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Interleukin-6 polymorphisms modify the risk of periodontitis: a systematic review and meta-analysis

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Abstract: Objective: To clarify the association of IL-6 polymorphisms and periodontitis, a meta-analysis of case-control studies and a systemic review were conducted. Material and methods: We performed a literature search using PubMed and Medline database to May 2009, with no restrictions. We also reviewed references from all retrieved articles. Six case-control studies involving 1093 periodontitis cases and 574 controls were selected for meta-analysis to assess the purported associations between IL-6 polymorphisms and the risk of periodontitis. IL-6 −174 G/C and −572 C/G polymorphisms were included in the present meta-analysis, and the association between IL-6 −6331 T/C polymorphism and the risk of periodontitis was adequately reviewed as well. Results and conclusion: The present meta-analysis indicates that the IL-6 −174 G allele could not modify the risk of chronic periodontitis, but increased the risk of aggressive periodontitis. And −572 C/G polymorphism is associated with the pathogenesis of periodontitis, including chronic periodontitis or aggressive periodontitis.

Key words: Interleukin-6, Polymorphism, Periodontitis, Risk, Meta-analysis
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INTRODUCTION

Periodontitis is an opportunistic inflammatory disease of the periodontium. It is widely regarded as one of the most common diseases worldwide, with a prevalence of 10%~15% (Albandar and Rams, 2002). The pathogenesis of periodontitis involves more than virulent microorganisms (D'Aiuto *et al*., 2005; Kornman, 2008; Salvi *et al*., 2008). The systemic immune response, genetic factors, and environmental factors also affect the risk of developing periodontitis (Agrawal *et al*., 2006; Corey *et al*., 1993; van Dyke and Sheilesh, 2005). In recent years, studies have demonstrated that periodontitis is associated with elevated levels of a variety of inflammatory biomarkers (Loos, 2005; Loos *et al*., 2000; Shi *et al*., 2008; Slade *et al*., 2003). Furthermore, genetic variants of some cytokines confer susceptibility to periodontitis (Holla *et al*., 2008; Nibali *et al*., 2008a; Trevilatto *et al*., 2003).

Interleukin-6 (IL-6) is produced by many types of cells and is one of the most important mediators of the inflammatory response, in which it may play proinflammatory or anti-inflammatory role (Seruga *et al*., 2008; Woods *et al*., 2000). Several inflammatory diseases, including periodontitis, have been associated with elevated levels of IL-6 (Kishimoto, 2006; Loos *et al*., 2000). As the −174 G/C and −572 C/G polymorphisms of the IL-6 gene increase IL-6 expression (Fishman *et al*., 1998; Gu *et al*., 2008), they may be associated with susceptibility to periodontitis.

Trevilatto *et al*.(2003) first investigated the association between the IL-6 −174 G/C polymorphism and chronic periodontitis, and observed differences between controls and periodontitis patients in genotype and allele frequencies. Subsequently, many studies were published on whether the IL-6 −174 G/C polymorphism predisposes to periodontitis, but the results are contradictory (Babel *et al*., 2006; Moreira *et al*., 2007; Nibali *et al*., 2009). A similar issue exists

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concerning the relationship between the −572 C/G polymorphism and periodontitis (Holla *et al*., 2004; Komatsu *et al*., 2005; Nibali *et al*., 2009). As statistical power is limited by small sample sizes in case-control studies, we conducted a meta-analysis using pooled published data to examine the association between IL-6 polymorphisms and the risk of periodontitis.

MATERIALS AND METHODS

Search strategy

To identify all studies on the association between IL-6 polymorphisms and periodontitis, we conducted a systematic search of literature published before May 2009 using the Medline database (US National Library of Medicine, Bethesda, Maryland) and PubMed (National Center for Biotechnology, National Library of Medicine) and the key words, interleukin-6, IL-6, polymorphism, polymorphisms, and periodontitis. The full texts of the candidate articles were examined to determine whether they contained sufficient information on the association of the IL-6 −174 G/C and −572 C/G polymorphisms and the risk of periodontitis. Furthermore, references cited in the retrieved articles were screened to trace additional relevant studies.

Inclusion criteria

Articles were included in the meta-analysis if they met all the following criteria: (1) unrelated case-control experimental design, (2) genotype frequency documentation, and (3) frequency distributions for genotypes that do not deviate from Hardy-Weinberg equilibrium (HWE).

Data extraction

Two investigators independently reviewed all studies and extracted the data using a standardized form. Data were collected on the authors, year of publication, country of origin, study design (hospital based, population based, or nested), numbers of cases and controls with GG, GC, or CC genotypes, and type of periodontitis (Table 1).

Statistical analysis

HWE analysis for genotype distribution among cases and controls was carried out using Pearson's goodness-of-fit chi-squared test. The chi-square-based Q statistic test (Lau *et al*., 1997), which measures homogeneity between studies, was used to assess the presence of heterogeneity. A value of 0% for I^2 indicates the absence of heterogeneity and increasing percentages indicate increasing heterogeneity (Higgins *et al*., 2003). When heterogeneity was not significant, the results were pooled using a fixed effect model and the Mantel-Haenszel method, or a random effect model and the DerSimonian and Laird method (Petitti, 1994). Funnel plots were used to examine the publication bias of reported associations. The strength of associations between IL-6 polymorphisms and periodontitis was assessed according to the odds ratio (OR), which was calculated using the method of Woolf (1955). The significance of the pooled OR was determined using the *Z*-test. Analyses were performed using Review Manage version 5.0 software (RevMan, Oxford, England). A P value of <0.05 was considered statistically significant.

RESULTS

Eleven studies focusing on the topic of the association of IL-6 polymorphisms with priodontitis were detected (Babel *et al*., 2006; Brett *et al*., 2005; Holla *et al*., 2004; Jansson *et al*., 2006; Komatsu *et al*., 2005; Moreira *et al*., 2007; Nibali *et al*., 2008b; 2009; Tervonen *et al*., 2007; Trevilatto *et al*., 2003; Wohlfahrt *et al*., 2006). Of the 11 studies, 10 containing genotyping data were listed in Table 1. However, three studies were excluded from the present analysis because the number of individuals who carried a GG or CC genotype was not supplied (Babel *et al*., 2006; Jansson *et al*., 2006; Tervonen *et al*., 2007). Two studies (Brett *et al*., 2005; Nibali *et al*., 2008b) were also excluded, as they have overlapping patients with the study performed by Nibali *et al*.(2009), as clearly described in the paper. Finally, six studies composing of 1093 periodontitis patients and 574 controls met the inclusion criteria. None of the frequency distributions for the genotypes studied deviated from the HWE of the controls (data not shown).

IL-6 −174 G/C polymorphism and periodontitis

For IL-6 −174 G/C polymorphism, there are overlapping patients among three studies (Brett *et al*., 2005; Nibali *et al*., 2008b; 2009). Therefore, only the

Table 1 Characteristics of studies focusing on IL-6 polymorphisms and periodontitis

Study	Country	Study design	Case (GG/GC/CC)	Control (GG/GC/CC)	Form of disease
−174 polymorphism					
Trevilatto *et al*.(2003)	Brazil	Hospital c/c	29/15/4	12/21/3	Chronic
Holla *et al*.(2004)	Czech	Population c/c	43/71/34	37/53/17	Chronic
Brett *et al*.(2005)	UK	Population c/c	52/37/17	55/19/25	Aggressive, chronic
Wohlfahrt *et al*.(2006)	US	Hospital c/c	48/63/26	32/36/14	Chronic
Babel *et al*.(2006)	Germany	Population c/c	72*/52	84*/32	Chronic
Moreira *et al*.(2007)	Brazil	Hospital c/c	98/48/9	30/18/6	Aggressive, chronic
Tervonen *et al*.(2007)	Finland	Population c/c	11/40†	37/141†	Chronic
Nibali *et al*.(2008b)	UK	Hospital c/c	139/66/17	110/91/30	Aggressive
Nibali *et al*.(2009)	UK	Hospital c/c	273/166/54	102/87/29	Aggressive, chronic
−572 polymorphism					
Holla *et al*.(2004)	Czech	Population c/c	139/9/0	86/21/0	Chronic
Komatsu *et al*.(2005)	Japan	Hospital c/c	5/36/71	4/32/41	Chronic
Nibali *et al*.(2009)	UK	Population c/c	11/36/220	3/30/185	Chronic

c/c: case/control; * Total number of individuals carrying GG or GC genotype; † Total number of individuals carrying GC or CC genotype

last study (Nibali *et al*., 2009) was included in the overall analysis, as it contains information on all of the above patients. Finally, five studies (Holla *et al*., 2004; Moreira *et al*., 2007; Nibali *et al*., 2009; Trevilatto *et al*., 2003; Wohlfahrt *et al*., 2006) composing of 981 periodontitis patients and 497 controls were involved in the following meta-analysis. Overall, no significant difference in allele frequency was found between the periodontitis cases and the healthy controls (OR: 1.13, 95% confidence interval (CI): 0.85~1.51). However, the pooled analysis showed strong heterogeneity (P=0.04, I^2=61%). This was caused by the study of Holla *et al*.(2004), which is an outlier in the funnel plots. After excluding this study, the heterogeneity of the meta-analysis decreased to be unremarkable (P=0.19, I^2=37%). As shown in Fig.1, the −174 G allele frequency was significantly higher for the pooled cases than for the corresponding controls (Z=2.30, P=0.02). The pooled OR was 1.24 (95% CI: 1.03~1.49), suggesting that carriers of the −174 G allele have a significantly higher risk of being predisposed to periodontitis. This result was confirmed by pooled analyses of genotypic frequency. Individuals carrying the GG genotype have a remarkably increased risk of periodontitis compared with individuals with the GC or CC genotype (OR: 1.35, 95% CI: 1.06~1.73, P=0.02), and there was no significant difference between study heterogeneities (P=0.12, I^2=49%). Of the 981 periodontitis patients and 497 controls mentioned above, 806 patients and 423 controls were Caucasians. The meta-analysis data were similar to the overall result: the −174 G allele frequency was significantly higher for the pooled cases than for the controls (P=0.04, OR: 1.24, 95% CI: 1.01~1.51). Furthermore, a significantly increased risk was associated with the GG genotype compared with the GC or CC genotype (OR: 1.39, 95% CI: 1.05~1.85, P=0.10 for heterogeneity). Overall, the IL-6 −174 G allele increased the risk of periodontitis.

Chronic periodontitis (CP) and aggressive periodontitis (AP) have distinct pathophysiological differences and may have different genetic predispositions; therefore, we performed separate analyses for the two forms of periodontitis. The data of Moreira *et al*.(2007) were excluded in the following meta-analysis, as they did not report the genotype data of CP subgroup or AP subgroup. Therefore, four studies (including 604 patients and 443 controls) were involved in the analysis of the association of IL-6 −174 G/C polymorphism with risk of CP. The pooled OR of the G allele versus the C allele was 0.98 (95% CI: 0.82~1.17, P=0.82) and there was no significant difference between study heterogeneities (P=0.11, I^2=50%) in the CP subgroup. In Caucasian population, the pooled analyses of allele frequency between CP cases and controls are similar (OR: 1.02, 95% CI: 0.84~1.23, P=0.07 for heterogeneity). Furthermore, there was no significant difference in genotypic frequency between the healthy controls and the CP cases (data not shown), suggesting that the −174 G allele

and the −174 G/C polymorphism do not confer a risk of CP. Up to date, only one case-control study on 224 AP patients and 231 healthy controls was performed in order to detect the association of IL-6 −174 G/C polymorphism with risk of AP (Nibali *et al*., 2008b). The genotyping result indicated that IL-6 −174 G allele significantly increased the risk of AP in comparison with −174 C allele in subjects of all ethnicities (OR: 1.69, 95% CI: 1.26~2.27, *P*=0.0005). Compared with GC or CC genotype, the genotype GG was associated with a diagnosis of AP (OR: 1.86, 95% CI: 1.28~2.70, *P*=0.001). Similar results were obtained in Caucasian patients and controls (data not shown). In brief, the IL-6 −174 G allele could not modify the risk of CP, but increased the risk of AP. The association of IL-6 −174 G/C polymorphism and periodontitis risk is summarized in Table 2.

IL-6 −572 C/G polymorphism and periodontitis

Three original investigations, including 527 patients and 402 controls, were involved in studies on the IL-6 −572 C/G polymorphism among CP cases (Holla *et al*., 2004; Komatsu *et al*., 2005; Nibali *et al*., 2009). The meta-analysis showed that individuals carrying the GG genotype have a remarkably increased risk of developing CP compared with individuals with the GC or CC genotype (OR: 2.65, *P*=0.002; 95% CI: 1.44~4.87, *P*=0.18 for heterogeneity). No significant difference was detected in allelic frequency distribution between CP cases and controls. However, the pooled analysis was strongly heterogeneous (*P*=0.004). This was caused by the study of Holla *et al*.(2004), which is an outlier in the funnel plots with a high OR. When this study was excluded, the heterogeneity of the meta-analysis decreased (*P*=0.07). The second meta-analysis also showed that there was no significant difference in allelic frequency distribution and genotypic frequency distribution (under the dominant or co-dominant genetic model) between CP patients and controls (data not shown). We did not conduct a meta-analysis of the association between the −572 C/G polymorphism and AP because only one relevant study was detected (Nibali *et al*., 2008b).

Study or subgroup	Experimental		Control		Weight (%)	OR (95% CI)
	Events	Total	Events	Total		
Trevilatto *et al*.(2003)	73	96	45	72	6.1	1.90 (0.98~3.72)
Wohlfahrt *et al*.(2006)	159	274	100	164	26.0	0.88 (0.60~1.31)
Moreira *et al*.(2007)	244	310	78	108	12.2	1.42 (0.86~2.35)
Nibali *et al*.(2009)	712	986	291	436	55.6	1.29 (1.02~1.65)
Total (95% CI)		1666		780	100.0	1.24 (1.03~1.49)
Total events	1188		514			

Heterogeneity: chi^2=4.79, *df*=3 (*P*=0.19); I^2=37%
Test for overall effect: *Z*=2.30 (*P*=0.02)

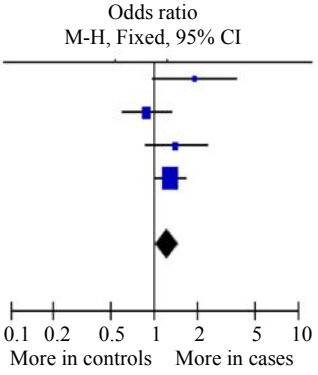

Fig.1 Meta-analysis of the association between the IL-6 −174 G allele and periodontitis
The numbers depicted under "events" and "total" represent the number of −174 G alleles out of all allele

Table 2 Association of IL-6 −174 G/C polymorphism and state of periodontitis*

State of periodontitis	G vs C				GG vs GC/CC			
	OR (95% CI)	*P*	I^2 (%)	$P^\dagger$	OR (95% CI)	*P*	I^2 (%)	$P^\dagger$
Chronic periodontitis	0.98 (0.82~1.17)	0.82	50	0.11	0.84 (0.59~1.18)	0.32	0	0.78
Aggressive periodontitis	1.69 (1.26~2.27)	0.0005	−	−	1.86 (1.28~2.70)	0.001	−	−
Overall	1.24 (1.03~1.49)	0.02	37	0.19	1.35 (1.06~1.73)	0.02	49	0.12

* Most subjects involved in meta-analysis were Caucasians; † *P* value for heterogeneity

DISCUSSION

It is widely accepted that cytokines and oral pathogens play central roles in the inflammatory process associated with the etiology of periodontitis (Taylor *et al*., 2004; Wu *et al*., 2007; He and Shi, 2009). As a pleiotropic cytokine, IL-6 is induced in response to several inflammatory stimuli (Terry *et al*., 2000). IL-6 also regulates the expression of cytokines such as IL-1, IL-10, and tumor necrosis factor-α (Opal and DePalo, 2000; Woods *et al*., 2000). IL-6 is present in endothelial cells, fibroblasts, and macrophages in periodontitis patients, but is absent from these cells in healthy individuals (Takahashi *et al*., 1994). Clinical studies indicate that IL-6 plays a crucial role in the inflammatory response to Gram-negative bacteria (Dalrymple *et al*., 1996) by affecting the composition of the subgingival microbiota and increasing the susceptibility to colonization with periodontopathogenic bacteria (Cooke and Hill, 2001; Ishihara *et al*., 1997; Coats *et al*., 2009). IL-6 is also a potent stimulator of osteoclast differentiation and bone resorption (Gemmell *et al*., 1997; Hughes *et al*., 2006), which are typical of periodontitis. The intensity of IL-6 expression is positively correlated with attachment loss (Moreira *et al*., 2007) and IL-6 is associated with continuous tissue destruction in periodontitis (McCauley and Nohutcu, 2002). Furthermore, levels of IL-6 are elevated in periodontitis patients (Loos, 2005; Loos *et al*., 2000) and decreased after successful periodontal therapy (D'Aiuto *et al*., 2004a; 2004b). Thus, the available evidence indicates that IL-6 is involved in the pathogenesis of periodontitis.

Because of the notion that genetic factors may predispose to periodontitis, several studies had been conducted to determine whether IL-6 gene polymorphisms predispose to periodontitis. However, the results of studies on the associations between these polymorphisms and clinical forms of periodontitis are contradictory. By and large, our meta-analysis showed that carriers of the −174 G allele had an increased risk of developing periodontitis. And specifically, the IL-6 −174 G allele could not modify the risk of CP, but increased the risk of AP. Furthermore, compared with −572 C allele carriers (genotypes GC and CC), individuals carrying the GG genotype had a significantly higher risk of CP. These findings suggest that common polymorphisms in the IL-6 promoter region modify the risk of periodontitis.

A recent study also reviewed the association between the IL-6 −174 G/C polymorphism and periodontitis (Nikolopoulos *et al*., 2008). It is difficult to compare their results directly with ours for three reasons. First, the previous review focused on the pathogenesis of CP, whereas we paid more attention to whether common IL-6 polymorphisms increase the risk of aggressive or chronic types of periodontitis. Second, our review is the more comprehensive one of the two, as we identified 6 studies on the association between IL-6 polymorphisms and periodontitis compared with four studies in the other report. Finally, our study is the first to summarize the association between the IL-6 −572 C/G polymorphism and periodontitis.

After the initial meta-analysis of the −174 G/C polymorphism and CP, a case-control study (Nibali *et al*., 2009) involving 271 CP patients and 144 controls was published and was included in a subsequent analysis. There was no significant association between this polymorphism and CP, which is in accordance with the results of the previous analysis (Nikolopoulos *et al*., 2008). Notably, in the study Caucasian Brazilians genotype GG was statistically associated with susceptibility to severe form of CP (Trevilatto *et al*., 2003). The result reminds us that the association of IL-6 −174 G/C polymorphism with severe form of CP should be paid more attention to in subsequent investigations. Moreover and interestingly, one association study performed by Nibali *et al*. (2008b), which involved 224 AP patients and 231 controls, revealed that the −174 G allele is statistically significantly associated with an increased risk of AP. Although the sample size was not large enough, the results nevertheless indicated that the IL-6 −174 G allele is associated with susceptibility to AP. Compared with the −174 C allele, the −174 G allele significantly increased the expression of IL-6 (Fishman *et al*., 1998), which explains the positive relationship between the −174 G/C polymorphism and AP. Furthermore, Nibali *et al*.(2008c) revealed that the IL-6 −174 GG genotype is associated with an increased OR of concomitant detection of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. These results imply that the IL-6 −174 G/C polymorphism cause periodontitis by altering immune competence.

The IL-6 −572 C/G polymorphism has also been studied in the context of susceptibility to periodontitis (Holla *et al*., 2004; Komatsu *et al*., 2005; Nibali *et al*., 2009). When we pooled data from previous studies, there was no remarkable difference in allelic frequency distribution between CP cases and controls. However, compared with C allele carriers (genotypes GC and CC), the GG genotype significantly increased the risk of CP, suggesting that the −572 G allele is associated with the pathogenesis of CP under the recessive genetic model. A meta-analysis of the association between the −572 C/G polymorphism and AP was not conducted because only one relevant study was detected (Nibali *et al*., 2008b). According to genotyping data (Nibali *et al*., 2008b), the −572 G allele significantly increased the risk of developing AP compared with the −572 C allele (OR: 1.79, 95% CI: 1.18~2.72, P=0.006). Therefore, on the basis of the aforementioned evidence, we concluded that the −572 C/G polymorphism is associated with the pathogenesis of periodontitis, as it predisposes to either CP or AP. However, as one study has shown that the −572 G polymorphism reduces the transcriptional activity of the IL-6 promoter (Gu *et al*., 2008), the mechanism of this polymorphism predisposed to periodontitis seems too complicated. Herein, functional investigations on the association of IL-6 −572 C/G polymorphism with periodontitis are warranted.

Besides the well-discussed IL-6 −174 G/C and −572 C/G polymorphisms, the −6331 T/C polymorphism in the promoter region of the gene could also alter the transcriptional activity of IL-6 (Smith *et al*., 2008). Actually, the IL-6 −6331 T/C polymorphism is in complete linkage disequilibrium with the −6106 A/T polymorphism. Nibali *et al*.(2009; 2008b) recently reported that −6106 TT increased the risk of localized AP in Caucasian population. Therefore, we could conclude that IL-6 −6331 T/C polymorphism is involved in the pathogenesis of periodontitis among Caucasians. However, the association of IL-6 −6331 T/C polymorphism with risk of periodontitis requires to be confirmed in other ethnics.

The power of mapping and characterization analyses of disease-causing genes is significantly increased when association studies are based on haplotypes instead of genotypes (Akey *et al*., 2001). Holla *et al*.(2004) and Nibali *et al*.(2009) hypothesized that IL-6 haplotypes confer susceptibility to periodontitis. As a result, some haplotypes of IL-6 gene were discovered to be associated with the risk of developing periodontitis. However, it is difficult to determine the role of a particular haplotype in disease susceptibility using meta-analysis.

In conclusion, our meta-analysis indicates that the IL-6 −174 G allele could not modify the risk of CP, but increased the risk of AP. And −572 C/G polymorphism is associated with the pathogenesis of periodontitis, as it predisposes to either CP or AP. The association of IL-6 −174 G/C polymorphism with severe form of CP should be paid more attention to in future investigations.

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