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The association of Fcγ receptor IIIb genetic polymorphism and susceptibility to periodontitis in Taiwanese individuals

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Abstract

Aim: The allelic polymorphism of FcγRIIIb, the neutrophil-specific receptor involved in the phagocytosis of immunoglobulin G-opsonized bacteria, has functionally distinct capacities that are important in host defence mediated by neutrophils. The aim of this study was to identify whether the polymorphism of FcγRIIIb is associated with periodontitis in Taiwanese individuals.

Materials and methods: This case–control study included of 93 aggressive periodontitis (AgP) patients, 372 chronic periodontitis (CP) patients and 158 healthy controls (HC). The FcγRIIIb genotypes were determined by PCR using allele-specific primers. The risk for periodontitis associated with genotypes was calculated as the odds ratio (OR).

Results: A significant difference was observed in the distribution of the FcγRIIIb genotype between either AgP and HC, or AgP and CP, but not between CP and HC. The OR for carriage of the NA2 allele (NA1NA2+NA2NA2 *versus* NA1NA1) in AgP was 3.27 [95% confidence interval (CI) = 1.57–7.51, $p = 0.0027$] and 2.94 (95% CI = 1.49–6.48, $p = 0.0037$), as compared with HC and CP. After adjusting for possible confounding factors, the association was still significant.

Conclusions: The results of the present study suggest that subjects carrying at least one copy of the FcγRIIIb-NA2 allele might be associated with susceptibility to AgP. However, the clinical implications of the FcγRIIIb allelic polymorphism should be determined by further studies.

Key words: FcγRIIIb; genetic polymorphism; susceptibility to periodontitis; Taiwanese

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Periodontitis is an inflammatory disease caused by bacteria in the periodontal pocket. Polymorphonuclear neutrophils (PMNs) can migrate beyond the junctional epithelium into the periodontal pocket to form a barrier between the epithelium and bacterial plaque and constitute the first line of defence against bacterial infection. They are the most numerous leucocytes among the cellular infiltrates in gingival crevicular fluid (GCF), and are also found in the apical part of the pocket epithelium and in its adjacent connective tissue (Yuan et al. 1999). The functional impairment of PMNs in the diseased periodontium would enhance and promote the development of periodontitis.

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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The Fc gamma receptor (FcγR) is the molecule on the cell surface interacting with the constant part of immunoglobulin G (IgG). FcγR-bearing cells can catch the IgG immune complexes. Therefore, FcγRs are considered as the crucial links between the cellular and the humoral immune systems. Binding of IgG-opsonized particles to leucocytes triggers a variety of potent effects or functions of leucocytes, including phagocytosis, antibody-dependent cellular cytotoxicity, augmentation of antigen presentation, release of inflammatory mediators, degra-

nulation, superoxide production, and regulation of antibody production (Brunkhorst et al. 1992, Kushner and Cheung 1992). Besides the antibacterial functions, these responses also induce inflammatory reactions and consequently result in tissue destruction.

Leucocyte FcγRs have been divided into three main classes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). Twelve transcripts of FcγRs have been identified. FcγRIIIb, which is neutrophil-specific, belongs to the low-affinity receptor (Nimmerjahn and Ravetch 2006). In humans, only complexed or aggregated IgG1 and IgG3 can bind to FcγRIIIb. It is the most abundant FcγR on neutrophils (about 100,000–300,000 copies/cell) (Huizinga et al. 1990, De Haas et al. 1995b) and is important in modulating the immune response of neutrophil.

FcγRIIIb bears the neutrophil antigen (NA) polymorphism, designated as NA1 and NA2. The allelic polymorphism in FcγRIIIb can have significant consequences for the effector function of PMNs (Salmon et al. 1990). The DNA that encodes the NA1 allele is different from that which encodes the NA2 allele at five nucleotides (positions 141, 147, 227, 277, and 349), resulting in differences in four amino acids (positions 36, 65, 82 and 106) in the membrane distal Ig-like domain, which is the Fc-binding region. The amino acid differences at positions 65 and 82 result in two extra glycosylation sites in FcγRIIIb-NA2 (Ory et al. 1989). Glycosylation has a significant inhibitory effect on the interaction of FcγRIIIb with hIgG3 (Galon et al. 1997). The genetic polymorphism of FcγRIIIb results in a receptor structural difference due to a variation in the glycosylation pattern, and as a consequence, the different IgG-binding affinity. In addition, the two isoforms of FcγRIIIb display different phagocytic capacities (Salmon et al. 1992, Bredius et al. 1994). PMNs from homozygous NA2 subjects exhibited a significantly lower phagocytosis of IgG1- and IgG3-opsonized *Porphylomonas gingivalis* and lower oxidative burst, compared with those from homozygous NA1 donors (Kobayashi et al. 2000b). Therefore, the functions of FcγRIIIb display an allelic variation. Inappropriate expression of the FcγRIIIb function is considered to play an important role in the pathogenesis of periodontitis.

It is well known that microbial factors cannot solely be held responsible for periodontitis. Accumulating evidences suggest that host immune responses to bacterial challenge and susceptibility to these infections may be under genetic control (Michalowicz 1994). The allelic polymorphism of FcγRIIIb has functionally distinct capacities that are important in receptor-mediated phagocytosis of IgG immune complexes. When the FcγRIIIb-mediated leucocyte functions are less efficient due to genetic polymorphism in the FcγR-IIIB genes, the susceptibility and/or progression of infectious diseases, including periodontitis, considerably are affected. Several studies have been performed on the association of FcγRIIIb polymorphism and periodontal diseases (Kobayashi et al. 2000a, 2001, Meisel et al. 2001, Sugita et al. 2001, Yoshihara et al. 2001, Chung et al. 2003, Loos et al. 2003, De Souza and Colombo 2006, Wolf et al. 2006), but the results are inconsistent. The possible explanations for the inconsistent results are the different ethnicities and the relatively small sample size in previous studies. The evaluation of the FcγRIIIb genotype as a risk factor for periodontal diseases should be examined in a distinct ethnic background and should be carried out in a large population. The present study aimed to determine the distribution of the FcγRIIIb genotype and the allele frequency in Taiwanese individuals with enrolment of larger numbers of study subjects, and to evaluate the association of the FcγRIIIb polymorphism and susceptibility to periodontal disease.

Materials and Methods

Study subjects

This case–control study consisted of 93 aggressive periodontitis (AgP) patients (40 females, 53 males), 372 chronic periodontitis (CP) patients (164 females, 208 males), and 158 ethnically matched periodontally healthy (HC) subjects (82 females, 76 males). They were recruited from patients who visited the Department of Periodontics of Kaohsiung Medical University Hospital. None of them had a history or clinical manifestations of systemic diseases that could affect the progression or expression of periodontal diseases. Subjects with <18 remaining teeth, those who are pregnant, currently breastfeeding, or needed antibiotic prophylaxis before periodontal treatment were excluded from this study. The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University. Informed consent was signed by all subjects.

The study subjects were diagnosed as having AgP, CP, and HC on the basis of clinical examinations [probing depth (PD) and attachment loss (AL)] and radiographic patterns of alveolar bone destruction. The diagnostic criteria for AgP and CP were defined in accordance with the classification agreed on at the World Workshop for Periodontics and The American Academy of Periodontology (1999). AgP was inclusive of more than eight teeth with AL $\geqslant$ 5 mm and PD $\geqslant$ 6 mm. The level of attachment loss was not consistent with the plaque level or local contributing factors. CP was indicated in subjects >35 years of age, with AL $\geqslant$ 5 mm at more than one tooth, more than three sites with PD $\geqslant$ 6 mm, and lesions distributed at more than two teeth in each quadrant. The level of attachment loss must appear consistent with the plaque level or local contributing factors. HC was indicated by no evidence of AL at more than one site or PD >3 mm. Almost all AgP patients were <35 years of age at the time of the initial diagnosis. The recruited healthy controls (HC) were older than 35 years so that they could not be too young to have periodontitis. The study subjects were classified as current smokers, former smokers, and non-smokers. Subjects who still had the smoking habit or had quit smoking within the last 6 months before enrolling in this study were designated as current smokers. Former smokers were subjects who had quit smoking for at least 6 months.

Sample collection and DNA extraction

Twenty millilitres of heparin-anticoagulated peripheral blood was collected from each study subject. Genomic DNA was extracted from the peripheral blood leucocytes by standard phenol/chloroform extraction techniques and precipitation with ethanol (Blin and Stafford 1976). The DNA concentration was determined by ultraviolet (UV) spectrophotometry.

FcγRIIIb allotyping (NA1 and NA2)

FcγRIIIb allotyping was carried out using allele-specific primers (Bux et al. 1995). Two microlitres of genomic DNA was added to a 25 μl reaction mixture containing 1 $\times$ reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1.25 U

Taq polymerase (JMP Holdings, London, UK), and 1.0 μM of each primer. In the NA1-ASPA (allele-specific primer annealing), the sense primer was 5′-CAG TGG TTT CAC AAT GTG AA-3′ and the antisense primer was 5′-CAT GGA CTT CTA GCT GCA CCG-3′. In the NA2-ASPA, the sense primer was 5′-CTC AAT GGT ACA GCG TGC TT -3′ and the antisense primer was 5′-CTG TAC TCT CCA CTG TCG TT-3′. The PCR procedure consisted of initial denaturation at 95°C for 9 min., followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. The products for NA1 and NA2 were 141 and 169 bp, respectively. The genomic DNA samples from two subjects representing NA1NA1 and NA1NA2 genotypes, which were identified by DNA sequencing, were used as the internal controls in each PCR procedure. All PCR products were analysed by electrophoresis on 2% polyacrylamide gels containing ethidium bromide and were visualized under UV light.

Statistical analysis

Statistical analyses were performed using a JMP statistical software package (SAS Institute, Cary, NC, USA). Comparisons of descriptive statistics among three groups were expressed as mean (± SD) and within-group proportions. Differences in the demographic factors among the three groups were tested with the χ^2-test and the ANOVA test. The distribution of the FcγRIIIb genotype and its allele frequencies in each group were compared by the χ^2-test. The risk association of genotypes and periodontal diseases was computed by logistic regression and expressed as an odds ratio (OR) with a 95% confidence interval (CI). The significance of all analyses was accepted at $p < 0.05$. To control the possible confounding effects, age, gender, and smoking status were used as independent variables of multiple logistic regression for adjustment.

Results

Table 1 presents the demographic characteristics of the study subjects. The gender distribution was similar for these three groups. The mean age of the AgP patients was significantly lower than that of the CP patients and the HC. A significant difference was observed in the smoking status either between CP patients and HC ($p = 0.0008$), or between CP and AgP patients ($p = 0.036$), but not between AgP patients and HC ($p = 0.091$). The CP patients were more likely to be current smokers.

Table 1. Demographic characteristics of study subjects classified as patients with aggressive periodontitis (AgP), chronic periodontitis (CP), and healthy controls (HC)

	AgP $n = 93$	CP $n = 372$	HC $n = 158$	Total $n = 623$	p-value*
Gender (n) (%)					0.209
Female	40 (43.0)	164 (44.1)	82 (51.9)	286 (45.9)	
Male	53 (57.0)	208 (55.9)	76 (48.1)	337 (54.1)	
Age (mean ± SD)	37.7 ± 6.9	52.4 ± 7.5	50.9 ± 9.5		<0.0001
Smoking (n) (%)†					0.003
None	67 (72.0)	269 (72.3)	132 (83.5)	468 (75.1)	
Former	13 (14.0)	25 (6.7)	14 (8.9)	52 (8.3)	
Current	13 (14.0)	78 (21.0)	12 (7.6)	103 (16.5)	

*Comparisons performed by χ^2-test or ANOVA
†Smoking status: $p = 0.091$ for AgP *versus* HC; $p = 0.036$ for AgP *versus* CP; $p = 0.0008$ for CP *versus* HC

Table 2. FcγRIIIb genotype distribution and allelic frequencies in patients with aggressive periodontitis (AgP), chronic periodontitis (CP), and healthy controls (HC)

	AgP $n = 93$	CP $n = 372$	HC $n = 158$	Total $n = 623$
Genotype (n) (%)*				
NA1NA1	9 (9.7)	89 (23.9)	41 (26.0)	139 (22.3)
NA1NA2	75 (80.6)	241 (64.8)	88 (55.7)	404 (64.8)
NA2NA2	9 (9.7)	42 (11.3)	29 (18.4)	80 (12.8)
Allelic frequency (n) (%)†				
NA1	93 (50.0)	419 (56.3)	170 (53.8)	682 (54.7)
NA2	93 (50.0)	325 (43.7)	146 (46.2)	564 (45.3)

*Distribution of FcγRIIIb genotype (3 × 2 contingency table): $\chi^2 = 17.26$, $p = 0.0002$, for AgP *versus* HC; $\chi^2 = 11.38$, $p = 0.0034$, for AgP *versus* CP; $\chi^2 = 5.59$, $p = 0.0612$, for CP *versus* HC.
†Allelic frequency among groups: the difference was not significant.
Comparisons performed by χ^2–test.

Table 2 presents the distribution of the FcγRIIIb genotype and allelic frequencies. The genotype distribution of FcγRIIIb in HC satisfied Hardy–Weinberg equilibrium ($p > 0.05$). In this study population, heterozygote (the NA1NA2 genotype) was the most abundant (64.8%), followed by the NA1 homozygote (NA1NA1 genotype, 22.3%) and NA2 homozygote (NA2NA2 genotype, 12.8%). The genotype distribution in AgP was significantly different from that in HC and that in CP ($p = 0.0002$ for AgP *versus* HC, $p = 0.0034$ for AgP *versus* CP), while the genotype distribution did not show a significant difference between CP and HC ($p = 0.0612$). No significant difference was observed in the allelic frequency among groups.

Because the genotype distribution in AgP was significantly different from HC and CP, we analysed further the association of AgP and the FcγRIIIb genotype (Table 3). The proportion of FcγRIIIb-NA2 carriers was higher in AgP patients as compared with the CP patients and HC. The crude OR for NA2 carrier (NA1NA2 and NA2NA2 genotypes combined compared with the NA1NA1 genotype) associated with AgP was 3.27 (95% CI = 1.57–7.51, $p = 0.0027$) and 2.94 (95% CI = 1.49–6.48, $p = 0.0037$) as compared with HC and CP, respectively. After adjustment for age, gender, and smoking status, the association was still significant (adjusted OR = 2.74, 95% CI = 1.07–7.67, $p = 0.043$ for AgP *versus* HC; adjusted OR = 2.65, 95% CI = 1.06–7.29, $p = 0.046$ for AgP *versus* CP). This means that the NA2 carriers (NA1NA2 or NA2NA2 genotype) had a higher risk of having AgP than the non-NA2 carriers (NA1NA1 genotype). No significant difference was observed in the distribution of NA2 carriers between CP and HC.

Discussion

The results of this study indicate that the FcγRIIIb polymorphism is associated with susceptibility to AgP in Taiwanese individuals. The FcγRIIIb-NA2 carriers

Table 3. The association of FcγRIIIb genotype and risk of periodontitis, comparisons performed by logistic regression analysis

	NA1NA2+NA2NA2*	NA1NA1*	Crude OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
AgP	84 (90.3)	9 (9.7)	3.27 (1.57–7.51)	0.0027	2.74 (1.07–7.67)	0.043
HC	117 (74.1)	41 (25.9)	1		1	
AgP	84 (90.3)	9 (9.7)	2.94 (1.49–6.48)	0.0037	2.65 (1.06–7.29)	0.046
CP	283 (76.1)	89 (23.9)	1		1	
CP	283 (76.1)	89 (23.9)	1.11 (0.72–1.70)	0.62	1.08 (0.70–1.67)	0.716
HC	117 (74.1)	41 (25.9)	1		1	

*Expressed as case number (%)

OR, odds ratio; CI, confidence interval; AgP, aggressive periodontitis; CP, chronic periodontitis; HC, healthy controls; Adjusted OR, adjusted for gender, sex, and smoking status for logistic regression analysis.

were found to be over presented in AgP patients as compared with the HC and CP patients . The results are consistent with the findings in Japanese by Yoshihara et al. (2001) and Kobayashi et al. (2000a) and in Caucasians by De Souza and Colombo (2006) that carrying at least one FcγRIIIb-NA2 allele exhibited increased susceptibility to AgP, but are in disagreement with the findings of Nibali et al. (2006), who reported that the NA1NA1 genotype was associated with AgP.

Although the association of the FcγRIIIb polymorphism and susceptibility to periodontitis in Taiwanese individuals had been evaluated (Chung et al. 2003), the sample size was small (28 AgP, 50 CP, and 74 HC) in the study. Because sample size is critical in an association study, we carried out this case–control study with a relatively larger sample size (93 AgP, 372 CP, and 158 HC) in order to obtain a the more reliable result. Our results showed that the FcγRIIIb-NA2 carrier was associated with an increased risk of AgP, and 12.8% of our study subjects had NA2 homozygotes. On the contrary, no association could be observed between FcγRIIIb polymorphism and periodontitis, and no NA2 homozygote could be detected in the aforementioned study. Another study that surveyed the FcγRIIIb genotype distribution in 583 Taiwanese individuals showed that 15.1% of the study subjects had the NA2NA2 genotype (Chen et al. 2006). The genotype distribution and allelic frequencies of our study population (623 subjects, NA1NA1 22.3%, NA1NA2 64.8%, NA2NA2 12.8%; NA1 allele 54.7%, NA2 allele 45.3%) were similar to that found in a southern Chinese population (413 subjects, NA1NA1 28.1%, NA1NA2 56.9%, NA2NA2 14.5%; NA1 allele 56.5%, NA2 allele 43.0%) (Tong et al. 2003). The reason why no NA2NA2 genotype could be observed in Chung's study could be related to the small sample size.

Host immune reactions triggered by periodontal pathogens result in periodontal tissue destruction. High levels of IgG1 and IgG3 subclass were found in GCF taken from periodontitis sites (Wilton et al. 1993, Takahashi et al. 1997). The efficient catch and phagocytosis of IgG1- and IgG3-opsonized bacteria via FcγRIIIb, then clearance of immune complexes are crucial and critical for host defence in periodontal tissue. Neutrophils bearing the FcγRIIIb-NA2 allele have lower receptor affinity to IgG3, which is the predominant anti-*Porphyromonas gingivalis* serum IgG for rapidly progressive periodontitis (it has been changed to AgP) patients (Whitney et al. 1992), and lower phagocytic capacity. It is considered that neutrophils expressing the NA2 allele are less efficient in the capture of IgG-opsonized periodontal pathogens (Kobayashi et al. 2000b), and triggering less potent effector functions of neutrophils, being indicative of the humoral immune response of the FcγRIIIb-NA2 carrier, might be ineffective in clearing the *P. gingivalis* or other periodontal pathogens. The relative impairment of IgG-immune complexes clearance by the NA2 allele is supposed to favour deposition of immune complexes, persistent bacterial infections and consequently prolonged proinflammatory reactions in periodontal tissue. The functional depression of the first line of host-defensive cell population in the periodontium of the FcγRIIIb-NA2 carrier would initiate the development early and enhance the progression of periodontitis.

Genetic studies of the association between periodontitis and the FcγRIIIb polymorphism exhibit inconsistent findings (Table 4). Some investigations have associated the FcγRIIIb-NA2 allele with recurrence (Kobayashi et al. 1997), severity (Kobayashi et al. 2001), severity and extent of bone loss (Meisel et al. 2001) of CP, and progression of periodontitis (Yoshihara et al. 2005). The FcγRIIIb-NA2 allele (Yoshihara et al. 2001) and FcγRIIIb NA2NA2 genotype were overrepresented in generalized AgP patients (Kobayashi et al. 2000a, De Souza and Colombo 2006), and were associated with susceptibility to localized AgP in African Americans (Fu et al. 2002). In contrarst, a study reported that the FcγRIIIb NA1NA1 genotype was significantly associated with generalized AgP in Caucasians (Nibali et al. 2006). Several reports pointed out that the FcγRIIIb polymorphism did not contribute to periodontitis (Kobayashi et al. 1997, Chung et al. 2003), likely due to the very small number of NA2NA2 homozygotes in some ethnic populations. FcγRIIIb genotype was reported not to be associated with the severity of periodontitis (Meisel et al. 2001, Loos et al. 2003, Wolf et al. 2006) and the treatment response of periodontitis (Colombo et al. 1998, Wolf et al. 2006). In addition, the FcγRIIIb genotype had also been reported to be related to interindividual differences in the resistance to periodontitis. The NA1 allele was significantly overrepresented in the periodontitis-resistant group compared with the periodontitis-susceptible group (Sugita et al. 2001).

Several issues might account for the conflicting results between the different studies: variations at the ethnic backgrounds of the study populations, the different definitions and classifications of periodontal disease, and the relatively small sample size. The NA1 and NA2 allelic frequencies observed in different ethnic groups were quite different (Hessner et al. 1996). In Caucasians, NA2 is the most frequent allele (NA1:NA2 = 0.37:0.63). On the other hand, NA1 is more prevalent in Asian populations, including Taiwanese individuals and Japanese (NA1:NA2 = 0.68:0.32). Based on the literature, the prevalence of the FcγRIIIb genotype differs widely among distinct ethnic backgrounds, making comparisons of the findings among studies quite difficult. Therefore, the evaluation of any FcγR genotype as the risk factor for periodontal diseases should be examined in different ethnic populations. Sample size is critical in association

Table 4. Studies on the association of FcγRIIIb genetic polymorphism and periodontitis

Ethnicity	Subjects	Genotype distribution (%)			Allele frequency		*p*-value	Authors
		NA1/NA1	NA1/NA2	NA2/NA2	NA1	NA2		
Brazilian	49 healthy	91.8	8.2	0.0	0.96	0.04	OR = 32.5, 95% CI = 10.6–99.8	De Souza and Colombo (2006)
	31 GAgP	19.4	38.7	41.9	0.42	0.58	$p < 0.001$	
Caucasian	73 healthy	11	55	34	0.38	0.62	Association not found	Wolf et al. (2006)
	132 periodontitis	16	47	37	0.39	0.61	(disease severity and response to therapy)	
Taiwanese	74 healthy	44.6	55.4	0.0	0.72	0.28	Association not found	Chung et al. (2003)
	50 CP	38.0	62.0	0.0	0.69	0.31		
	30 GAgP	42.9	57.1	0.0	0.71	0.29		
Caucasian	61 healthy	8	48	44	0.32	0.68	Association not found	Loos et al. (2003)
	68 periodontitis	12	49	40	0.36	0.64		
Japanese	64 healthy	43.8	45.3	10.9	0.66	0.34	Association not found	Kobayashi et al. (2001)
	50 severe CP	32.0	48.0	20.0	0.56	0.44		
	39 moderate CP	46.2	41.0	12.8	0.67	0.33		
Caucasian	154 subjects	11.0	51.3	37.7	0.37	0.63	Not associated with disease severity	Meisel et al. (2001)
Japanese	55 healthy	43.6	45.5	11.1	0.66	0.34	EOP *versus* CP $p = 0.027$	Yoshihara et al. (2001)
	42 GEOP	16.7	52.8	30.6	0.43	0.57	EOP *versus* HC $p = 0.006$	
	52 AP	42.3	42.3	15.4	0.63	0.37	OR for NA2 carrier EOP *versus* CP OR = 3.67 EOP *versus* HC OR = 3.87	
Japanese	46 p-resistant	43.5	56.5	0.0	0.72	0.28	OR = 1.87, 95% CI = 1.07–3.28	Sugita et al. (2001)
	73 p-susceptible	26.0	63.0	11.0	0.58	0.42	$p = 0.03$	
Japanese	104 healthy	35.6	52.9	11.5	0.62	0.38	NA2 carrier	Kobayashi et al. (2000a)
	83 AP	36.1	48.2	15.7	0.60	0.40	GEOP *versus* HC: OR = 2.4 95% CI = 1.0–6.8, $p = 0.04$	
	38 GEOP	18.4	52.6	29.0	0.45	0.55	GEOP *versus* AP: OR = 2.5 95% CI = 1.0–7.1, $p = 0.04$	
Japanese	105 healthy	36.2	52.4	11.4	0.62	0.38	Association not found	Kobayashi et al. (1997)
	100 AP	37	43	20	0.59	0.41		
Caucasian	144 healthy	9.7	57.6	32.6	0.22	0.78	NA1NA1 associated with GagP	Nibali et al. (2006)
	88 GAgP	21.6	43.2	35.2	0.40	0.6	OR = 2.73, 95% CI = 1.24–6.04, $p = 0.013$	

GagP, generalized aggressive periodontitis; CP, chronic periodontitis; GEOP, generalized early onset periodontitis AP, adult periodontitis; p-resistant, periodontitis resistant p-susceptible: periodontitis susceptible.

studies. The number of study subjects in several previous studies was relatively small. The interpretation of conclusions from these studies should be made with caution.

Smoking is a known risk factor for periodontitis. In this study, we just used the smoking status as an independent variable for adjustment, and did not stratify the study subjects by their smoking status, because the current smokers in AgP patients (13 in 93) and HC (12 in 158) were quite few.

Our study has several limitations. There are three subclasses of FcγRs (FcγRIIa, FcγRIIIa, and IIIb) bearing functionally allelic polymorphisms that can interact with IgG1 and IgG3 subclasses. The IgG-mediated leucocyte effector function is regulated by the interaction of FcγRs. Human FcγRs genes are clustered in very close proximity on chromosome 1q21-24. The findings of previous studies supported the fact that the FcγR polymorphisms might be non-randomly distributed (Van der Pol et al. 2003, Torkildsen et al. 2005). Linkage disequilibrium may exist between the FcγRIIIb gene and the other FcγRs genes (Hatta et al. 1999) and/or other adjacent genes. Cross-linking of FcγRIIa and FcγRIIIb on neutrophils showed a synergistic intracellular Ca^{2+} response (Vossebeld et al. 1995), and increased phagocytosis and respiratory burst (Kocher et al. 1997). FcγRIIIb can enhance FcγRIIa function in an allele-sensitive manner, that is, NA1 homozygotes of FcγRIIIb showed greater activation of FcγRIIa than the NA2 homozygotes (Salmon et al. 1995). Therefore, in the investigation of FcγR polymorphisms and susceptibility to periodontal disease, FcγR combined genotypes may represent more relevant risk factors than a single FcγR genotype. Some composite genotypes of FcγRs have been reported to be associated with periodontal diseases. For instance, the FcγRIIIa-158 V and FcγRIIIb-NA2 composite genotype was associated with

the severity of CP in Japanese (Kobayashi et al. 2001); the FcγRIIIb NA2NA2 and FcγRIIa H/H131 composite genotype may be associated with generalized AgP in Caucasians (De Souza and Colombo 2006). We did not investigate the combination effect among FcγRs. We cannot rule out the possibility that the association of the FcγRIIIb polymorphism with susceptibility to AgP may result from the linkage disequilibrium with the real susceptibility genes located in the neighbouring FcγR genes.

In summary, we have identified that the FcγRIIIb allelic polymorphism is associated with susceptibility to AgP. However, the clinical implications of the FcγRIIIb polymorphism are not yet clear (De Haas et al. 1995a). Further investigations about the clinical implications of the FcγRIIIb polymorphism and the effects of FcγR composite genotypes on neutrophils are needed to clarify the significance of the findings of the present study.

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References

Blin, N. & Stafford, D. W. (1976) A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Research* **3**, 2303–2308.

Bredius, R. G. M., Fijen, C. A. P., De Haas, M., Kuijper, E. J., Weening, R. S., Van de Winkel, J. G. J. & Out, T. A. (1994) Role of neutrophil FcγRIIa (CD32) and FcγRIIIb (CD16) polymorphic forms in phagocytosis of human IgG1- and IgG3-opsonized bacteria and erythrocytes. *Immunology* **83**, 624–630.

Brunkhorst, B. A., Strohmeier, G., Lazzari, K., weil, G., Melnick, D., Fleit, H. B. & Simons, E. R. (1992) Differential roles of the FcrRII and FcrRIII in immune complex stimulation of human neutrophils. *The Journal of Biological Chemistry* **267**, 20659–20666.

Bux, J., Stein, E. L. & Mueller-Ekhardt, C. (1995) NA gene frequencies in the German populatiom, determined by polymerase chain reaction with sequence-specific primers. *Transfusion* **35**, 54–57.

Chen, J. Y., Wang, C. M., Wu, J. M., Ho, H. H. & Luo, S. F. (2006) Association of rheumatoid factor production with Fc gamma receptor IIIa polymorphism in Taiwanese rheumatoid arthritis. *Clinical and Experimental Immunology* **144**, 10–16.

Chung, H.-Y., Lu, H.-C., Chen, W.-L., Lu, C.-T., Yang, Y.-H. & Tsai, C.-C. (2003) Gm(23) allotypes and Fcγreceptor genotypes as risk factors for various forms of periodontitis. *Journal of Clinical Periodontology* **30**, 954–960.

Colombo, A. P., Eftimiadi, C., Haffajee, A. D., Cugini, M. A. & Socransky, S. S. (1998) Serum IgG2 level, Gm(23) allotype and FcγRIIa and FcγRIIIb receptors in refractory periodontal disease. *Journal of Clinical Periodontology* **25**, 465–474.

De Haas, M., Kleijer, M., Van Zwieten, R., Roos, D. & Von dem Borne, A. E. G. K. (1995a) Neutrophil FcγRIIIb deficiency, nature, and clinical consequences: a study of 21 individuals from 14 families. *Blood* **86**, 2403–2413.

De Haas, M., Vossebeld, P. J. M., Von dem Borne, A. E. G. K. & Roos, D. (1995b) Fcγ receptors of phagocytes. *Journal of Laboratory and Clinical Medicine* **126**, 330–341.

De Souza, R. C. & Colombo, A. P. V. (2006) Distribution of FcγRIIa and FcγRIIIb genotypes in patients with generalized aggressive periodontitis. *Journal of Periodontology* **77**, 1120–1128.

Fu, Y., Korostoff, J. M., Fine, D. H. & Wilson, M. E. (2002) Fc gamma receptor genes as risk markers for localized aggressive periodontitis in African-Americans. *Journal of Periodontology* **73**, 517–523.

Galon, J., Robertson, M. W., Galinha, A., Mazières, N., Spagnoli, R., Fridman, W.-H. & Sautès, C. (1997) Affinity of the interaction between Fc gamma receptor type III (FcγRIII) and monomeric human IgG subclasses. Role of FcγRIII glycosylation. *European Journal of Immunology* **27**, 1928–1932.

Hatta, Y., Tsuchiya, N., Ohashi, J., Matsushita, M., Fujiwara, K., Hagiwara, K., Juji, T. & Tokunaga, K. (1999) Association of Fc gamma receptor IIIB, but not Fc gamma receptor IIA and IIIA polymorphisms with systemic lupus erythematosus in Japanese. *Genes and Immunity* **1**, 53–60.

Hessner, M. J., Curtis, B. R., Endean, D. J. & Aster, R. H. (1996) Determination of neutrophil antigen gene frequencies in five ethnic groups by polymerase chain reaction with sequence-specific primers. *Transfusion* **36**, 895–899.

Huizinga, T. W. J., de Hass, M., Kleijer, M., Nuilens, J. H., Roos, D. & von dem Borne, A. E. G. K. (1990) Soluble Fc gamma receptor III in human plasma originates from release by neutrophils. *Journal of Clinical Investigation* **86**, 416–423.

Kobayashi, T., Sugita, N., Van der Pol, W.-L., Nunokawa, Y., Westerdaal, N. A. C., Yamamoto, K., Van de Winkel, J. G. J. & Yoshie, H. (2000a) The Fcγ receptor genotype as a risk factor for generalized early-onset periodontitis in Japanese patients. *Journal of Periodontology* **71**, 1425–1432.

Kobayashi, T., Van der Pol, W.-L., Van de Winkel, J. G. J., Hara, K., Sugita, N., Westerdaal, N. A. C., Yoshie, H. & Horigome, T. (2000b) Relevance of IgG receptor IIIb (CD16) polymorphism to handling of Porphyromonas gingivalis: implications for the pathogenesis of adult periodontitis. *Journal of Periodontal Research* **35**, 65–73.

Kobayashi, T., Westerdaal, N. A. C., Miyazaki, A., Van der Pol, W.-L., Suzuki, T., Yoshie, H., Van de Winkel, J. G. J. & Hara, K. (1997) Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. *Infection and Immunity* **65**, 3556–3560.

Kobayashi, T., Yamamoto, K., Sugita, N., Van der Pol, W.-L., Yasuda, K., Kaneko, S., Van de Winkel, J. G. J. & Yoshie, H. (2001) The Fcγreceptor genotype as a severity factor for chronic periodontitis in Japanese patients. *Journal of Periodontology* **72**, 1324–1331.

Kocher, M., Siegel, M. E., Edberg, J. C. & Kimberly, R. P. (1997) Cross-linking of Fc gamma receptor IIa and Fc gamma receptor IIIb induces different proadehesive phenotypes on human neutrophils. *The Journal of Immunology* **159**, 3940–3948.

Kushner, B. H. & Cheung, N.-K. V. (1992) Absolute requirement of CD11/CD18 adhesion molecules, FcRII, and the phosphatidylinositol-linked FcRIII for monoclonal antibody-mediated neutrophil antihuman tumor cytotoxicity. *Blood* **79**, 1484–1490.

Loos, B. G., Leppers-Van de Straat, F. G. J., Van de Winkel, J. G. J. & Van de Velden, U. (2003) Fcγreceptor polymorphisms in relation to periodontitis. *Journal of Clinical Periodontology* **30**, 595–602.

Meisel, P., Carlsson, L. E., Sawaf, H., Fanghaenel, J., Greinacher, A. & Kocher, T. (2001) Polymorphisms of Fcγ-receptors RIIa, RIIIa, and RIIIb in patients with adult periodontal diseases. *Genes and Immunity* **2**, 258–262.

Michalowicz, B. S. (1994) Genetics and heritable risk factors in periodontal disease. *Journal of Periodontology* **65**, 479–488.

Nibali, L., Parkar, M., Brett, P., Knight, J., Tonetti, M. S. & Griffiths, G. S. (2006) NADPH oxidase (CYBA) and Fcγpolymorphisms as risk factors for aggressive periodontitis. *Journal of Clinical Periodontology* **33**, 529–539.

Nimmerjahn, F. & Ravetch, J. V. (2006) Fcγ receptors: old friends and new family members. *Immunity* **24**, 19–28.

Ory, P. A., Clark, M. R., Kwoh, E. E., Clarkson, S. B. & Goldstein, I. M. (1989) Sequences of complementary DNAs that encode the NA1 and NA2 forms of Fc receptor III on neutrophils. *The Journal of Clinical Investigation* **84**, 1688–1691.

Salmon, J. E., Edberg, J. C., Brogle, N. L. & Kimberly, R. P. (1992) Allelic polymorphisms of human Fcγ receptor IIA and Fcγ receptor IIIB. Independent mechanisms for differences in human phagocyte function. *The Journal of Clinical Investigation* **89**, 1274–1281.

Salmon, J. E., Edberg, J. C. & Kimberly, R. P. (1990) Fcγ receptor III on human neutrophils. Allelic variants have functionally distinct capacities. *The Journal of Clinical Investigation* **85**, 1287–1295.

Salmon, J. E., Millard, S. S., Brogle, N. L. & Kimberly, R. P. (1995) Fc gamma receptor IIIb enhances Fc gamma receptor IIa function

in an oxidant dependent and allele-sensitive manner. *Journal of Clinical Investigation* **95**, 2877–2885.

Sugita, N., Kobayashi, T., Ando, Y., Yoshihara, A., Yamamoto, K., Van de Winkel, J. G. J., Miyazaki, H. & Yoshie, H. (2001) Increased frequency of FcγRIIIb-NA1 allele in periodontitis-resistant subjects in an elderly Japanese population. *Journal of Dental Research* **80**, 914–918.

Takahashi, K., Mooney, J., Frandsen, E. V. G. & Kinane, D. F. (1997) IgG and IgA subclass mRNA-bearing plasma cells in periodontitis gingival tissue and immunoglobulin levels in the gingival crevicular fluid. *Clinical and Experimental Immunology* **107**, 158–165.

Tong, Y., Jin, J., Yan, L. X., Neppert, J., Marget, M. & Flesh, B. K. (2003) FCGR3B gene frequencies and FCGR3 variants in a Chinese population from Zhejiang Province. *Annals of Hematology* **82**, 574–578.

Torkildsen, Ø., Utsi, E., Mellgren, S. I., Harbo, H. F., Vedeler, C. A. & Myhr, K.-M. (2005) Ethnic variation of Fcγ receptor polymorphism in Sami and Norwegian populations. *Immunology* **115**, 416–421.

Van der Pol, W. L., Jansen, M. D., Sluiter, W. J., Van de Sluis, B., Leppers-ven de Straat, F. G., Kobayashi, T., Westendorp, R. G., Huizinga, T. W. & Van de Winkel, J. G. J. (2003) Evidence for non-random distribution of Fc gamma receptor genotype combinations. *Immunogenetics* **55**, 240–246.

Vossebeld, P. J. M., Kessler, J., Von dem Borne, A. E. G. K., Roos, D. & Verhoeven, A. J. (1995) Heterotypic Fc gamma receptor clusters evoke a synergistic Ca^{+2} response in human neutrophils. *The Journal of Biological Chemistry* **270**, 10671–10679.

Whitney, C., Ant, J., Moncla, B., Johnson, B., Page, R. C. & Engel, D. (1992) Serum immunoglobulin G antibody to *Porphyromonas gingivalis* in rapidly progressive periodontitis: titer, avidity, and subclass distribution. *Infection and Immunity* **60**, 2194–2200.

Wilton, J. M. A., Bampton, J. L. M., Hurst, T. J., Caves, J. & Powell, J. R. (1993) Interleukin-1 beta and IgG subclass concentrations in gingival crevicular fluid from patients with adult periodontitis. *Archs of Oral Biology* **38**, 55–60.

Wolf, D. L., Neiderud, A. M., Hinckley, K., Dahlén, G., Van de Winkel, J. G. J. & Papapanou, P. N. (2006) Fcγ receptor polymorphisms and periodontal status: a prospective follow-up study. *Journal of Clinical Periodontology* **33**, 691–698.

Yoshihara, A., Sugita, N., Yamamoto, K., Kobayashi, T., Hirotomi, T., Ogawa, H., Miyazaki, H. & Yoshie, H. (2005) FcγRIIIb genotypes and smoking in periodontal disease progression among community-dwelling older adults in Japan. *Journal of Periodontology* **76**, 250–255.

Yoshihara, A., Sugita, N., Yamamoto, K., Kobayashi, T., Miyazaki, H. & Yoshi, H. (2001) Analysis of vitamin D and Fc gamma receptor polymorphisms in Japanese patients with generalized early-onset periodontitis. *Journal of Dental Research* **80**, 2051–2054.

Yuan, Z.-N., Schreurs, O., Gjermo, P., Helgeland, K. & Schenck, K. (1999) Topical distribution of FcγRI, FcγRII and FcγRIII in inflamed human gingiva. *Journal of Clinical Periodontology* **26**, 441–447.

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Clinical Relevance

Scientific rationale for the study: FcγRIIIb plays an important role in the neutrophil defence against invading microorganisms. Because the allelic polymorphism of FcγRIIIb can affect the receptor affinity and phagocytic capacity of neutrophils, we carried out this study to evaluate the association between the FcγRIIIb allelic polymorphism and susceptibility to periodontitis.

Principal findings: Carriage of FcγRIIIb-NA2 allele was associated with increased susceptibility to AgP patients as compared with the HC and CP in this Taiwanese individuals population.

Practical implications: Subjects who are FcγRIIIb-NA2 carriers (either the NA1NA2 genotype or the NA2NA2 genotype) had a higher risk of having AgP.