Journal of
PERIODONTAL RESEARCH

J Periodont Res 2011; 46: 292–302

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2010.01338.x

Fcγ RIIB polymorphisms, periodontitis and preterm birth in Japanese pregnant women

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Iwanaga R, Sugita N, Hirano E, Sasahara J, Kikuchi A, Tanaka K, Yoshie H. FcγRIIB *polymorphisms, periodontitis and preterm birth in Japanese pregnant women. J Periodont Res 2011; 46: 292–302. © 2011 John Wiley & Sons A/S*

Background and Objective: Recently, numerous studies have investigated the association of preterm birth with periodontitis. FcγRIIb is a human low-affinity receptor for immunoglobulin G (IgG). We have previously demonstrated single nucleotide polymorphisms (SNPs) of FcγRIIb to be associated with periodontitis and the serum-specific IgG level against periodontopathic bacteria. In this study, we investigated whether *FcγRIIB* gene polymorphisms were associated with periodontitis and/or pregnancy outcome.

Material and Methods: We assessed the periodontal conditions of 122 Japanese pregnant women within 5 d of delivery, and polymorphisms in *FcγRIIB* and in other Fcγ receptors were detected from the genomic DNA. Using clinical and genomic data, we analyzed the relationship between periodontitis, preterm birth and Fcγ receptor polymorphisms.

Results: A significant difference was observed in the distribution of *FcγRIIB*-nt645 + 25A/G (rs2125685) between preterm and term birth groups, with a higher prevalence of nt645 + 25AA in the preterm birth group ($p = 0.032$). Additionally, the *FcγRIIB*-nt645 + 25GG carrier showed significantly higher results for the prevalence of periodontitis ($p = 0.048$), mean pocket depth ($p = 0.021$), mean clinical attachment level ($p = 0.010$), percentage of sites with pocket depth ≥ 4 mm ($p = 0.005$) and percentage of sites with clinical attachment level ≥ 3 mm ($p = 0.007$) than the AA carrier. An association between preterm birth and periodontitis was not observed in this study.

Conclusion: These findings suggest that *FcγRIIB*-nt645 + 25AA carriers are more likely to experience preterm birth than *FcγRIIB*-nt645 + 25AG and GG carriers. Also, women with *FcγRIIB*-nt645 + 25G exhibited a greater tendency to have periodontitis than those with nt645 + 25A.

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Key words: *FcγRIIB*; polymorphism; periodontitis; preterm birth

Accepted for publication November 14, 2010

Preterm birth (PTB) represents a major problem for modern obstetrics because of its increasing frequency and accompanying socioeconomic impact. It is generally found that premature babies have more cognitive, behavioral, socioemotional and academic problems than full-term babies, even in the absence of major neurosensory or motor impairment (1). Infections play a significant role in spontaneous preterm labor and birth as well as in related neonatal complications (2). It has been reported that birth canal infection plays a key role in the etiopathogenesis of PTB (3). Therefore, it is possible that inflammation caused by bacterial infection affects PTB. In addition, it is generally accepted that periodontitis is an infectious disease caused by the direct effect of periodontopathic bacteria, along with the specific host immune response (4–6). It is therefore suggested that PTB and

periodontitis are alike in the perspective that periodontitis is an infectious disease and PTB is sometimes caused by infection. Many studies suggest an association between PTB and periodontitis (7–10). Recent studies have suggested a relationship between infection caused by one species of periodontopathic bacteria, and preterm low birth weight (11).

FcγRIIb (CD32b) is a human type II low-affinity receptor for immunoglobulin G (IgG). FcγRII is encoded by three highly homologus genes – FcγRIIA, *FcγRIIB* and *FcγRIIC* – that are clustered on chromosome 1q23 (12–14). FcγRIIa and FcγRIIc contain an activatory signal motif, immunoreceptor tyrosine-based activation motif. On the other hand, FcγRIIb contains a unique immunoreceptor tyrosine-based inhibition motif (ITIM). Co-ligation of FcγRIIb with the immunoreceptor tyrosine-based activation motif-containing receptor induces the phosphorylation of the ITIM tyrosine by Lyn, a member of the Src family of kinases (14). Three transcripts (Fcγ RIIb1, FcγRIIb2 and FcγRIIb3) have been identified in *FcγRIIB* as a result of alternative splicing. FcγRIIb1 is exclusively expressed on B cells and contains complete domains from all exons. In addition, FcγRIIb1 has been shown to act as a negative-feedback regulator by inhibiting B-cell antigen receptor-elicited activation signals via ITIM through IgG immune complexes (15–17).

We have previously suggested that a low level of production of IgG against the periodontal bacterium *Porphyromonas gingivalis* in early pregnancy is associated with intrauterine growth retardation and some instances of PTB (18). Moreover, we indicated the association of *FcγRIIB* gene polymorphisms with susceptibility to periodontitis in Japanese subjects (19). One of the polymorphisms, *FcγRIIB*-I232T, in patients with periodontitis was associated with an increase in the serum-specific IgG2 level against the outer membrane protein from *P. gingivalis* (20). Therefore, we hypothesized that *FcγRIIB* gene polymorphisms may mediate the relationship between periodontitis and PTB.

Accordingly, we investigated the relationship between the single nucleotide polymorphisms (SNPs) in Fcγ receptors and clinical data of both periodontitis and PTB in Japanese pregnant women (PW) and performed statistical analyses to identify any significant associations.

Material and methods

Subjects

The study group consisted of 122 Japanese PW [mean age (range): 31.9 (19–43) years] who were referred to the Department of Obstetrics and Gynecology, Niigata University Medical and Dental Hospital, and delivered live infants between October 2006 and February 2008.

The study was confined to limit the effect of confounding variables. Women were excluded from the study if they had any systemic medical problems, such as essential hypertension, hepatitis B, anemia, gestational diabetes, anxiety, depressive illness, renal diseases, or other systemic or genetic diseases and obstetric problems, such as multiple fetuses, an incompetent cervix and placental or uterine abnormalities (21). Women with a history of alcohol or drug abuse, or who were malnourished, were also excluded. Data on smoking habits and history were obtained from interviewing each woman. Subjects who continued smoking until confirmed as pregnant were regarded as smokers. These criteria were adopted because they were determined to be confounders and/or risk factors for adverse pregnancy outcomes.

One thousand and ninety-nine women were invited to participate in the study: 424 refused to participate; 545 were excluded because of the above-mentioned criteria; and the genomic DNA obtained from eight women was insufficient in quantity or of too poor quality to determine the Fcγ receptor genotype. Therefore, the final sample was composed of 122 women.

Before delivery, informed consent was obtained from each subject by means of a signed form. This form was previously reviewed and approved by the Ethical Committee of Niigata University Faculty of Dentistry in accordance with the Helsinki Declaration.

Clinical assessment

Obstetric data, including length of gestation and the presence or absence of PTB, were obtained from the Department of Obstetrics and Gynecology, Niigata University Medical and Dental Hospital. PTB was defined as delivery after week 22 but before week 37 of gestation. Term birth (TB) was defined as a delivery no earlier than week 37 and no later than week 41 of gestation. Gestational age at delivery was calculated from the first day of the last menstrual period and ascertained by a crown–rump length of between approximately 14 and 41 mm. The gestational age was corrected using the crown–rump length measurement data when the date determined by the crown–rump length measurement was more than 7 d earlier than the date of the last menstrual period.

Women with threatened PTB between the completed 24th and 37th gestational weeks, including obstetric events during pregnancy, such as premature rupture of membranes or necessity for treatment of premature uterine activity and/or cervical dilation, received special obstetric treatment to maintain their pregnancy for as long as possible. The therapy consisted of, first, bed rest, and then tocolytic agents. Antibiotic administration was included when premature rupture of membranes or onset of clinical chorioamnionitis occurred. Clinical chorioamnionitis was diagnosed by a body temperature of $\geq 38°C$ in the presence of other clinical findings, including maternal or fetal tachycardia, uterine tenderness, or a malodorous vaginal discharge.

Within 5 d after labor, clinical periodontal parameters were evaluated. Periodontal parameters, including probing depth, clinical attachment level, plaque control record and bleeding on probing were measured at six sites per tooth. Third molar teeth were excluded. The average for

whole-mouth probing depth, clinical attachment level, and the number of sites with bleeding on probing divided by the total number of sites per mouth and multiplied by 100 (i.e. percentage of sites with bleeding on probing), were calculated for each subject. The definition criterion for periodontitis in this study was > 60% of sites with a clinical attachment level of ≥ 3 mm (22). The measurements of probing depth and clinical attachment level were made to the nearest millimeter using a calibrated color-coded periodontal probe (CP-12; Hu-Friedy, Chicago, IL, USA). The examinations were carried out by three calibrated examiners (N.S., Y.S. and E.H.) who were masked from the obstetric data.

Determination of *FcγRIIB*, *FcγRIIA*, *FcγRIIIA* and *FcγRIIIB* genotypes

Genomic DNA was isolated from the venous blood obtained from all subjects. To determine *FcγRIIB*-nt645+25G/A (rs2125685) and *FcγRIIB*-nt645+7C/A (rs2125684) genotypes, nested PCRs for *FcγRIIB* and *FcγRIIB*-intron 4 were performed as described previously (19). Briefly, we first performed an *FcγRIIB*-specific PCR with the primer set on introns 3 and 6 because the *FcγRIIC* gene is highly similar to the *FcγRIIB* gene. After purification of the *FcγRIIB*-specific fragment, PCR with primers specific for exon 4 and intron 4 was performed using the purified fragment as a template. The *FcγRIIB*-intron 4 PCR was performed in a 25-μL reaction mixture containing 2 U of Ex Taq™ (TAKARA BIO INC., Ootsu, Japan), and 2.5 μL of the purified PCR product was denatured at 94°C for 5 min before being subjected to 35 cycles of amplification (94°C for 1 min, 64°C for 1 min and 72°C for 1 min) and a final extension at 72°C for 5 min.

Subsequently, to determine *FcγRIIB* −343G/C (rs3219018) genotype, we first performed long-range PCR designed to specifically amplify *FcγRIIB* and *FcγRIIC* from genomic DNA for nested PCR. A 15-kb region was amplified using a sense primer annealing in the common *FcγRIIB*/*C* promoter region and an antisense primer specific for exon 7 of *FcγRIIB* (23). PCR amplification of the 15-kb region was performed in a 50-μL reaction mixture containing 1.25 U of LA Taq™ (TAKARA BIO INC). The PCR conditions were 94°C for 1 min, 30 cycles at 98°C for 10 s and 70°C for 15 min, followed by a 10-min extension at 72°C. Amplification of the *FcγRIIB* promoter region was performed in a 25-μL reaction mixture containing 1.25 U of Ex Taq™, and 2.5 μL of the purified 15-kb PCR products was denatured at 94°C for 1 min before 30 cycles of amplification (94°C for 1 min, 68°C for 1 min, and 72°C for 1 min) and a final extension at 72°C for 5 min. Nucleotide sequences of these amplified fragments were determined using the ABI PRISM Big Dye Terminator Cycle Sequencing kits v1.1 and the ABI PRISM 377 sequencer (Applied Biosystems, Foster City, CA, USA).

A 6-mL sample of venous blood was also sent to the BML Corporation for DNA isolation and genotyping of the following SNPs: *FcγRIIB* nt646-184A/G (rs57420706), *FcγRIIB*-I232T (rs1050501), *FcγRIIA*-R131H (rs1891274), *FcγRIIIA*-V158F (rs396991) and *FcγRIIIB* (NA1/NA2). Determination of genotypes was performed using a nano-Invader DNA chip system. Owing to technical difficulties, *FcγRIIB*-nt646-184A/G and *FcγRIIIA*-V158F were genotyped using a PCR-Invader assay.

Statistical analysis

Chi-square tests were used to analyze the association of the seven *FcγRIIB*, *FcγRIIA*, *FcγRIIIA* and *FcγRIIIB* polymorphisms (rs2125684, rs2125685, rs57420706 and rs1050105) with susceptibility to periodontitis and to PTB. When the expected values in any of the cells of a contingency table were below 5, Fisher's exact test was used. Differences in clinical parameter values between the *FcγRIIB* SNPs were assessed using 2×2 or 3×2 contingency tables, the Mann–Whitney exact test, the Mann–Whitney *U*-test and the Kruskal–Wallis test. Chi-square tests, Fisher's exact tests, Mann–Whitney *U*-tests and Kruskal–Wallis tests were performed using StatView® ver5.0 (SAS Institute Inc, Cary, NC, USA). Yates' correction was used in chi-square tests. Mann–Whitney exact tests were performed using SPSS 16.0 Family for Windows containing SPSS Exact tests (SPSS Japan Inc., Tokyo, Japan).

Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) parameters analyses were performed using the ethnicity-matched 48 TB/nonperiodontitis PW. They were unrelated to each other.

HWE, haplotype frequencies and LD parameters were analyzed in 48 TB/nonperiodontitis PW and with exact *p*-values. Haplotype case–control studies were assessed using permutation tests. HWE, haplotype frequencies, LD parameters and haplotype case–control studies were estimated from genotyping results using the software SNPAlyze® v6.0.1 (Dynacom Co., Ltd, Yokohama, Japan). Statistical significances in all analyses were accepted at a *p*-value of < 0.05. The genotype frequency of *FcγRIIB* did not deviate from HWE without *FcγRIIB*-nt646-184A/G because no *FcγRIIB*-nt646-184G/G carriers were observed in the healthy controls (p-value $= 4.26 \times 10^{-12}$). The SNP was included in haplotype analyses and LD calculations.

Results

Clinical features of cases and controls (TB vs. PTB, and nonperiodontitis vs. periodontits)

Obstetric and periodontal clinical assessments (age, history of smoking, gestational weeks at delivery, mean probing depth, mean clinical attachment level, percentage of sites with probing depth ≥ 4 mm and percentage of sites with clinical attachment level ≥ 3 mm) of the 122 Japanese PW were separated into cases and controls (TB vs. PTB, and nonperiodontitis vs. periodontitis). Significant differences were found between PTB and TB groups in age ($p = 0.0007$) and gestational weeks at delivery ($p < 0.0001$). Significant differences were also found between periodontitis and nonperiodontitis groups in mean probing depth ($p < 0.0001$), percentage of sites

Table 1. Clinical characteristics of Japanese pregnant women

	TB (n = 71)	PTB (n = 51)	p-value	Nonperiodontitis (n = 81)	Periodontitis (n = 41)	p-value
Age (years)	33.3 ± 5.1	30.0 ± 4.9	0.0007*	31.7 ± 5.3	35.5 ± 4.9	0.512
History of smoking (%)	28.2	33.3	0.575	31.3	28.2	0.890
Gestational weeks at delivery	39.1 ± 1.3	31.8 ± 3.7	< 0.0001*	36.3 ± 4.3	35.5 ± 4.9	0.453
Percentage of plaque control record	33.0 ± 19.4	28.1 ± 22.5	0.162	24.8 ± 18.6	43.8 ± 19.5	< 0.0001*
Percentage of bleeding on probing	12.1 ± 13.5	15.7 ± 19.6	0.807	11.6 ± 14.3	18.0 ± 20.0	0.026*
Mean probing pocket depth (mm)	2.39 ± 0.44	2.43 ± 0.33	0.559	2.27 ± 0.35	2.49 ± 0.37	< 0.0001*
Mean clinical attachment level (mm)	2.49 ± 0.33	2.42 ± 0.42	0.295	2.29 ± 0.28	2.85 ± 0.22	< 0.0001*
Percentage of sites with a pocket depth of ≥ 4 mm	5.1 ± 8.5	6.3 ± 11.7	0.521	3.2 ± 6.7	10.2 ± 12.2	0.007*
Percentage of sites with a clinical attachment level of ≥ 3 mm	50.4 ± 19.1	43.6 ± 22.7	0.744	38.0 ± 14.2	71.8 ± 7.6	< 0.0001*

*p value < 0.05.
Values are given as mean ± SD, unless specified otherwise.
PTB, preterm birth; TB, term birth.
The p-value was calculated using the chi-square test 2 × 2 contingency table between subjects with and without a history of smoking. When the expected values in any of the cells of a contingency table were below 5, Fisher's exact probability tests were used. Statistical analyses between other comparisons were performed using the Mann–Whitney U-test.

with probing depth ≥ 4 mm (p = 0.007), mean clinical attachment level (p < 0.0001) and percentage of sites with clinical attachment level ≥ 3 mm (p < 0.0001), as expected. There were significant differences between periodontitis and nonperiodontitis groups in plaque control record and bleeding on probing (p = 0.001 and p = 0.026, respectively). The data are shown in Tables 1 and 2.

Distributions of *FcγRIIB*, *FcγRIIA*, *FcγRIIIA* and *FcγRIIIB* polymorphisms in cases and controls (TB vs. PTB, and nonperiodontitis vs. periodontitis)

Five SNPs in the *FcγRIIB* gene were detected in Japanese PW, all of which were confirmed to be *FcγRIIB*-specific. Of these SNPs, one resulted in an amino-acid substitution in exon 5 (rs1050501). The other three SNPs (rs2125684, rs2125685 and rs57420706) were detected in intron 4. The previously reported polymorphism rs3219018 in the promoter region was not detected in our subjects; it was therefore not included in further statistical analyses in this study. The genotypes of *FcγRIIA*-R131H (rs1801274), *FcγRIIIA*-V158F (rs396991) and *FcγRIIIB*-NA1/NA2 were also determined in the same PW and controls. As shown in Table 3, a significant overrepresentation of the *FcγRIIB*-nt645 + 25A allele was observed in the PTB group compared with the TB group (χ^2 = 6.1; p = 0.048). There was no significant difference in the distributions of genotypes and alleles of the other Fcγ receptor polymorphisms in the comparison between PTB and TB.

The distribution of four SNPs in *FcγRIIB* and three other Fcγ receptors polymorphisms in periodontitis and nonperiodontitis groups were also compared. As shown in Table 4, the carriage rate of the *FcγRIIB*-nt645 + 25G allele was significantly higher in the periodontitis group compared with the nonperiodontitis group (χ^2 = 4.4; p = 0.027). There was a significantly higher representation of the *FcγRIIA*-131R allele in the periodontitis group compared with the nonperiodontitis group (χ^2 = 5.9; p = 0.012). No other difference was observed in the distribution of genotypes and alleles in the comparison between periodontitis and nonperiodontitis groups.

We also analyzed the association between *FcγRIIB*-nt645 + 25A/G (rs2125685) and obstetric/periodontal clinical parameters (Tables 5 and 6). There was no significant difference between *FcγRIIB*-nt645 + 25A/G alleles or genotypes in the plaque control record or in bleeding on probing. The *FcγRIIB*-nt645 + 25AA carriers had a significantly shorter gestational period and a higher PTB rate compared with the AG and GG carriers (p = 0.028, Mann–Whitney exact test; Fig. 1A, p = 0.032, 2 × 2 contingency table). Additionally, the *FcγRIIB* nt645 + 25GG carriers had significantly higher outcomes for the prevalence of periodontitis (p = 0.048, 2 × 2 contingency table), mean probing pocket depth (p = 0.021, Mann–Whitney U-test), sites with percentage of probing depth ≥ 4 mm (p = 0.005, Mann–Whitney U-test), mean clinical attachment levels (p = 0.010, Mann–Whitney exact test; Fig. 1B) and sites with percentage of clinical attachment level ≥ 3 mm (p = 0.007, Mann–Whitney exact test) compared with the AA carriers.

Case–control statistics in dominant, recessive, genotype and allele models for *FcγRIIB*, *FcγRIIA*, *FcγRIIIA* and *FcγRIIIB* polymorphisms

We also analyzed case–control (TB vs. PTB, and nonperiodontitis vs. periodontitis) statistics for *FcγRIIB*, *FcγRIIA*, *FcγRIIIA* and *FcγRIIIB* polymorphisms in four genetic models and performed a comparative consideration for the four models. In a dominant model, the *FcγRIIB*-nt645 + 25AA carriers had significantly higher prevalence in PTB compared to the AG and GG carriers (p = 0.032). In the recessive and allele models, the *FcγRIIB*-nt645 + 25GG carrier and G

Table 2. Clinical characteristics of Japanese pregnant women, stratified by birth status (term or preterm) and periodontitis

	TB (n = 71)			PTB (n = 51)		
	Nonperiodontitis (n = 49)	Periodontitis (n = 22)	p-value	Nonperiodontitis (n = 34)	Periodontitis (n = 17)	p-value
Age (years)	33.10 ± 5.37	33.68 ± 4.44	0.886	29.62 ± 4.58	30.77 ± 5.53	0.361
History of smoking (%)	26.5	31.8	0.776	38.2	23.5	0.358
Gestational weeks at delivery	39.14 ± 1.26	39.14 ± 1.36	0.990	32.29 ± 3.77	30.71 ± 3.35	0.058
Percentage of plaque control record	27.9 ± 17.6	44.0 ± 19.0	0.001*	20.5 ± 19.4	43.4 ± 20.7	0.001*
Percentage of sites with bleeding on probing	11.4 ± 12.4	13.7 ± 16.0	0.430	11.9 ± 16.8	23.4 ± 22.9	0.0189*
Mean probing pocket depth (mm)	2.29 ± 0.27	2.75 ± 0.20	< 0.0001*	2.16 ± 0.30	2.86 ± 0.27	< 0.0001*
Mean clinical attachment level (mm)	2.34 ± 0.25	2.84 ± 0.20	< 0.0001*	2.21 ± 0.30	2.86 ± 0.26	< 0.0001*
Percentage of sites with a pocket depth of ≥ 4 mm	2.39 ± 3.41	7.12 ± 7.73	0.004*	3.20 ± 8.48	12.05 ± 14.85	0.003*
Percentage of sites with a clinical attachment level of ≥ 3 mm	39.95 ± 13.82	70.65 ± 7.17	< 0.0001*	33.07 ± 15.54	71.18 ± 7.65	< 0.0001*

*p value < 0.05.
Values are given as mean ± SD, unless specified otherwise.
PTB, preterm birth; TB, term birth.
The p-value was calculated using the chi-square test 2 × 2 contingency table between subjects with and without a history of smoking. When the expected values in any of the cells of a contingency table were below 5, Fisher's exact probability tests were used. Statistical analyses between other comparisons were performed using the Mann–Whitney U-test.

allele were significantly more prevalent in periodontitis compared with the AG and AA alleles or the A allele (p = 0.023 and p = 0.021, respectively). There were no significant differences between cases and controls (TB vs. PTB, and nonperiodontitis vs. periodontitis) in the other SNPs of *FcγRIIB* or in the genotype model. In addition, the *FcγRIIA*-131R allele had a significantly higher prevalence in subjects with periodontitis compared with the 131H allele (p = 0.023). We did not find significant differences between cases and controls (TB vs. PTB, and nonperiodontitis vs. periodontitis) in rs396991 and *FcγRIIIB*-NA1/NA2 (p > 0.05).

Haplotype distributions of *FcγRIIB* SNPs in cases and controls (TB vs. PTB, and nonperiodontitis vs. periodontitis)

Case–control (TB vs. PTB, and nonperiodontitis vs. periodontitis) statistics in the haplotypes for four *FcγRIIB* SNPs were analyzed in Japanese PW (Table 7).

There were no significant differences in the prevalence of haplotypes for four *FcγRIIB* SNPs between TB and PTB groups. On the other hand, the prevalence of the major haplotype C-G-A-T (haplotype frequency = 0.293) was significantly higher in periodontitis compared with nonperiodontitis (p = 0.030).

LD characterization with Fcγ receptor

Haplotype frequencies were estimated for *FcγRIIB* SNPs (rs2125684, rs2125685, rs57420706 and rs1050501) in 48 TB/nonperiodontitis PW. Furthermore, two-locus LD analyses were conducted between the seven Fcγ receptor SNP combinations (rs2125684, rs2125685, rs57420706, rs1050501, rs1801274 as *FcγRIIA*-131H/R, and rs396991 as *FcγRIIIA*-176F/V and *FcγRIIIB*-NA1/NA2). The data are shown in Table 8. We found a significantly positive LD between two pairs for *FcγRIIB* SNPs (rs2125684 vs. rs2125685, $p = 3.66 \times 10^{-5}$; and rs2125685 vs. rs1050501, $p = 8.16 \times 10^{-5}$), three pairs for *FcγRIIB*/other Fcγ receptor polymorphisms (rs2125684 vs. rs396991, p = 0.047; rs2125684 vs. *FcγRIIIB*-NA1/NA2, p = 0.013; and rs1050501 vs. *FcγRIIIB*-NA1/NA2, $p = 4.95 \times 10^{-11}$), but no pairs for Fcγ receptor polymorphisms without *FcγRIIB*.

Discussion

FcγRIIb, the only known inhibitory Fcγ receptor, was the first discovered and is the best-studied example of an ITIM-containing receptor. FcγRIIb is ubiquitously expressed on immune cells, including B cells, monocytes, polymorphonuclear neutrophils, myeloid dendritic cells and plasmacytoid dendritic cells (24). FcγRIIb1 functions as a negative regulator of B cells (14,25) and has been suggested to play an important role in maintaining peripheral tolerance (26). Therefore, the genetically determined polymorphism of *FcγRIIB* and the other Fcγ receptors may contribute to interindividual differences in susceptibility to inflammatory diseases such periodontitis and conditions aggravated by host-induced inflammation, such as PTB.

The inhibitory functions of *FcγRIIB*-232T have been reported to be stronger than those of *FcγRIIB*-232I (27), although other authors (28,29) have suggested the opposite. In our previous study, a patient with periodontitis who was also a carrier of *FcγRIIB*-232T had a lower level of serum IgG2 against *P. gingivalis* outer membrane protein compared with noncarriers of *FcγRIIB*-232T (20). LD analyses in the present study identified the haplotype frequencies of *FcγRIIB*-232I/-nt645 + 25G and *FcγRIIB*-232T/-nt645 + 25A to be significantly higher than expected. Therefore, the *FcγRIIB*-nt645 + 25A allele

Table 3. FcγRIIB, *FcγRIIA*, *FcγRIIIA* and *FcγRIIIB* polymorphisms in Japanese pregnant women stratified according to birth status (term or preterm)

Gene	SNP	Genotype or allele		TB (n = 71)	PTB (n = 51)	χ^2	p-value
FcγRIIB	−343 G/C rs3219018	Genotype	GG	71 (1.00)	51 (1.00)	–	–
			GC	0 (0)	0 (0)		
			CC	0 (0)	0 (0)		
		Allele	G/C	71 (1.00)/0 (0)	102 (1.00)/0 (0)	–	–
	Nt 645 + 7C/A rs2125684	Genotype	AA	1 (0.01)	1 (0.02)	1.1	0.568
			AC	14 (0.20)	8 (0.16)		
			CC	56 (0.79)	42 (0.82)		
		Allele	A/C	15 (0.11)/126 (0.89)	9 (0.11)/92 (0.89)	0.6	0.530
	Nt 645 + 25A/G rs2125685	Genotype	AA	20 (0.28)	24 (0.47)	6.1	0.048*
			AG	39 (0.55)	17 (0.33)		
			GG	12 (0.17)	10 (0.20)		
		Allele	A/G	79 (0.56)/63 (0.44)	65 (0.64)/37 (0.36)	1.6	0.236
	Nt 646-184A/G rs57420706	Genotype	AA	66 (0.93)	49 (0.96)	0.5	0.698
			AG	5 (0.07)	2 (0.04)		
			GG	0 (0)	0 (0)		
		Allele	A/G	137 (0.96)/5 (0.04)	100 (0.98)/2 (0.02)	0.5	0.702
	I232T (nt695T/C) rs1050501	Genotype	TT	43 (0.60)	29 (0.56)	2.5	0.289
			TC	25 (0.35)	16 (0.31)		
			CC	3 (0.04)	6 (0.12)		
		Allele	T/C	111 (0.78)/31 (0.22)	74 (0.72)/28 (0.28)	1.0	0.364
FcγRIIA	131H/R rs1801274	Genotype	HH	39 (0.55)	31 (0.61)	0.4	0.807
			HR	26 (0.37)	16 (0.31)		
			RR	6 (0.08)	4 (0.08)		
		Allele	H/R	104 (0.73)/38 (0.27)	78 (0.76)/24 (0.24)	0.3	0.655
FcγRIIIA	176F/V rs396991	Genotype	FF	40 (0.56)	27 (0.53)	1.5	0.472
			VF	26 (0.37)	17 (0.33)		
			VV	5 (0.07)	7 (0.14)		
		Allele	F/V	106 (0.75)/36 (0.25)	71 (0.70)/31 (0.30)	0.8	0.388
FcγRIIIB	NA1/NA2	Genotype	NA1NA1	26 (0.37)	21 (0.41)	0.5	0.763
			NA1NA2	34 (0.48)	21 (0.41)		
			NA2NA2	11 (0.15)	9 (0.18)		
		Allele	NA1/NA2	86 (0.61)/56 (0.39)	63 (0.60)/39 (0.40)	< 0.1	0.85

*p-value < 0.05.
The p-value was calculated using the chi-square test 3 × 2 (between genotypes) or 2 × 2 (between alleles) contingency table. When the expected values in any of the cells of a contingency table were below 5, Fisher's exact probability tests were used.
PTB, preterm birth; SNP, single nucleotide polymorphism; TB, term birth.

might be associated with a greater inhibitory function and lower IgG levels compared with the *FcγRIIB*-nt645+25G allele.

In this study, the *FcγRIIB*-nt645 + 25AA carriers had a significantly shorter gestational period and a higher rate of PTB, compared with the AG and GG carriers. Furthermore, a significant over-representation of the *FcγRIIB*-nt645+25A allele was observed in the PTB group compared with the TB group. These facts may suggest that the nt645+25A allele is a factor for the susceptibility of PTB, although further investigation with a large number of PW should be performed to confirm this result.

As it has been reported that both periodontitis and PTB are caused by infection aggravated by host-induced inflammation (30) and a lower level of serum IgG against periodontopathic bacteria was associated with PTB compared with TB (18), it was presumed that the susceptibility allele of the *FcγRIIB*-nt645+25A/G polymorphism was consistent in periodontitis and in PTB. However, in this study, we found conflicting results in that Japanese PW carriers of the *FcγRIIB*-nt645+25G allele/GG genotype had a significantly higher prevalence of periodontitis and higher levels of periodontal clinical parameters than carriers of the A allele/AA genotype.

We allowed for this, and proposed that *FcγRIIB*-nt645+25A allele carriers may have a tendency to suffer from conditions induced by high levels of inflammation, such as severe periodontitis and PTB; in contrast, *FcγRIIB*-nt645+25G allele carriers may be likely to suffer from conditions induced by mild inflammation (such as mild periodontitis), but unlikely to develop conditions induced by severe inflammation. The degree of periodontitis in Japanese PW in this study was mild (mean clinical attachment level = 2.55 ± 0.35). Additionally, the mean age (range) of the subjects was 31.9 (19–43) years, comparatively younger than patients with periodontitis in our previous study (19). These facts suggest that the subjects had been exposed to low levels of periodontal bacteria for a brief time-period. The clinical, pathological and immunolog-

Table 4. FcγRIIB, *FcγRIIA*, *FcγRIIIA*, and *FcγRIIIB* polymorphisms in Japanese pregnant women stratified according to periodontitis status (nonperiodontitis and periodontitis)

Gene	SNP	Genotype or allele		Nonperiodontits (n = 83)	Periodontitis (n = 39)	χ^2	p-value
FcγRIIB	−343 G/C rs3219018	Genotype	GG	83 (1.00)	39 (1.00)	–	–
			GC	0 (0)	0 (0)		
			CC	0 (0)	0 (0)		
		Allele	G/C	163 (1.00)/0 (0)	78 (1.00)/0 (0)	–	–
	Nt 645 + 7C/A rs2125684	Genotype	AA	2 (0.02)	0 (0)	1.1	0.565
			AC	14 (0.17)	8 (0.21)		
			CC	67 (0.81)	31 (0.79)		
		Allele	A/C	18 (0.11)/148 (0.89)	18 (0.23)/148 (0.77)	0.0	> 0.99
	Nt 645 + 25A/G rs2125685	Genotype	AA	34 (0.41)	10 (0.26)	5.0	0.081
			AG	38 (0.46)	18 (0.46)		
			GG	11 (0.13)	11 (0.28)		
		Allele	A/G	106 (0.64)/60 (0.36)	38 (0.49)/40 (0.51)	4.4	0.027*
	Nt 646-184A/G rs57420706	Genotype	AA	79 (0.95)	36 (0.92)	0.0	0.679
			AG	4 (0.05)	3 (0.08)		
			GG	0 (0)	0 (0)		
		Allele	A/G	162 (0.98)/4 (0.02)	75 (0.96)/3 (0.04)	0.0	0.683
	I232T (nt695T/C) rs1050501	Genotype	TT	4 8 (0.58)	24 (0.62)	0.2	0.901
			TC	29 (0.35)	12 (0.31)		
			CC	6 (0.07)	3 (0.07)		
		Allele	T/C	125 (0.75)/41 (0.25)	60 (0.77)/18 (0.23)	0.0	0.873
FcγRIIA	131H/R rs1801274	Genotype	HH	53 (0.64)	17 (0.43)	6.2	0.044*
			HR	26 (0.31)	16 (0.42)		
			RR	4 (0.05)	6 (0.15)		
		Allele	H/R	132 (0.80)/34 (0.20)	50 (0.64)/28 (0.36)	5.9	0.012*
FcγRIIIA	176F/V rs396991	Genotype	FF	26 (0.31)	17 (0.44)	1.9	0.378
			VF	49 (0.59)	18 (0.46)		
			VV	8 (0.10)	4 (0.10)		
		Allele	F/V	101 (0.61)/65 (0.39)	52 (0.67)/26 (0.33)	0.5	0.398
FcγRIIIB	NA1/NA2	Genotype	NA1NA1	34 (0.41)	13 (0.33)	0.7	0.721
			NA1NA2	36 (0.43)	19 (0.49)		
			NA2NA2	13 (0.16)	7 (0.18)		
		Allele	NA1/NA2	104 (0.63)/62 (0.37)	45 (0.58)/33 (0.42)	0.4	0.484

*p value < 0.05.
The p-value was calculated using the chi-square test 3 × 2 (between genotypes) or 2 × 2 (between alleles) contingency table. When the expected values in any of the cells of a contingency table were below 5, Fisher's exact probability tests were used.
PTB, preterm birth; SNP, single nucleotide polymorphism; TB, term birth.

ical aspects of periodontal tissue differ with severity of periodontitis (31). Polymorphonuclear leukocytes (PMNs) and monocytes are highly activated in the primary inflammatory stage. As mentioned above, *FcγRIIB*-nt645 + 25G might have a weaker inhibitory function than *FcγRIIB*-nt645 + 25A and therefore the presence of *FcγRIIB*-nt645 + 25G on PMNs and monocytes might activate these cells more strongly compared with *FcγRIIB*-nt645 + 25A (32,33) and initiate inflammation promptly, resulting in mild periodontitis. In contrast, in the later phase of inflammation, weak humoral immune responses may occur in *FcγRIIB*-nt645 + 25A allele carriers, in contrast to the normal humoral immune responses in *FcγRIIB*-nt645 + 25G allele carriers (34).

It is still not clear, however, if there would be functional difference of response against infection in carriers of the *FcγRIIB*-nt645 + 25A/G SNP *in vitro*. Further studies should be undertaken to confirm this hypothesis.

Van der Pol *et al*. (35) reported that *FcγRIIA*, *FcγRIIB*, *FcγRIIIA* and *FcγRIIIB* mapped within 3.5cR on chromosome 1, band q23-24. As expected from this proximity, their haplotype analyses of *FcγRIIA*131H/R, *FcγRIIIA*-176F/V and *FcγRIIIB*-NA1/NA1 in Japanese donors revealed an increased frequency of the *FcγIIA*131H/H-*FcγRIIIB*-NA1/NA1 combination, as observed in Caucasians, but no statistical difference in the FcγRIIA-*FcγRIIIA* or in the *FcγRIIA-FcγRIIIB* from the expected frequencies. In our analysis of 48 Japanese TB/nonperiodontitis PW, significantly positive LDs were found between the *FcγRIIB*-nt645 + 7C and the *FcγRIIIA*-176V, the *FcγRIIB*-nt645 + 7C and the *FcγRIIIB*-NA2, and the *FcγRIIB*-232T and the *FcγRIIIB*-NA2. In contrast, *FcγRIIA*-131H/R had no significantly positive LD with other Fcγ receptor gene polymorphisms. The discrepancy between the reports may be a result of the relatively low frequency of *FcγRIIA*-131R in Japanese people and the limited number of TB/nonperiodontitis PW.

Table 5. Comparison between *FcγRIIB*-nt645 + 25A/G genotypes in Japanese pregnant women

	Genotypes				
Diagnosis	AA (n = 44)	AG (n = 56)	GG (n = 22)	AG + GG (n = 78)	AA + AG (n = 99)
Preterm birth (%)	54.5	30.4	45.5	34.6	52.5
Gestational week at delivery	35.0 ± 4.7	36.9 ± 4.1	36.1 ± 4.7	36.7 ± 4.3	36.1 ± 4.4
Periodontitis (%)	25.6	32.1	50.0	50.0	28.3
Percentage of sites with a plaque control record	29.97 ± 19.21	29.78 ± 2.77	36.34 ± 23.70	31.63 ± 21.68	30.14 ± 19.91
Percentage of sites with bleeding on probing	10.43 ± 12.58	13.37 ± 14.59	19.68 ± 23.99	15.18 ± 17.87	12.16 ± 13.81
Mean probing pocket depth (mm)	2.33 ± 0.37	2.42 ± 0.39	2.57 ± 0.33	2.46 ± 0.37	2.38 ± 0.36
Percentage of sites with a probing pocket depth of ≥ 4 mm	2.79 ± 4.26	5.799 ± 11.67	8.49 ± 12.42	6.57 ± 11.87	4.54 ± 9.36
Mean clinical attachment level (mm)	2.38 ± 0.35	2.46 ± 0.40	2.64 ± 0.29	2.51 ± 0.37	2.43 ± 0.37
Percentage of sites with a clinical attachment level of ≥ 3 mm	43.58 ± 20.46	47.25 ± 21.56	57.05 ± 13.66	50.01 ± 20.07	45.86 ± 21.07

Data are expressed as the percentage of disease (preterm labor or periodontitis) and mean ± SD of obstetric or periodontal clinical parameters.

Table 6. Statistical significances in the comparison between *FcγRIIB*-nt645 + 25A/G genotypes in Japanese pregnant women

	p-value[a]			
	Genotype	AA vs. GG	AA vs. AG + GG	AA + AG vs. GG
Preterm birth	0.048*	0.603	0.032*	0.640
Gestational week at delivery	0.083	0.281	0.028*	0.968
Periodontitis	0.081	0.048*	0.111	0.085
Percentage of sites with a plaque control record	0.466	0.274	0.647	0.386
Percentage of sites with bleeding on probing	0.228	0.123	0.117	0.271
Mean probing pocket depth (mm)	0.099	0.021*	0.095	0.090
Percentage of sites with a probing pocket depth of ≥ 4 mm	0.031*	0.005*	0.120	0.018*
Mean clinical attachment level (mm)	0.043*	0.010*	0.126	0.034*
Percentage of sites with a clinical attachment level of ≥ 3 mm	0.035*	0.007*	0.95	0.032*

*p-value < 0.05.

[a]Statistical analyses between genotypes were performed using the Kruskal–Wallis test. Statistical analyses between gestational weeks and mean clinical attachment level were performed using the Mann–Whitney exact test. Statistical analyses between the percentage of sites with a clinical attachment level of ≥ 3 mm were performed using the Mann–Whitney U-test.

Additionally, in two-locus LD analyses with four *FcγRIIB* polymorphisms, we found a significantly positive LD for *FcγRIIB*-nt645 + 25A with *FcγRIIB*-nt645 + 7C and with *FcγRIIB*-232T. Furthermore, the major haplotype C-G-A-T (haplotype frequency = 0.293) of *FcγRIIB* was significantly increased in subjects with periodontitis compared with nonperiodontitis subjects (p = 0.030). These results suggested two possible explanations, namely that the *FcγRIIB*-nt645 + 25A/G was a true PTB/periodontitis susceptibility SNP or a marker SNP located around a true PTB/periodontitis susceptibility SNP. However, *FcγRIIB*-nt645 + 7A/C, *FcγRIIB*-232I/T, *FcγRIIIA*-176F/V and *FcγRIIB*-NA1/NA2 had no significant association with PTB and periodontitis, as mentioned previously (Tables 3 and 4), which supports the notion of considering *FcγRIIB*-nt645 + 25A/G as an independent factor for the susceptibility for PTB/periodontitis.

There was a significant association between lower maternal age and PTB (Tables 1 and 2). Women who are younger than 18 or older than 35 are more likely to have PTB than women in the 19–34 years age-range (36,37). In our study, the mean age was 33.3 ± 5.1 years in PTB. Sexual activity may be higher in younger women and related to the risk of PTB (38).

There was no significant association between PTB and periodontitis. Previous studies have suggested that several periodontal pathogens are associated with PTB (39). The reason for such discrepancies between previous reports and our result may be attributable to differing parities and severities of periodontitis. Offenbacher *et al*. (22) reported that the mean clinical attachment level of the women was 3.10 ± 0.74 mm for PTB with a low birth-weight infant and 2.80 ± 0.61 mm for TB with a normal birth-weight infant, whereas in this study the respective clinical attachment level results were 2.42 ± 0.45 mm and 2.50 ± 0.32 mm. We feel that the

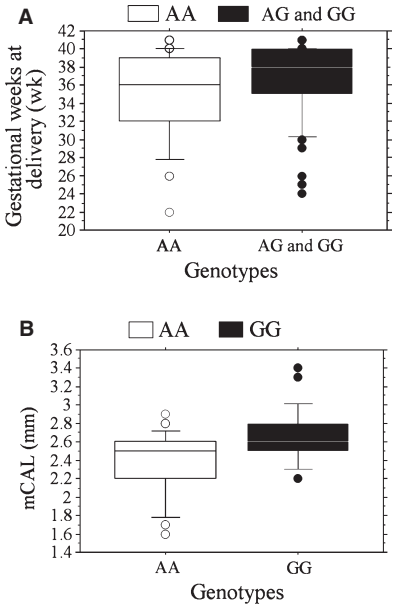

Fig. 1. Box plots for obstetric and periodontal clinical parameters between *Fcγ RIIB*-nt645 + 25A/G genotypes. mCAL, mean clinical attachment level.

Table 7. Case–control (preterm birth/term birth and periodontitis/nonperiodontitis) haplotype analysis for *Fcγ RIIB* single nucleotide polymorphisms in Japanese pregnant women

Haplotype[a]	Haplotype frequency			Permutation *p*-value
	Overall	Case	Control	
Preterm birth/ term birth				
C-A-A-T	0.353	0.371	0.342	0.667[b]
C-G-A-T	0.293	0.250	0.323	0.215[b]
C-A-A-C	0.221	0.252	0.201	0.393[b]
Periodontitis/ nonperiodontitis				
C-A-A-T	0.353	0.293	0.382	0.238[c]
C-G-A-T	0.293	0.381	0.254	0.030*[,c]
C-A-A-C	0.221	0.187	0.236	0.403 [c]

*Permutation p value < 0.05.
[a]Haplotype pair: rs2125684-rs2125685-rs57420706-rs1050501. Minor alleles were abbreviated because these haplotype frequencies were very small (haplotype frequencies < 0.10).
[b]Preterm birth vs. term birth.
[c]Periodontitis vs. nonperiodontitis.

Table 8. Linkage disequilibrium among Fcγ receptor polymorphisms in term birth/nonperiodontitis pregnant women ($n = 48$)

Haplotype pair	*p*-value[a]
Fcγ RIIB-IIB	
nt645 + 7C/A (rs2125684) – nt645 + 25A/G (rs2125685)	3.66×10^{-5}*
nt645 + 7C/A (rs2125684) – nt646-184A/G (rs57420706)	0.732
nt645 + 7C/A (rs2125684) – I232T (rs1050501)	0.097
nt645 + 25A/G (rs2125685) – nt646-184A/G (rs57420706)	0.415
nt645 + 25A/G (rs2125685) – I232T (rs1050501)	8.16×10^{-5}*
nt646-184A/G (rs57420706) – I232T (rs1050501)	0.616
IIB – *IIA*131H/R (rs1801274)	
nt645 + 7C/A (rs2125684) – *IIA* (rs1801274)	0.945
nt645 + 25A/G (rs2125685) – *IIA* (rs1801274)	0.113
nt646-184A/G (rs57420706) – *IIA* (rs1801274)	0.05
I232T (rs1050501)-*IIA* (rs1801274)	0.546
IIB – *IIIA*176F/V (rs396991)	
nt645 + 7C/A (rs2125684) – *IIIA* (rs396991)	0.013*
nt645 + 25A/G (rs2125685) – *IIIA* (rs396991)	0.961
nt646-184A/G (rs57420706) – *IIIA* (rs396991)	0.090
I232T (rs1050501) – *IIIA* (rs396991)	0.256
IIB – *IIIB*-NA1/NA2	
nt645 + 7C/A (rs2125684) – *IIIB*-NA1/NA2	0.013*
nt645 + 25A/G (rs2125685) – *IIIB*-NA1/NA2	0.131
nt646-184A/G (rs57420706) – *IIIB*-NA1/NA2	0.457
I232T (rs1050501) – *IIIB*	4.95×10^{-11}*
IIA-131H/R (rs1801274) – *IIIA*-176F/V (rs396991)	0.905
IIA-131H/R (rs1801274) – *IIIB*-NA1/NA2	0.314
IIIA-176F/V (rs396991) – *IIIB*-NA1/NA2	0.577

*p-value < 0.05.
[a]Two-locus linkage disequilibrium analyses were conducted between the seven Fcγ receptor single nucleotide polymorphism combinations (rs2125684, rs2125685, rs57420706, rs1050501, rs1801274 as *Fcγ RIIA*-131H/R, and rs396991 as *Fcγ RIIIA*-176F/V and *Fcγ RIIIB*-NA1/NA2).

differences in socioeconomic status between previous reports and our study may be another factor contributing to the discrepancies in the results. In a systematic review, Xiong *et al.* (40) indicated socioeconomic status and access to dental care as reasons for the discrepancies among previous reports. Significant associations between periodontitis and adverse pregnancy outcomes were reported in studies conducted in developing countries, which tend to include African–American women and women from economically disadvantaged families. In contrast, studies conducted in developed countries, for example, European countries or Canada (all of which offer their citizens universal health care), did not find an association between periodontitis and adverse pregnancy outcomes. Although we failed to collect socioeconomic data from each subject in this study, Japan has been placed on development levels in the range of highly developed (41).

In summary, as discussed above, our study indicated that *Fcγ RIIB*-nt645 + 25A/G was a susceptibility SNP for both PTB and periodontitis. The genetic scenario of periodontitis and PTB was different for subjects with the *Fcγ RIIB*-nt645 + 25A/G polymorphism, and no significant association was observed between periodontitis and PTB in this study. Both results might have been related to the mildness of periodontitis in women in this study. The analyses suggest that *Fcγ RIIA*-131R is independently associated with

susceptibility to periodontitis in Japanese PW. Identification of specific genetic susceptibility factors to PTB and periodontitis will promote the accuracy of diagnosis and the therapeutic strategies. Further genetic studies among different ethnic subjects and in functional analyses of Fcγ receptor genotypes connected with PTB and periodontitis should be performed.

Acknowledgements

This work was supported by Grants-in-Aid for scientific Research (19592385) and for Challenging Exploratory Research (21659480) from the Japan Society for the Promotion of Science (JSPS), Tokyo, Japan. The authors are not aware of any conflicts of interest related to this study.

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