Interleukin-8 -251A/T polymorphism and periodontitis susceptibility: a meta-analysis

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ABSTRACT. The -251A/T polymorphism in the anti-inflammatory cytokine interleukin-8 (*IL-8*) gene has been implicated in susceptibility to periodontitis; however, this correlation has not been elucidated. In this meta-analysis, we investigated the association between the *IL-8* -251A/T polymorphism and the risk of periodontitis. All eligible case-control studies published until August 2014 were identified and extracted from PubMed, Web of Science, EMBASE, China National Knowledge Internet, and WanFang databases. The strength of this association was accessed by pooled odds ratios (ORs) with 95% confidence intervals (CIs), using either a fixed- or random-effect model. Nine case-control studies, including 1811 cases and 2043 controls, were identified. Overall, no significant associations were found between the *IL-8* -251A/T polymorphism and the risk of periodontitis. The results of the analysis of periodontitis subgroup revealed similarities between chronic periodontitis and aggressive periodontitis. An additional analysis based on ethnicity revealed an association between the *IL-8* -251A/T polymorphism and periodontitis among Asians (dominant model, OR = 1.784, 95%CI = 1.130-2.817) and a mixed population (AA

vs TT, OR = 0.667, 95%CI = 0.471-0.974). The results of this meta-analysis suggest that the *IL-8* -251A/T polymorphism may increase the risk of periodontitis in Asian and mixed populations. However, larger and well-designed studies are warranted to validate our findings.

Key words: IL-8; Polymorphism; Periodontitis; Meta-analysis

INTRODUCTION

Periodontitis represents a set of inflammatory diseases affecting tissues that surround and support the teeth (Armitage, 1999). In cases of bacterial infection, periodontal tissues become inflamed and are destroyed by the inflammatory process. If left untreated, the infected teeth lose the support of the alveolar bone, become mobile, and are eventually lost (Loos et al., 2005). Periodontitis can be divided into chronic periodontitis (CP) and aggressive periodontitis (AgP), both of which affect a large percentage of the population (Dye, 2012).

Periodontitis is a complex human disease, among the other diseases such as Alzheimer's disease, Crohn's disease, diabetes, and cardiovascular disease (Tabor et al., 2002). The phenotype of complex diseases is a result of both genetic and environmental factors. Although pathogenic bacteria and various environmental factors, such as smoking and stress, are involved in the pathogenesis of periodontitis, genetic factors are also represented in its etiology (Michalowicz et al., 1991, 2000; Borrell and Papapanou, 2005). Several researchers have attempted to elucidate the role of genes and their variants (polymorphisms) in host responses to periodontitis and disease progression (Laine et al., 2010). Cytokines play an imperative role in tissue deterioration in periodontal diseases, and genetic polymorphisms within these genes are considered to be key inducers of periodontal disease (Yoshie et al., 2007).

Interleukin-8 (IL-8) is a chemokine often associated with inflammation. Many studies have shown that IL-8 expression is increased in affected periodontal tissues; moreover, high levels of IL-8 have been found in the crevicular fluid of patients with periodontitis (Tamura et al., 1992; Takigawa et al., 1994; Tsai et al., 1995; Dongari-Bagtzoglou and Ebersole, 1998). These results suggest that the *IL-8* gene may be an important candidate gene for periodontitis association studies. Several human diseases, including oral squamous cell carcinoma, breast cancer, bronchiolitis, macular degeneration, gastric cancer, and prostate cancer, are associated with a single nucleotide polymorphism in the *IL-8* gene at position -251A/T (rs4073) (Hull et al., 2000; Garza-Gonzalez et al., 2007; Kamali-Sarvestani et al., 2007; Vairaktaris et al., 2007; Goverdhan et al., 2008). A previous case-control study attempting to identify the differences in frequencies of the *IL-8* -251A/T polymorphism between patients and healthy controls revealed no such associations (Kim et al., 2009). This was followed by several epidemiological studies assessing the association between the *IL-8* -251A/T polymorphism and either CP or AgP, with conflicting results (Andia et al., 2011; Jing et al., 2011; Li et al., 2012; Andia et al., 2013; Borilova Linhartova et al., 2013; Khosropanah et al., 2013; Sippert et al., 2013; Zhang, et al., 2014). This could be attributed to the relatively small sample size or geographic difference in each of the published studies. Hence, we performed a meta-analysis to investigate the association between the *IL-8* -251A/T polymorphism and susceptibility to chronic/aggressive periodontitis.

MATERIAL AND METHODS

Search strategy

PubMed, Web of Science, EMBASE, China National Knowledge Internet, and WanFang databases (http://www.wanfangdata.com.cn) were searched for studies prior to August 27, 2014 pertaining to the following key subjects: "periodontal diseases", "periodontitis", "IL-8", "interleukin-8", ''polymorphism", "variant", and a combination of these phrases. Additional studies were identified through a search of references in the extracted original or review articles. Moreover, the search results were restricted to human populations and language restrictions were not applied in order to avoid selection bias. The eligibility criteria were as follows: a) studies evaluating the -251A/T polymorphism within *IL-8* and chronic/aggressive periodontitis risk; b) studies with a case-control design; and c) studies providing sufficient data for the estimation of the odds ratio (OR). Studies were excluded: a) in case of case-only studies, case reports, or review articles; b) when the number of wild and null genotypes could not be ascertained; and c) when the genotypic distribution of the control population was not in accordance with the Hardy-Weinberg equilibrium (HWE).

Data extraction

The following data was collected from each study: name of the first author, published data, country of origin, ethnicity (Caucasian, Asian, mixed), periodontitis type (chronic or aggressive), genotyping methods, sample size (cases/controls), genotypic distributions, and the HWE status of the controls. The required data was extracted from each article individually by two authors using a structured sheet, and entered into a database. Discrepancies were adjudicated by an additional author until a consensus was reached.

Statistical analysis

The correlation between risk of periodontitis and the *IL-8* -251A/T polymorphism was estimated for each study after determining the ORs and 95%CIs. Four ORs were calculated: dominant model (AT+AA *vs* TT), recessive model (AA *vs* AT+TT), heterozygote comparison (AT *vs* TT), and homozygote comparison (AA *vs* TT). In the secondary analysis, the ORs and 95%CIs were calculated with respect to the ethnic group (Caucasian, Asian, mixed) and periodontitis type (CP, AgP). The between-study heterogeneity was assessed using Cochran's Q statistic (Higgins and Thompson, 2002). When a significant Q test result ($P > 0.1$) indicated homogeneity across studies, the fixed-effect model (Mantel-Haenszel method) was used; otherwise, the random-effect model (DerSimonian and Laird method) was applied (DerSimonian and Laird, 1986). The effect of heterogeneity was also quantified by an I^2 test (Higgins and Thompson, 2002). The conformance of controls to the HWE was assessed by the chi-squared test. The stability of the meta-analysis results was assessed by a one-way sensitivity analysis (Ramos-Corpas and Santiago, 2006). Possible publication bias was evaluated using the Egger linear regression test by visual inspection of the funnel plot (Egger et al., 1997). All analyses were performed using the STATA 11.0 software (College Station, Texas, USA), using two-sided P values.

RESULTS

Study characteristics

Our search strategy led to the retrieval of 88 potentially relevant studies. Nine studies that corresponded to the selection criteria (Kim et al., 2009; Andia et al., 2011; Jing et al., 2011; Li et al., 2012; Andia et al., 2013; Borilova Linhartova et al., 2013; Khosropanah et al., 2013; Sippert et al., 2013; Zhang et al., 2014) were recruited for the final analysis (Figure 1). The name of the first author, published data, country of origin, ethnicity, periodontitis type, genotyping methods, sample size (cases/controls), genotypic distributions, and HWE status among controls of each study are listed in Table 1. Two studies (Andia et al. 2011, 2013) examined the same control population. Therefore, these two studies were combined for the overall analysis. In total, 9 studies comprising 1,811 cases and 2,043 controls were included in the meta-analysis of the *IgG* -251A/T polymorphism of either CP or AgP (Kim et al., 2009; Jing et al., 2011; Li et al., 2012; Andia et al., 2011, 2013; Borilova Linhartova et al., 2013; Khosropanah et al., 2013; Sippert et al., 2013; Zhang et al., 2014). Among the nine studies, six (Kim et al., 2009; Andia et al., 2011; Li et al., 2012; Khosropanah et al., 2013; Sippert et al., 2013; Zhang et al., 2014) described CP cases, two studies (Jing et al., 2011; Andia et al., 2013) described AgP cases, and the remaining (Borilova Linhartova et al., 2013) was conducted in both CP and AgP cases. Four of the nine studies (Kim et al., 2009; Andia et al., 2011, 2013; Sippert et al., 2013) focused on a Brazilian population (mixed), three (Jing et al., 2011; Li et al., 2012; Zhang et al., 2014) focused on a Chinese population (Asian), and the remaining two studies (Borilova Linhartova et al., 2013; Khosropanah et al., 2013) focused on Caucasians. Genotypic distributions among the controls of all studies were consistent with HWE ($P > 0.05$) (Kim et al., 2009; Jing et al., 2011; Li et al., 2012; Andia et al., 2011, 2013; Borilova Linhartova et al., 2013; Khosropanah et al., 2013; Sippert et al., 2013; Zhang et al., 2014).

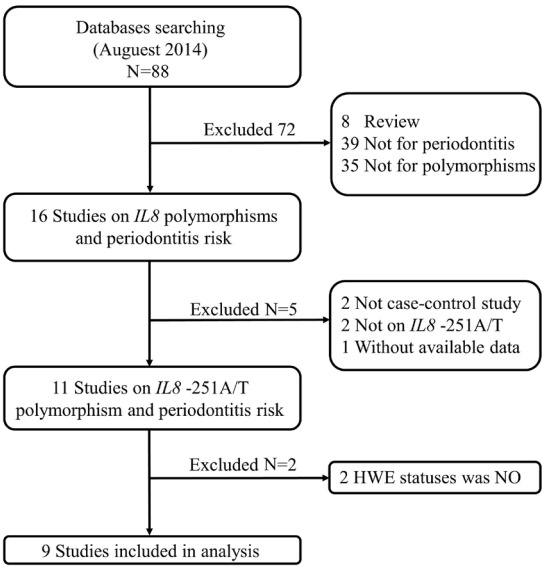

Figure 1. Flow chart depicting the study selection procedure.

Table 1. Characteristics of studies included in the meta-analysis.

First author name	Year	Country	Ethnicity	Periodontitis type	Genotyping method	Case	Control	Genotypes distribution						HWE
								Case			Control			
								AA	AT	TT	AA	AT	TT	
Kim	2009	Brazil	Mixed	CP	SSP-PCR	268	220	56	146	66	36	120	64	Y
Andia	2011	Brazil	Mixed	CP	PCR-RFLP	181	108	21	135	25	13	57	38	Y
Li	2011	China	Asian	AgP	PCR-RFLP	77	50	15	36	26	11	20	19	Y
Li	2012	China	Asian	CP	PCR-RFLP	122	532	35	27	60	121	264	147	Y
Khosropanah1	2013	Iran	Caucasian	CP	ASPCR	227	40	41	101	85	12	17	11	Y
Andia	2013	Brazil	Mixed	AgP	PCR-RFLP	76	108	11	50	15	13	57	38	Y
Sippert	2013	Brazil	Mixed	CP	PCR-RFLP	124	187	28	62	34	42	92	53	Y
Linhartova	2013	Czech Republic	Caucasian	CP	TaqMan	278	156	63	120	95	29	78	49	Y
				AgP		58	156	13	27	18	29	78	49	
Zhang	2014	China	Asian	CP	MALDI-TOF MS	400	750	7	177	152	140	387	223	Y

AgP = aggressive periodontitis; CP = chronic periodontitis; SSP-PCR = sequence-specific primer polymerase chain reaction; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; ASPCR = allele-specific polymerase chain reaction; MALDI-TOF MS = matrix-assisted laser desorption/ionization time of flight mass spectrometry; HWE = Hardy-Weinberg equilibrium; Y = yes.

Quantitative data synthesis

Overall, we observed no significant associations between the *IL-8* -251A/T polymorphism and periodontitis risk (dominant model, OR = 1.095, 95%CI = 0.701-1.708; recessive model, OR = 0.781, 95%CI = 0.453-1.347; AT *vs* TT: OR = 1.095, 95%CI = 0.685-1.752; AA *vs* TT: OR = 1.321, 95%CI = 0.682-2.557) (Table 2). Upon examination of the periodontitis type subgroup, similar results were observed in both CP and AgP in all genetic models (Table 2). Subgroup analysis with respect to ethnicity revealed that the *IL-8* -251A/T polymorphism is associated with periodontitis in Asians (dominant model, OR = 1.784, 95%CI = 1.130-2.817) (Table 2) (Figure 2A) and a mixed population (AA *vs* TT, OR = 0.667, 95%CI = 0.471-0.974) (Table 2) (Figure 2B).

Heterogeneity and sensitivity analyses

Substantial heterogeneities were observed among studies aiming to demonstrate an association between the *IL-8* -251A/T polymorphism and periodontitis risk under all genetic models (dominant model, $I^2 = 87.2\%$, $P < 0.001$; recessive model, $I^2 = 85.3\%$, $P < 0.001$; AT *vs* TT: $I^2 = 86.7\%$, $P < 0.001$; AA *vs* CC: $I^2 = 87.3\%$, $P < 0.001$) (Table 2). Next, we assessed the source of heterogeneity in all genetic models by comparing the ethnicity and periodontitis type. The heterogeneity was partly decreased in the mixed population, Caucasians, and AgP in some models. However, significant heterogeneity was observed in Asians and those with CP (Table 2).

The stability of the results of this meta-analysis were validated by conducting a sensitivity analysis after removing studies individually. The statistical significance of the results was not altered when any single study was omitted, confirming the stability of the results (Figure 3).

Publication bias

Begg's funnel plot and the Egger test were performed to determine any publication bias among the included studies. Shapes of the funnel plot for the dominant model of *IL-8* -251A/T did not reveal any obvious asymmetry (Figure 4). The Egger test also confirmed no statistical significance for a publication bias (dominant model, P = 0.211; recessive model, P = 0.517, AT *vs* TT: P = 0.211, AA *vs* TT: P = 0.534).

DISCUSSION

IL-8 is a member of the chemokine family that is mainly involved in the initiation and amplification of acute inflammatory reactions and chronic inflammatory processes (Tamura et al., 1992; Takigawa et al., 1994; Dongari-Bagtzoglou and Ebersole, 1998; Campa et al., 2005). Therefore, it is biologically plausible that genetic variations within *IL-8* could modulate the risk of periodontitis. A study published in 2009 first reported that the *IL-8* -251A/T polymorphism lacked any association with periodontitis susceptibility (Kim et al., 2009). Several investigators then duplicated the work in different populations. However, the results were contradictory. No consensus has been reached regarding the correlation between *IL-8* -251A/T and periodontitis risk, even within the same population.

Table 2. Association between *IL8* -251A/T polymorphism and risk of periodontitis.

	N[a]	AT/AA *vs* TT (dominant)			AT/TT *vs* AA (recessive)			AT *vs* TT			AA *vs* TT		
		OR (95%CI)	P[b]	I^2	OR (95%CI)	P[b]	I^2	OR (95%CI)	P[b]	I^2	OR (95%CI)	P[b]	I^2
Total	8	1.095 (0.701-1.708)	0.000	87.2	0.781 (0.453-1.347)	0.000	85.3	1.095 (0.685-1.752)	0.000	86.7	1.321 (0.682-2.557)	0.000	87.3
Ethnicity													
Mixed	3	0.643 (0.358-1.156)	0.010	78.2	1.161 (0.849-1.587)	0.680[c]	0.0	0.645 (0.341-1.220)	0.007	79.9	**0.667 (0.471-0.974)**	0.290[c]	19.2
Asian	3	**1.784 (1.130-2.817)**	0.035	70.2	0.483 (0.081-2.864)	0.000	95.1	1.726 (0.773-3.853)	0.000	87.5	2.689 (0.543-13.319)	0.000	93.1
Caucasian	2	1.205 (0.844-1.720)	0.412[c]	0.0	0.852 (0.350-2.074)	0.045	74.0	1.240 (0.846-1.818)	0.897[c]	0.0	1.314 (0.527-3.281)	0.078	67.8
Periodontitis type													
CP	7	1.120 (0.688-1.822)	0.000	88.6	0.760 (0.414-1.397)	0.000	87.4	1.130 (0.673-1.897)	0.000	88.4	1.370 (0.656-2.861)	0.000	89.0
AgP	3	0.716 (0.482-1.064)	0.252[c]	27.6	1.121 (0.698-1.800)	0.774[c]	0.000	0.708 (0.466-1.077)	0.234[c]	31.2	0.742 (0.433-1.272)	0.537[c]	0.0

OR = odds ratio; CI = confidence interval. [a]Number of comparison. [b]P value of Q-test for heterogeneity test. [c]Fixed-effect model was used when P value for heterogeneity test > 0.1; otherwise, random-effect model was used. Bold values represent significant values of OR.

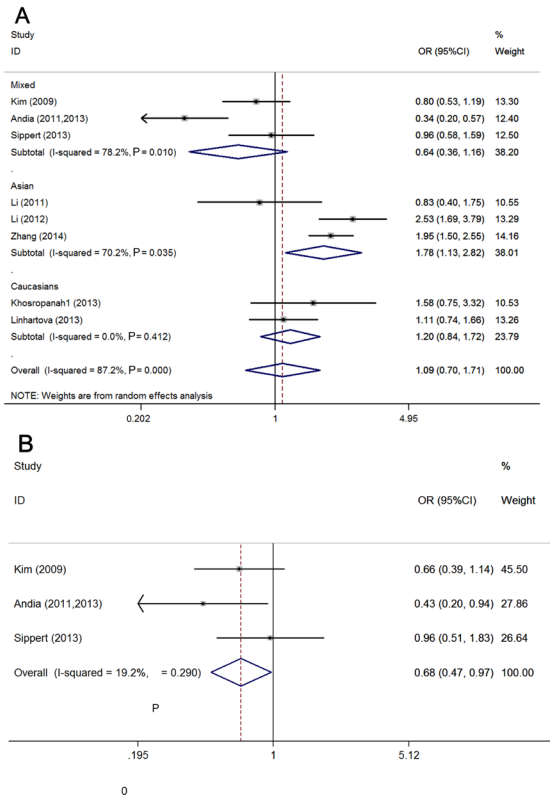

Figure 2. Meta-analysis of the association between *IL-8* -251A/T and periodontitis susceptibility. **A.** Forest plot of published case-control association studies of the -251A/T polymorphism (dominant model) used in the overall analysis. **B.** Forest plot of published case-control association studies of the -251A/T polymorphism (AA *vs* TT) in a mixed population.

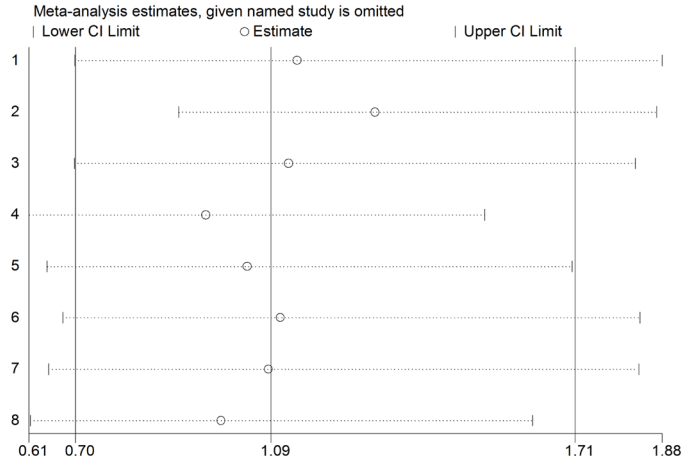

Figure 3. Sensitive analyses in the overall analysis (dominant model).

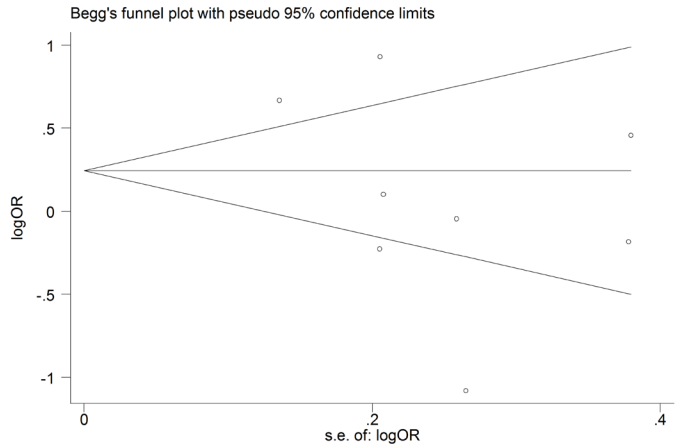

Figure 4. Begg's funnel plot for publication bias (dominant model).

A meta-analysis, which may be able to overcome the shortcomings of individual studies by systematically combining results from each study, increases the power to detect an association, as well as the precision of the magnitude of effect, and could shed light on the reasons for discrepancy among results by exploring the heterogeneity among the results (Ioannidis et al., 2001). Thus, we performed a meta-analysis of all eligible studies to derive a precise estimation of the association between *IL-8* -251A/T and periodontitis risk.

A total of 1811 cases and 2043 controls were analyzed to further investigate the possible association between the *IL-8* -251A/T polymorphism and periodontitis risk. The pooled data revealed no significant association between the *IL-8* -251A/T polymorphism and periodontitis risk. Furthermore, we failed to detect any association between the -251A/T polymorphism and risk of CP or AgP in the subgroup analysis by periodontitis type. However, when stratified by ethnicity, the *IL-8* -251A/T polymorphism appeared to be associated with periodontitis in Asians (dominant model, OR = 1.784, 95%CI = 1.130-2.817) and a mixed population (AA *vs* TT, OR = 0.667, 95%CI = 0.471-0.974). However, the same association was not observed in Caucasians. These results could be attributed to two reasons: the frequencies of the genetic marker of interest may also show large heterogeneity between races (Ioannidis et al., 1998); and the difference between the -251A/T allele frequency in different study populations may result in its association with periodontitis in Asians and mixed populations, and not in Caucasians. This may be due to a lack of statistical power, as only two studies conducting a subgroup analysis on Caucasians was included in this study. These results may change with the increase in number of studies in the future.

The statistical synthesis of gene-periodontitis association studies is subject to many biases, caused by the limited number of subjects enrolled in individual studies, an inappropriate selection of controls, heterogeneity in the definition of periodontitis, and the performance of multiple tests (Borrell and Papapanou, 2005). Similar to other complex diseases, it is estimated that 10 to 50 genes with several major master genes may be involved in periodontitis. Therefore, it would be more efficient to simultaneously analyze a large sample size for multiple gene polymorphisms (Yoshie et al., 2007). Meta-analyses cannot correct all biases of individual studies; however, it could be possible to generate a statistical conclusion with larger power and precision. Although no evidence of publication bias was found by Begg's rank correlation

or Egger weighted regression methods, great effort was taken to limit bias by avoiding any form of quality scoring, searching for reports not included in electronic databases, assessing the effect of HWE violations, applying multivariate meta-analytic techniques, performing statistical tests for detecting publication bias, and evaluating the existence of a time trend in the summary estimates (Deng et al., 2011).

Some shortcomings of this analysis should be discussed. First, periodontitis is a multifactorial disease, and interactions between gene-gene, gene-environment, and even different polymorphic loci of the same gene may have an effect on the genetic association with periodontitis phenotypes (Dimou et al., 2010). However, we were unable to conduct genotype-stratified analyses because of the lack of detailed original data in the eligible studies. Secondly, a difference in diagnostic criteria and severity of disease among eligible studies could influence the results described herein. Therefore, these results should be interpreted with caution.

In conclusion, our study provides the first indication that the *IL-8* -251A/T polymorphism may be associated with periodontitis in Asian and mixed populations. These findings may help increase our understanding of *IL-8* in the etiology of periodontitis. However, larger and better-designed studies are warranted to validate our findings. Moreover, additional gene-gene and gene-environment interactions should also be considered, which could ensure a better, more comprehensive understanding of the association between *IL-8* polymorphisms and periodontitis risk.

Conflicts of interest

The authors declare no conflict of interest.

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