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Association between tumor necrosis factor-α promoter −308 A/G, −238 A/G, interleukin-6 −174 G/C and −572 G/C polymorphisms and periodontal disease: a meta-analysis

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Abstract The aim of this study was to determine whether tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) promoter polymorphisms confer susceptibility to periodontitis in ethnically different populations. A literature search was performed using PubMed and Embase and a meta-analysis of the identified studies was conducted to explore the associations between TNF-α −308 A/G, −238 A/G, IL-6 promoter −174 G/C and −572 G/C polymorphisms and periodontitis. Seventeen comparison studies for the TNF-α −308 A/G polymorphism and three studies for the TNF-α −238 A/G polymorphism were included in the meta-analysis. And 16 separate studies for the IL-6 −174 G/C polymorphism and 10 studies for the IL-6 −572 G/C polymorphism were considered in our meta-analysis. Analysis after stratification by ethnicity indicated that the TNF-α −308 A allele was associated with periodontitis in Brazilian, Asian, and Turkish populations (OR = 0.637, 95 % CI = 0.447–0.907, $p = 0.013$; OR = 0.403, 95 % CI = 0.204–0.707, $p = 0.009$; OR = 1.818, 95; % CI = 1.036–3.189, $p = 0.037$). The meta-analysis showed no association between the TNF-α −238 A/G polymorphism and periodontitis. The meta-analysis indicated an association of the IL-6 −174 G/C polymorphisms with periodontitis in Brazilian populations (OR for GG + GC = 2.394, 95 % CI = 1.081–5.302, $p = 0.031$). Stratification by ethnicity and disease type indicated an association between the IL-6 −572 G allele and chronic periodontitis (OR = 1.585, 95 % CI = 1.030–2.439, $p = 0.036$), and periodontitis in Europeans (OR = 2.118, 95 % CI = 1.254–3.577, $p = 0.005$). This meta-analysis demonstrates that the TNF-α −308 A/G polymorphism confers susceptibility to periodontitis in Brazilian, Asian and Turkish populations. The IL-6 −174 G/C polymorphism may confer susceptibility to periodontitis in Brazilians, and the IL-6 −572 G/C polymorphism may be associated with susceptibility to periodontitis in Europeans, and chronic periodontitis.

Keywords TNF-α · IL-6 · Polymorphism · Periodontitis · Meta-analysis

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Introduction

Periodontitis is considered as a chronic inflammatory disease by bacterial infection and it is classified into two categories, namely chronic (CP) and aggressive (AP) [1]. Periodontitis is caused by microorganisms that adhere to and grow on tooth surfaces, along with an overly aggressive immune response against these microorganisms [1]. In some patients, periodontitis may develop with malfunctioning of some components of the immunity system (i.e. under the situation of impaired/insufficient immune response).

Tumor necrosis factor-α (TNF-α) is a potent pro-inflammatory cytokine that plays important roles in inflammatory and immune responses, including periodontitis [2]. Several single nucleotide polymorphisms have been identified in its promoter [3]. Of these polymorphisms, G-to-A substitutions at positions −308 and −238 have been intensively studied; these allelic variations could be of functional significance [4]. TNF-α has been found at high levels in the gingival crevicular fluid and inflamed

tissues in periodontitis [5]. TNF-α stimulates bone resportion by osteoclast [6] and induces tissue destruction by releasing of the matrix metalloproteinases, which are destructive to the extracellular matrix of ginviva, peritodontal ligaments and alveolar bone [7]. Numerous studies have examined the potential contributions made by TNF-α promoter polymorphisms to periodontitis susceptibility [8–20].

Interleukin-6 (IL-6) is a multifunctional cytokine involved in the inflammatory response and in the modulation of immune responses, including B cell and T cell differentiation [21]. IL-6 is regulated mainly at the transcriptional level by regulatory elements in the 5′ flanking region of the gene [22]. In this region, the −174 G/C and −572 G/C polymorphisms are important regulators of transcription [23]. For example, a luciferase reporter vector assay showed that the −174 G construct was expressed significantly higher than the corresponding −174 C construct [24]. The IL-6 −572 G/C polymorphism lies near a potential glucocorticoid receptor element at position −557 to −552 [25]. Furthermore, the −572 G/C polymorphism has been reported to influence levels of circulating C-reactive protein and bone resorption markers in postmenopausal women, and to be associated with several diseases [26, 27]. Subsequently many studies did examine relationships between IL-6 and TNF-α polymorphisms and periodontitis [16–22, 24–26], but studies have produced diverse results.

A meta-analysis provides a powerful means of overcoming the problem of small sample size and inadequate statistical power, especially in genetic studies of complex traits [28–31]. In the present study, we investigated whether the TNF-α promoter −308 A/G and −238 A/G, IL-6 −174 G/C and −572 G/C polymorphisms contribute to the susceptibility of periodontitis by performing a meta-analysis using published data.

Materials and methods

Identification of eligible studies and data extraction

A literature search was conducted for studies that examined associations between the TNF-α promoter −308 A/G and −238 A/G polymorphisms and IL-6 polymorphisms and periodontitis. We utilized the PubMed and Embase citation indexes to identify articles in which the TNF-α polymorphisms were determined in periodontitis patients and controls (up to July 2012). In addition, all references mentioned in the identified articles were reviewed to identify additional studies not indexed by PubMed and Embase. The following key words and subject terms were searched: 'Tumor necrosis factor', 'TNF-alpha', 'polymorphism', "interleukin-6," "IL-6," and 'periodontitis'. The following information was extracted from each study: author, year of publication, ethnicity of the study population, demographics, numbers of cases and controls, and the A allele frequencies of the TNF-α promoter −308 A/G and −238 A/G polymorphisms. Allele frequencies were calculated from the corresponding genotype distributions.

Evaluation of statistical associations

A Chi squared test was used to determine whether the observed genotype frequencies conformed to Hardy–Weinberg (H–W) expectations (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Meta-analyses were performed using allelic contrast, homozygote contrast, recessive model, and homozygote model. Point estimates of risk, odds ratios (ORs), and 95 % confidence intervals (CIs) were determined for each study. Cochran's Q-statistic was used to assess within and between study variations and heterogeneities [32]. When the heterogeneity test assessed the probability of the null hypothesis, all studies were evaluating the same effect. If a significant Q-statistic ($p < 0.10$) indicated heterogeneity across studies, the random effects model was used for the meta-analysis, and when heterogeneity was not indicated across studies, the fixed effects model was used. The fixed effects model assumes that genetic factors have similar effects on periodontitis susceptibility across all studies, and that observed variations between studies are caused by chance alone [33]. The random effects model assumes that different studies show substantial diversity, and assesses both within-study sampling errors and between-study variances [34]. The random effects model was used in the presence of significant between-study heterogeneity. The effect of heterogeneity was quantified using a recently developed measure, $I^2 = 100\ \% \times (Q - df)/Q$, [35] where I^2 ranges between 0 and 100 % and represents the proportion of inter-study variability attributable to heterogeneity rather than chance. I^2 values of 25, 50, and 75 % were defined as low, moderate, and high estimates, respectively. Statistical manipulations were undertaken using the Comprehensive Meta-Analysis computer program (Biosta, Englewood, NJ, USA).

Evaluation of publication bias

Funnel plots are used to detect publication bias, but they require a range of studies of varying sizes and subjective judgments. Thus, in the present study, we evaluated publication bias using Egger's linear regression test [36], which measures funnel plot asymmetry using a natural logarithm scale of ORs.

Results

Studies included in the meta-analysis

Thirteen studies met the inclusion criteria [8–20]. However, one of these studies contained data of two different groups such as aggressive or chronic periodontitis, and they were treated independently [10]. Fourteen and three separate comparisons of the TNF-α −308 A/G and −238 A/G polymorphisms were considered in the meta-analysis. A total of 17 separate comparisons were considered in our meta-analysis consisting of 1,120 patients with periodontitis and 1,592 controls (14 studies with 884 cases and 1,160 controls for the TNF-α −308 A/G polymorphism; three studies with 236 cases and 432 controls for the TNF-α −238 A/G polymorphism), involving six European, five Asian, four Brazilian, and two Turkish populations. Ethnicity-specific meta-analysis was conducted on these populations. Selected characteristics of the relationships found between the TNF-α polymorphisms and periodontitis are summarized in Table 1.

Seventeen studies met the inclusion criteria [8, 9, 14, 37–50]. However, one of these studies contained data on three different groups for the IL-6 −174 G/C polymorphism and the IL-6 −572 G/C polymorphism [41], and three studies contained the data of both the IL-6 −174 G/C and −572 G/C polymorphisms [16, 17, 27]; these studies were treated independently. A total of 26 separate comparisons were considered in our meta-analysis consisting of 3,323 patients with periodontitis and 2,488 controls (16 studies with 1,887cases and 1,490 controls for the IL-6 −174 G/C polymorphism; 10 studies with 1,436 cases and 998 controls for the IL-6 −572 G/C polymorphism), involving seven European, three Asian, two Brazilian, two Indian, one African, and one Turkish population for the −174 G/C polymorphism and two European, six Asian, one Indian, and one African population for the −572 G/C polymorphism. Ethnicity-specific meta-analysis was conducted on these populations. Selected characteristics of the relationships found between the IL-6 polymorphisms and periodontitis are summarized in Table 2.

Frequencies of the A alleles of the TNF-α promoter −308 A/G polymorphism in the different control groups

The study by Babel et al. showed AA and AG + GG genotype data, but not allele data [14]. Thus, the allele data from the study by Babel et al. was not included in Table 2. The mean frequency of the A allele of the TNF-α −308 A/G polymorphism was 16.0 % among all controls. Asian controls had a lower A allele prevalence (11.6 %) than the controls of the other ethnic groups, in which frequencies varied from 12.9 to 27.1 %. The Brazilian control population had the highest A allele frequency (27.1 %) (Table 3).

Meta-analysis of the association between the TNF-α −308 A/G polymorphism and periodontitis

Meta-analysis was performed on all periodontitis patients and on the patients in each ethnic group. A summary of our meta-analysis findings of the relation between the TNF-α −308 A/G polymorphism and periodontitis is provided in Table 4. Meta-analysis showed no association between the A allele and periodontitis in all subjects (OR = 0.846, 95 % CI = 0.629–1.138, $p = 0.269$) with between-study heterogeneity ($I^2 = 49.0$ %) (Table 4; Fig. 1). Analysis after stratification by ethnicity indicated that the A allele was associated with periodontitis in Brazilian, Asian and Turkish populations (in respective order: OR = 0.637, 95 % CI = 0.447–0.907, $p = 0.013$; OR = 0.403, 95 % CI = 0.204–0.707, $p = 0.009$; OR = 1.818, 95 % CI = 1.036–3.189, $p = 0.037$), but not in Europeans (OR = 1.034, 95 % CI = 0.772–1.385, $p = 0.822$) (Table 4; Fig. 2). Stratification by disease type indicated no association between the −308 A allele and aggressive or chronic periodontitis (Table 4).

Meta-analysis of the association between the TNF-α −238 A/G polymorphism and periodontitis

Meta-analysis showed no association between the A allele of the TNF-α −238 A/G polymorphism and periodontitis in all patients (OR 0.757, 95 % CI 0.309–1.740, $p = 0.512$) without between-study heterogeneity ($I^2 = 8.56$ %) (Table 5). Meta-analyses of the TNF-α −238 A/G polymorphism using the dominant, additive model, or homozygote model showed the same pattern as meta-analysis of the TNF-α −238 A allele, indicating no significant association with periodontitis (Table 5).

Meta-analysis of the IL-6 −174 G/C polymorphism and periodontitis susceptibility

Meta-analysis containing studies in HWE revealed an association between periodontitis and the IL-6 −174 G allele (OR = 1.387, 95 % CI = 1.040–1.849, $p = 0.026$) (Fig. 3; Table 6). Stratification by ethnicity and disease type indicated no association between the IL-6 −174 G allele and periodontitis in each ethnic group and chronic periodontitis (Table 6). Meta-analysis using the recessive and the homozygote models showed an association of the IL-6 −174 G/C polymorphisms with periodontitis in

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

Author (Ref)	Country	Ethnicity	Disease type	Polymorphism	Numbers		Case			Control			Association *p* value	HWE *p* value
					Case	Control	GG	AG	AA	GG	AG	AA		
Erciyas et al. [8]	Turkey	Turkish	AP	−308 G/A	35	85	20	15	0	71	12	2	0.014	0.112
Costa et al. [9]	Brazil	Brazilian	CP	−308 G/A	38	27	33	1	4	18	6	3	0.118	0.063
Menezes-1 and Colombo [10]	Brazil	Brazilian	CP	−308 G/A	74	51	51	17	6	28	17	6	0.105	0.196
Menezes-2 and Colombo [10]	Brazil	Brazilian	AP	−308 G/A	38	51	25	11	2	28	17	6	0.185	0.196
Guzeldemir et al. [11]	Turkey	Turkish	AP	−308 G/A	31	31	17	12	2	24	0	7	0.658	2.5e−08
Freitas et al. [12]	Brazil	Brazilian	AP	−308 G/A	30	70	17	12	1	38	26	6	0.574	0.610
Zhu et al. [13]	China	Asian	AP	−308 G/A	64	28	47	17	0	16	12	0	0.166	0.148
Babel et al. [14]	Germany	European	CP	−308 G/A	122	110	116*		6	102*		8	0.455	NA
Folwczny et al. [15]	Germany	European	CP	−308 G/A	81	80	52	27	2	58	22	0	0.194	0.153
Fassmann et al. [16]	Czech	European	CP	−308 G/A	132	114	105	26	1	87	26	1	0.560	0.531
Soga et al. [17]	Japan	Asian	P	−308 G/A	64	64	63	1	0	62	2	0	0.569	0.898
Craandijk et al. [18]	Netherlands	European	P	−308 G/A	90	264	66	21	3	187	71	6	0.818	0.807
Endo et al. [19]	Japan	Asian	P	−308 G/A	46	140	44	2	0	100	40	0	0.006	0.075
Galbraith et al. [20]	USA	European	P	−308 G/A	39	45	31	6	2	34	11	0	0.907	0.350
Soga et al. [17]	Japan	Asian	P	−238 G/A	100	64	100	0	0	62	2	0	0.183	0.898
Craandijk et al. [18]	Netherlands	European	P	−238 G/A	90	264	85	4	1	248	15	1	0.941	0.151
Endo et al. [19]	Japan	Asian	AP	−238 G/A	46	104	45	1	0	99	4	1	0.361	0.071

Ref reference, *USA* United States of America, *AP* aggressive periodontitis, *CP* chronic periodontitis, *P* unclassified periodontitis, *HWE* Hardy–Weinberg equilibrium, *NA* not available

* GG + GA

Table 2 Characteristics of the studies included in the meta-analysis

Author (Ref)	Country	Ethnicity	Disease type	Polymorphism	Numbers		Case			Control			Association *p* value	HWE *p* value
					Case	Control	CC	GC	GG	CC	GC	GG		
Kalburg et al. [37]	India	Indian	CP	−174 G/C	15	15	2	3	10	9	4	2	0.000	0.217
Fan et al. [38]	China	Asian	CP	−174 G/C	178	130	0	1	177	0	1	129	0.824	0.964
Franch-Chillida et al. [39]	UK	Indian	P	−174 G/C	103	141	6	18	79	7	28	106	0.919	0.010
Erciyas et al. [8]	Turkey	Turkish	AP	−174 G/C	35	85	1	12	22	5	31	49	0.491	0.973
Costa et al. [9]	Brazil	Brazilian	CP	−174 G/C	68	27	0	37	31	3	12	12	0.402	1.000
Xiao et al. [40]	China	Asian	CP	−174 G/C	157	132	0	1	156	0	0	132	0.570	0.000
Nibali-1 [41]	UK	European	P	−174 G/C	318	144	52	142	124	28	74	42	0.064	0.651
Nibali-2 [41]	UK	African	P	−174 G/C	90	45	0	9	81	0	7	38	0.366	0.571
Nibali-3 [41]	UK	Asian	P	−174 G/C	85	29	2	15	68	1	6	22	0.595	0.483
Tervonen et al. [42]	Finland	European	CP	−174 G/C	51	178	40*		11	141*		37	0.101	NA
Moreira et al. [43]	Brazil	Brazilian	CP	−174 G/C	261	106	14	83	164	11	35	60	0.001	0.106
Babel et al. [14]	Germany	European	CP	−174 G/C	124	116	52		72**	32		84**	0.772	NA
Jansson et al. [44]	Sweden	European	CP	−174 G/C	100	100	32	26	42	13	32	55	0.158	0.025
Brett et al. [45]	UK	European	P	−174 G/C	106	99	17	37	52	25	19	55	0.059	0.000
Holla et al. [46]	Czech	European	CP	−174 G/C	148	107	34	71	43	17	53	37	0.000	0.783
Trevilatto et al. [47]	Brazil	European	CP	−174 G/C	48	36	4	15	29	3	21	12	0.824	0.142
Fan et al. [38]	China	Asian	CP	−572 G/C	178	130	6	52	120	3	32	95	0.268	0.875
Franch-Chillida et al. [39]	UK	Indian	P	−572 G/C	105	130	22	50	33	21	56	53	0.121	0.344
Jingjin et al. [50]	China	Asian	CP	−572 G/C	153	96	6	98	49	1	30	65	0.000	0.220
Xiao [40]	China	Asian	CP	−572 G/C	159	134	3	48	108	2	36	96	0.500	0.502
Nibali-1 [41]	UK	European	P	−572 G/C	318	144	2	31	285	0	11	133	0.278	0.633
Nibali-2 [41]	UK	African	P	−572 G/C	86	45	5	13	68	0	6	39	0.107	0.631
Nibali-3 [41]	UK	Asian	P	−572 G/C	84	29	13	38	33	3	13	13	0.468	0.924
Guan et al. [49]	China	Asian	CP	−572 G/C	93	96	6	38	49	1	30	65	0.017	0.220
Holla et al. [46]	Czech	European	CP	−572 G/C	148	107	139	9	0	86	21	0	0.002	0.260
Komatsu et al. [48]	Japan	Asian	CP	−572 G/C	112	77	5	36	71	4	32	41	0.216	0.478

Ref reference, *UK* United Kingdom, *AP* aggressive periodontitis, *CP* chronic periodontitis, *P* unclassified periodontitis, *HWE* Hardy–Weinberg equilibrium, *NA* not available

* CC + GC, ** GC + GG

Brazilian populations (OR for GG + GC = 2.394, 95 % CI = 1.081–5.302, $p = 0.031$; OR for GG vs. CC = 2.498, 95 % CI = 1.108–5.628, $p = 0.027$, respectively), but not in European, Asian, and Indian populations (Table 6).

Table 3 Prevalences of the A allele of the TNF-α −308 G/A polymorphism

Population	No. of studies	Numbers		A allele (%)	
		Case	Control	Case	Control
European	4	342	503	14.0	14.3
Brazilian	4	180	199	18.6	27.1
Asian	3	183	232	7.92	11.6
Turkish	2	66	116	23.5	12.9
Overall	13	771	1,050	14.5	16.0

Meta-analysis of the IL-6 −572 G/C polymorphism and periodontitis susceptibility

Meta-analysis stratified by ethnicity and disease type indicated an association between the IL-6 −572 G allele and chronic periodontitis (OR = 1.585, 95 % CI = 1.030–2.439, $p = 0.036$), and periodontitis in the European group (OR = 2.118, 95 % CI = 1.254–3.577, $p = 0.005$) (Fig. 4; Table 7). Analysis using the recessive model showed the same pattern for the IL-6 −174 G allele, showing an association between the IL-6 −572 GG genotype and chronic periodontitis, and periodontitis in the European group (Table 7).

Heterogeneity and publication bias

Between-study heterogeneity was found during analyses of the TNF-α −308 A/G polymorphism, such as −308 A

Table 4 Meta-analysis of the association between the TNF-α −308 G/A polymorphism and periodontitis

Polymorphism	Population	No. of studies	Test of association			Test of heterogeneity		
			OR	95 % CI	*p* value	Model	*p* value	I^2
TNF −308 A vs. G	Overall	13	0.846	0.629–1.138	0.269	R	0.023	49.0
	European	4	1.034	0.772–1.385	0.822	F	0.564	0
	Brazilian	4	0.637	0.447–0.907	0.013	F	0.826	0
	Asian	3	0.403	0.204–0.707	0.009	F	0.232	31.6
	Turkish	2	1.818	1.036–3.189	0.037	F	0.170	46.9
	AP	6	0.788	0.421–0.475	0.456	R	0.004	70.6
	CP	4	0.847	0.617–1.162	0.303	F	0.110	50.2
AA vs. GA + GG (recessive)	Overall	11	0.721	0.433–1.202	0.210	F	0.707	0
	European	5	1.123	0.520–2.426	0.767	F	0.526	0
	Brazilian	4	0.604	0.279–1.307	0.200	F	0.869	0
	Asian	NA	NA	NA	NA	NA	NA	NA
	Turkish	2	0.277	0.064–1.191	0.085	F	0.699	0
	AP	4	0.338	0.127–0.899	0.030	F	0.952	0
	CP	5	0.792	0.402–1.519	0.499	F	0.797	0
AA + GA vs. GG (dominant)	Overall	13	0.833	0.557–1.244	0.172	R	0.002	61.9
	European	4	0.969	0.699–1.343	0.850	F	0.572	0
	Brazilian	4	0.602	0.388–0.935	0.024	F	0.545	0
	Asian	3	0.331	0.157–0.700	0.004	F	0.249	28.0
	Turkish	2	3.385	1.702–6.734	0.001	F	0.679	0
	AP	6	0.891	0.368–2.159	0.799	R	0.009	79.3
	CP	4	0.757	0.430–1.332	0.334	R	0.087	54.2
AA vs. GG	Overall	10	0.730	0.406–1.311	0.291	F	0.755	0
	European	4	1.841	0.619–5.475	0.273	F	0.698	0
	Brazilian	4	0.514	0.234–1.128	0.297	F	0.938	0
	Asian	NA	NA	NA	NA	NA	NA	NA
	Turkish	2	0.458	0.104–2.014	0.301	F	0.760	0
	AP	4	0.409	0.152–1.104	0.078	F	0.987	0
	CP	4	0.754	0.313–1.815	0.528	F	0	0

OR odds ratio, *CI* confidence interval, *F* fixed model, *R* random model, *AP* aggressive periodontitis, *CP* chronic periodontitis, *NA* not available

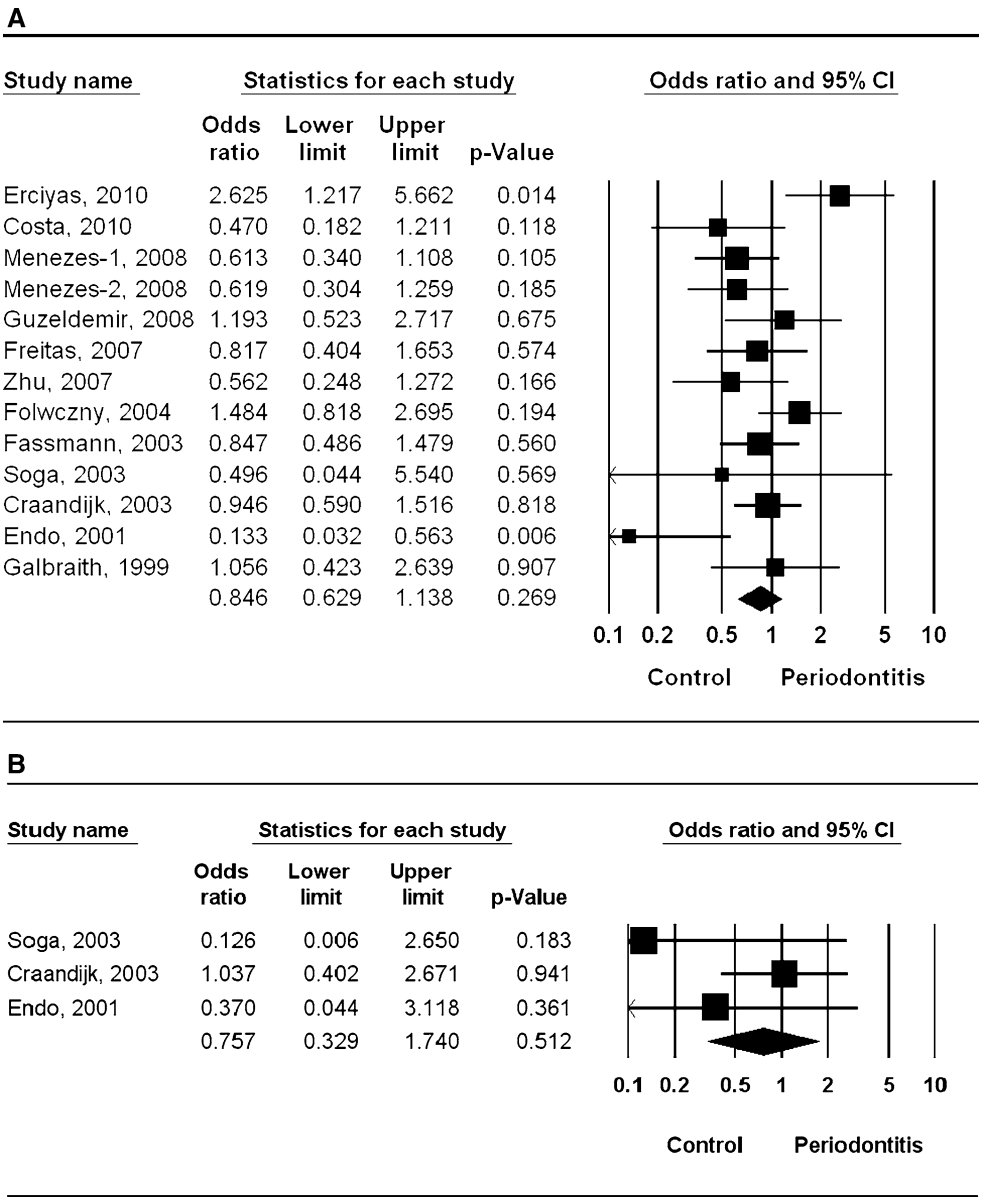

Fig. 1 ORs and 95 % CIs of individual studies and pooled data for the association between the A allele of the TNF-α −308 (**a**) and TNF-α −238 (**b**) and periodontitis in all study subjects

allele and the AA + GA genotype in overall and aggressive periodontitis, the AA +GA genotype in chronic periodontitis (Table 4). However, there was no between-study heterogeneity during analyses of the TNF-α −238 A/G polymorphisms (Table 4). The distribution of the TNF-α −308 A/G polymorphism in normal controls was not consistent with H–W equilibrium in one study [11]. Deviation from H–W equilibrium among controls implies potential bias during control selection or genotyping errors. However, excluding the study did not materially affect our results.

The distribution of genotypes of the IL-6 polymorphisms in control groups was consistent with the HWE, except for four studies on the IL-6 −174 G/C polymorphism [39, 40, 44, 45]. There were two studies that did not show HWE [14, 42]. When we excluded these studies, overall results were substantially affected (OR = 1.387, 95 % CI = 1.040–1.849, $p = 0.026$) (Table 6). Between-study heterogeneity for the IL-6 −174 G/C polymorphism was found among all study subjects and some subgroup analyses. Accordingly, meta-analysis was performed using a random effects model when heterogeneity was present in a population (Table 7). The funnel plot, which is usually used to detect publication bias, was difficult to interpret because we included a relatively small number of studies. Egger's regression test showed no evidence of publication bias in the meta-analysis (Egger's regression test p values > 0.1).

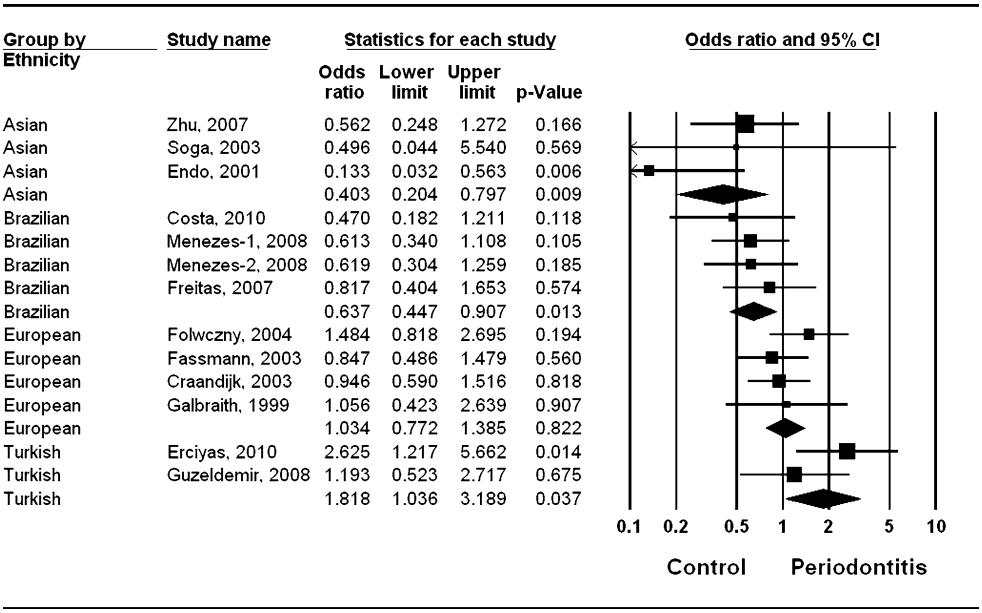

Fig. 2 ORs and 95 % CIs of individual studies and of pooled data for the association between the A allele of the TNF-α –308 and periodontitis in each ethnic group

Table 5 Meta-analysis of the association between the TNF-α −238 G/A polymorphism and periodontitis

Polymorphism	Population	No. of studies	Test of association			Test of heterogeneity		
			OR	95 % CI	*p* value	Model	*p* value	I^2
TNF −238 A vs. G	Overall	3	0.757	0.309–1.740	0.512	F	0.335	8.56
	European	1	1.037	0.402–2.671	0.941	NA	NA	NA
	Asian	2	0.260	0.045–1.489	0.130	F	0.571	0
AA vs. GA + GG (recessive)	Overall	2	1.637	0.199–13.42	0.646	F	0.524	0
	European	1	2.955	0.183–47.73	0.445	NA	NA	NA
	Asian	1	0.742	0.030–18.55	0.856	NA	NA	NA
AA + GA vs. GG (dominant)	Overall	3	0.680	0.278–1.661	0.397	F	0.438	0
	European	1	0.912	0.324–2.564	0.861	NA	NA	NA
	Asian	2	0.287	0.049–1.691	0.168	F	0.509	
AA vs. GG	Overall	2	1.612	0.196–13.23	0.656	F	0.523	0
	European	1	2.918	0.181–47.15	0.451	NA	NA	NA
	Asian	1	0.729	0.029–18.24	0.847	NA	NA	NA

OR odds ratio, *CI* confidence interval, *F* fixed model, *R* random model, *NA* not available

Discussion

In the present study, we addressed associations between the TNF-α promoter −308 and −238 A/G polymorphisms and periodontitis susceptibility. Ethnicity-specific meta-analysis showed an association between the TNF-α −308 A allele and periodontitis in Brazilian, Asian, and Turkish populations but not in Europeans. Stratification by disease type indicated no association between the −308 A allele and aggressive or chronic periodontitis and no association was found between periodontitis and the TNF-α −238 A/G polymorphism. We found an association an association between periodontitis and the IL-6 −174 G allele in all study subjects in HWE (OR = 1.387, 95 % CI = 1.040–1.849, $p = 0.026$). Ethnicity-specific meta-analysis showed an association of the IL-6 −174 G/C polymorphisms with periodontitis in Brazilian populations. We identified an association of the IL-6 −572 G/C polymorphisms with periodontitis in all study subjects (OR for GG + GC = 1.507, 95 % CI = 1.050–2.162). Furthermore, stratification by ethnicity and disease type indicated an association between the IL-6 −572 G allele and chronic periodontitis, and periodontitis in Europeans.

TNF-α is increased in the gingival crevicular fluid and inflamed tissues in periodontitis. Our meta-analysis showed

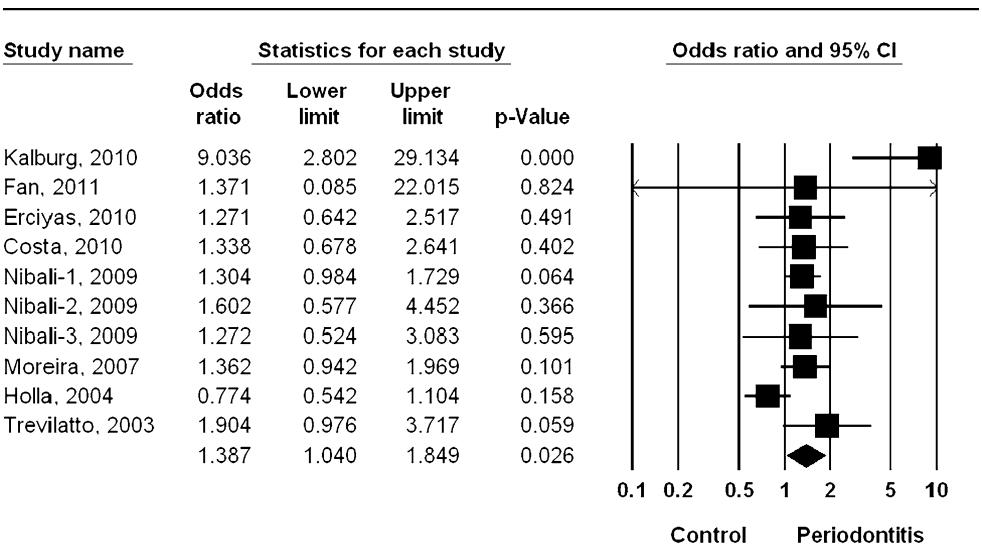

Fig. 3 ORs and 95 % CIs of the individual studies and pooled data for the association between the G allele of the IL-6 promoter −174 G/C and periodontitis in all study subjects in HWE

Table 6 Meta-analysis of the association between the IL-6 promoter −174 G/C polymorphism and periodontitis

Polymorphism	Population	No. of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95 % CI	*p* value	Model	*p* value	I^2	*p* value
G vs. C allele	Overall	14	1.189	0.911–1.551	0.203	R	0.000	64.9	0.392
	Overall in HWE	10	1.387	1.040–1.849	0.026	R	0.018	55.0	
	CP	8	1.261	0.753–2.112	0.378	R	0.000	79.2	
	European	5	0.974	0.655–1.449	0.897	R	0.001	79.8	
	Asian	3	1.186	0.525–2.683	0.681	F	0.784	0	
	Brazilian	2	1.356	0.981–1.876	0.066	F	0.964	0	
	Indian	2	2.849	0.340–23.91	0.335	R	0.001	91.0	
GG + GC vs. CC (dominant)	Overall	12	1.110	0.684–1.800	0.672	R	0.000	67.0	0.130
	Overall in HWE	8	1.550	0.857–2.804	0.148	R	0.049	50.4	
	CP	7	1.064	0.505–2.240	0.870	R	0.000	76.3	
	European	6	0.770	0.458–1.294	0.324	R	0.004	70.7	
	Asian	1	1.482	0.129–16.97	0.752	NA	NA	NA	
	Brazilian	2	2.394	1.081–5.302	0.031	F	0.154	50.6	
	Indian	2	2.576	0.237–28.05	0.437	R	0.024	80.2	
GG vs. GC + CC (recessive)	Overall	15	1.158	0.896–1.495	0.262	R	0.046	41.6	0.340
	Overall in HWE	10	1.349	1.080–1.685	0.008	F	0.119	36.1	
	CP	7	1.187	0.760–1.853	0.451	R	0.015	58.0	
	European	6	1.043	0.681–1.586	0.845	R	0.010	66.8	
	Asian	3	1.169	0.473–2.889	0.736	F	0.786	0	
	Brazilian	2	1.240	0.824–1.866	0.300	F	0.678	0	
	Indian	2	3.209	0.288–35.80	0.343	R	0.012	84.2	
GG vs. CC	Overall	11	1.358	0.764–2.416	0.297	R	0.001	66.3	0.170
	Overall in HWE	8	1.909	0.962–3.791	0.065	R	0.025	56.2	
	CP	6	1.665	0.559–4.960	0.360	R	0.000	80.7	
	European	5	0.887	0.448–1.753	0.729	R	0.005	72.8	
	Asian	1	1.543	0.134–17.87	0.727	NA	NA	NA	
	Brazilian	2	2.498	1.108–5.628	0.027	F	0.190	41.7	
	Indian	2	3.864	0.161–92.65	0.404	R	0.009	85.4	

IL-6 interleukin-6, *OR* odds ratio, *CI* confidence interval, *F* fixed model, *R* random mode, *NA* not available

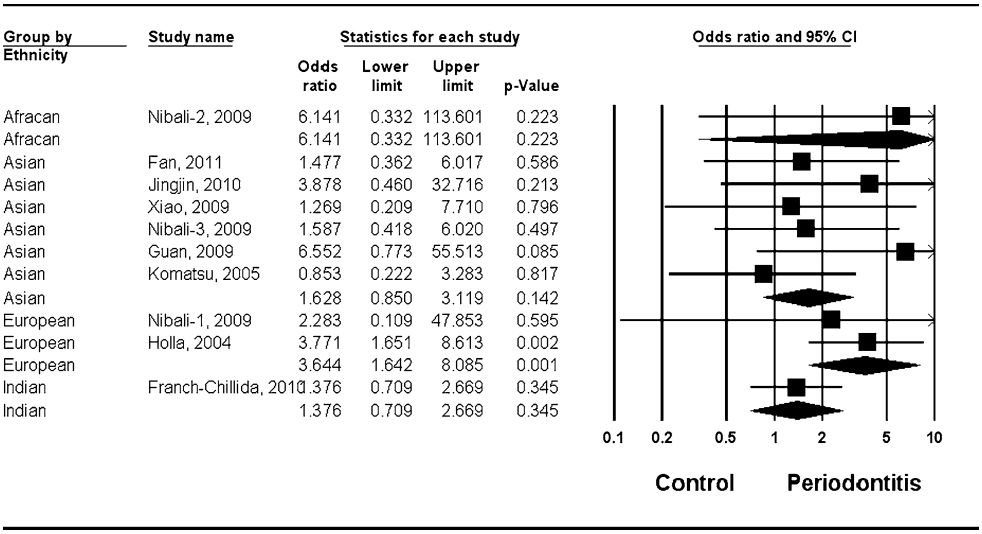

Fig. 4 ORs and 95 % CIs of individual studies and pooled data for the association between the GG genotype of the IL-6 promoter −572 G/C and periodontitis in each ethnic group

Table 7 Meta-analysis of the association between the IL-6 promoter −572 G/C polymorphism and periodontitis

Polymorphism	Population	No. of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95 % CI	*p* value	Model	*p* value	I^2	*p* value
G vs. C allele	Overall	10	1.523	1.159–2.001	0.003	R	0.005	61.8	0.393
	CP	6	1.585	1.030–2.439	0.036	R	0.000	77.7	
	Asian	6	1.395	0.958–2.033	0.083	R	0.003	72.4	
	European	2	2.118	1.254–3.577	0.005	F	0.111	60.6	
GG + GC vs. CC (dominant)	Overall	10	1.507	1.050–2.162	0.026	R	0.002	67.5	
	CP	6	1.541	0.846–2.804	0.157	R	0.000	83.5	
	Asian	6	1.498	0.887–2.531	0.130	R	0.000	79.6	
	European	1	1.502	0.875–2.578	0.140	NA	NA	NA	
GG vs. GC + CC (recessive)	Overall	10	1.919	1.290–2.825	0.001	F	0.569	0	0.477
	CP	6	2.383	1.371–4.143	0.002	F	0.370	7.30	
	Asian	6	1.628	0.850–3.119	0.142	F	0.662	0	
	European	2	3.644	1.642–8.085	0.001	F	0.755	0	
GG vs. CC (homozygote)	Overall	9	1.811	1.120–2.928	0.015	F	0.614	0	0.171
	CP	5	1.801	0.850–3.816	0.125	F	0.238	27.5	
	Asian	6	1.780	0.917–3.453	0.088	F	0.355	9.52	
	European	1	2.338	0.111–49.03	0.584	NA	NA	NA	

IL-6 interleukin-6, *OR* odds ratio, *CI* confidence interval, *F* fixed model, *R* random model, *NA* not available

that the TNF-α −308 A/G polymorphism may influence the susceptibility to periodontitis in various ethnic groups. However, it needs to be explained why the TNF-α –308 A/G polymorphism is not associated with periodontitis in European population and plays a different role in different ethnic populations. It can be related with genetic heterogeneity in different populations. Clinical heterogeneity may also explain the discrepancy. Different linkage disequilibrium (LD) patterns may contribute to the discrepancy. This polymorphism may be in LD with a nearby causal variant in one ethnic group but not in another [51]. The difference might arise purely by chance, such as type I error, or because of multiple testing which inflates the type I error. TNF polymorphisms may occur with distinct patterns in ethnically distinct populations [52]. The difference of the allele frequency of the TNF-α −308 A/G polymorphism exists among ethnic groups. In our study, Asian controls had a lower A allele prevalence (11.6 %) than the controls of the other ethnic groups, in which frequencies varied from 12.9 to 27.1 %. The Brazilian control population had the highest A allele frequency (27.1 %).

As IL-6 −174 G/C and −572 G/C polymorphisms increase IL-6 expression, they may be associated with

susceptibility to periodontitis [5–7]. IL-6 transcription is influenced by complex interactions determined by these haplotypes. The IL-6 promoter region influences IL-6 gene transcription not by a simple mechanism but rather through complex interactions determined by the haplotype [21]. Because IL-6 polymorphisms and haplotypes are associated with an increased inflammatory response, specifically in the presence of periodontopathogenic bacteria, IL-6 gene may play a key role in the pathogenesis of periodontitis.

This meta-analysis differs from a previous meta-analysis on the relation between the IL-6 polymorphisms and periodontitis risk [53], because in the present study seven more studies were included, and meta-analysis stratified by ethnicity was further conducted. The result of this meta-analysis regarding an association between the IL-6 −174 G/C and −572 G/C polymorphisms and susceptibility to periodontitis is in agreement with the previous study. In addition, our meta-analysis revealed an association between IL-6 −174 G/C polymorphism and periodontitis in Brazilians, and between IL-6 −572 G/C polymorphism and periodontitis in Europeans.

The present study had some limitations that should be considered. First, publication bias and heterogeneity may have distorted the meta-analysis. Second, our ethnicity-specific meta-analysis included data only from European, Brazilian, Asian, and Turkish patients. Thus, our results are applicable only to these ethnic groups. Additional studies are required in other ethnic groups to determine whether the TNF-α polymorphisms confer a risk of periodontitis. Third, the numbers of subjects and studies included in the ethnicity-specific meta-analysis were small. There were only two studies for Turkish population. This analysis may not have enough power to explore the association between TNF-α –308 A/G polymorphism and periodontitis in each ethnic group. Small sample size may have distorted the meta-analysis of the TNF-α −238 A/G polymorphism. There were only three studies available for the meta-analysis of the TNF-α −238 A/G polymorphism. The number of studies included in the meta-analysis was too small to draw definitive conclusion. Interpretation of the data should be done cautiously. Fourth, data were not stratified by sex, disease severity, or environmental variables because of insufficient data.

In conclusion, this meta-analysis demonstrates that the TNF-α −308 A/G polymorphism confers susceptibility to periodontitis in Brazilian, Asian, and Turkish populations, and the IL-6 −174 G/C polymorphism may confer susceptibility to periodontitis in Brazilians, and that the IL-6 −572 G/C polymorphism is associated with susceptibility to periodontitis in Europeans, and chronic periodontitis. Further larger scale studies are required to explore the roles played by the TNF-α and IL-6 genes in the pathogenesis of periodontitis.

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Conflict of interest The authors have no potential conflict of interest to declare.

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