ARCHIVES OF ORAL BIOLOGY 60 (2015) 533–539

Available online at www.sciencedirect.com

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journal homepage: http://www.elsevier.com/locate/aob

Relationships between IL-6 gene polymorphism, low BMD and periodontitis in postmenopausal women

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ARTICLE INFO

Article history:
Accepted 5 December 2014

Keywords:
IL-6
Polymorphism
Low BMD
Periodontitis

ABSTRACT

Objective: IL-6 plays critical roles in bone resorption and the pathogenesis of periodontitis in both inflammation and alveolar bone loss. A negative correlation was observed between periodontitis and truncal bone mineral density (BMD) in postmenopausal women. The C allele carriers of a genetic polymorphism IL-6-572G/C have higher levels of serum IL-6 compared to G allele carriers. We investigated the possible effect of IL-6-572G/C polymorphism on the relationship between low BMD and periodontitis in postmenopausal women.

Subjects and methods: A total of 300 postmenopausal Japanese women who lived in Yokogoshi area of Niigata City, Japan, participated in this study. Genomic DNA was extracted from peripheral blood. The IL-6-572G/C genotypes were determined by the restriction fragment length polymorphism method. Bone mineral density (BMD) of right femoral neck and serum bone metabolism markers were measured. Low BMD was defined to have the BMD $<$ 80% of the mean for young adults. Periodontal parameters at two sites per tooth were measured.

Results: Serum osteocalcin levels were significantly lower in the IL-6-572G/G genotype ($p = 0.025$). In the -572G allele non-carriers, percentages of PPD $\geq$ 4 mm sites were significantly higher in low BMD group compared with the healthy control group ($p = 0.021$). Logistic regression analysis revealed low BMD to be associated with periodontitis (Odds ratio = 1.736, $p = 0.027$) after adjusted with IL-6-572G carriage, age, serum albumin level.

Conclusions: IL-6-572G/C polymorphism was not an independent risk factor of low BMD or periodontitis, but may affect the relationship between the two diseases in postmenopausal Japanese women.

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http://dx.doi.org/10.1016/j.archoralbio.2014.12.005
0003–9969/

1. Introduction

Interleukin-6 (IL)-6 is a pleiotropic cytokine that plays a central role in immune, inflammatory, acute phase responses and several endocrine and metabolic disorders.[1] IL-6 is produced by many cell types such as stimulated monocytes, fibroblasts, endothelial cells and T and B lymphocytes.[2] Likewise, within the periodontal tissue, a variety of cell types have been found to express the IL-6 gene, and IL-6 is present at higher levels in inflamed tissue compared to controls.[3]

The human IL-6 gene has been assigned to chromosome 7p21 with five exons and four introns.[4,5] Polymorphisms in the IL-6 gene have been reported to be associated with periodontitis.[6] There was a relationship between genetic polymorphism IL-6-174G/C and chronic periodontitis in geriatric patients.[7] Another study reported that IL-6-174G/C and -572G/C polymorphisms were associated with susceptibility to chronic periodontitis.[8] Enzyme-linked immunosorbent assay confirmed that the -572G and -373[A9T11] haplotypes were associated with lower serum IL-6 levels in healthy Japanese.[9]

The natural decline in oestrogen production after menopause leads to increased bone resorption in part because of increased production of pro-inflammatory cytokines suppressed by oestrogen.[10] IL-6 produced by osteoblasts, monocytes, and T cells promotes osteoclast differentiation and activation.[11,12] Some studies have claimed that the balance of hormone was a factor of IL-6 fluctuation.[13] It was evident from this that IL-6 was one of the reasons of bone loss fraught with menopause.[14] IL-6 is also a possible mediator of oestrogen-deficient bone loss in mice.[15] A clinical study has shown that IL-6 mRNA expression in bone was enhanced in 95% of patients with osteoporotic vertebral fracture, compared with an enhancement in 50% of postmenopausal controls.[16] Many factors influence the risk of low BMD including diet, physical activity, medication use, coexisting disease, ageing, and reduced sex steroid production. However, one of the most important clinical risk factors is a positive family history of osteoporosis, emphasizing the importance of genetics in the pathogenesis of osteoporosis.[17]

Decreased skeletal bone mineral density may be a risk factor of alveolar bone loss which is a clinical feature of periodontitis.[18] Additionally, serum bone biomarkers such as pyridinoline-crosslink fragment of the type I collagen are associated with not only systemic BMD loss, but with alveolar bone loss.[19] However, conclusions from association studies between BMD and periodontitis have been controversial. Some studies failed to find significant associations between tooth loss or periodontits and BMD.[20,21] One of the causes of this discrepancy might be the variance of genetic background among research subjects.

Although a large number of reports have demonstrated that IL-6 affects periodontitis and low BMD, few studies have been done to elucidate the potential role of IL-6 in the relationship between periodontitis and systemic bone mineral reduction.

The present study was undertaken in order to determine whether IL-6-572G/C polymorphism affects the relationship between periodontitis and low BMD in postmenopausal women.

2. Materials and methods

2.1. Subjects

The protocol of the study was approved by the Ethics Committee of Niigata University School of Medicine. As described in our previous report by Wang et al,[22] all 1310 women aged 55–75 year who lived in the Yokogoshi area of Niigata City, Japan were invited to participate in a cross-sectional, epidemiological study for postmenopausal women in 2005. Six-hundred seventy-four of them agreed to participate in the study with written informed consent. Exclusion criteria were as follows: a history of bilateral oophorectomy, corticosteroid therapy, treatment for suspected osteoporosis with bisphosphonates, selective oestrogen receptor modulators, active vitamin D analogues, vitamin K, oestrogen, or calcitonin. There was no dialytic patient. The women diagnosed with kidney disease were excluded, but there were 8 women having serum creatinine levels little over (8.1–8.3 mg/dl) of reference value (0.4–0.8 mg/dl). Of the remaining 600 women, 407 came to the follow-up examinations in 2010 and agreed to participate in the present study with written informed consents. Three edentulous women were excluded. The women of 59 who were smoker and having history of smoking were excluded. The genomic DNA samples from 45 women were insufficient in quantity or quality for the genotyping. The final number of subjects in this study was 300. None of them had history of smoking.

2.2. Genotyping of IL-6-572G/C polymorphism

The genotyping were carried out blind to clinical status and in duplicates. Genomic DNA was extracted from peripheral blood. The IL-6-572G/C (rs1800796) genotype was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) as described by Ferrari et al.[23] PCR was carried out using 50 ng genomic DNA with annealing temperature 60 °C, for 30 cycles using forward primer 5′-GGAGACGCCTTGAAGTAACTGC-3′ and reverse primer 5′-GAGTTTCCTCTGACTCCATCGCAG-3′. The purified PCR product was subjected to restriction digestion by *MbiI* enzyme at 37 °C. Genotypes were determined by examining DNA fragments on 2.0% agarose gel electrophoresis. The genomic DNA samples from healthy volunteers with IL-6-572GG, GC and CC genotypes were used as controls in each PCR-RFLP and electrophoresis. The sequencing was performed using the Big Dye Terminator Cycle Sequencing Kits v1.1 and the ABI PRISM 310 sequencer (Applied Biosystems, Foster City, CA, USA). The genotypes of all 300 women were successfully obtained.

2.3. Periodontal examination

Periodontal parameters at the buccal and mesial sides (NHANESIII, 1988–1994) per tooth were measured including probing pocket depth (PPD), clinical attachment loss (CAL), and bleeding on probing (BOP) as described in a previous report.[24] The measurements of PPD and CAL were taken to the nearest millimetre using a calibrated colour-coded periodontal probe

(CP-12, Hu-Friedy, Chicago, IL, USA). The averages for whole-mouth PPD and CAL, and the percentages of sites with PPD $\geq$ 4 mm, CAL $\geq$ 4 mm, BOP were calculated for each subject. The definition criterion for periodontitis in this study was having more than four teeth on which more than one site with CAL $\geq$ 4mm, PPD $\geq$ 4mm and BOP concurrently (modified criterion by[36]).

2.4. Measurement of bone mineral density (BMD)

BMDs of the right femoral neck were measured as previously described by Nakamura et al.[25] Dual-energy X-ray absorptiometry (DXA) using a QDR4500a absorptiometer (Hologic Inc., Bedford, MA, USA) was performed by a single, trained X-ray technician. The *in vivo* coefficients of variation (CVs) of the BMD measurements were 0.6% for the femoral neck. Long-term CV value of the BMD measurements using a quality control phantom were within 1.2%. The definition criteria for healthy, low BMD, osteopenia and osteoporosis groups were women with BMD of proximal femur (t-score) $\geq$ 80% YAM (the mean for young adults aged 20–44 years), 80%YAM < BMD, 70% $\leq$ BMD < 80%YAM and BMD < 70%YAM, respectively.

2.5. Serum biochemical measurements

As described in a previous study,[25] serum type I collagen cross-linked *N*-telopeptides (NTX) concentration, a marker of bone resorption, was determined by ELISA (Osteomark NTX Serum; Ostex International, Inc., Seattle, WA, USA; reference value: 10.7–24.0 nmol BCE/l), which had an inter-assay CV of 2.8%. Serum osteocalcin concentration, an indicator of bone formation, was determined by an immunoradiometric assay (Mitsubishi Kagaku Medical, Inc., Tokyo, Japan; reference value: 3.1–12.7 ng/ml) with an inter-assay CV of 6.6%. Serum vitamin D concentration, measured as 25-hydroxyvitamin D (25(OH)D), was determined by RIA (DiaSorin, Stillwater, MN, USA) with an inter-assay CV of 9.9%. The serum albumin concentration was measured by a BCG method.[26]

2.6. Interview and nutritional information

Demographic and nutritional information was obtained at interview. Vitamin D and calcium intake was assessed with a previously validated food frequency questionnaire.[27] The correlation coefficients between values measured by this method and the conventional 3 day diet record were 0.413 for vitamin D and 0.668 for calcium.

2.7. Statistical analysis

Periodontal parameters, BMD, osteopenia and serum bone metabolism markers were compared between the IL-6-572C/G genotypes with Kruskal–Wallis test, chi-square tests or Mann–Whitney *U*-tests. Correlations between periodontal parameters and serum NTX or osteocalcin were analyzed with Spearman rank for correlation analysis. Multiple logistic regression analysis for periodontitis as an outcome was conducted. Computations were performed using the SPSS Statistics software version 19.0 for Windows (SPSS Inc., IBM, Chicago, IL, USA). The statistical powers to detect differences between groups were calculated using the software G*Power version 3.1 (Institute for Experimental Psychology, Dusseldorf, Germany). Differences were considered significant at $p < 0.05$.

3. Results

The determined IL-6-572G/C genotypes of 300 women in this study were all consistent between each duplicate analysis. The distribution was in Hardy–Weinberg equilibrium. Because of a relatively small number of GG homozygotes, comparisons by G allele carriage were also performed.

First, we compared the characteristics between IL-6-572G/C genotypes and between G-carrier and non-carrier groups as shown in Table 1. There was no significant difference in age, BMI, vitamin D intake, calcium intake and number of teeth among IL-6-572C/G genotypes. Periodontal parameters also turned out to have no significant difference among IL-6-572C/G genotypes. Femoral bone mineral density and prevalence of low BMD showed no significant difference among the genotypes and between G carrier and non-carriers. The concentrations of serum osteocalcin in women with the GG genotype were significantly lower than those in the other genotypes. The other serum markers of bone metabolism showed similar concentrations in three genotypes.

Next, as shown in Table 2, correlations between periodontal parameters and serum NTX or osteocalcin levels were analyzed in the total subjects included in this study or separately in G allele carrier group and non-carrier group. There were very weak positive correlations between percentages of sites with PPD $\geq$ 4 mm and NTX levels in the total subjects and in the G allele carriers. The other periodontal parameters showed no significant correlation with serum NTX levels. There was also a positive correlation between percentages of site with PPD $\geq$ 4 mm and osteocalcin levels in total subjects. Serum osteocalcin levels did not correlate with any other periodontal parameters.

As shown in Table 3, we next compared periodontal parameters between women with healthy BMD and those having low BMD divided into the G carriers and non-carriers. In the comparison, the power (1-β) was 0.85 *post hoc*. There was no difference in the periodontal parameters between the healthy and low BMD group in G carriers. Only in G non-carriers, percentages of sites with PPD $\geq$ 4 mm were significantly higher in the low BMD group than in the healthy group ($p = 0.021$, Table 3). There was no significant difference of periodontal parameters between healthy and osteopenia, or between healthy and osteoporosis.

Multiple logistic regression analysis with periodontitis (+) as an outcome was performed (Table 4). Age, IL-6-572G allele carriage, low BMD and serum albumin levels were entered as variables. Fitness of the model was $p = 0.019$. Low BMD showed significant association with periodontitis after adjustment with the confounding factors ($p = 0.027$, Table 4).

4. Discussion

In this study, we performed an association analysis among IL-6-572G/C genotypes, periodontitis and low BMD, in a total of

Table 1 – Characteristics of IL-6-57G/C genotypes.

	GG	CG	CC	*p*-Values	G allele carrier	G allele non-carrier	*p*-Values
	n = 14	*n* = 130	*n* = 156		*n* = 144	*n* = 156	
Age	64.42 ± 4.79	63.91 ± 5.53	63.05 ± 5.30	0.343	63.90 ± 5.41	63.05 ± 5.30	0.233
Body mass index	23.17 ± 4.05	23.13 ± 3.53	22.70 ± 3.51	0.530	23.17 ± 3.56	22.70 ± 3.51	0.190
Vitamin D intake (μg)	12.03 ± 2.27	11.50 ± 2.64	11.75 ± 2.86	0.688	11.75 ± 2.85	11.75 ± 2.86	0.719
Calcium intake (mg)	534.01 ± 77.04	522.02 ± 156.82	524.42 ± 137.57	0.786	524.87 ± 137.24	524.42 ± 137.57	0.603
Number of teeth	22.57 ± 4.43	22.11 ± 6.10	22.92 ± 5.70	0.538	22.13 ± 5.96	22.92 ± 5.70	0.255
Periodontitis (+)	10 (71%)	79 (61%)	83 (53%)	0.242	89 (62%)	83 (53%)	0.161
Mean PPD (mm)	1.89 ± 0.38	1.85 ± 0.39	1.96 ± 0.53	0.423	1.86 ± 0.39	1.96 ± 0.53	0.175
Mean CAL (mm)	2.44 ± 0.49	2.43 ± 0.61	2.52 ± 0.74	0.919	2.44 ± 0.60	2.52 ± 0.74	0.714
Sites with PPD ≥ 4 mm (%)	5.34 ± 0.45	8.32 ± 0.10	7.08 ± 0.10	0.282	8.01 ± 0.10	7.08 ± 0.10	0.392
Sites with CAL ≥ 4 mm (%)	19.99 ± 0.22	16.15 ± 0.16	15.70 ± 0.18	0.597	16.56 ± 0.17	15.70 ± 0.18	0.404
Bleeding on probing (%)	7.83 ± 0.07	11.66 ± 0.11	10.22 ± 0.10	0.318	11.26 ± 0.11	10.22 ± 0.10	0.467
Bone mineral density (g/cm^2)	0.72 ± 0.08	0.67 ± 0.09	0.69 ± 0.09	0.217	0.66 ± 0.09	0.69 ± 0.09	0.135
Low BMD	4 (29%)	48 (37%)	42 (27%)	0.098	52 (36%)	42 (27%)	0.087
Osteopenia	4 (29%)	38 (29%)	37 (24%)	0.422	42 (29%)	37 (24%)	0.196
Osteoporosis	0 (%)	10 (7.7%)	5 (3.3%)	0.152	10 (6.9%)	5 (3.2%)	0.138
Serum 25(OH)D (nmol/l)	20.36 ± 4.12	20.09 ± 6.35	19.94 ± 5.57	0.898	20.12 ± 6.18	19.94 ± 5.57	0.905
Serum NTX (nmol BCE/l)	16.22 ± 4.22	19.58 ± 6.07	19.31 ± 6.32	0.142	19.23 ± 6.00	19.31 ± 6.32	0.864
Serum albumin (g/dl)	4.48 ± 0.20	4.46 ± 0.25	4.47 ± 0.24	0.849	4.46 ± 0.25	4.46 ± 0.25	0.738
Serum osteocalcin (ng/ml)	6.13 ± 1.09	7.69 ± 2.84	8.04 ± 2.46	0.025	7.52 ± 2.80	8.04 ± 2.46	0.082

Mean ± SD. *p*-Values < α, α = 0.05. The Kruskal–Wallis tests were performed among IL-6 genetic polymorphism. The Mann–Whitney *U* tests were performed comparing IL-6-572G-carriers and non-carriers. Chi-square test was performed with IL-6 genetic polymorphism or IL-6 G-carriers and non-carriers for periodontitis or low BMD. The definition criterion for periodontitis(+) was having more than four teeth on which more than one site with CAL ≥ 4 mm, PPD ≥ 4 mm, and BOP concurrently. The definition criteria for low BMD, osteopenia and osteoporosis were BMD < 80%YAM, 70%≤BMD < 80%YAM and BMD < 70%YAM, respectively.

300 postmenopausal Japanese women. Multiple logistic regression analysis demonstrated that low BMD was significantly associated with periodontitis after adjustment with age, IL-6-572G/C polymorphism and serum albumin level. Since IL-6 has biological effects on both systemic bone resorption and periodontal tissue destruction in periodontitis, the result corresponds to previous studies. IL-6-572G/C polymorphism may influence the relationship between BMD and periodontitis in postmenopausal women.

IL-6 is a proinflammatory cytokine and plays a key role in chronic periodontitis. Fibroblasts from periodontal lesions produce greater amounts of IL-6 and IL-8 constituently than those from healthy controls.[28] Serum IL-6 was associated with teeth with deepened periodontal pocket.[29] A meta-analysis

Table 2 – (A) Correlations between serum NTX level and periodontal parameters. (B) Correlations between serum osteocalcin level and periodontal parameters.

(A)	Total (*n* = 300)		G allele carrier (*n* = 144)		G allele non-carrier (*n* = 156)	
	r	*p*-Values	*r*	*p*-Values	*r*	*p*-Values
Number of teeth	−0.091	0.116	−0.041	0.637	−0.145	0.071
Mean PPD (mm)	0.044	0.446	0.041	0.629	0.041	0.610
Mean CAL (mm)	0.060	0.304	0.048	0.568	0.057	0.479
Sites with PPD ≥ 4 mm (%)	0.119	0.039	0.165	0.048	0.064	0.426
Sites with CAL ≥ 4 mm (%)	0.025	0.662	0.052	0.534	−0.001	0.985
Bleeding on probing (%)	0.080	0.165	0.128	0.126	0.036	0.653
(B)	Total (*n* = 300)		G allele carrier (*n* = 144)		G allele non-carrier (*n* = 156)	
	r	*p*-Values	*r*	*p*-Values	*r*	*p*-Values
Number of teeth	0.030	0.956	0.031	0.711	−0.030	0.707
Mean PPD (mm)	0.093	0.093	0.158	0.060	0.033	0.648
Mean CAL (mm)	0.056	0.333	0.106	0.209	0.018	0.826
Sites with PPD ≥ 4 mm (%)	0.027	0.047	0.049	0.556	0.031	0.703
Sites with CAL ≥ 4 mm (%)	0.047	0.415	0.085	0.311	0.025	0.757
Bleeding on probing (%)	0.054	0.353	0.023	0.787	0.096	0.232

Spearman's rank correlation coefficient.

Table 3 – (A) Comparison of periodontal parameters between healthy and low BMD groups in IL-6-572G allele carriers. (B) Comparison of periodontal parameters between healthy and low BMD groups in IL-6-572G allele non-carriers.

(A) G allele carrier ($n = 144$)

	Healthy ($n = 92$)	Low BMD			p-Value 1	p-Value 2	p-Value 3	p-Value 4	p-Value 5
		Total ($n = 52$)	Osteopenia ($n = 42$)	Osteoporosis ($n = 10$)					
Age (years)	62.91 ± 5.00	64.41 ± 5.95	64.48 ± 5.62	65.89 ± 4.49	0.336	0.320	0.643	0.195	0.163
Number of teeth	23.27 ± 5.21	21.90 ± 6.32	21.79 ± 6.17	19.30 ± 7.76	0.385	0.434	0.463	0.204	0.279
Periodontitis (+)	62 (67%)	27 (52%)	23 (55%)	4 (40%)	0.130	0.161	0.406	0.087	0.076
Mean PPD (mm)	1.94 ± 0.49	1.88 ± 0.57	1.83 ± 0.41	1.96 ± 0.40	0.610	0.670	0.255	0.475	0.880
Mean CAL (mm)	2.53 ± 0.75	2.46 ± 0.74	2.37 ± 0.55	2.57 ± 0.58	0.689	0.668	0.331	0.562	0.412
Sites with PPD ≥ 4 mm (%)	6.10 ± 0.10	8.70 ± 0.10	8.70 ± 0.11	7.94 ± 0.10	0.960	0.243	0.761	0.795	0.109
Sites with CAL ≥ 4 mm (%)	14.70 ± 0.17	17.30 ± 0.18	16.35 ± 0.20	13.89 ± 0.18	0.455	0.388	0.515	0.311	0.232
Bleeding on probing (%)	10.92 ± 0.13	11.96 ± 0.08	11.97 ± 0.12	11.91 ± 0.18	0.691	0.820	0.402	0.434	0.927

(B) G allele non-carrier ($n = 156$)

	Healthy ($n = 114$)	Low BMD			p-Value 1	p-Value 2	p-Value 3	p-Value 4	p-Value 5
		Total ($n = 42$)	Osteopenia ($n = 37$)	Osteoporosis ($n = 5$)					
Age (years)	62.47 ± 4.81	64.62 ± 6.12	64.68 ± 6.09	64.15 ± 7.02	0.197	0.074	0.923	0.650	0.074
Number of teeth	22.77 ± 5.59	20.89 ± 6.89	21.03 ± 6.90	25.60 ± 3.85	0.129	0.104	0.110	0.296	0.163
Periodontitis (+)	58 (51%)	25 (60%)	23 (62%)	2 (40%)	0.411	0.233	0.349	0.635	0.370
Mean PPD (mm)	1.86 ± 0.40	1.85 ± 0.39	2.02 ± 0.62	1.78 ± 0.18	0.800	0.983	0.587	0.487	0.933
Mean CAL (mm)	2.52 ± 0.70	2.51 ± 0.81	2.53 ± 0.76	2.16 ± 0.43	0.455	0.709	0.221	0.242	0.840
Sites with PPD ≥ 4 mm (%)	6.00 ± 0.09	9.00 ± 0.12	8.70 ± 0.11	5.63 ± 0.07	0.538	0.287	0.528	0.836	0.021
Sites with CAL ≥ 4 mm (%)	15.50 ± 0.19	16.10 ± 0.16	18.78 ± 0.22	10.99 ± 0.09	0.496	0.248	0.534	0.937	0.216
Bleeding on probing (%)	9.90 ± 0.10	11.11 ± 0.10	11.78 ± 0.15	6.13 ± 0.08	0.426	0.570	0.186	0.274	0.846

Mean ± SD. Mann–Whitney U test. The definition criterion for healthy in this study was BMD ≥ 80% YAM (young adult mean). The definition criteria for low BMD, osteopenia and osteoporosis were BMD < 80%YAM, 70% ≤ BMD < 80%YAM and BMD < 70%YAM, respectively. The definition criterion for periodontitis (+) was having more than four teeth on which more than one site with CAL ≥ 4 mm, PPD ≥ 4 mm, and BOP concurrently.

* Skewing of distributions between periodontitis and low BMD, osteopenia or osteoporosis were assessed by chi-square test. p-Value 1: Comparison between healthy, osteopenia and osteoporosis. p-Value 2: Comparison between healthy and osteopenia. p-Value 3: Comparison between osteoporosis and osteopenia. p-Value 4: Comparison between osteoporosis and healthy. p-Value 5: Comparison between healthy and low BMD.

with 10 studies indicated an association between the IL-6-572G allele and periodontitis in Europeans.[8] It seems to be inconsistent with the other reports suggesting higher levels of serum IL-6 in -572C allele carriers.[9,30] In the present study, IL-6-572G/C polymorphism did not show significant associations with the prevalence of periodontitis or periodontal parameters. The difference of the IL-6 production level derived from the polymorphism may not be strong enough to induce clinical difference of periodontal parameters independently. Additionally, in a previous study reported IL-6-572G allele as a risk for chronic periodontitis, no CC genotype was found in Caucasians.[31] On the contrary, it was 52% of Japanese women in this study. The difference may suggest that ethnic background has modulated the clinical effects of genetic polymorphisms.

Table 4 – Multiple logistic regression analysis with periodontitis (+) as an outcome ($n = 300$).

Variable	p-Value	Exp (B)	95% CI
Age	0.182	1.031	0.986–1.078
IL-6-572G allele carriage	0.084	0.658	0.410–1.057
Low BMD	0.027	1.736	1.065–2.825
Serum albumin level	0.104	0.446	0.168–1.181

Fitness of the model: $p = 0.019$, Forced entry.

Postmenopausal women with the IL-6-572GG genotype presented significantly lower serum osteocalcin levels compared to those with other genotypes in this study. Osteocalcin is an extracellular matrix protein abundant in bone[32] and a negative regulator of bone formation.[33] A high concentration of serum osteocalcin results in low BMD with a high turn-over type. A previous study has reported significant effects of the IL-6-572C allele in both serum biological markers and low BMD in postmenopausal women.[23] However, we could not find a significant association between IL-6-572G/C polymorphism and BMD values or the prevalence of low BMD. Some confounding factors, such as PPARγ polymorphism,[22] may

have concealed the effect of the IL-6-572G/C polymorphism on BMD in this study.

NTX is a very specific marker of bone resorption because of its cross-linked α-2 peptide.[34] We found a positive but very weak correlation between serum NTX levels and the percentages of sites with PPD ≥ 4 mm in IL-6-572G allele carriers and total subjects included in this study. No other correlations were significant between periodontal parameters and serum NTX or osteocalcin levels. Unfortunately, we have no data of periodontal bone loss. In any case, most of the systemic amount of NTX or osteocalcin might have originated from organs other than alveolar bone, which could limit the significance of the potential correlations between periodontitis and systemic NTX or osteocalcin levels. We expected but obtained no strong evidence that IL-6-572G/C polymorphism would have enhanced correlations between periodontitis and serum NTX.

Only in IL-6-572G allele non-carriers, low BMD group showed significantly higher percentage of sites with PPD ≥ 4 mm compared to healthy controls. The result may suggest a role of the G allele to protect postmenopausal women from complication of both periodontitis and low BMD. This hypothesis was enhanced by the result from the logistic regression analysis. However, the other periodontal parameters did not show significant differences between healthy and low BMD, osteopenia or osteoporosis, probably because of small numbers of women. Further studies with larger numbers of subjects would verify the hypothesis.

There are many reports for the relationships between IL-6-174C/G or -572G/C polymorphisms and periodontitis. However, frequency of IL-6-174C allele is very low in Asia. We found no polymorphism of IL-6-174C/G in a previous study with Japanese pregnant women.[35] Although haplotype analysis in future will increase the scope and relevance of the IL-6 gene on clinical phenotypes, we analyzed a single polymorphism which limited view of this study.

In conclusion, IL-6-572G/C polymorphism may affect the relationship between low BMD and periodontitis in postmenopausal Japanese women, though it did not show a significant association with low BMD or periodontitis independently. Further studies are required to determine the role of all functional polymorphisms in IL-6 gene including the measurement of serum IL-6 levels.

Conflict of interest statement

The authors declare that they have no conflict of interests.

Funding

The work was supported by Grants in Aid for Scientific Research (24593121) from Japan Society for the Promotion of Science (JSPS), Tokyo, Japan.

Competing interests

None declared.

Ethical approval

The protocol in this study approver by the Institutional Review Board of the Faculty of Medicine, Niigata University (Approval No. 303).

Author contributions

Yuki Hanai performed all genotyping and prepared the manuscript. Noriko Sugita designed genotyping and statistical analysis, and advised preparation of the manuscript. Yanming Wang extracted and adjusted genomic DNA for genotyping. Akihiro Yoshihara, Masanori Iwasaki and Hideo Miyazaki planned and performed the collection of medical and periodontal data and blood samples, and additionally, advised statistical analyses. Kazutoshi Nakamura managed recruitment of subjects and advised regarding medical aspects. Hiromasa Yoshie gave scientific advices on the study.

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