PDs were significantly higher in subjects with the FcγRIIa-H/H131 polymorphism at baseline. However, the longitudinal changes in these parameters did not vary in subjects with different FcγRIIa or RIIIb genotypes. Therefore, the present data do not provide strong evidence for the use of these polymorphisms as severity factors for periodontitis. This observation is in accordance with Kobayashi et al. (2001), who concluded that neither the FcγRIIa nor the FcγRIIIb polymorphisms alone could serve as severity factors. However, these authors reported that a composite genotype comprised of FcγRIIIa-V158 and FcγRIIIb-NA2 was strongly associated with severe periodontitis, defined as having more than seven inter-proximal sites with >50% bone loss and a total mean bone loss of >34% of the root length.

One of the limitations of the present study is that no uniformly standardized periodontal therapy was carried out in all study participants. Instead, the study participants received a periodontal treatment regime deemed appropriate to their individual status by their care-taking periodontist. However, given the fact that the baseline clinical characteristics were largely similar in subjects belonging to the various genotype groups, the possibility for a polymorphism/treatment interaction seems unlikely. In fact, a post hoc analysis confirmed that the used therapies (surgical/non-surgical, with/without adjunc-

tive antibiotics) occurred in similar frequencies in all polymorphism groups (data not shown).

In conclusion, in view of the present data, it is questionable whether FcγR polymorphisms have utility as either risk indicators or severity markers for periodontitis in Caucasian populations. This conclusion is not necessarily in contrast with the findings reported above stemming from non-Caucasian populations, as both the prevalence and the biological significance of any given polymorphism may conceivably vary in racially/ethnically distinct populations. While genetic influences on the periodontitis phenotype are decisive, periodontitis is a multifactorial disease most likely involving multiple genes. Thus, assessment of genetic risk based on single gene polymorphisms appears to be a simplistic approach, given the abundant redundancy of the biologic systems that determine disease susceptibility. Instead, a genetic approach that simultaneously examines multiple candidate genes may be more successful in the identification of those at risk for destructive periodontal disease before the development of irreversible tissue damage.

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Clinical Relevance

Scientific rationale: In an effort to expand our knowledge on the genetic determinants of susceptibility to periodontal infections, we examined whether specific polymorphisms in human phagocytic cell receptors (FcγR) confer risk for periodontitis in a northern European subject sample.

Principal findings: There was no difference in the occurrence of the examined polymorphisms among subjects with and without periodontitis. There were few, marginal associations between FcγR genotypes and clinical periodontal status, and no associations with microbiologic or serologic markers and therapeutic responses.

Practical implications: There appears to be little value in the use of the investigated FcγR genotypes as severity markers for periodontitis in northern European populations.