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Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls

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Abstract

Aim: We conducted a systematic review and a meta-analysis, in order to investigate the potential association of cytokine gene polymorphisms with either aggressive or chronic periodontal disease.

Material and Methods: A comprehensive literature search was performed. We retrieved a total of 53 studies summarizing information about 4178 cases and 4590 controls. Six polymorphisms were included in our meta-analysis which are the following: IL-1A G[4845]T, IL-1A C[– 889]T, IL-1B C[3953/4]T, IL-1B T[– 511]C, IL-6 G[– 174]C and TNFA G[– 308]A. Random effect methods were used for the analysis. We calculated the specific odds ratios along with their 95% confidence intervals to compare the distribution of alleles and genotypes between cases and controls.

Results and Conclusions: Using random effect methods we found statistically significant association of IL-1A C[– 889]T and IL-1B C[3953/4]T polymorphisms with chronic periodontal disease without any evidence of publication bias or significant statistical heterogeneity. A weak positive association was also found concerning IL-1B T[– 511]C and chronic periodontal disease. No association was found for all the cytokines examined as far as the aggressive form of the disease is concerned. Future studies may contribute to the investigation of the potential multigenetic predisposition of the disease and reinforce our findings.

Key words: cytokine; periodontities; polymorphism; meta-analysis

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Periodontitis is an infection of the supporting tissues of the teeth and it is classified into two broad categories, namely chronic (CP) and aggressive (AP) (Armitage 1999). CP is rather common affecting up to 30% of adults, while 7–13% of the adult population will develop severe forms of destructive periodontal disease (Nares 2003). AP, formerly described as early onset periodontitis, seems to be a less frequent occurring form of periodontitis (Papapanou 1999). Periodontal disease is initiated by the accumulation of plaque bacteria in the gingival sulcus, which induces an inflammatory response (Heitz-Mayfield 2005). While gingivitis is generally reversible, periodontitis destroys the periodontium and consists of one of the dominant causes of tooth loss in adults (Kinane & Hart 2003). The destruction of the underlying ligament and the alveolar bone is influenced by the host immune response to the microbial challenge (Heitz-Mayfield 2005). While pathogenic microflora and various other environmental risk factors are involved in the pathogenesis of the disease (Borrell & Papapanou 2005), evidence suggests also a genetic component to the aetiology (Michalowicz et al. 1991, 2000, Kinane & Hart 2003). Cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour

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necrosis factor-a (TNF-a) seem to play an important role in the focal immunopathology of periodontal disease. Thus, the genetic control of the cytokine function may affect the appearance or the severity of periodontitis.

IL-1 is composed of two distinct but functionally similar molecules, IL-1a and IL-1b. Both molecules are produced by macrophages, monocytes and dendritic cells. IL-1a is largely a regulator of intracellular events and a mediator of local inflammation, whereas IL-1b is primarily an extracellular protein released from cells (Dinarello 1996). IL-1 single nucleotide gene polymorphisms (SNP) such as the C/T single base-pair substitution in the IL-1A promoter (−889) (which is more than 99% concordant with IL-1A G4845T at exon 5) and in the IL-1B+3954 locus (formerly referred as IL-1B+3953) have been early related to periodontitis (Kornman et al. 1997) and triggered the subsequent publication of numerous epidemiological studies (Kinane & Hart 2003). Furthermore, even though not definitely convincing (Shapira et al. 2005), functional studies on these SNPs have shown interesting results such as the association of the −889T variant with a fourfold increase in IL-1a levels in the gingival crevicular fluid (GCF) (Shirodaria et al. 2000) or the elevated GCF concentration of IL-1b in shallow sites of patients carrying the risk alleles (Engebretson et al. 1999).

IL-6 is a pleiotropic cytokine released by many cell types (Klein et al. 2001) and its secretion levels are determined by the producing cell type, the nature of the stimulus and the genetic background of the source cells (Taylor et al. 2004). A SNP, G[−174]C, located within the IL-6 promoter, was found to influence IL-6 expression. G allele carriers have increased plasma levels of IL-6, present higher IL-6 gene transcriptional activity and develop higher inducible IL-6 responses when compared with CC individuals (Fishman et al. 1998, Hulkkonen et al. 2001). Previous research has assessed the role of IL-6 G[−174]C polymorphism in individuals with periodontitis (Trevilatto et al. 2003, D'Aiuto et al. 2004).

TNF-a is secreted as a response to bacterial stimulation by a variety of cell types (Taylor et al. 2004) and exhibits its biologic properties by binding two high-affinity receptors (Tervahartiala et al. 2001). TNF-a stimulates osteoclasts differentiation and together with IL-1a may result in bone resorption (Kobayashi et al. 2000, Loos et al. 2005). Furthermore, it promotes the release of collagenases (MMP) that destruct the extracellular matrix (Brenner et al. 1989) and has been excessively produced in inflamed periodontal tissues (Stashenko et al. 1991, Graves & Cochran 2003). Genetic analyses have revealed a large number of polymorphic sites within the TNF-a locus. One of the best-described polymorphisms is the G to A transition at position −308 within the promoter region (Wilson et al. 1993). The rare allele 2 (A−308) has been connected with a higher transcriptional activity (Wilson et al. 1997) and an elevated TNF production (Braun et al. 1996). In the TNF-A gene, many other variants have also been identified (Higuchi et al. 1998).

Several research groups have explored the involvement of cytokine SNPs in the risk of periodontal disease. However, individual studies with small sample sizes may lack the power to detect mild gene effects. Therefore, we conducted a meta-analysis to summarize the findings of separate studies in this scientific field and evaluate possible sources of heterogeneity.

Material and Methods

Retrieval of published studies

A comprehensive Pubmed search up to October 2007 was conducted. The following MESH headings, keywords and text words were used: ''periodontitis'' or ''periodontal disease'' combined with ''interleukin'' or ''IL'' or ''tumor necrosis factor-A'' or ''TNF-A''. After an initial screening of titles and abstracts, only relevant articles remained. The qualifying full text publications and their reference lists were carefully read to decide whether information on the topic of interest was included. We complemented the search by reviewing special meeting issues of journals in order to find abstracts not included in computer indices.

Inclusion and exclusion criteria

Population-based case–control designs were included in the meta-analysis if: (i) they examined the effect of genetic variability in IL-1, IL-6 or TNF-a genes (SNPs) on the occurrence of periodontitis, and (ii) they provided sufficient data to estimate a measure of the association in the form of odds ratio (OR). To avoid selection bias, published manuscripts were considered for review without any language or quality restrictions (Stroup et al. 2000, Pan et al. 2005). Furthermore, to eliminate bias resulting from the ''grey literature'' (Conn et al. 2003), we decided to include also studies published in conference proceedings or as short abstracts.

Data extraction

Two reviewers (N. D., G. N.) independently extracted the necessary information using a standardized form and discrepancies were resolved by consensus. The following data were sought from each study: (i) authors, journal, year of publication, ethnicity of participants and geographical setting; (ii) number of eligible and genotyped cases and controls; (iii) the polymorphism under investigation and the disease form (AP or CP); (iv) frequency of genotypes and alleles in cases and controls; (v) genotyping procedures; (vi) average characteristics of the participants (age, sex, severity of the disease, smoking status, matching details, presence of a systemic disease or a medical condition) that could be used as covariates in a meta-regression. Information concerning these variables was not always available for all studies and in particular concerning the severity of the disease we used a dichotomous variable indicating whether more than 50% of the patients had the severe form of the disease.

Statistical analysis

The OR was used to compare the distribution of alleles and genotypes between cases and controls. We computed the genetic contrast of the mutant allele *versus* the wild type, the contrasts of homozygous genotypes against the others and the contrast of heterozygotes against the remaining persons. In secondary analyses, we calculated specific ORs according to the racial descent of subjects (separate analyses for Caucasians and Asians) and the Hardy–Weinberg equilibrium (HWE) status of the control population. Deviations from the HWE were calculated by the χ^2 method. The between-studies heterogeneity was assessed using the Cochran's Q statistic (Petiti 1994) and the inconsistency index I^2 (Higgins et al. 2003). The combined ORs along with their 95% confidence intervals (CIs) were estimated applying both fixed and random-

effects methods (DerSimonian & Laird 1986). In case of heterogeneity, random-effects models are more appropriate as they estimate the between-study variance (τ^2); however, when heterogeneity is absent, random- and fixed-effects methods coincide.

Publication bias or other small study-related bias was evaluated using the rank correlation method of Begg (Begg & Mazumdar 1994), the Egger's regression method (Egger et al. 1997) and its random-effects analogue (Thompson & Sharp 1999). Influential studies were identified by checking the effect of removing an individual study each time on the overall significance of the estimate or on the heterogeneity statistic. Cumulative meta-analysis (Lau et al. 1992, 1995) was performed in order to investigate the possible trend of the effect over time (Ioannidis & Trikalinos 2005). For all analyses performed here, the statistical package Stata 8 (Stata Corporation, College Station, TX, USA) was used. Statistically significant results were declared those with a p value <0.05.

Results

After a careful screening of the published literature, 53 studies involving 4178 cases and 4590 controls (counting each study's cases and controls only once) were eligible for the meta-analysis (Table 1). Among them, 29 studies genotyped more than one variant. One of the papers contained information about distinct independent populations (Gonzales et al. 2003) and it was counted twice. Four publications lacked the necessary data to assess the HWE and compute ORs in all contrasts (Kornman et al. 1997, Lin et al. 2003, Babel et al. 2006, Tervonen et al. 2007). The polymorphisms occurred in frequencies inconsistent with the HWE in the control arm of nine studies (Parkhill et al. 2000, Qian et al. 2002, Zhong et al. 2002, Sakellari et al. 2003, Brett et al. 2005, Komatsu et al. 2005, Agrawal et al. 2006, Goncalves Lde et al. 2006, Tian et al. 2006). Several of the eligible articles that were written in languages other than English (i.e. Russian, Chinese) were retrieved and translated in order to avoid the ''local literature bias'' (Pan et al. 2005). Concerning smoking, an established risk factor for periodontitis, limited information was available. In particular, of the 19 studies on aggressive periodontitis, 11 (58%) included pieces of relevant information and only four (21%) of them stratified genotypes according to smoking habits. Similarly, among 41 studies on CP, 24 (59%) contained some kind of information and only seven (17%) of them presented the distribution of genotypes separately by smoking status. Thus, there was a lack of necessary data for a proper and comprehensive analysis, even though neither one of the attempted meta-regressions yielded a significant finding.

Studies excluded from the meta-analysis are presented in Table 2. The detailed results concerning AP are listed in Table 3 and Fig. 1, whereas those for CP in Table 4 and Fig. 2.

IL-1A G[4845]T polymorphism (rs17561).

Totally, five studies examined the effect of the IL-1A G[4845]T polymorphism on the development of CP. Among them, two studies were conducted in Caucasian populations (Sakellari et al. 2003, 2006). The per-allele OR of the 4845T variant for CP was 1.162 (95% CI: 0.930, 1.452). A much higher and significant risk for CP was observed only in the TT *versus* GG+GT comparison (OR: 1.898, 95% CI: 1.167, 3.090). However, in the same contrast, meta-analysis of studies enrolling Caucasians or being in HWE produced insignificant results. Heterogeneity was not detected in most analyses and there was no evidence for publication bias.

Altogether, eight reports assessed the association between the IL-1A G[4845]T polymorphism and AP. Half of these studies recruited Caucasians (Gonzales et al. 2003, Scapoli et al. 2005, Sakellari et al. 2006, Havemose-Poulsen et al. 2007). The pooled OR for the T allele *versus* the G allele was 1.115 with a 95% CI 0.861–1.443. Insignificant estimates were derived from all comparisons. Heterogeneity was consistently absent in all analyses whereas publication bias was only detected in the GT *versus* GG+TT contrast.

IL-1A C[– 889]T polymorphism (rs1800587)

In total, 13 studies provided data on the association between IL-1A C[– 889]T polymorphism and CP. Among them, eight studies were performed in populations of Caucasian descent (Kornman et al. 1997, Gore et al. 1998, Shirodaria et al. 2000, Laine et al. 2001, Rogers et al. 2002, Brett et al. 2005, Tsarev & Nikolaeva 2007, Tervonen et al. 2007) and four recruited Asian individuals (Duan et al. 2002, Zhong et al. 2002, Anusaksathien et al. 2003, Huang & Zhang 2004). Two reports lacked sufficient data to calculate ORs in all contrasts (Kornman et al. 1997, Tervonen et al. 2007). Overall, the relative risk of the T allele for CP was 1.310 (95% CI:

Table 1. The list and the characteristics of studies that were included in the meta-analysis

Authors	Year	Country	Cases	Controls	Form of disease	Gene, polymorphism
Tai et al.	2002	Japan	47	97	Aggressive	IL-1A (G[4845]T), IL-1B (C[3953/4]T), IL-1B (T[– 511]C)
Li et al.	2004	China	122	95	Aggressive	IL-1A (G[4845]T), IL-1B (C[3953/4]T), IL-1B (T[– 511]C)
Sakellari et al.	2006	Greece	102	90	Aggressive, chronic	IL-1A (G[4845]T), IL-1B (C[3953/4]T), TNFA (G[– 308]A), Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Gonzales et al.	2003	Germany	28	33	Aggressive	IL-1A (G[4845]T), IL-1B (C[3953/4]T), Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Gonzales et al.	2003	El Salvador	16	14	Aggressive	IL-1A (G[4845]T), IL-1B (C[3953/4]T), Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Scapoli et al.	2005	Italy	40	96	Aggressive	IL-1A (G[4845]T), IL-1B (C[3953/4]T), IL-1B (T[– 511]C)
Havemose-Poulsen et al.	2007	Denmark	45	25	Aggressive	IL-1A (G[4845]T), IL-1A (C[– 889]T), IL-1B (C[3953/4]T), IL-1B (T[– 511]C)
Walker et al.	2000	USA	37	104	Aggressive	IL-1A (G[4845]T), IL-1B (C[3953/4]T), Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)

Table 1. (Contd.)

Authors	Year	Country	Cases	Controls	Form of disease	Gene, polymorphism
Anusaksathien et al.	2003	Thailand	80	43	Aggressive, chronic	IL-1A (C[-889]T), IL-1B (C3953/4T), Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
Hodge et al.	2001	UK	56	56	Aggressive	IL-1A (C[-889]T), IL-1B (C[3953/4]T), Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
Rogers et al.	2002	Australia	69	60	Aggressive, chronic	IL-1A (C[− 889]T), IL-1B (C3953/4T), Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
Brett et al.	2005	UK	107	100	Aggressive, chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T), IL-1B (T[− 511]C), IL-6 (G[− 174]C), TNFA (G[− 308]A)
Quappe et al.	2004	Chile	36	75	Aggressive	IL-1A (C[− 889]T), IL-1B (C[3953/4]T), Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
Moreira et al.	2007	Brazil	122	41	Aggressive, chronic	IL-1A (C[− 889]T)
Drozdzik et al.	2006	Poland	52	52	Aggressive, chronic	IL-1B (C[3953/4]T)
Moreira et al.	2005	Brazil	97	31	Aggressive, chronic	IL-1B (C[3953/4]T)
Agrawal et al.	2006	India	60	60	Chronic	IL-1A (G[4845]T), IL-1B (C[3953/4]T)
Sakellari et al.	2003	Greece	45	110	Chronic	IL-1A (G[4845]T), IL-1B (C[3953/4]T), Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Goncalves Lde et al.	2006	Brazil	58	47	Chronic	IL-1A (G[4845]T), IL-1B (C[3953/4]T), Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Thomson et al.	2001	New Zealand	61	800	Chronic	IL-1A (G[4845]T), IL-1B (C[3953/4]T), Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Duan et al.	2002	China	30	94	Chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T)
Huang & Zhang	2004	China	182	89	Chronic	IL-1A (C[-889]T), IL-1B (C[3953/4]T), IL-1B (T[− 511]C)
Zhong et al.	2002	China	133	92	Chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T)
Laine et al.	2001	The Netherlands	105	53	Chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T), Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
Gore et al.	1998	USA	32	32	Chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T), IL-1B (T[− 511]C)
Kornman et al.	1997	USA	85	49	Chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T), IL-1B (T[− 511]C), TNFA (G[− 308]A), Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
Soga et al.	2003	Japan	64	64	Chronic	IL-1B (C[3953/4]T), IL-1B (T[− 511]C), TNFA (G[− 308]A)
Galbraith et al.	1999	USA	20	45	Chronic	IL-1B (C[3953/4]T), TNFA (G[− 308]A)
Lin et al.	2003	China	124	172	Chronic	IL-1B (C[3953/4]T), TNFA (G[− 308]A)
Babel et al.	2006	Germany	122	114	Chronic	IL-6 (G[− 174]C), TNFA (G[− 308]A)
Parkhill et al.	2000	UK	70	72	Aggressive	IL-1B (C[3953/4]T)
Shirodaria et al.	2000	UK	83	27	Chronic	IL-1A (C[− 889]T)
Tian et al.	2006	China	36	36	Chronic	IL-1B (C[3953/4]T)
Gustafsson et al.	2006	Sweden	13	13	Chronic	IL-1B (C[3953/4]T)
Komatsu et al.	2005	Japan	112	77	Chronic	IL-6 (G[− 174]C)
Holla et al.	2004	Chech	148	107	Chronic	IL-6 (G[− 174]C)
Trevlatto et al.	2003	Brazil	48	36	Chronic	IL-6 (G[− 174]C)
Wohlfahrt et al.	2006	USA	137	82	chronic	IL-6 (G[− 174]C)
Qian et al.	2002	China	65	96	Chronic	TNFA (G[− 308]A)
Fassmann et al.	2003	Czech	132	114	Chronic	TNFA (G[− 308]A)
Folwaczny et al.	2004	Germany	81	80	Chronic	TNFA (G[− 308]A)
Donati et al.	2005	Sweden	60	39	Chronic	TNFA (G[− 308]A)
Craandijk et al.	2002	The Netherlands	90	264	Chronic	TNFA (G[− 308]A)
Pang et al.	2005	China	166	80	Chronic	TNFA (G[− 308]A)
Galbraith et al.	1998	USA	32	32	Chronic	TNFA (G[− 308]A)
Tervonen et al.	2007	Finland	51	178	Chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T), IL-6 (G[− 174]C), TNFA (G[− 308]A)
Tsarev & Nikolaeva	2007	Russia	75	20	Chronic	IL-1A (C[− 889]T), IL-1B (C[3953/4]T), Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
Maria de Freitas et al.	2007	Brazil	30	70	Aggressive	IL-1A (C[− 889]T), TNFA (G[− 308]A)
Endo et al.	2001	Japan	46	104	Aggressive	TNFA (G[− 308]A)
Nastri & Caruso	2003	Italy	20	10	Chronic	Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)
McDevitt et al.	2000	Atlanta, Georgia	44	46	Chronic	Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Papapanou et al.	2001	Sweden	132	73	Chronic	Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T)
Lopez et al.	2005	Chile	330	101	Chronic	Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T)

Table 2. The list and the characteristics of studies that were excluded from the meta-analysis

Authors	Year	Country	Cases	Controls	Form of disease	Gene, polymorphism
Brett et al.	2005	UK	107	100	Aggressive, chronic	IL-10 (C[− 627]A), IL-10 (G[− 1082]A)
Scarel-Caminaga et al.	2004	Brazil	67	43	Chronic	IL-10 (C[− 819]T), IL-10 (C[− 592]A)
Babel et al.	2006	Germany	118	114	Chronic	IL-10 (G[− 1087]A)
Scarel-Caminaga et al.	2004	Brazil	67	43	Chronic	IL-10 (G[− 1087]A)
Berglundh et al.	2003	Sweden	60	39	Chronic	IL-10 (G[− 1087]A)
Sumer et al.	2007	Turkey	75	73	Chronic	IL-10 (C[− 597]A), IL-10 (C[− 824]T)
Endo et al.	2001	Japan	46	104	Aggressive	TNFA (G[− 238]A), TNFA (T[− 1031]C), TNFA (C[− 863]A), TNFA (C[− 857]T)
Soga et al.	2003	Japan	64	64	Aggressive, chronic	TNFA (G[− 238]A), TNFA (T[− 1031]C), TNFA (C[− 863]A), TNFA (C[− 857]T)
Craandijk et al.	2002	The Netherlands	90	264	Chronic	TNFA (G[− 238]A), TNFA (G[− 376]A), TNFA (G[489]A)
Galbraith et al.	1998	USA	32	32	Chronic	TNFA (G[− 238]A)
Gonzales et al.	2004	Japan	31	30	Aggressive	IL-4 (C[− 590]T), IL-4 70bp repeat
Gonzales et al.	2004	German	30	33	Aggressive	IL-4 (C[− 590]T), IL-4 70bp repeat
Scarel-Caminaga et al.	2003	Brazil	69	44	Chronic	IL-4 (C[− 590]T)
Kang et al.	2003	Korea	32	150	Chronic	IL-4 (C[− 590]T), IL-4 70bp repeat
Donati et al.	2005	Sweden	60	39	Chronic	IL-4 RA Q551R
Galicia et al.	2006	Japan	212	210	Aggressive, chronic	IL-6R (A[48892]C), IL-6R (G[− 183]A)
Berdeli et al.	2006	Turkey	103	190	Aggressive chronic	IL-1RN
Folwaczny et al.	2005b	Germany	123	122	Chronic	IL-16 (T[− 295]C)
Folwaczny et al.	2005a	Germany	123	121	ND	IL-18 (G[− 656]T), IL-18 (C[− 607]A), IL-18 (G[− 137]C), IL-18 (T[113]G), IL-18 (C127T), IL-18 codon 35 A/C
Gonzales et al.	2007	Germany	58	51	Aggressive	IL-4 (C[− 590]T), IL-4 (C[− 34]T), IL-13 (C[− 1112]T), IL-13 (A[− 1512]C)
Kara et al.	2007	Turkey	75	73	Chronic	IL-4 (C[− 590]T), IL-4 70bp repeat

0.959, 1.789) with a strong evidence for between-study heterogeneity ($p = 0.003$, $I^2 = 62.80\%$). However, the subgroup analysis of Caucasians yielded a significant measure of effect (OR: 1.314, 95% CI: 1.031, 1.674) without statistical diversity across studies ($p = 0.525$, $I^2 = 0\%$). An enhanced risk for CP was also demonstrated in the comparison of subjects carrying the TT or CT genotype *versus* the CC homozygotes (overall OR: 1.570, 95% CI: 1.110, 2.221, p for heterogeneity = 0.001, $I^2 =$ 62.3%; Caucasians OR: 1.664, 95% CI: 1.269, 2.181, no heterogeneity). Synthesizing data from studies involving mostly patients (>50% of total number of patients) with severe CP (Kornman et al. 1997, Shirodaria et al. 2000, Laine et al. 2001, Duan et al. 2002, Rogers et al. 2002, Moreira et al. 2007, Tsarev & Nikolaeva 2007) also produced significant estimates, without evidence of heterogeneity, in the comparison of alleles (OR: 1.312, 95% CI: 1.007, 1.710), in the CT *versus* TT+CC contrast (OR: 1.439, 95% CI: 1.025, 2.019) and in the TT+CT *versus* CC comparison (OR: 1.639, 95% CI: 1.206, 2.229).

Previous research has shown the remarkable concordance (>99%) between the IL-1A C[− 889]T and the IL-1A G[4845]T polymorphism. Therefore, we combined primary studies also addressing either of these two SNPs. The per-allele OR was 1.266 (95% CI: 1.033, 1.552) with a moderate evidence of heterogeneity ($I^2 = 48.9\%$). The summary analysis of studies conducted among Caucasian populations yielded a significant estimate (OR: 1.325, 95% CI: 1.083, 1.622) as well but the zero value of I^2 testified to the absence of statistical heterogeneity.

The meta-analysis of eight studies showed no indication of an association between the − 889T variant and AP. The per-allele pooled OR was 0.994 with a 95% CI 0.773–1.277. The insignificance of the estimates sustained and the heterogeneity was absent in all comparisons. Synthesizing the estimates from studies investigating the effect either of the IL-1A C[− 889]T or of the IL-1A G[4845]T polymorphism provided non-significant results as well. No statistical test was suggestive of publication bias in meta-analyses related to the IL-1A C[− 889]T polymorphism.

IL-1B C[3953/4]T polymorphism (rs1143634)

Twenty-three studies evaluated the role of the IL-1B C[3953/4]T polymorphism for susceptibility to CP. More than half of them enrolled subjects of Caucasian origin (Kornman et al. 1997, Gore et al. 1998, Galbraith et al. 1999, Laine et al. 2001, Rogers et al. 2002, Sakellari et al. 2003, 2006, Brett et al. 2005, Drozdzik et al. 2006, Gustafsson et al. 2006, Tsarev & Nikolaeva 2007, Tervonen et al. 2007). Three publications had inadequate information to calculate ORs in all contrasts (Kornman et al. 1997, Lin et al. 2003, Tervonen et al. 2007). The carriage of the T allele conferred a 45% relative increase in the risk for CP (OR: 1.447, 95% CI: 1.129, 1.854) and more than doubled the hazard in populations of Asian origin (OR: 2.185, 95% CI: 1.218, 3.917) (Duan et al. 2002, Zhong et al. 2002, Anusaksathien et al. 2003, Soga et al. 2003, Huang & Zhang 2004, Tian et al. 2006, Agrawal et al. 2006). A significant per-allele OR was also derived from patients having the severe form of CP (OR: 2.020, 95% CI: 1.229, 3.322) (Kornman et al. 1997, Galbraith

Table 3. Results of the meta-analysis for the studies of aggressive periodontitis

Contrast	Category	Number of studies	Number of cases/controls	Odds ratio (random effects)	95% confidence interval		I^2 (%)
T allele *versus* G allele (IL-1A G[4845]T, rs17561)							
	All	8	381/551	1.115	0.861	1.443	0.00
	Caucasians	4	159/241	1.046	0.751	1.456	2.30
	Asians	2	169/192	1.232	0.702	2.160	0.00
	Other	2	53/118	1.251	0.659	2.377	0.00
	HWE	8	381/551	1.115	0.861	1.443	0.00
T allele *versus* C allele (IL-1A C[− 889]T, rs1800587)							
	All	8	319/470	0.994	0.773	1.277	7.30
	Caucasian	5	202/311	0.869	0.651	1.158	0.00
	Asian	1	26/43	0.633	0.188	2.134	–
	Other	2	91/116	1.488	0.933	2.374	0.00
	HWE	7	269/370	1.121	0.856	1.468	0.00
T allele *versus* C allele (IL-1B C[3953/4]T, rs1143634)							
	All	16	705/1041	0.944	0.750	1.189	22.10
	Caucasian	9	375/582	0.893	0.689	1.159	26.50
	Asian	3	195/235	1.531	0.367	6.387	47.80
	Other	4	135/224	1.052	0.628	1.761	12.10
	HWE	15	635/969	0.991	0.778	1.262	18.60
C allele *versus* T allele (IL-1B T[− 511]C, rs16944)							
	All	5	303/413	1.114	0.891	1.394	0.00
	Caucasian	3	134/221	1.299	0.930	1.815	0.00
	Asian	2	169/192	0.983	0.727	1.329	0.00
	HWE	5	303/413	1.114	0.891	1.394	0.00
A allele *versus* G allele (TNFA G[− 308]A, rs1800629)							
	All	4	172/361	1.044	0.704	1.550	0.00
	Caucasian	3	126/257	1.011	0.626	1.632	24.90
	Asian	1	46/104	1.133	0.204	6.300	–
	HWE	3	122/264	0.889	0.518	1.527	0.00

HWE, Hardy–Weinberg equilibrium.

et al. 1999, Laine et al. 2001, Duan et al. 2002, Rogers et al. 2002, Soga et al. 2003, Moreira et al. 2005, Tsarev & Nikolaeva 2007). There was statistically significant heterogeneity in these analyses. Both TT *versus* CT+CC and TT+CT *versus* CC contrasts provided evidence of an association between the IL-1B C[3953/4]T polymorphism and CP (OR: 1.604, 95% CI: 1.113, 2.311, no between-study heterogeneity and OR: 1.498, 95% CI: 1.164, 1.928, p for heterogeneity = 0.006, respectively). A stronger association was seen comparing subjects having the TT or CT genotype with the CC homozygotes in Asian populations (OR: 2.424, 95% CI: 1.493, 3.937, no between-study heterogeneity). Furthermore, TT homozygotes had an elevated risk to develop severe CP (OR: 2.499, 95% CI: 1.142, 5.472, no between-study heterogeneity).

On the contrary, the quantitative synthesis of 16 studies was not supportive of a relationship between the IL-1B C[3953/4]T polymorphism and AP. The per-allele OR was 0.944 (95% CI: 0.750, 1.189). Insignificant pooled estimates were obtained from most analyses and heterogeneity was not observed. The Egger test ($p = 0.052$) suggested a marginal evidence for publication bias only in the CT *versus* CC+TT contrast among patients with CP.

Composite genotype IL-1A (C[− 889]T or G[4845]T)/IL-1B (C[3953/4]T)

The effect of the composite genotype on the occurrence of CP has been extensively researched in 13 studies (Table 1). Most investigators (nine) had recruited Caucasian populations (Kornman et al. 1997, Laine et al. 2001, Papapanou et al. 2001, Rogers et al. 2002, Nastri & Caruso 2003, Sakellari et al. 2003, 2006, Lopez et al. 2005, Tsarev & Nikolaeva 2007). The presence of the T allele at IL-1A − 889 or +4845 and at IL-1B 3953/4 loci (positive genotype) was more frequent among periodontitis cases compared with controls, even though the estimate was marginally insignificant (OR: 1.422, 95% CI: 0.981, 2.061). However, Caucasians carrying the positive genotype had a statistically significant elevated risk of developing CP (OR: 1.664, 95% CI: 1.065, 2.602). The I^2 was >55% in the above-mentioned analyses. Confining the analysis to seven studies (Kornman et al. 1997, Laine et al. 2001, Rogers et al. 2002, Anusaksathien et al. 2003, Nastri & Caruso 2003, Lopez et al. 2005, Tsarev & Nikolaeva 2007) addressing the effect of the T allele at IL-1A − 889 (not considering +4845) and at IL-1B 3953/4, we obtained a significant overall estimate (OR: 2.097, 95% CI: 1.120, 3.924). Individuals of Caucasian origin (Kornman et al. 1997, Laine et al. 2001, Rogers et al. 2002, Nastri & Caruso 2003, Lopez et al. 2005, Tsarev & Nikolaeva 2007) carrying the positive genotype (not considering +4845) had also an increased risk of progressing to CP (OR: 2.251, 95% CI: 1.207, 4.199). Non-significant estimates were derived when we focused the analysis on studies examining the role of the T allele at positions IL-1A+4845 (not considering

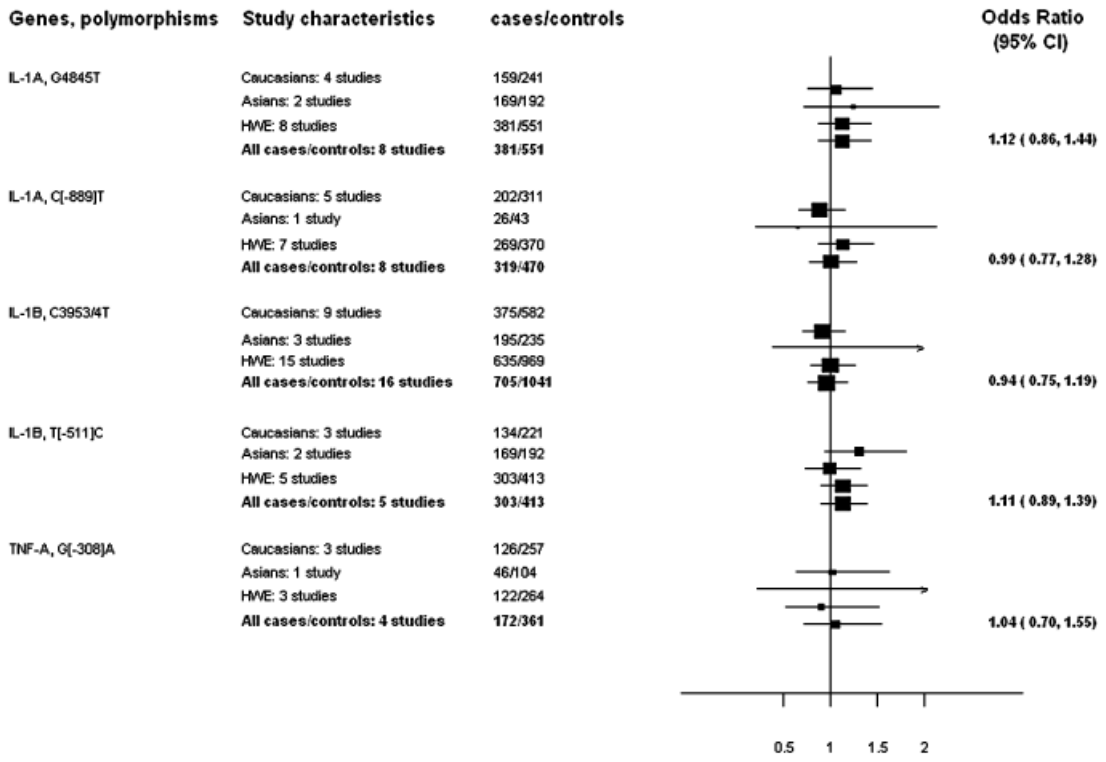

Fig. 1. The results of the meta-analysis for the studies concerning aggressive periodontitis.

– 889) and IL-1B 3953/4. Finally, the findings of meta-analysis do not support any contributory effect of the composite genotype to the initiation of aggressive periodontitis. The results concerning the association between the composite genotype and periodontal disease are presented in Table 5 and they are graphically depicted in Fig. 3.

IL-1B T[– 511]C polymorphism (rs16944)

Five research groups focused on the potential association between the IL-1B T[– 511]C polymorphism and CP. The combined OR of the T allele *versus* the C allele was 1.481 (95% CI: 0.941, 2.332). The elevated risk for CP of Asian subjects having the T allele was based only on two studies (OR: 1.987, 95% CI: 1.223, 3.228) (Soga et al. 2003, Huang & Zhang 2004). Regarding genotype contrasts, heterozygotes and CC homozygotes had a modest increased risk for developing CP (OR: 1.438, 95% CI: 1.031, 2.007), which was higher for subjects of Asian origin (OR: 1.889, 95% CI: 1.122, 3.181). There was no statistically significant between-study heterogeneity for any of these analyses.

The synthesis of five studies addressing the influence of the 511C variant on AP yielded insignificant findings. The per-allele OR of the C allele was 1.114 with a 95% CI 0.891–1.394. Heterogeneity was consistently absent in this meta-analysis. Formal statistical testing was not in favor of the existence of publication bias in most comparisons.

IL-6 G[– 174]C polymorphism (rs1800795)

Totally, seven studies investigated the possible differential risk for CP of the 174C allele carriers. Among them, four teams of investigators recruited Caucasians (Holla et al. 2004, Brett et al. 2005, Babel et al. 2006, Tervonen et al. 2007). Two reports had limited data to calculate effect estimates for all comparisons (Babel et al. 2006, Tervonen et al. 2007). The pooled OR of the C allele *versus* the G allele was 1.083 (95% CI: 0.814, 1.442) with corresponding results in the subgroup analyses. The genotype contrasts showed insignificant findings. Bias diagnostics failed to reveal the presence of publication or other small study-related bias.

TNFA G[– 308]A polymorphism (rs1800629)

A relatively large number of studies (15) have examined the relation between CP and the TNFA G[– 308]A polymorphism. The great majority of studies were carried out in Caucasian populations (Kornman et al. 1997, Galbraith et al. 1998, 1999, Craandijk et al. 2002, Fassmann et al. 2003, Folwaczny et al. 2004, Brett et al. 2005, Donati et al. 2005, Babel et al. 2006, Sakellari et al. 2006, Tervonen et al. 2007). There were missing data on allele or genotype distributions in four reports reducing the available for analysis pool of studies in some contrasts (Kornman et al. 1997, Lin et al. 2003, Babel et al. 2006, Tervonen et al. 2007). The per-allele relative risk for CP of the mutant (A) allele was 0.878 (95% CI: 0.635, 1.213). Generally, all summary estimates indicated the lack of association between the TNFA G[– 308]A polymorphism and the susceptibility to CP.

Similar non-significant results were observed in the meta-analysis of four studies that evaluated the role of the aforementioned TNF-A polymorphism on the occurrence of aggressive periodontitis. The pooled OR for the allele comparison was 1.044 with a 95% CI: 0.704, 1.550. Publication bias was not detected in any of these combined analyses.

Discussion

The current meta-analysis of 53 studies is the first quantitative evaluation of the

Table 4. Results of the meta-analysis for the studies of chronic periodontitis

Contrast	Category	Number of studies	Number of cases/controls	Odds ratio (random effects)	95% confidence interval		I^2 (%)
T allele *versus* G allele (IL-1A G[4845]T, rs17561)							
	All	5	280/1107	1.162	0.930	1.452	0.00
	Caucasians	2	101/200	1.351	0.939	1.945	0.00
	Asians	1	60/60	1.225	0.735	2.040	–
	Other	2	119/847	0.998	0.712	1.399	0.00
	HWE	2	117/890	1.076	0.784	1.477	0.00
T allele *versus* C allele (IL-1A C[− 889]T, rs1800587)							
	All	11	1002/878	1.310	0.959	1.789	62.80
	Caucasian	6	536/519	1.314	1.031	1.674	0.00
	Asian	4	399/318	1.055	0.426	2.612	86.00
	Other	1	67/41	1.869	0.981	3.560	–
	HWE	10	866/551	1.272	0.894	1.808	65.50
	Severe $\geqslant$50%	6	408/295	1.312	1.007	1.710	0.00
T allele *versus* C allele (IL-1B C[3953/4]T, rs1143634)							
	All	20	1470/2328	1.447	1.129	1.854	52.40
	Caucasian	10	617/800	1.224	0.940	1.594	32.00
	Asian	7	683/650	2.185	1.218	3.917	53.70
	Other	3	170/878	1.355	0.700	2.625	64.10
	HWE	17	983/1754	1.446	1.088	1.921	57.90
	Severe $\geqslant$50%	7	393/367	2.020	1.229	3.322	57.20
C allele *versus* T allele (IL-1B T[− 511]C, rs16944)							
	All	4	420/334	1.481	0.941	2.332	69.10
	Caucasian	2	174/184	1.033	0.683	1.563	0.00
	Asian	2	246/153	1.987	1.223	3.228	60.10
	HWE	4	335/285	1.481	0.941	2.332	69.10
C allele *versus* G allele (IL-6 G[− 174]C, rs1800795)							
	All	5	677/695	1.083	0.814	1.442	31.80
	Caucasian	2	380/500	1.282	0.965	1.705	0.00
	Asian	1	112/77	0.688	0.014	34.869	–
	Other	2	185/118	0.809	0.384	1.705	73.30
	HWE	3	333/225	0.995	0.645	1.534	63.60
A allele *versus* G allele (TNFA G[− 308]A, rs1800629)							
	All	11	1202/1508	0.878	0.635	1.213	47.70
	Caucasian	8	783/1098	0.970	0.763	1.233	0.00
	Asian	3	419/410	0.611	0.151	2.468	81.20
	HWE	9	700/806	1.030	0.805	1.317	0.30

HWE, Hardy–Weinberg equilibrium.

relevance to either CP or CP of six cytokine gene polymorphisms. Previous attempts to summarize the existing evidence have always been in the context of a narrative literature review (Greenstein & Hart 2002, Nares 2003, Loos et al. 2005, Shapira et al. 2005, Huynh-Ba et al. 2007). The following SNPs were the focus of the analysis because an adequate number of primary studies provided sufficient information to calculate pooled effect estimates for the strength of the postulated associations: IL-1A G[4845]T, IL-1A C[− 889]T, IL-1B C[3953/4]T, IL-1B T[-511]C, IL-6 G[-174]C, TNFA G[-308]A. Data on other cytokine gene polymorphic markers were sparse (Table 2) and clearly further research is needed concerning these polymorphisms in order to obtain reliable estimates.

The findings of the meta-analysis indicate a moderate positive association between the IL-1A − 889T variant and CP in Caucasians. The current report also documents an elevated risk for CP of IL-1B 3953/4T carriers, particularly in Asian-descent patients. Furthermore, the simultaneous carriage of the T allele at IL-1A − 889 and at IL-1B 3953/4 loci seems to confer an additional risk compared with the separate effect of each SNP. Based on a smaller number of studies compared with the latter SNPs, the analysis provided an indication of a weak positive effect of the IL-1B 511C variant on CP. On the contrary, the remaining polymorphisms were not found to determine significantly the susceptibility to CP. Furthermore, the − 889T and the 3953/4T allele seemed to be related to the development of the severe form of the disease (i.e. when analysing separately studies with more than 50% severe CP patients). This fact is an indication of a dose–response effect, and according to the Hill's criteria of causality (Rothman & Greenland 2005), should be a strong indication for a true aetiologic relationship concerning the particular genetic variants and the disease. Finally, the accumulation of existing genetic data suggested no association of aggressive

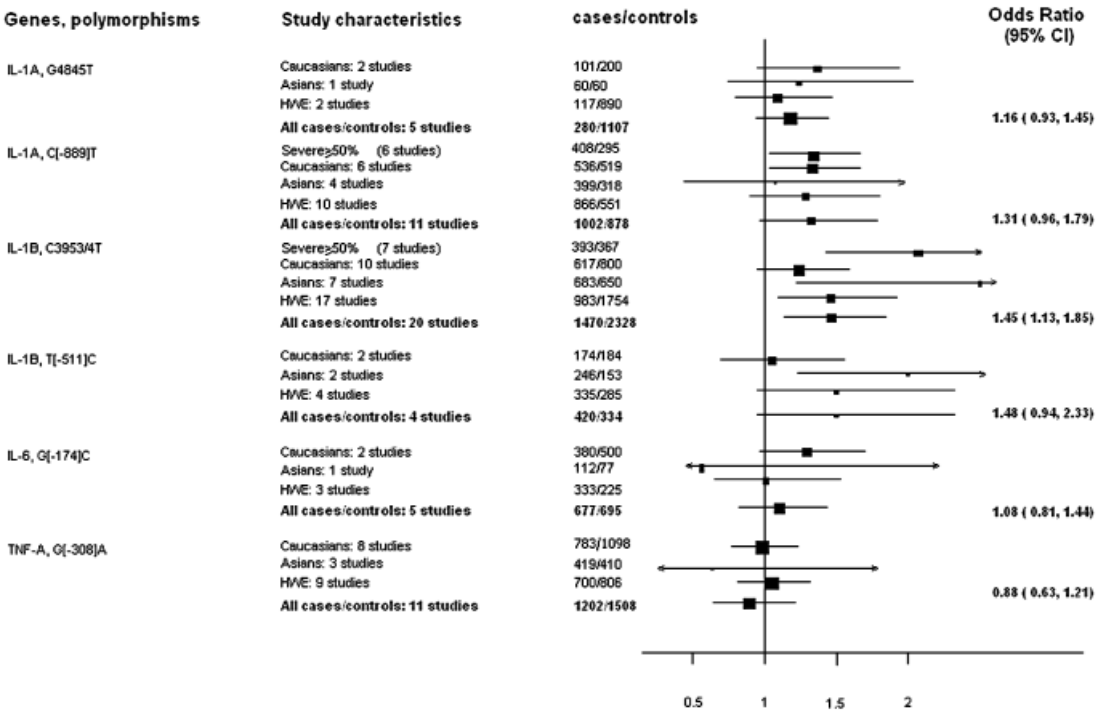

Fig. 2. The results of the meta-analysis for the studies concerning chronic periodontitis.

Table 5. The results of the meta-analysis concerning the association of the composite genotype with either aggressive or chronic periodontitis

Contrast (Positive vs negative genotype)	Category	Number of studies	Number of cases/controls	Odds ratio (random effects)	95% confidence interval		I^2 (%)
Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T) in aggressive periodontitis	All	4	139/234	1.259	0.749	2.114	0.00
	Caucasians	2	77/116	0.979	0.529	1.810	0.00
	Asians	1	26/43	1.680	0.101	28.064	0.0
	Other	1	36/75	2.444	0.876	6.825	0.0
Composite IL-1A (G4845T)/IL-1B (C[3953/4]T) in aggressive periodontitis	All	4	127/241	1.047	0.617	1.776	0.00
	Caucasians	2	74/123	1.198	0.647	2.216	0.00
	Other	2	53/118	0.715	0.254	2.012	0.00
Composite IL-1A (C[− 889]T)/IL-1B (C[3953/4]T) in chronic periodontitis	All	7	687/331	2.097	1.120	3.924	54.90
	Caucasians	6	633/288	2.251	1.207	4.199	57.00
	Asians	1	54/43	0.260	0.010	6.543	0.0
	Severe 50%	4	248/143	1.854	0.688	4.999	65.20
Composite IL-1A (G4845T)/IL-1B (C[3953/4]T) in chronic periodontitis	All	6	396/1166	1.004	0.752	1.341	0.00
	Caucasians	3	233/273	1.066	0.730	1.555	0.00
	Other	3	163/893	0.962	0.546	1.694	28.40

periodontitis with the six SNPs examined in the present meta-analysis.

Based on this summary analysis, there is remarkable evidence, without publication bias or significant statistical heterogeneity across studies that IL-1A C[− 889]T and IL-1B C[3953/4]T are risk factors for CP. Several arguments can reinforce the positive findings of the meta-analysis. IL-1a and IL-1b have pro-inflammatory properties and they are found in increased levels in diseased periodontal tissues. Furthermore, the polymorphisms in the IL-1 genes have direct functional significance by altering the gene transcription or the protein production and they have also been connected with other complex diseases (Pociot et al. 1992, Shirodaria et al. 2000, Loos et al. 2005, Shapira et al. 2005). However, population-based gene–periodontitis association studies are subject to limitations such as the limited size of subjects sample, the poor matching for potential confounders, the existence of heterogeneity in periodontitis definition, the small size of the effect estimates, the multiple testing and the inappropriate selection of controls (Borrell & Papapanou 2005). Thus, a significant association could be due to population stratification, to a genotyping or simply to a statistical sampling error. Furthermore, a true relationship between an allele and a disease may be attributed to the physically close location of the associated allele to the actual disease susceptibility locus that is currently unknown (Kinane & Hart 2003).

Meta-analysis cannot correct all the biases of individual studies but it generates a statistical conclusion with larger power and precision (Petiti 1994). Many efforts have been gone into conducting appropriately the meta-analysis and avoiding any possible source of bias. We did not apply any form of quality scoring (Greenland 1998), we searched

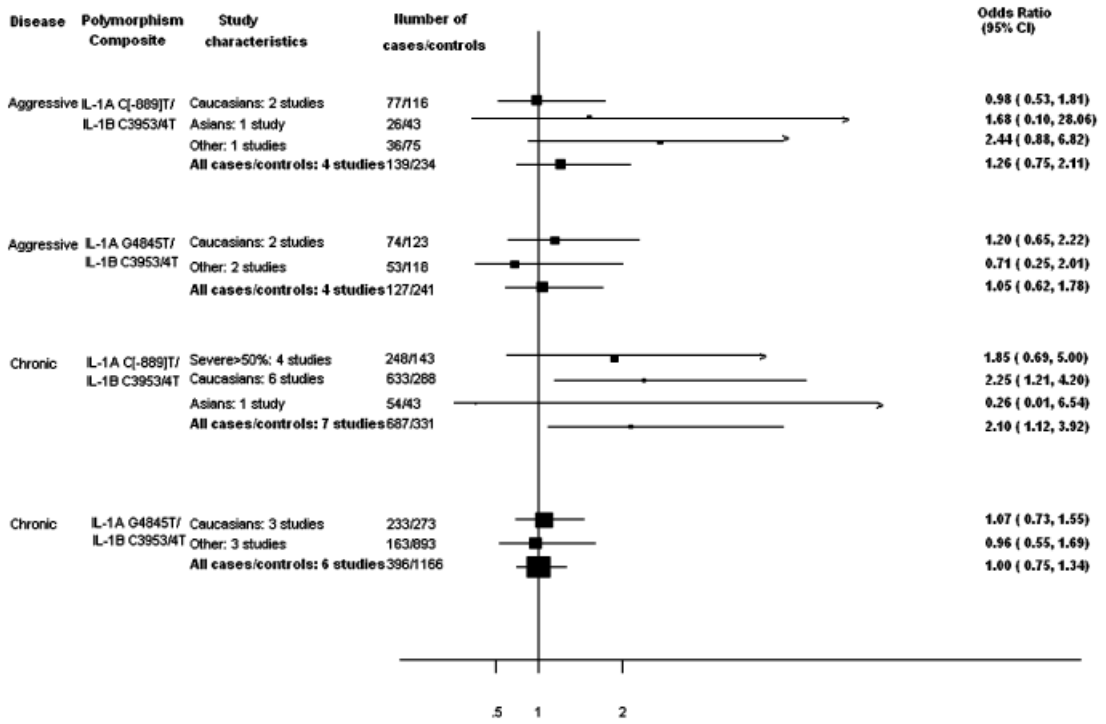

Fig. 3. The results of the meta-analysis concerning the association of the composite genotype with either aggressive or chronic periodontitis.

for works not listed in the publicly available databases (Conn et al. 2003), we identified, retrieved and included in the analysis non-English articles in order to avoid the local literature bias (Pan et al. 2005), we assessed the impact of deviations from the HWE (Trikalinos et al. 2006), we performed appropriate tests for detecting publication bias or other small study-related bias (Egger et al. 1997, Sterne et al. 2000) and we addressed the problem of the early extreme estimates appearing in the meta-analysis of genetic association studies (Ioannidis & Trikalinos 2005) that correlate with the replication validity of studies in genetic epidemiology (Ioannidis et al. 2001). While smoking is widely considered a risk factor for periodontitis, sparse data on smoking habits across studies did not allow the comprehensive investigation of smoking's confounding effect. However, meta-regression analyses based on few studies did not reveal an association between the summary estimates and the smoking status.

A combined estimate with enhanced statistical power is extremely useful for gene–disease association research where small effects are usually anticipated. Furthermore, a primary advantage of meta-analysis is the exploration of the heterogeneity across studies using direct statistical tests (Petiti 1994). Therefore, the number of published meta-analyses of genetic studies continuously rises and there are increasing numbers of scientific articles addressing the proper methodology of pooling genetic evidence (Munafo & Flint 2004, Thakkinstian et al. 2005, Bagos & Nikolopoulos 2007). An example of the increasing power provided by a meta-analytic approach is the insignificant measure of association derived for the IL-1A G[4845]T polymorphism among Caucasians and the significant estimate obtained for the IL-1A C[−889]T in the same population group. The aforementioned loci are in almost complete linkage disequilibrium and the results should have been comparable. The magnitude of the estimates was small but similar in both cases (1.351 for IL-1A G[4845]T and 1.314 for IL-1A C[−889]T). However, the first analysis enrolled only two studies yielding a marginally insignificant result (95% CI: 0.939, 1.945) whereas in the second case, with six studies included, the results reached significance along with a narrower CI (95% CI: 1.031, 1.674). Indeed, using the method proposed by Barrowman et al. (2003), we estimate that only one additional study on Caucasian subjects would suffice to produce a significant estimate for the G[4845]T polymorphism. Meta-analysis obviously increases the likelihood of identifying a true effect but there is also need for a sufficient number of eligible primary studies to be included. Thus, future research should concentrate on SNPs such the IL-1B T[−511]C for which meta-analysis based on relatively small number of patients was suggestive, despite not convincing, of an association.

Periodontitis is a serious and prevalent chronic inflammatory disease in humans and it is important to elucidate its aetiology. Environmental factors such infection by specific bacteria at high levels, smoking and poorly controlled diabetes mellitus are implicated largely in the occurrence of periodontal disease but a potential multigenic predisposition seems to exist and needs investigation (Borrell & Papapanou 2005, Heitz-Mayfield 2005, Loos et al. 2005, Palmer et al. 2005). Future studies should carefully address such interactions, i.e. performing stratified analyses that could be used in a subsequent meta-analysis. On the other hand, the list of candidate susceptibility loci is continuously expanding but most of the reported associations are not replicated in subsequent research (Loos et al. 2005). Even if genetic effects are repeatedly observed, their small impact may not result in routine screening recommendations and clinical

benefits in the near future. However, the knowledge of the genetic determinants helps to understand better and deeply the causal pathways of a multifactorial disease such as periodontitis. One other suggestion for future research thus, concerns the conduction of studies that address simultaneously the combined effect of two or more SNPs perhaps by haplotype analysis.

In conclusion, we found that some cytokine gene polymorphisms are associated with modest increases in the probability of periodontal disease developing. Future studies concerning the distribution and dynamics of genetic variation at many loci simultaneously might decipher the direct and epistatic (interaction among multiple alleles) genetic involvement in periodontitis (Ioannidis et al. 2006). Combining a few genetic variants, even with moderate effects, may improve substantially our ability to predict periodontitis risks and the clinical utility of genetic research (Ioannidis et al. 2006). Furthermore, the gene–environment interaction should also be considered and recently employed approaches in other scientific areas such as the whole-genome association studies or the mendelian randomization approach (Minelli et al. 2005) must have a place in the field of periodontology (Borrell & Papapanou 2005, Loos et al. 2005, Shapira et al. 2005).

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Clinical Relevance

Scientific rationale for the study: Conflicting results have been presented in the past concerning the role of a genetic component in the susceptibility to periodontitis and various cytokine gene SNPs have been implicated.

Principal findings: With this meta-analysis, we present for the first time strong evidence that IL-1A C[−889]T and IL-1B C[3953/4]T polymorphisms are significantly associated with chronic periodontal disease and the association is stronger for patients having the severe form of the disease. On the other hand, none of the tested polymorphisms showed any significant association with aggressive periodontitis.

Practical implications: The results presented here may have implications in screening and prevention of periodontal disease.