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Detection of association between periodontitis and polymorphisms of IL-1β + 3954 and TNF-α -863 in the Korean population after controlling for confounding risk factors

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Abstract

Background and Objective: Interleukin (IL)-1 and tumor necrosis factor (TNF)-α are inflammatory cytokines that play an important role in periodontitis, and their genetic variations have been suggested to be associated with increased risk of periodontitis. Focusing on three single nucleotide polymorphisms (SNPs) of IL-1α + 4845, IL-1β + 3954, and TNF-α −863, we aimed to investigate the relationship between periodontitis risk and the polymorphisms of IL-1 α/β and TNF-α in Koreans.

Material and Methods: Mouthwash samples from 548 subjects (135 controls without periodontitis, 387 generalized chronic periodontitis patients, and 26 generalized aggressive periodontitis patients) were collected for isolation of genomic DNA. Genotyping of selected SNPs was performed using real-time PCR. Univariable associations between the polymorphisms and periodontitis were assessed by chi-squared test or Fisher's exact test. To evaluate the association after controlling for confounding effects of various risk factors, we stratified the subjects according to the presence or absence of self-reported diseases and employed multiple logistic regression model to adjust for age, smoking status, and oral hygiene indices and behaviors.

Results: Significant association of IL-1β + 3954 and TNF-α −863 polymorphisms with periodontitis was observed after adjusting for the confounding risk factors, but not in univariable association analysis. The significant association between genotype CT of IL-1β + 3954 and increased risk of advanced periodontitis was consistently detected regardless of the status of self-reported diseases. In the polymorphism of TNF-α −863, the genotype with minor allele (CA + AA) was significantly associated with periodontitis susceptibility, which was observed only in the subjects with self-reported diseases.

Conclusion: The results suggest that genetic variations of IL-1β + 3954 and TNF-α −863 are associated with increased risk of periodontitis in Koreans. In addition, our

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findings underscore the importance of controlling for confounding risk factors to detect significant association between genetic factors and risk of periodontitis. A further well-designed large-scale study is needed to warrant our results.

KEYWORDS

interleukin-1, periodontitis, single nucleotide polymorphism, tumor necrosis factor-α

1 | INTRODUCTION

Periodontitis is an inflammatory disease that begins with dysbiosis in which destruction of the homeostatic balance occurs in periodontal microbiomes, which can cause host response alteration.[1] Polymicrobial synergy and dysbiosis in susceptible hosts break the host-microbe homeostasis and eventually cause periodontitis.[2] Although periodontitis is initiated by pathogenic bacteria, it is a complex disease in which various etiologic factors are involved at numerous levels: host responses, environmental factors, and systemic conditions. Inflammatory and immune responses of the host against the subgingival biofilm are regarded as important determinants of severity and progression of periodontitis.[3]

When macrophages, epithelial cells, neutrophils, and lymphocytes are stimulated by microbial components, cellular secretion of inflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, is elevated.[4] These pro-inflammatory cytokines directly induce osteoclastogenesis, which finally results in alveolar bone resorption.[5] The cytokines also stimulate release of tissue-derived enzymes, matrix metalloproteinases, which are destructive to the extracellular matrix of tooth-supporting soft and hard tissues.[6] The association between progression of periodontitis and elevated levels of IL-1 and TNF-α is well established, and the expression levels of IL-1 and TNF-α are known to be significantly correlated with clinical parameters of periodontitis.[7,8]

On the other hand, many studies have suggested that genetic polymorphisms including single nucleotide polymorphisms (SNPs) in IL-1 and TNF-α genes are associated with susceptibility to periodontitis or severity of periodontal disease.[9-14] Among the SNPs known to be associated with periodontitis, IL-1α + 4845, IL-1β + 3954, and TNF-α −863 have been extensively investigated,[11,15-18] and several comprehensive meta-analyses reported that risk of periodontitis is significantly associated with SNPs of IL-1 (IL-1α + 4845 and IL-1β + 3954)[9,19-24] or TNF-α −863 polymorphism.[10] However, the previous studies were examined mainly in Caucasians and several Asian populations including Chinese, Japanese, and Indians. In addition, allele frequency of SNP varies considerably in populations with different ancestry. For example, only 2.3% of Chinese were observed to harbor a composite genotype of allele 2 of IL-1α + 4845 and IL-1β + 3954, whereas approximately 30% of European population carry the genotype with an increased risk of severe periodontitis.[25]

To date, the relationships between the three SNPs (IL-1α + 4845, IL-1β + 3954, and TNF-α −863) and susceptibility or severity of periodontitis have been scarcely investigated in the Korean population. Accordingly, we evaluated the associations in 548 Koreans comprised of 413 patients with chronic or aggressive periodontitis and 135 controls without periodontitis. In addition, we examined the relationships separately in two groups of patients stratified by the presence or absence of self-reported diseases.

2 | MATERIAL AND METHODS

2.1 | Study subjects and clinical examination

This study was designed as a case-control study and included 548 subjects (135 controls without periodontitis, 387 generalized chronic periodontitis patients, and 26 generalized aggressive periodontitis patients) who visited the Department of Periodontics, Pusan National University Dental Hospital, between August 2016 and April 2018. The study protocol was approved by the Institutional Review Board of Pusan National University Dental Hospital (PNUDH-2016-019).

The diagnosis of controls without periodontitis, generalized chronic periodontitis patients, and generalized aggressive periodontitis patients was performed according to the classification established in 1999 International Workshop of the American Academy of Periodontology (APP) for a Classification of Periodontal Diseases and Conditions.[26] The following patients were excluded: (a) those who received periodontal treatment within the past 6 months; (b) women who were pregnant or breastfeeding; and (c) those who refused to sign the informed consent form. The subjects received complete information regarding the objectives and procedures of this study and provided written informed consent. The severity of chronic periodontitis was categorized on the basis of clinical attachment loss as follows: slight = 1 or 2 mm; moderate = 3 or 4 mm; and severe ≥ 5 mm. Although some of the control subjects showed CAL ≈2.5 mm, all controls did not show any clinical manifestations of periodontitis or inflammation such as gingival swelling, redness, or bleeding of probing. The CAL and plaque index (PI) were measured during the clinical evaluation. The CAL was measured using a periodontal probe (PGF-W, Osung, Kwangmyong, South Korea). The PI is an indicator of oral hygiene and is determined by the O'Leary plaque index.[27] All measurements were performed by two experienced periodontists. Medical, dental, and smoking status were gathered by questionnaires including present medical status, additional oral care (dental floss or mouthwash), and toothbrushing frequency per day. Smoking status was categorized into three classes to distinguish

present smokers, former (those who had quit ≥ 6 months ago) smokers, and those who had never smoked.

2.2 | Genotyping

Mouthwash samples were collected by rinsing the mouth with 12 mL of solution for 30 seconds (E-zen Gargle; JN Pharm, Korea).[28] Genomic DNA was extracted from mouthwash samples using an Exgene™ Clinic SV DNA extraction kit according to the manufacturer's instructions (GeneAll). The DNA concentration was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Primer and probe sequences for IL-1α and IL-1β polymorphisms (IL-1α + 4845: rs17561; IL-1β + 3954: rs1143634) were proprietary and were obtained from Applied Biosystems (assay ID for rs17561: C_9546471_10; assay ID for rs1143634: C_9546517_10). Primer and probe sequences for TNF-α polymorphism (rs1800630) were designed to target nucleotide substitutions at position −863 (C->A). The primer and probe sequences of the target site for the TNF-α (rs1800630) were as follows: Forward primer, 5′-GAGATGTGACCACAGCAATG-3′; Reverse primer, 5′- AGGTCCTGGAGGCTCTTTCA-3′; Probe, FAM-AG TATGGGGACCCCCACTTAA-MGB, VIC-AGTATGGGGACCCCCCC TTAA-MGB. Polymorphism detection was done using the QuantStudio™ 6 Flex Real-Time PCR System (Thermo Fisher Scientific). All qPCR reactions were performed in a total volume of 20 μL using the 2 × TaqMan® Genotyping Master Mix (Applied Biosystems), containing 2 μL of template, 900 nmol/L primers, and 200 nmol/L probes. qPCR conditions were as follows: denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 1 minutes, and 60°C for 30 seconds.

2.3 | Statistical analysis

The reproducibility of two separate investigator and intra-investigator assessments was evaluated using Cohen's kappa index. Intra- and inter-examiner agreements were 0.90 and 0.83, respectively. All statistical analyses were performed using SigmaPlot 13.0 software (Systat Software Inc) or R statistical software (https://www.R-project.org/). The Hardy-Weinberg equilibrium (HWE) was evaluated using a chi-squared test for each SNP among the controls and cases separately. Univariable associations between genetic variations and periodontitis susceptibility or severity were assessed using chi-squared or Fisher's exact tests. Fisher's exact tests were performed when criteria for the chi-squared test were not fulfilled. The association between the polymorphisms and periodontitis susceptibility was examined by estimating odds ratio (OR) for heterozygous and homozygous genotypes with minor allele to homozygous genotype with the major allele of each SNP by assigning all controls without periodontitis as "Control" status and all chronic and aggressive periodontitis as "Case" status. The association between the polymorphisms and periodontitis severity was investigated in two different models. In the first model (Model I), both controls and slight periodontitis were assigned as "Control" status and moderate/severe chronic periodontitis and aggressive periodontitis were assigned as "Case" status. In the second model (Model II), the analysis was

TABLE 1 Self-reported diseases in periodontitis patients and controls without periodontitis

		Periodontitis				
		Chronic periodontitis				
	Controls without periodontitis (n = 135)	Slight (n = 86)	Moderate (n = 172)	Severe (n = 129)	Aggressive periodontitis (n = 26)	Total (n = 413)
Subjects without self-reported disease	122 (90.4%)	54 (62.8%)	65 (37.8%)	65 (49.6%)	17 (65.4%)	201 (48.7%)
Subjects with self-reported diseases[a]	13 (9.6%)	32 (33.7%)	107 (59.9%)	64 (46.5%)	9 (26.9%)	212 (51.3%)
Cardiovascular	6 (4.4%)	15 (17.4%)	52 (30.2%)	32 (24.8%)	3 (11.5%)	102 (24.7%)
Hyperlipidemia	2 (1.5%)	10 (11.6%)	27 (15.7%)	9 (7.0%)	1 (3.8%)	47 (11.4%)
Diabetes	0 (0.0%)	2 (2.3%)	22 (12.8%)	16 (12.4%)	1 (3.8%)	41 (9.9%)
Gastritis	5 (3.7%)	5 (5.8%)	14 (8.1%)	6 (4.7%)	0 (0.0%)	25 (6.1%)
Thyroid disease	3 (2.2%)	5 (5.8%)	6 (3.5%)	4 (3.1%)	1 (3.8%)	16 (3.9%)
Osteoporosis	0 (0.0%)	1 (1.2%)	8 (4.7%)	2 (1.6%)	0 (0.0%)	11 (2.7%)
Arthritis/Gout	0 (0.0%)	1 (1.2%)	4 (2.3%)	4 (3.1%)	1 (3.8%)	10 (2.4%)
Allergy/Inflammation/Infection	1 (0.7%)	1 (1.2%)	2 (1.2%)	4 (3.1%)	0 (0.0%)	7 (1.7%)
Cancer	1 (0.7%)	0 (0.0%)	3 (1.7%)	3 (2.3%)	0 (0.0%)	6 (1.5%)
etc.[b]	0 (0.0%)	4 (4.7%)	5 (2.9%)	3 (2.3%)	2 (7.7%)	14 (3.4%)

[a]Many subjects self-reported to have multiple diseases simultaneously.

[b]Parkinson, depression, panic disorder, tinnitus, anemia, herniated disk, dizziness, glaucoma, prostate hyperplasia, and pituitary tumor.

confined to the chronic periodontitis defining the slight periodontitis as "Control" status and moderate/severe periodontitis are "Case" status. Finally, the associations between genetic variations and periodontitis susceptibility or severity were further investigated by multiple logistic regression analysis after adjusting for various risk factors of periodontitis including age, sex, smoking history, number of teeth, plaque index, additional oral care, and toothbrushing frequency per day. *P*-values were considered statistically significant when *P*-value was less than .05.

3 | RESULTS

A total of 413 cases (387 chronic and 26 aggressive periodontitis) and 135 controls without periodontitis were enrolled in our study. Approximately 51.3% of 413 subjects with periodontitis self-reported to have various diseases including cardiovascular (24.7%), hyperlipidemia (11.4%), diabetes (9.9%), gastritis (6.1%), thyroid disease (3.9%), osteoporosis (2.7%), arthritis/gout (2.4%), allergy/inflammation/infection (1.7%), and cancer (1.5%) (Table 1). In contrast, only thirteen subjects (9.6%) of 135 controls answered to have self-reported diseases. For decades, potential associations between periodontal disease and various systemic diseases and conditions (eg, cardiovascular disease, rheumatoid arthritis, obesity, and metabolic syndrome) have been investigated vigorously.[29-42] Thus, all further analyses were performed with three separated datasets comprised of all subjects (n = 548), subjects without self-reported disease (n = 323), and those with self-reported diseases (n = 225) to adjust for any effect of such diseases or conditions on the association analysis.

The characteristics of the study population are presented in Table 2 including demographics, clinical information, and oral hygiene indices and behaviors. The patients with periodontitis showed considerably older age, higher level of plaque index, and less number of teeth compared to the controls with statistical significance (all *P*-values < .001 in Mann-Whitney U tests). Oral hygiene behaviors and smoking history of the periodontitis patients were also significantly different from those of the controls (all *P*-values < .001 in chi-squared tests). Most of the characteristics were also different between controls and periodontitis cases in the subjects without self-reported disease. In contrast, except for CAL and plaque index, distributions of most other characteristics including age were not significantly different between controls and periodontitis patients among the subjects with self-reported diseases. In addition, the subjects with self-reported diseases were considerably older than those without self-reported disease in both controls and periodontitis cases.

Genotype distributions of the polymorphisms of the three cytokine genes, IL-1α + 4845 G/T (rs17561), IL-1β + 3954 C/T (rs1143634), and TNF-α −863 C/A (rs1800630), were not significantly different from those expected from the Hardy-Weinberg equilibrium in both the control and case groups (data not shown). In Table 3, the genotype distributions of the three SNPs are presented with the ORs and *P*-values calculated from univariable association analyses with periodontitis susceptibility or severity.

In polymorphisms of IL-1α + 4845 G/T (rs17561), frequency of T allele was 8.5%, which is slightly higher than the frequency of East Asians (7.5%) but much lower than the global population allele frequency (26.8%) in The Genome Aggregation Database (gnomAD). Genotype TT was very rare and only one of the 548 subjects (0.2%) carried the genotype TT, whereas genotype GT accounted for 17.8, 16.5, and 11.5% in controls, chronic (17.4% in slight, 18.0% in moderate, and 14.0% in severe periodontitis), and aggressive cases, respectively. In univariable association analyses, no statistical significance was observed between IL-1α + 4845 polymorphism and periodontitis susceptibility or severity in all three datasets regardless of the presence or absence of self-reported diseases (Table 3).

For IL-1β + 3954 C/T (rs1143634) polymorphisms, T allele frequency was 3.3%, which is almost two times higher than the frequency of East Asians (1.9%) but much lower than the global frequency of T allele (19.2%) in gnomAD. In a previous study, the T allele frequency of IL-1β + 3954 C/T (rs1143634) was reported to be 4.7% in 311 Koreans,[43] which also indicates a relatively high frequency of T allele of IL-1β + 3954 in Koreans compared to other East Asian populations. Approximately 6.6% of the subjects (36/548) carried the allele T, and all of the subjects who carried the IL-1β + 3954 polymorphisms were heterozygous, carrying genotype CT. The genotype CT accounted for 7.4, 6.2, and 7.7% in controls, chronic (2.3% in slight, 9.3% in moderate, and 4.7% in severe periodontitis), and aggressive periodontitis, respectively. No significant difference was observed in univariable association analyses between T allele frequency of IL-1β + 3954 and periodontitis susceptibility or severity. However, relatively smaller p-values (0.15-0.18) with higher ORs (3.30-4.39) were obtained in the univariable associations when the analysis was confined to chronic periodontitis (Table 3), implying a possible relationship between IL-1β + 3954 C/T (rs1143634) polymorphisms and severity of chronic periodontitis.

The polymorphisms of TNF-α −863 C/A (rs1800630) were detected in a much higher proportion of the study population compared to the IL-1 polymorphisms. The frequency of A allele was 17.2%, which is congruent to the frequency of East Asians (17.6%) and slightly higher than global frequency (14.3%) in gnomAD. Out of the 548 subjects, 159 subjects (29.0%) carried heterozygous genotype CA and 15 subjects (2.7%) showed homozygous genotype AA. The homozygous genotype AA was rare in both controls (3.7%) and periodontitis cases (2.1 and 7.7% in chronic and aggressive periodontitis, respectively). Genotype CA accounted for 31.1, 28.2, and 30.8% in controls, chronic (31.4% in slight, 25.0% in moderate, and 30.2% in severe chronic periodontitis), and aggressive periodontitis, respectively. No significant univariable association was recognized between TNF-α −863 C/A (rs1800630) polymorphisms and periodontitis susceptibility or severity.

In order to investigate the associations between the polymorphisms of the three SNPs after controlling for confounding effects of other risk factors of periodontitis, multiple logistic regression analysis

TABLE 2 Characteristics of periodontitis patients and controls without periodontitis

Characteristics		Controls without periodontitis (n = 135)	Periodontitis					Mann-Whitney U test or chi-squared test (*P*-value)[a]
			Chronic periodontitis			Aggressive periodontitis	Total (n = 413)	
			Slight (n = 86)	Moderate (n = 172)	Severe (n = 129)	(n = 26)		
All subjects (n = 548)								
Sex	Male	68 (50.4%)	41 (47.7%)	93 (54.1%)	81 (62.8%)	15 (57.7%)	230 (55.7%)	.33
	Female	67 (49.6%)	45 (52.3%)	79 (45.9%)	48 (37.2%)	11 (42.3%)	183 (44.3%)	
Age (y)	median (IQR)	29 (27-33)	46.5 (36-59)	58 (51-63)	53 (48-57)	40 (36-45)	53 (45-60)	<.001
Clinical attachment level (mm)	median (IQR)	2.5 (2.2-2.6)	2.8 (2.5-3.0)	3.4 (3.0-3.7)	4.3 (3.7-4.9)	5.0 (4.5-5.3)	3.5(2.9-4.3)	<.001
Plaque index	median (IQR)	14.3 (8.5-23.6)	27.7 (11.6-42.9)	40.9 (22.1-56.2)	44.2 (27.0-61.2)	33.2 (23.9-46.7)	38.7 (22.4-54.5)	<.001
Number of teeth	median (IQR)	28 (27-28)	28 (27-28)	27 (25-28)	26 (24-28)	26.5 (25-28)	27 (25-28)	<.001
Smoking history	Never	112 (83.0%)	61 (70.9%)	96 (55.8%)	57 (44.2%)	13 (50.0%)	227 (55.0%)	<.001
	Former	18 (13.3%)	15 (17.4%)	40 (23.3%)	34 (26.4%)	3 (11.5%)	91 (22.0%)	
	Present	5 (3.7%)	10 (11.6%)	36 (20.9%)	38 (29.5%)	10 (38.5%)	95 (23.0%)	
Additional oral care	Yes	108 (80.0%)	61 (70.9%)	96 (55.8%)	60 (46.5%)	18 (69.2%)	235 (56.9%)	<.001
	No	27 (20.0%)	25 (29.1%)	76 (44.2%)	69 (53.5%)	8 (30.8%)	178 (43.1%)	
Toothbrushing frequency per day	≥3	107 (79.3%)	51 (59.3%)	74 (43.0%)	56 (43.4%)	13 (50.0%)	194 (47.0%)	<.001
	≤2	28 (20.7%)	35 (40.7%)	98 (57.0%)	73 (56.6%)	13 (50.0%)	219 (53.0%)	
Subjects without self-reported disease (n = 323)								
Sex	Male	64 (52.5%)	28 (51.9%)	28 (43.1%)	22 (33.8%)	8 (47.1%)	115 (57.2%)	.12
	Female	58 (47.5%)	26 (48.1%)	37 (56.9%)	43 (66.2%)	9 (52.9%)	86 (42.8%)	
Age (y)	median (IQR)	29 (27-31.8)	40 (32-47.8)	53 (45-59)	52 (46-57)	39 (36-43)	48 (40-56)	<.001
Clinical attachment level (mm)	median (IQR)	2.5 (2.2-2.6)	2.8 (2.5-3.0)	3.5 (3.0-3.8)	4.3 (3.5-4.9)	4.8 (4.5-5.0)	3.5 (2.9-4.4)	<.001
Plaque index	median (IQR)	14.5 (8.6-24.1)	28.2 (11.6-42.9)	44.2 (29.5-58.9)	46.7 (26.7-61.2)	34.8 (28.4-47.1)	38.9 (23.9-54.5)	<.001
Number of teeth	median (IQR)	28 (28-28)	28 (26-28)	28 (26-28)	26 (24-28)	28 (25-28)	27 (25-28)	<.001
Smoking history	Never	103 (84.4%)	38 (70.4%)	33 (50.8%)	27 (41.5%)	10 (58.8%)	108 (53.7%)	<.001
	Former	14 (11.5%)	10 (18.5%)	14 (21.5%)	17 (26.2%)	1 (5.9%)	42 (20.9%)	
	Present	5 (4.1%)	6 (11.1%)	18 (27.7%)	21 (32.3%)	6 (35.3%)	51 (25.4%)	
Additional oral care	Yes	97 (79.5%)	42 (77.8%)	40 (61.5%)	34 (52.3%)	14 (82.4%)	130 (64.7%)	.007
	No	25 (20.5%)	12 (22.2%)	25 (38.5%)	31 (47.7%)	3 (17.6%)	71 (35.3%)	

(Continues)

TABLE 2 (Continued)

Characteristics		Controls without periodontitis (n = 135)	Periodontitis				Total (n = 413)	Mann-Whitney U test or chi-squared test (*P*-value)[a]
			Chronic periodontitis			Aggressive periodontitis		
			Slight (n = 86)	Moderate (n = 172)	Severe (n = 129)	(n = 26)		
Toothbrushing frequency per day	≥3	98 (80.3%)	35 (64.8%)	33 (50.8%)	310 (47.7%)	12 (70.6%)	111 (55.2%)	<.001
	≤2	24 (19.7%)	19 (35.2%)	32 (49.2%)	34 (52.3%)	5 (29.4%)	90 (44.8%)	
Subjects with self-reported diseases (n = 225)								
Sex	Male	3 (23.1%)	17 (53.1%)	51 (47.7%)	26 (40.6%)	3 (33.3%)	115 (54.2%)	.19
	Female	10 (76.9%)	15 (46.9%)	56 (52.3%)	38 (59.4%)	6 (66.7%)	97 (45.8%)	
Age (y)	median (IQR)	55 (31-70)	59 (53.5-64.3)	60 (54-64)	53.5 (51-57)	45 (37-49)	57 (51-62.3)	.43
Clinical attachment level (mm)	median (IQR)	2.4 (2.3-2.5)	2.8 (2.5-3.0)	3.4 (3.1-3.7)	4.2 (3.8-4.9)	5.2 (4.9-5.5)	3.5 (3.0-3.7)	<.001
Plaque index	median (IQR)	10.0 (4.6-15.2)	27.7 (14.5-50.9)	38.8 (20.5-55.0)	42.2 (30.1-59.1)	29.8 (16.7-45.3)	38.6 (20.9-54.5)	<.001
Number of teeth	median (IQR)	28 (27-28)	28 (28-29)	27 (25-28)	27 (25-28)	26 (25-28)	27 (25-28)	.32
Smoking history	Never	9 (69.2%)	23 (71.9%)	63 (58.9%)	30 (46.9%)	3 (33.3%)	119 (56.1%)	.17[b]
	Former	4 (30.8%)	5 (15.6%)	26 (24.3%)	17 (26.55%)	1 (11.1%)	49 (23.1%)	
	Present	0 (0%)	4 (12.5%)	18 (16.8%)	17 (26.55%)	5 (55.6%)	44 (20.8%)	
Additional oral care	Yes	11 (84.6%)	19 (59.4%)	56 (52.3%)	26 (40.6%)	4 (44.4%)	105 (49.5%)	.03
	No	2 (15.4%)	13 (40.6%)	51 (47.7%)	38 (59.4%)	5 (55.6%)	107 (50.5%)	
Toothbrushing frequency per day	≥3	9 (69.2%)	16 (50%)	41 (38.3%)	25 (39.1%)	1 (11.1%)	83 (39.2%)	.064
	≤2	4 (30.8%)	16 (50%)	66 (61.7%)	39 (60.9%)	8 (88.9%)	129 (60.8%)	

[a]Comparisons were made between controls without periodontitis and all subjects with periodontitis.

[b]Fisher's exact test is applied.

TABLE 3 Distributions of genotypes of IL-1α + 4845, IL-1β + 3954, and TNF-α −863 polymorphisms and their associations with periodontitis susceptibility and severity

Polymorphisms	Distribution of polymorphisms						Association[a] OR (*P*-value)		
	Controls without periodontitis	Chronic periodontitis			Aggressive periodontitis	Total	Susceptibility[b]	Severity	
		Slight	Moderate	Severe				Model I[c]	Model II[d]
IL-1α + 4845 G/T (rs17561)									
All subjects (n = 548)									
GG	110 (81.5%)	71 (82.6%)	141 (82.0%)	111 (86.0%)	23 (88.5%)	456 (83.2%)	.85	.86	.92
GT	24 (17.8%)	15 (17.4%)	31 (18.0%)	18 (14.0%)	3 (11.5%)	91 (16.6%)	(.63)	(.58)	(.93)
TT	1 (0.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)			
Subjects without self-reported disease (n = 323)									
GG	99 (81.1%)	47 (87%)	56 (86.2%)	55 (84.6%)	15 (88.2%)	272 (84.2%)	.70	.81	1.15
GT	22 (18%)	7 (13%)	9 (13.8%)	10 (15.4%)	2 (11.8%)	50 (15.5%)	(.31)	(.60)	(.95)
TT	1 (0.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)			
Subjects with self-reported diseases (n = 225)									
GG	11 (84.6%)	24 (75%)	85 (79.4%)	56 (87.5%)	8 (88.9%)	184 (81.8%)	1.24	.73	.64
GT	2 (15.4%)	8 (25%)	22 (20.6%)	8 (12.5%)	1 (11.1%)	41 (18.2%)	(1.00)	(.57)	(.46)
TT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)			
IL-1β + 3954 C/T (rs1143634)									
All subjects (n = 548)									
CC	125 (92.6%)	84 (97.7%)	156 (90.7%)	123 (95.3%)	24 (92.3%)	512 (93.4%)	.84	1.38	3.30
CT	10 (7.4%)	2 (2.3%)	16 (9.3%)	6 (4.7%)	2 (7.7%)	36 (6.6%)	(.80)	(.38)	(.15)
TT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)			
Subjects without self-reported disease (n = 323)									
CC	113 (92.6%)	53 (98.1%)	59 (90.8%)	61 (93.8%)	16 (94.1%)	302 (93.5%)	.80	1.34	4.39
CT	9 (7.4%)	1 (1.9%)	6 (9.2%)	4 (6.2%)	1 (5.9%)	21 (6.5%)	(.79)	(.67)	(.18)
TT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)			
Subjects with self-reported diseases (n = 225)									
CC	12 (92.3%)	31 (96.9%)	97 (90.7%)	62 (96.9%)	8 (88.9%)	210 (93.3%)	.85	1.67	2.33
CT	1 (7.7%)	1 (3.1%)	10 (9.3%)	2 (3.1%)	1 (11.1%)	15 (6.7%)	(.60)	(.74)	(.70)
TT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)			
TNF-α −863 C/A (rs1800630)									
All subjects (n = 548)									
CC	88 (65.2%)	59 (68.6%)	125 (72.7%)	86 (66.7%)	16 (61.5%)	374 (68.3%)	.83	.88	.93

(Continues)

TABLE 3 (Continued)

Polymorphisms	Distribution of polymorphisms						Association[a] OR (*P*-value)		
	Controls without periodontitis	Chronic periodontitis			Aggressive periodontitis	Total	Susceptibility[b]	Severity	
		Slight	Moderate	Severe				Model I[c]	Model II[d]
CA	42 (31.1%)	27 (31.4%)	43 (25.0%)	39 (30.2%)	8 (30.8%)	159 (29.0%)	(.44)	(.53)	(.89)
AA	5 (3.7%)	0 (0%)	4 (2.3%)	4 (3.1%)	2 (7.7%)	15 (2.7%)			
Subjects without self-reported disease (n = 323)									
CC	78 (63.9%)	38 (70.4%)	48 (73.9%)	43 (66.2%)	11 (64.7%)	218 (67.5%)	.77	.85	1.02
CA	39 (32%)	16 (29.6%)	16 (24.6%)	20 (30.8%)	4 (23.5%)	95 (29.4%)	(.35)	(.59)	(1.00)
AA	5 (4.1%)	0 (0%)	1 (1.5%)	2 (3.1%)	2 (11.8%)	10 (3.1%)			
Subjects with self-reported diseases (n = 225)									
CC	10 (76.9%)	21 (65.6%)	77 (72%)	43 (67.2%)	5 (55.6%)	156 (69.3%)	1.50	.97	.81
CA	3 (23.1%)	11 (34.4%)	27 (25.2%)	19 (29.7%)	4 (44.4%)	64 (28.4%)	(.76)	(1.00)	(.68)
AA	0 (0%)	0 (0%)	3 (2.8%)	2 (3.1%)	0 (0%)	5 (2.2%)			

[a]Association p-values with odds ratio (OR) for heterozygous and homozygous minor allele to homozygous major allele were assessed using chi-squared tests or Fisher's exact tests

[b]All chronic and aggressive periodontitis cases are assigned as "Case" status in the association analyses.

[c]Controls without periodontitis and slight chronic periodontitis are assigned as "Control" status, and moderate/severe chronic and aggressive periodontitis are assigned as "Case" status in the association analyses.

[d]Slight chronic periodontitis cases are assigned as "Control" status, and moderate/severe periodontitis cases are assigned as "Case" status in the association analyses.

TABLE 4 Association of genotypes of IL-1α + 4845, IL-1β + 3954, and TNF-α −863 polymorphisms with periodontitis susceptibility and severity after adjustment for various confounding factors using multiple logistic regression model

	Susceptibility[b]		Severity			
			Model I[c]		Model II[d]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
All subjects	(n = 548)		(n = 548)		(n = 387)	
Sex (male vs female)	0.43 (0.21-0.85)	.017	0.58 (0.32-1.03)	.064	0.83 (0.41-1.67)	.60
Age (y)[a]	1.11 (1.08-1.14)	<.001	1.08 (1.06-1.11)	<.001	1.07 (1.04-1.09)	<.001
Plaque index[a]	1.05 (1.03-1.07)	<.001	1.04 (1.03-1.05)	<.001	1.03 (1.01-1.04)	<.001
Number of teeth[a]	0.92 (0.80-1.05)	.23	0.88 (0.79-0.96)	.009	0.86 (0.76-0.96)	.010
Smoking history (former and present vs. never)	3.46 (1.63-7.54)	.0015	3.28 (1.82-6.05)	<.001	2.47 (1.21-5.10)	.014
Toothbrushing frequency (≤2 vs ≥3)	1.93 (1.03-3.67)	.041	1.77 (1.09-2.86)	.019	1.60 (0.91-2.80)	.10
Additional oral care method (no vs yes)	2.16 (1.12-4.31)	.024	1.96 (1.20-3.25)	.008	1.72 (0.96-3.16)	.073
IL-1α (GT & TT vs GG)	0.80 (0.33-1.93)	.61	0.49 (0.23-1.03)	.059	0.47 (0.21-1.08)	.070
IL-1β (CT vs CC)	0.87 (0.23-3.42)	.84	3.46 (1.13-11.4)	.034	7.30 (1.63-52.5)	.019
TNF-α (CA & AA vs CC)	0.92 (0.50-1.68)	.77	1.01 (0.62-1.65)	.98	1.00 (0.55-1.82)	.99
Subjects without self-reported disease	(n = 323)		(n = 323)		(n = 184)	
Sex (male vs female)	0.67 (0.30-1.45)	.31	0.80 (0.36-1.72)	.57	1.16 (0.43-3.16)	.77
Age (y)[a]	1.12 (1.08-1.16)	<.001	1.11 (1.08-1.15)	<.001	1.12 (1.07-1.17)	<.001
Plaque index[a]	1.05 (1.03-1.07)	<.001	1.05 (1.03-1.07)	<.001	1.04 (1.02-1.06)	<.001
Number of teeth[a]	0.90 (0.76-1.05)	.19	0.96 (0.84-1.09)	.58	1.02 (0.87-1.19)	.79
Smoking history (former and present vs. never)	2.86 (1.21-6.96)	.018	3.24 (1.48-7.32)	.0039	2.54 (0.96-6.96)	.062
Toothbrushing frequency (≤2 vs ≥3)	1.66 (0.76-3.63)	.20	1.23 (0.62-2.41)	.56	1.46 (0.64-3.39)	0.38
Additional oral care method (no vs yes)	1.53 (0.69-3.46)	.29	1.77 (0.84-3.78)	.14	1.94 (0.76-5.26)	.18
IL-1α (GT & TT vs GG)	0.70 (0.22-2.10)	.53	0.36 (0.10-1.16)	.094	0.33 (0.08-1.35)	.11
IL-1β (CT vs CC)	0.93 (0.18-4.95)	.93	5.47 (1.09-30.3)	.043	9.90 (1.17-219.2)	.062
TNF-α (CA & AA vs CC)	0.52 (0.25-1.06)	.075	0.69 (0.34-1.38)	.30	0.93 (0.38-2.29)	.87
Subjects with self-reported diseases	(n = 225)		(n = 225)		(n = 203)	
Sex (male vs female)	0.04 (0.004-0.29)	.0034	0.46 (0.18-1.15)	.10	0.83 (0.29-2.46)	.74
Age (y)[a]	1.05 (0.99-1.13)	.10	1.00 (0.97-1.04)	.94	1.00 (0.95-1.05)	.90
Plaque index[a]	1.08 (1.03-1.14)	.0020	1.03 (1.01-1.05)	.0016	1.02 (1.00-1.04)	.10
Number of teeth[a]	0.92 (0.67-1.22)	.59	0.74 (0.61-0.88)	.0013	0.68 (0.54-0.84)	<.001
Smoking history (former and present vs never)	8.97 (1.62-67.4)	.018	3.27 (1.23-9.03)	.019	2.40 (0.76-7.92)	.14
Toothbrushing frequency (≤2 vs ≥3)	2.26 (0.50-11.3)	.29	2.14 (1.01-4.62)	.048	1.78 (0.76-4.18)	.18
Additional oral care method (no vs yes)	18.0 (2.80-212.7)	.008	2.27 (1.04-5.16)	.043	1.55 (0.66-3.78)	.32
IL-1α (GT & TT vs GG)	1.36 (0.17-29.4)	.80	0.40 (0.14-1.15)	.081	0.33 (0.11-1.02)	.047
IL-1β (CT vs CC)	0.38 (0.01-12.8)	.55	3.95 (0.70-32.1)	.14	6.48 (0.85-137.0)	.12
TNF-α (CA & AA vs CC)	11.7 (1.72-154.5)	.028	1.44 (0.63-3.46)	.39	0.93 (0.38-2.39)	.88

[a]These variables are added as continuous variables in the logistic regression model.

[b]All chronic and aggressive periodontitis cases are assigned as "Case" status in logistic regression analyses.

[c]Controls without periodontitis and slight chronic periodontitis are assigned as "Control" status, and moderate/severe chronic and aggressive periodontitis are assigned as "Case" status in logistic regression analyses.

[d]Slight chronic periodontitis cases are assigned as "Control" status, and moderate/severe periodontitis cases are assigned as "Case" status in logistic regression analyses.

was employed with the adjustment for age, sex, smoking history, number of teeth, plaque index, additional oral care, and toothbrushing frequency per day (Table 4). In the association with periodontitis susceptibility, heterozygous or homozygous genotype with minor allele (CA & AA) of TNF-α −863 SNP (rs1800630) showed a higher risk of susceptibility to periodontitis compared to homozygous genotype with the major allele (CC) (OR = 11.7, *P*-value = 0.028) with statistical significance only within the subjects with self-reported

diseases. On the other hand, plaque index and smoking status were consistently noticed as significant risk factors of periodontitis in all three datasets including all subjects or subjects with/without self-reported diseases. In the association analyses with periodontitis severity with Model I where both controls and slight chronic periodontitis were designated as "Control" status and all other periodontitis (moderated, severe, and aggressive) as "Case" status, a higher risk of more severe periodontitis was observed among the subjects with genotype CT of IL-1β + 3954 SNP (rs1143634) compared to those with genotype CC (OR = 3.46 and p-value = 0.034), which was consistently observed within the subjects without self-reported disease (OR = 7.30, *P*-value = .019). Furthermore, all these significant associations were consistently observed when we repeated the analysis after excluding 26 subjects with aggressive periodontitis (Table S1). In addition, the higher risk of advanced periodontitis with genotype CT of IL-1β + 3954 was also noticed in Model II where the analysis was confined to only the chronic periodontitis (Table 4).

On the other hand, although statistical significance was not achieved at 5% level in most of the association models, the genotype with minor allele T (GT or TT) of IL-1α + 4845 SNP (rs17561) was more likely to be associated with periodontitis severity with a reversed direction, that is, toward a lower risk of severe periodontitis (OR < 1) (Table 4), which was not congruent to previous studies.

4 | DISCUSSION

Although it has long been controversial, recent several huge meta-analyses including thousands of subjects stratified by ethnicity have suggested positive associations between periodontitis and polymorphisms of pro-inflammatory cytokine genes, IL-1α/β and TNF-α, in Asians.[9,10,23] Nevertheless, the relationship has not been extensively investigated in the Korean population. Thus, we evaluated the genetic association of periodontitis focusing on three SNPs, IL-1α + 4845, IL-1β + 3954, and TNF-α −863, in 548 Koreans including 135 controls without periodontitis, 387 patients with chronic periodontitis at various stages, slight to severe, and 26 aggressive periodontitis. In univariable association analysis, we did not observe any significant association between the polymorphisms and periodontitis. However, significant associations were detected between the polymorphisms of IL-1β + 3954 or TNF-α −863 and periodontitis after controlling for confounding risk factors by employing stratification according to self-reported disease status and multiple logistic regression model with adjustment for age, smoking status, and oral hygiene indices and behaviors (Table 4).

In the association of polymorphism of IL-1β + 3954 with periodontitis, the significant association was observed when the patients with slight chronic periodontitis were assigned to "Control" status together with controls without periodontitis, while more progressive periodontitis cases (moderate/severe chronic and aggressive periodontitis) were assigned to "Case" status (OR = 3.46 and *P*-value = .034 in Model I), which was consistently recognized when the analysis was confined to chronic periodontitis (OR = 7.30 and *P*-value = .019 in Model II) (Table 4). This might suggest that the genetic variation of IL-1β + 3954 is related to risk of more severe or advanced periodontitis. Alternatively, the result may imply that periodontally healthy state and slight periodontitis, occasionally being pathologically indistinguishable, might not be completely separated from each other because sometimes initial clinical attachment loss and alveolar bone loss are not sufficiently recognizable in slight periodontitis, making the diagnosis and evaluation difficult.[44]

Considering much younger age distribution in the controls (median age: 29) compared to the periodontitis cases (median age: 53) in the study population, the biased selection of controls might have nullified the effect of genetic factors, which was not fully overcome by statistical adjustment, resulting in no association between the investigated genetic variations and periodontitis in the association analysis of IL-1 with periodontitis susceptibility. Namely, it can be possibly speculated that a considerable proportion of the young controls with the risk allele may develop periodontitis later in their lives when they reach similar ages to the periodontitis subjects. This postulation seems to be partly supported by the result that significant association between periodontitis and polymorphism of TNF-α −863 was observed only in the analysis of the subjects with self-reported diseases, in which age distribution of controls (median age: 55) was not significantly different from that of periodontitis cases (median age: 57) although the sample size of controls was extremely small (n = 13) (Table 3). Thus, further studies with age-matched controls are needed to confirm the significant association of IL-1β + 3954 polymorphisms with periodontitis. In addition, considering the small proportion of subjects with self-reported diseases in the controls [7 in 23 controls with age ≥ 40 (30%)] compared to those in the periodontitis group [201 in 355 subjects with age ≥ 40 (57%)], a larger sample size is likely to be needed for the control group, that is, ratio of controls to cases ≈ 2, even after controlling the age factor with age-matched controls.

TABLE 5 Minor allele frequencies (MAFs) in various ethnic groups in The Genome Aggregation Database (gnomAD)

Population	East Asian	Ashkenazi Jewish	European (Finnish)	South Asian	European (non-Finnish)	Latino	African
IL-1α + 4845 G/T (rs17561)	0.0747	0.3398	0.3086	0.3079	0.2999	0.2336	0.1760
IL-1β + 3954 C/T (rs1143634)	0.0194	0.2805	0.2442	0.1636	0.2402	0.1133	0.1301
TNF-α −863 C/A (rs1800630)	0.1755	0.1207	0.1587	—	0.1551	0.1182	0.1088

On the other hand, the age of the patients with aggressive periodontitis (median age: 40) was younger than that with chronic periodontitis (median age: 54) (*P*-value < .001 in Mann-Whitney U test). It had been widely accepted that aggressive type periodontitis occurs at a young age. However, age itself was not suggested as an important criterion in diagnosis of aggressive periodontitis in the 1999 AAP classification because patients with aggressive type periodontitis were also found in an older age.[26] Furthermore, more recently, the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions agreed that chronic and aggressive periodontitis are to be grouped as a single disease category because both are regarded as a common end result from the pathophysiologic viewpoint.[45] Congruently, the age distribution of aggressive periodontitis in this study is far from young age of onset. Rather, it is close to the age distribution of slight periodontitis (Table 2) although the median age is younger than that of all patients with chronic periodontitis."

The IL-1β + 3954 T allele was observed to be related to higher cytokine production of IL-1β and greater severity of systemic inflammation.[46] In addition, significant associations have been reported between the polymorphisms of IL-1 or TNF-α genes and periodontitis in patients with a certain systemic disease. For example, several studies showed that T allele of IL-1β + 3954 has a trend toward association with a higher risk of chronic periodontitis in patients with vascular disease.[47,48] Similarly, genetic variants of TNF-α −308 and −238 were associated with periodontal conditions in patients with coronary heart disease.[49] Accordingly, we applied logistic regression analysis after stratifying the data into sub-datasets based on disease classification in Table 1. Due to small sample size of controls in the sub-datasets, the analysis was conducted only in two sub-datasets, cardiovascular disease and hyperlipidemia. We observed only a marginally significant association between TNF-α −863 and periodontitis severity in Model I (OR = 6.34, *P*-value = .075) within the subjects with hyperlipidemia. No significant result was detected in associations involving IL-1 SNPs (data not shown), implying that considerably large samples are required to explore the more detailed effect modification of disease factor in the association of genetic polymorphisms and periodontitis, particularly for IL-1 SNPs, considering the low allele frequencies in Koreans. In fact, the minor allele frequencies (MAFs) of the two IL-1 SNPs, IL-1α + 4845 G/T (rs17561) and IL-1β + 3954 C/T (rs1143634), in East Asians are observed to be much lower than those in other ethnic groups, whereas the MAF of TNF-α −863 C/A (rs1800630) in East Asians shows the highest frequency among all ethnic groups in gnomAD database (Table 5).

In conclusion, our findings suggest that genetic variations of IL-1β + 3954 and TNF-α −863 are associated with increased risk of periodontitis in Koreans. The statistically significant associations were detected only after controlling for important confounding risk factors of periodontitis including disease status, age, smoking status, and oral hygiene indices and behaviors. Considering the very low frequency of risk allele of IL-1β + 3954 and big difference in age distribution between controls and periodontitis cases in the current study population, future, large well-designed studies are recommended to validate the polymorphisms as potential genetic risk factors in the Korean population, which may provide a better understanding of complex interactions between genetic factors, periodontal status, and systemic diseases as well.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article is reported.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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