

The *IL18* Promoter Polymorphism, rs1946518, Is Associated with the Risk of Periodontitis in Japanese Women: The Kyushu Okinawa Maternal and Child Health Study

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Interleukin-18 (IL-18) is a proinflammatory cytokine that plays an important role in periodontitis and its polymorphisms might modulate the individual susceptibility to periodontitis. Only a limited number of studies on the association between *IL18* single-nucleotide polymorphisms (SNPs) and the risk of periodontitis have been realized, however. The aim of this case-control study among young post-partum Japanese women (18 to 45 years) was to determine the impact of SNPs, rs1946518 (–607 C/A) and rs187238 (–137G/C), on periodontitis. The two SNPs may be located within a transcription factor-binding element, thereby influencing transcription from the *IL18* promoter. Subjects were 131 cases who had at least one tooth with a probing pocket depth of ≥ 4.0 mm and 1,017 periodontally healthy controls. Probing pocket depth measurements were performed between 1 and 12 months post-partum. In this population, the A allele of rs1946518 and the C allele of rs187238 are more common. After adjustment for age, education, smoking, and use of an interdental brush, compared with subjects with the AA or AC genotype of SNP rs1946518, those with the CC genotype had a significantly reduced risk of periodontitis (adjusted odds ratio = 0.54, 95% confidence interval = 0.29–0.97). No significant association was observed between rs187238 and the risk of periodontitis. Our study did not reveal any evidence of interaction between the *IL18* polymorphisms and smoking. Our findings indicate that the *IL18* promoter SNP, rs1946518, is a potential risk factor of periodontitis among young Japanese women.

Keywords: case-control study; *IL18*; Japan; periodontal disease; polymorphism

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Introduction

Periodontal disease is a chronic inflammatory disease of the teeth-supporting structures, such as alveolar bone, periodontal ligaments, and gingiva. Microbial plaque, which accumulates in the gingival crevice region and induces an inflammatory response, initiates periodontal disease. Like other common multifactorial diseases, genetic and environmental risk factors influence the development and progression of periodontal disease (Zhang et al. 2011; Genco and Borgnakke 2013). Genetic host factors may be implicated in determining susceptibility to periodontal disease (Zhang et al. 2011), which varies widely among indi-

viduals (Genco and Borgnakke 2013).

Interleukin-18 (IL-18), a member of the IL-1 family of cytokines, is a potent proinflammatory cytokine that plays an important role in periodontal disease (Orozco et al. 2007). One of the main functions of IL-18 is to promote the production of interferon- γ in the presence of IL-12 and to promote Th1 responses (Dinarello and Fantuzzi 2003). However, IL-18 also promotes Th2 responses. IL-18 promotes the secretion of other proinflammatory cytokines such as tumor necrosis factor- α , IL-1 β , IL-8, and granulocyte-macrophage colony-stimulating factor, which enhances the expansion, migration, and activation of neutrophils during infections (Sahoo et al. 2011). The balance of the Th1/

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Th2 immune response contributes to initiation and progression of periodontal disease (Orozco et al. 2007). High levels of IL-18 are detected in inflammatory and autoimmune diseases such as rheumatoid arthritis, psoriasis, and systemic lupus erythematosus (Mühl and Pfeilschifter 2004). Increasing evidence indicated that IL-18 has the property of inhibiting bone resorption; therefore, IL-18 would be expected to contribute to the regulation of bone in inflammatory bone diseases, including periodontal disease (Silva et al. 2015). Thus, IL-18 might affect periodontal health and disease through various functions including the production, signaling pathways, and the inhibition of bone resorption (Orozco et al. 2007).

IL-18 protein expression is regulated by the *IL18* promoter gene. Several polymorphisms in the promoter region of *IL18* have been identified (Giedraitis et al. 2001). The two single-nucleotide polymorphisms (SNPs) at positions -607 and -137 have been extensively studied in relation to multifactorial diseases such as asthma, systemic lupus erythematosus, cardiovascular disease, and diabetes. Each of these two SNPs may be located within a transcription factor-binding element, thereby influencing transcription from the *IL18* promoter gene (Giedraitis et al. 2001; Thompson and Humphries 2007). To our knowledge, only four studies have examined the association between *IL18* polymorphism at positions -607 and -137 and periodontal disease; these were conducted in Italian adults > 50 years old (30% male patients) (Martelli et al. 2012) and in German subjects with mean ages of 52.9 years (54% male patients) (Folwaczny et al. 2005), 34.0 years (42% male patients) (Noack et al. 2008), and 57.3 years (44% male patients) (Noack et al. 2009) and yielded inconsistent results. According, however, to a meta-analysis based on only three studies in Germany, *IL18* polymorphism was associated with an increased risk of periodontal disease (Li et al. 2014). It is necessary to accumulate further evidence in order to clarify how *IL18* polymorphisms affect periodontal disease.

In the present study, we assessed associations between two *IL18* SNPs, namely, rs1946518 (-607 C/A) and rs187238 (-137G/C), and the risk of periodontal disease among young Japanese women, using the data set of the Kyushu Okinawa Maternal and Child Health Study (KOMCHS). Furthermore, haplotype analyses were performed. As smoking is an established risk factor for periodontal disease, we investigated the possibility of interactions between the SNPs and smoking.

Materials and methods

Study population

The KOMCHS is an ongoing prospective prebirth cohort study conducted in seven prefectures on Kyushu Island in southern Japan or Okinawa Prefecture. Details of the KOMCHS have been described previously (Miyake et al. 2013; Tanaka et al. 2013). In brief, at 423 obstetric hospitals, pregnant women were recruited between April 2007 and March 2008. Finally, a total of 1,757 pregnant women between the 5th and 39th week of pregnancy gave their fully

informed written consent to participate and completed the baseline survey, which included questions regarding on smoking habits, education, and use of an interdental brush. Of these, 1,590 mothers completed the second survey after birth, and of these, 1,198 women received oral examinations between 1 and 12 months post-partum. Around four months after delivery, 1,492 mothers gave informed consent for genotyping. The present study subjects consisted of 1,157 women who both received oral examinations and provided genetic samples. The ethics committees of the Faculty of Medicine, Fukuoka University and Ehime University Graduate School of Medicine approved the KOMCHS.

Selection of cases and control subjects

Dental hygienists performed oral examinations for periodontal status. Probing pocket depth (PPD) was assessed with a Community Periodontal Index probe (YDM Corp., Tokyo, Japan) at six sites per tooth for six teeth: the right first molar, right first incisor, and left first molar in the maxilla and the right first molar, left first incisor, and left first molar in the mandible. Periodontal disease was defined as a case where there is a PPD of 4.0 mm or more at any site. Under this definition, among the 1,157 subjects, 131 women had periodontal disease. The remaining subjects were eligible to be used as controls, but nine women were excluded due to missing data on the variables under study; thus 1,017 women were classified as control subjects.

DNA extraction and genotyping

Buccal specimens were collected with BuccalAmp swabs (Epicenter BioTechnologies, Madison, WI, USA). Genomic DNA was extracted using a QIAmp DNA mini kit (Qiagen, Inc., Valencia, CA, USA). Genotyping of *IL18* SNPs was performed using TaqMan SNP Genotyping Assays on a StepOnePlus machine (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Genotypes were determined by TaqMan probes assay ID C_2898460_10 and C_2408543_10 for rs1946518 and rs187238, respectively.

Statistical analysis

The chi-square test was used to test for departures from the Hardy-Weinberg equilibrium among the control subjects. Linkage disequilibrium was examined using Haploview software version 4.2 (Broad Institute, Cambridge, MA, USA) (Barrett et al. 2005). To test for genetic associations, logistic regression was used to compute crude odds ratios (ORs) and 95% confidence intervals (CIs), with the reference category being the homozygote of the major allele. The following variables were adjusted in the multivariable model: age at oral examination, education, smoking, and use of an interdental brush. The statistical power was calculated using QUANTO version 1.2 (Gauderman 2002). Haplotypes and their frequencies were inferred according to the expectation maximization algorithm. We estimated whether haplotype frequencies differ between the cases and control groups, using crude ORs and 95% CIs that were based on the frequency of each haplotype relative to all other haplotypes combined. We assessed multiplicative interactions between the SNPs under study and smoking with regard to the risk of periodontal disease, using a multiplicative term included in a multivariate model. Excluding the calculation of linkage disequilibrium and statistical power, all statistical analyses were performed using STATA/SE software version 12.0 (StataCorp, College Station, TX, USA).

Results

The demographic characteristics of study subjects are summarized in Table 1. There were no differences in distributions of age, education, smoking status, or use of an interdental brush between cases and controls.

In the controls, the genotype distributions of *IL18* SNPs rs1946518 and rs187238 were consistent with Hardy-Weinberg equilibrium ($P = 0.75$ and 0.55 , respectively). The two SNPs under study were in tight linkage disequilibrium ($D' = 1$, $r^2 = 0.09$).

No evident relationships were observed between SNP rs1946518 and periodontal disease under the co-dominant or dominant model (Table 2). In the recessive model, compared with subjects with the AA or AC genotype of SNP rs1946518, those with the CC genotype had a significantly reduced risk of periodontal disease (adjusted OR = 0.54, 95% CI = 0.29-0.97). No evident association was observed between rs187238 and the risk of periodontal disease under any genetic models. With regard to SNP rs187238, the statistical power calculation revealed that, using our sample size, we could detect the gene-disease association for an

Table 1. Characteristics of the study population.

Variable	n (%)	
	Cases (n = 131)	Controls (n = 1,017)
Age, years, mean ± SD (median, range)	32.9 ± 4.1 (33, 21-41)	32.3 ± 4.2 (32, 18-45)
Education, years		
< 13	23 (17.6)	210 (20.7)
13-14	50 (38.2)	337 (33.1)
≥ 15	58 (44.3)	470 (46.2)
Smoking		
Never	86 (65.7)	722 (71.0)
Ever	45 (34.4)	295 (29.0)
Use of an interdental brush		
No	64 (48.9)	545 (53.6)
Yes	67 (51.2)	472 (46.4)

Table 2. ORs and 95% CIs for periodontal disease associated with *IL18* polymorphisms in young Japanese women.

SNP	Genotype	Number (%) of		Crude OR (95% CI)	Adjusted OR (95% CI)*	P value
		Cases (n = 131)	Controls (n = 1,017)			
rs1946518	AA	46 (35.1)	344 (33.8)	1.00	1.00	
	AC	72 (55.0)	500 (49.2)	1.08 (0.73-1.60)	1.07 (0.72-1.58)	0.76
	CC	13 (9.9)	173 (17.0)	0.56 (0.30-1.07)	0.56 (0.29-1.06)	0.08
	AC + CC vs. AA			0.94 (0.64-1.38)	0.93 (0.64-1.37)	0.73
	CC vs. AA + AC			0.54 (0.30-0.98)	0.54 (0.29-0.97)	0.04
rs187238	CC	108 (82.4)	793 (78.0)	1.00	1.00	
	CG	21 (16.0)	208 (20.5)	0.74 (0.45-1.21)	0.73 (0.45-1.20)	0.22
	GG	2 (1.5)	16 (1.6)	0.92 (0.21-4.05)	0.86 (0.19-3.83)	0.85
	CG + GG vs. CC			0.75 (0.47-1.21)	0.74 (0.46-1.20)	0.22
	GG vs. CC + CG			0.97 (0.22-4.27)	0.91 (0.21-4.04)	0.90

*Adjusted for age at oral examination, education, smoking, and use of an interdental brush. CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table 3. Haplotype analysis of 2 *IL18* polymorphisms associated with periodontal disease in young Japanese women*.

Haplotype [†]	Frequency, <i>n</i> (%)		Crude OR (95% CI) [‡]	<i>P</i> value
	Cases (<i>n</i> = 262)	Controls (<i>n</i> = 2,034)		
AC	139 (53.1)	948 (46.6)	1.29 (0.99-1.69)	0.05
CC	98 (37.4)	846 (41.6)	0.84 (0.64-1.10)	0.19
AG	25 (9.5)	240 (11.9)	0.79 (0.49-1.22)	0.28

*Rare haplotypes (frequencies less than 1% in either cases or controls) were deleted.

[†]Haplotype order is rs1946518 and rs187238.

[‡]Crude OR for each haplotype is relative to all other haplotypes combined.

CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table 4. Association between *IL18* polymorphisms and periodontal disease, stratified by smoking history in young Japanese women.

SNP	Genotype	Smoking history						<i>P</i> for interaction
		No (<i>n</i> = 808)			Yes (<i>n</i> = 340)			
		No. cases/controls	Adjusted OR (95% CI) [*]	<i>P</i> value	No. cases/controls	Adjusted OR (95% CI) [*]	<i>P</i> value	
rs1946518	AA+AC	76/594	1.00		42/250	1.00		
	CC	10/128	0.60 (0.30-1.19)	0.15	3/45	0.37 (0.11-1.25)	0.11	0.16

*Adjusted for age at oral examination, education, and use of an interdental brush.

CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

OR of 0.495 with an accuracy of more than 80% at a significance level of 0.05 with a two-sided alternative hypothesis under the dominant model.

Given the haplotype order of rs1946518 and rs187238, AC, CC, and AG haplotypes were identified after haplotypes with a frequency of < 1% in either cases or controls had been deleted. None of these were significantly related to the risk of periodontal disease, however (Table 3).

We observed no significant interaction between SNP rs1946518 and smoking in terms of the risk of periodontal disease (Table 4).

Discussion

To our knowledge, this is the first study in a non-Western population to examine the association between *IL18* polymorphisms and periodontal disease. The present study showed that the CC genotype of *IL18* SNP rs1946518 was significantly associated with a reduced risk of the disease. There was no association between *IL18* SNP rs187238 and periodontal disease. A case-control study in Italy (109 aggressive cases, 104 chronic cases, and 100 controls) showed that the AA genotype of SNP rs1946518 was positively associated with chronic periodontal disease, while the CG genotype of SNP rs187238 was positively associated with aggressive periodontal disease (Martelli et al. 2012). In three other previous studies in Germany, no significant association was observed between *IL18* polymorphisms and chronic periodontal disease (108 cases and 76 controls in one study, 123 cases and 121 controls in another)

(Folwaczny et al. 2005; Noack et al. 2009) or aggressive periodontal disease (111 cases and 80 controls) (Noack et al. 2008). It should be noted, however, that these previous studies used different definitions of periodontal disease, study populations, and confounding factors, thus limiting the feasibility of inter-study comparisons. There are also several genome-wide association studies on periodontal disease (Teumer et al. 2013; Shaffer et al. 2014; Shimizu et al. 2015), but none of these has reported an association for *IL18* SNP. This is likely to be attributed to the low power of the studied sample sizes to reach genome-wide significance.

The frequency distribution of genetic variants is different between populations. In our study population, the A allele frequency of rs1946518 was 58.9% and the C allele frequency of rs187238 was 88.5%. On the other hand, previous studies in Western populations such as in Italy and Germany demonstrated that A allele frequency of rs1946518 and C allele frequency of rs187238 ranged from 38.5% to 40.7% and from 24.8% to 27.7%, respectively (Folwaczny et al. 2005; Noack et al. 2008, 2009; Martelli et al. 2012).

IL-18 contributes to both host defense and inflammation in a cascade of cytokines associated with innate responses. A case-control study in Mexico (18 cases and 9 controls) demonstrated that serum IL-18 levels were higher in subjects with chronic periodontal disease than in controls (Sánchez-Hernández et al. 2011). In a case control study in Turkey (22 cases and 21 controls), salivary levels of IL-18 were significantly higher in patients with chronic periodon-

tal disease than in healthy control subjects, although there was no difference in plasma IL-18 levels (Ozçaka et al. 2011). A meta-analysis based on six studies suggested that higher levels of plasma IL-18 were associated with periodontal disease, especially among Asian populations (Li et al. 2014).

The two SNPs rs1946518 and rs187238 may influence the gene expression of *IL18* at the transcriptional level by disrupting the cyclic adenosine monophosphate-responsive element binding site and by changing the H4TF-1 nuclear factor binding at the *IL18* promoter (Giedraitis et al. 2001). However, it is still unclear whether *IL18* polymorphisms are implicated in inter-individual serum IL-18 level variation (Thompson and Humphries 2007). Recently, a case-control study of bladder cancer in Northern India reported that the heterozygous variant genotype of *IL18* rs187238 was significantly associated with elevated IL-18 serum level (Jaiswal et al. 2013).

The methodological strengths in the study were as follows: study subjects were homogeneous in gender and age group and we were able to control for several confounding factors.

We also recognized certain limitations in the present study, however. First, the rate of participation cannot be calculated due to lack of information for the exact number of eligible pregnant women who were recruited at obstetric hospitals. Additionally, it was impossible to assess differences of personal characteristics between participants and non-participants, because demographic data were not available. It is unlikely that our subjects were a representative sample of Japanese women in the general population. In fact, our study subjects have higher educational levels than do those in the general population (Statistics Bureau, Ministry of Public Management, Home Affairs, Posts and Telecommunications 2002). Additionally, according to the Survey of Dental Disease conducted in 2011 (Japanese Society for Dental Health 2013), the proportion of women aged 30 to 34 years having one or more periodontal sites with a PPD of 4.0 mm or deeper was 14.2%. The corresponding figure for our subjects was 11.4%. It was expected that our study population would have had a greater awareness and knowledge of health than the general population has. However, the distribution of both SNPs investigated was consistent with the Hardy-Weinberg equilibrium.

Second, in the current study, dental hygienists performed oral examinations. The hygienists were given detailed criteria for carrying out the examinations, but in order to standardize the procedure no specific training was provided. Further, no reliability assessment of measurements was carried out in the present study. Thus, it is uncertain whether intra- and inter-examiner consistency was established. Further, because data on partial-mouth examinations was used, the periodontal disease prevalence is likely to be underestimated. In the present study, no radiographic evaluation of alveolar bone loss was made. In addition, PPD measurements were performed between 1

and 12 months post-partum. Therefore, we could not exclude the possibility that periodontal changes such as increasing PPD depth and bleeding on probing or mechanical stimulation during pregnancy might remain. Thus, we need to be aware of the possibility of false positive errors in our outcome.

Third, although adjustment was made for some confounders, residual confounding cannot be ruled out.

Fourth, we did not confirm the Japanese ancestry of the study participants. Because admixture is a well-known confounder in genetic association studies, observed associations in the present study might be related to ancestry rather than disease.

Within the limits of the present study, our results demonstrate that the CC genotype of SNP rs1946518 was significantly associated with a reduced risk of periodontal disease in young post-partum Japanese women. None of the *IL18* haplotypes tested in the present study were associated with increased risk of periodontal disease, and there was no interaction of any of the *IL18* polymorphisms with smoking. Additional research is required to confirm these findings and to identify the mechanisms behind the observed association.

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Conflict of Interest

The authors declare no conflict of interest.

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