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Review Article

TLR4 as a risk factor for periodontal disease: a reappraisal

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Abstract

Aim: To determine whether genetic variants of the *TLR4* gene are associated with either chronic or aggressive periodontitis.

Methods: A systematic electronic search of literature was conducted to identify all published studies without any language restriction on the association between *TLR4* and periodontal diseases, including chronic periodontitis and aggressive periodontitis. All case–control studies evaluating the *TLR4* Asp299Gly and Thr399Ile polymorphisms in chronic or aggressive periodontitis were identified. A meta-analysis of the studies that fulfilled the inclusion criteria was performed.

Results: Seven studies comprising 744 chronic periodontitis cases and 855 controls and four studies consisting of a total of 295 aggressive periodontitis cases and 456 controls were included in the meta-analysis. In the pooled analysis, the *TLR4* 299Gly allele (*TLR4*+896 A>G) appeared to be a genetic risk factor for susceptibility to chronic periodontitis with a random effects and fixed effects odds ratio (OR) of 1.43 [95% confidence interval (CI):1.04–1.97; $p = 0.03$]. On the other hand, the *TLR4* 399Ile polymorphism (*TLR4*+1196 C>T) showed a protective effect against aggressive periodontitis with a random effects OR of 0.29 (95% CI: 0.13–0.61; $p = 0.001$).

Conclusion: Our results suggest that the alleles 299Gly and 399Ile in *TLR4* can be a potential genetic marker for periodontal disease.

Key words: association; meta-analysis; periodontal diseases; polymorphisms

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Both environmental and genetic factors have been implicated in the aetiology of periodontal diseases (Kinane et al. 2006a, b). Twin studies have suggested that as much as 50% of the risk for periodontal disease may be due to genetic factors (Michalowicz et al. 2000). Analogous to other complex diseases, it was estimated that 10–20 disease-modifying genes may be involved in the pathogenesis of periodontal disease (Loos et al. 2005). Toll-like receptor (TLR) 4, a member of the TLR superfamily and also described as ''immunity's eye'' (Travis 2001) or ''bug detectors'' (Kaisho & Akira 2001), is considered a good candidate gene for periodontal disease because of its role in the immune system (Hajishengallis et al. 2002, Janeway Jr. & Medzhitor 2002). Several lines of evidence support the biological relevance of TLR4 in the pathogenesis of periodontal disease. TLRs are involved in the recognition of Gram-negative bacteria such as the lipopolysaccharide (LPS) from *Porphyromonas gingivalis*, a major periodontopathogen (Poltorak et al. 1998, Takeuchi et al. 1999). The recognition of microbial components by gingival fibroblast TLRs leads to the release of critical pro-inflammatory cytokines that are necessary to activate potent immune responses (Wang et al. 2000). The expression of *TLR4* in epithelial gingiva (Mori et al. 2003, Ren et al. 2005) and human gingival fibroblast (Wang et al. 2003) supports its role in periodontal disease. The role of *TLR4* in periodontal disease is further supported by the work of Kinane et al. (2006a, b), which shows that gingival epithelial cells heterozygous for the *TLR4* polymorphisms Asp299Gly and Thr399Ile are hyporesponsive to *P. gingivalis* and produce lower levels of pro-inflammatory cytokines. Furthermore, *Tlr4-d*eficient mice showed hyporesponsiveness to LPS (Qureshi et al. 1999). Despite the perceived importance of *TLR*4 in the aetiology of periodontal disease, the results have been contradictory. Differences in the populations studied, difficulty in defining the phenotype, lack of statistical power, and the extent of linkage disequilibrium between genetic variants are potential factors that could underlie the discrepant results of these studies. Meta-analysis has been proposed as a tool to address the contradictory results of association studies (Emahazion et al. 2001, Ioannidis et al. 2001a, b, Lohmueller et al. 2003). This method can

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deal with these ambiguities and also overcome the problem of the small sample sizes and inadequate statistical power of genetic studies of complex traits. In this study, we conducted a meta-analysis to assess the association between genetic variants of *TLR4* and periodontal diseases.

Methods

Selection of studies

A systematic search of the literature was carried out using electronic databases including the Medline, PubMed, Embase, and Science Citation Index for all articles that were published by June 2008 on the association between *TLR4* polymorphisms and periodontal disease risk. The following terms were used in this search: ''Toll-Like Receptor 4'', ''TLR4'', concatenated with ''periodontal diseases'', ''periodontitis'', ''polymorphisms'', and ''Restriction Fragment Length Polymorphism.'' Additional articles were searched through the references cited in the articles selected. No language restrictions were applied. If any overlapping studies were identified, only the one that contained the original data was included. The reports with incomplete genotyping data were not included. The allele and genotype frequencies of *TLR4* reported from all selected studies were recorded and used as primary data for further analysis. The following information was extracted from each study: year of publication, ethnicity of the study population, and the number of cases and controls for each Asp299Gly and Thr399Ile genotype (Table 1). Studies were eligible if they had determined the distribution of alleles and/or genotypes for any of these polymorphisms in unrelated cases and controls with either chronic periodontitis or aggressive periodontitis.

Statistical analysis

Allele and genotype frequencies were described for each study. Hardy–Weinberg Equilibrium (HWE) was assessed using the χ^2 test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for major allele *versus* minor allele carriers for both Asp299Gly and Thr399Ile polymorphisms. The pooled OR was estimated using fixed effects (Mantel–Haenszel) and random-effects (DerSimonian & Laird 1986) models (Lau et al. 1997). Fixed-effects models assume that all

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

Author (year)/country			*TLR4* Asp299Gly						*TLR4* Thr399Ile					
	case	control	RR	OR	95% CI	W (%)	MAF case	MAF control	RR	OR	95% CI	W (%)	MAF case	MAF control
Chronic periodontitis	*N*	*N*				Random						Random		
Folwaczny et al. (2004)/Germany	122	122	–	1.30	0.49;3.42	10.95	0.04	0.03	–	1.27	0.51;3.20	14.63	0.05	0.04
Brett et al. (2005)/England	57	97	–	1.74	0.55;5.50	7.73	0.06	0.04	–	0.31	0.10;0.99	12.24	0.04	0.09
Laine et al. (2005)/the Netherland	100	99	–	1.30	0.43;2.91	11.34	0.05	0.05	–	1.13	0.43;2.91	14.31	0.05	0.05
Schröder et al. (2005)/Germany	116	116	+	2.22	1.07;4.61	19.22	0.21*	0.05*	+	6.57	2.56;16.86	14.41	0.21*	0.05*
Berdeli et al. (2007)/Turkey	83	106	–	0.84	0.23;3.07	6.07	0.02	0.03	–	0.75	0.17;3.23	9.61	0.02	0.02
Izakovicova Holla et al. (2007)/Czech	171	218	–	1.46	0.79;2.70	26.99	0.07	0.05	–	1.54	0.83;2.88	18.03	0.07	0.05
James et al. (2007)/England	90	94	–	1.18	0.55;2.53	17.7	0.1	0.09	–	1.26	0.60;2.62	16.76	0.12	0.1
Pooled Data				1.43	1.04;1.97	$p = 0.028$				1.31	0.72;2.39	$p = 0.38$		
Quantifying heterogeneity				$I^2 = 0\%$	0%;34.5%					$I^2 = 66.6\%$	[25.4%;85%]			
Test of heterogeneity				$Q = 2.67$; $p = 0.85$						$Q = 17.95$; $p = 0.006$				
Aggressive Periodontitis														
Brett et al. (2005)/England	51	97	–	3.27	1.09;9.82	24.96	0.09	0.04	-	0.25	0.07;0.91	24.5	0.03	0.09
Schröder et al. (2005)/Germany	81	81	–	2.65	1.05;6.70	26.71	0.2*	0.1*	-	2.65	1.06;6.70	27.79	0.2*	0.1*
Emingil et al. (2007)/Turkey	90	155	–	0.85	0.25;2.90	23.6	0.02	0.03	-	0.47	0.10;2.31	21.67	0.01	0.02
James et al. (2007)/England	73	123	+	0.26	0.08;0.78	24.73	0.03	0.08	+	0.25	0.08;0.78	26.04	0.03	0.08
Pooled Data				1.20	0.38;3.80	$p = 0.76$				0.56	0.15;2.01	$p = 0.37$		
Quantifying heterogeneity				$I^2 = 78.2\%$	41.2%; 91.9%					$I^2 = 78.3\%$	[41.5%; 91.9%]			
Test of heterogeneity				Q = 13.73; $p = 0.003$						$Q = 13.81$; $p = 0.003$				

*Schröder et al. (2005) reported genotype frequencies of Asp299Gly and Thr399Ile only in association.
RR, reported results; +, positive association; –, no association; W, study weight; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency.
OR (95% CI) calculated by the authors of the present study under random-effects model.

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studies aim at evaluating a common truth and results differ by chance alone. Random-effects models anticipate that the studies may have genuine differences in their results (Cooper & Heddges 1994).

The heterogeneity between studies was tested using the Cochrane Q test and results were considered significant when p-values were <0.10 (Cochran 1954, Lau et al. 1998, Zintzaras & Ioannidis 2005). We quantified the extent of heterogeneity using I2, which represents the proportion of variability between studies attributable to true variability rather than chance (Higgins et al. 2003, Higgins & Thompson 2004). Publication bias was examined by plotting a funnel of the reported effect assessed with the OR against the standard error of the natural logarithm of the OR ($p \geqslant 0.10$; Begg & Mazumdar 1994, Harbord et al. 2006). The meta-analysis was performed with the Meta package (http://www.cran.r-project.org/bin/windows/base).

Results

The combined search yielded 10 papers that provided information on the association of either chronic or aggressive periodontitis with *TLR4* Asp299Gly and Thr399Ile polymorphisms. Only two studies were from Asia (Fukusaki et al. 2007, Zhu et al. 2008). The rest were conducted in European countries (Folwaczny et al. 2004, Brett et al. 2005, Laine et al. 2005, Schröder et al. 2005, Berdeli et al. 2007, Izakovicova Holla et al. 2007, James et al. 2007). One study (D'Aiuto et al. 2004) was excluded because no genotyping information was available for the controls. Asp299Gly and Thr399Ile were not polymorphic in the Asian population (Fukusaki et al. 2007, Zhu et al. 2008). From those seven case–control studies in European Caucasians, 744 patients and 855 controls were evaluated. The overall frequency of the Asp299Gly wild-type alleles in cases and controls was 93.4 and 95.1, respectively. For the Thr399Ile variant, the wild-type allele frequency in cases and controls was 93.3 and 94.9, respectively. The overall frequency of the wild-type allele in the cases tended to be lower than the frequency in controls (Asp299Gly; $p = 0.04$, and Thr399Ile; $p = 0.06$).

In each study, we tested for HWE using the χ^2 test. The genotype distribution of the two *TLR4* variants studied among cases and controls in each study did not deviate from the expected HWE. The overall genotyping distribution of the two variants in all studies was also in HWE equilibrium.

For chronic periodontitis, seven studies of European origin were available (Table 1). The heterogeneity test for the pooled data sets was not significant (I2 = 0%, $p = 0.85$) for the *TLR4* Asp299Gly polymorphism, indicating the robustness of the meta-analysis for chronic periodontitis. The combined OR with a fixed effects and a random-effects model for chronic periodontal disease for the Asp299Gly polymorphism was 1.43 (95% CI: 1.04–1.97; $p = 0.03$). The *TLR4* Thr399Ile polymorphism showed significant evidence of genetic heterogeneity between studies (I2 = 66.6 percent, $p = 0.006$). Random-effects models are more appropriate when heterogeneity is present (DerSimonian and Laird 1986, Trikalinos et al. 2001). Thus, the pooled effect was calculated with a random-effects model for the *TLR4* Thr399Ile and resulted in an insignificant association (OR: 1.31; 95% CI: 0.72–2.39; $p = 0.38$).

Only four studies (Brett et al. 2005, Schröder et al. 2005, Emingil et al. 2007, James et al. 2007) were available that studied the relationship of aggressive periodontitis and the *TLR4* Asp299Gly and Thr399Ile polymorphisms, consisting of a total of 295 cases and 456 controls (Table 1). Both markers showed significant evidence of genetic heterogeneity ($I^2 = 78.3\%$, $p = 0.003$ and $I^2 = 78.3\%$ $p = 0.003$, respectively). The combined OR with a random-effects model for aggressive periodontitis did not reveal any association with *TLR4* Asp299Gly (OR 1.20; 95% CI: 0.37–3.83; $p = 0.76$), or *TLR4* Thr399Ile (OR 0.55; 95% CI: 0.15–2.01; $p = 0.37$) (Fig. 1).

Additionally, we performed a sensitivity analysis by removing one data set at a time. The aim of this analysis was to evaluate the influence of individual studies. The pooled OR was recalculated in the absence of each study. Iterative sensitivity analysis revealed that Schröder et al. (2005) accounted for the heterogeneity that was observed in aggressive periodontitis and the *TLR4* Thr399Ile polymorphism (Table 2, Fig. 2). Excluding this study changed genetic heterogeneity from 78.3% to 0% among studies and resulted in a significant change in the summary (OR 0.29; 95% CI: 0.13–0.61; $p = 0.001$). As per the *TLR4* Asp299Gly polymorphism and aggressive periodontitis and *TLR4* Thr399Ile in chronic periodontitis, excluding any individual study did not resolve heterogeneity among studies. Likewise, the summary OR was not significantly changed.

Furthermore, the Begg–Mazumdar test and the modified Egger test (Egger et al. 1997, Harbord et al. 2006) for funnel plot asymmetry for all study groups did not show any indication of publication bias (for *TLR4* Asp299Gly: chronic periodontitis, $p = 0.45$ and 0.34; aggressive periodontitis, $p = 0.50$ and 0.69; and for *TLR4* Thr399Ile: chronic periodontitis, $p = 0.10$ and 0.34; and aggressive periodontitis, $p = 1.0$ and 0.77) (Fig. 3).

Discussion

Periodontal disease is a complex disorder influenced by multiple genes and environmental factors, and each genetic factor contributes in varying degrees to the development of the disease. In this study, we focused on two co-segregating functional polymorphisms of *TLR4*: Asp299Gly and Thr399Ile. On the basis of seven case–control studies in Caucasian subjects, our meta-analysis revealed that *TLR4* Asp299Gly was associated with a significant increase in the risk of chronic periodontal disease (OR: 1.43; 95% CI: 1.04–1.97; $p = 0.03$). After exclusion of one study (Schröder et al. 2005) that accounted for study heterogeneity, the association between *TLR4* Thr399Ile and aggressive periodontitis became statistically significant (OR:0.29; 95% CI: 0.13–0.61; $p = 0.001$). The German study of Schröder et al. (2005) reports a control group well matched by age, gender, and smoking status. Six examiners determined the periodontal status of the participants in this study; however, all of them were trained at the same programme at the Charité University Medical Center, Berlin. Finally, among the 197 cases, there were 21 individuals from Croatia (10.7%), and among controls, 27 individuals (13.7%) were from Konstanz and not Berlin. Konstanz is located in the southwest corner of Germany, bordering Switzerland. We may speculate that undetected population stratification may have affected the results presented in this study.

Several studies have examined the role of *TLR4* genetic variation in chronic and aggressive periodontitis, but the results have been equivocal. Although *TLR4* is biologically a good candidate

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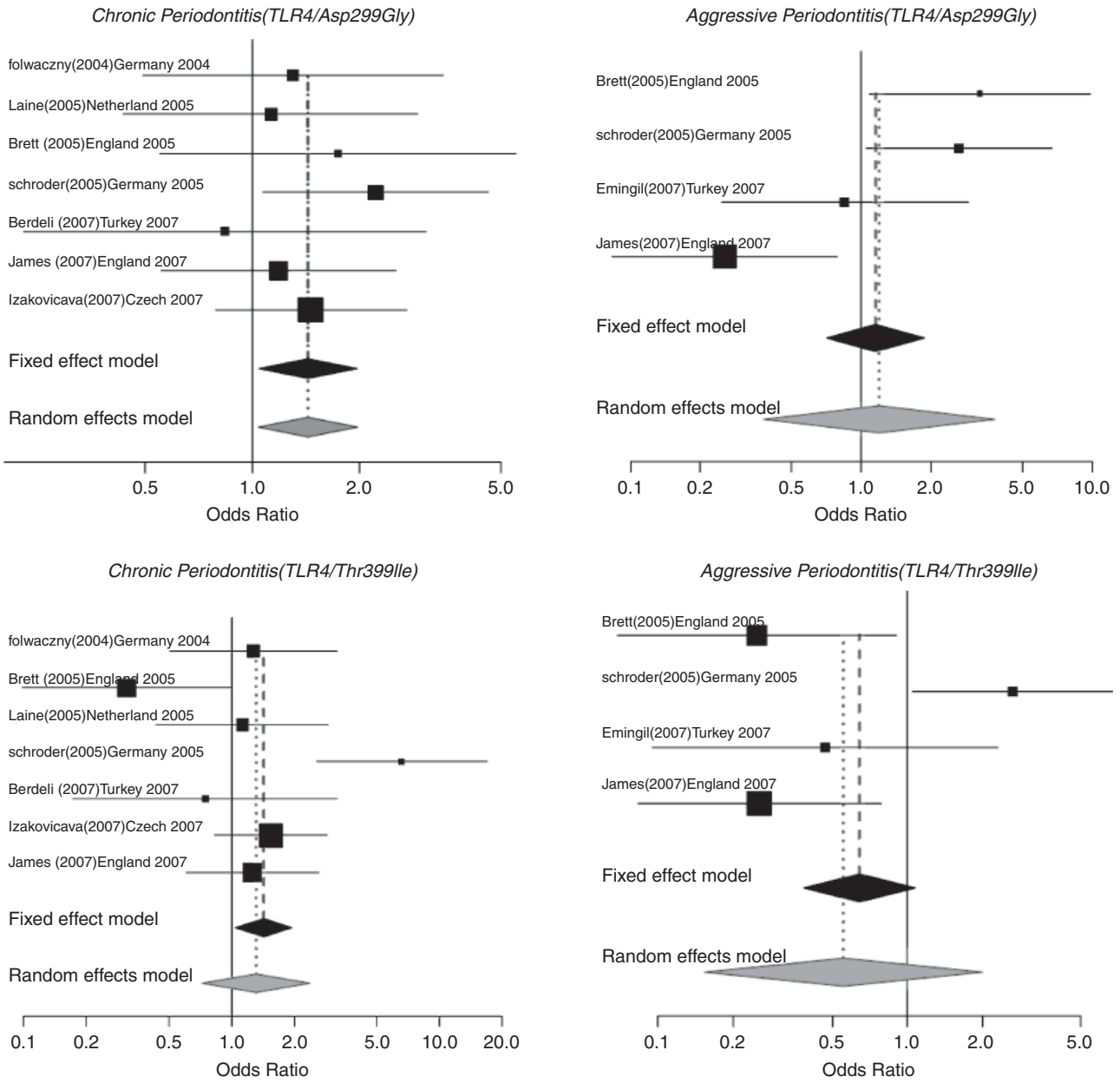

Fig. 1. Forest plot of published case–control association studies of *TLR4* Asp299Gly and Thr399Ile in European populations. Lines represent 95% confidence intervals (CI) for odds ratio of each study. Box sizes are proportional to inverse-variance weights. Meta-analysis by fixed-effects model and random-effects model is shown by diamonds (black and grey colours, respectively). The two ends of the diamonds indicate 95% CI.

Table 2. Summary results of the influential analysis (random-effects model)

Author (year)/country	OR	95% CI	*p*-value	I^2 (%)
Omitting Emingil et al. (2007)/Turkey	0.5755	[0.1099; 3.0130]	0.513	85.3
Omitting James et al. (2007)/England	0.7245	[0.1494; 3.5131]	0.6891	79.3
Omitting Brett et al. (2005)/England	0.7145	[0.1465; 3.4854]	0.6776	81.5
Omitting Schröder et al. (2005)/Germany	0.2898	[0.1373; 0.6118]	0.0012	0.0

CI, confidence interval; OR, odds ratio.

gene in the aetiology of periodontal disease, only one study (Schröder et al. 2005) showed a statistically significant association between chronic periodontal disease and *TLR4* Asp299Gly. The study by Folwaczny et al. (2004) and subsequent studies did not support the association with the risk of chronic periodontal disease and *TLR4* polymorphisms in Caucasian populations. In our meta-analysis, we found evidence of an association between chronic periodontal disease and *TLR4* Asp299Gly (Fig. 1). Although there was limited information on the *TLR4* Asp299Gly and Thr399Ile polymorphisms in Asians (Fukusaki et al. 2007, Zhu et al. 2008), the Japanese study (Fukusaki et al. 2007) found that another variant in *TLR4* was associated with a risk of chronic periodontal disease.

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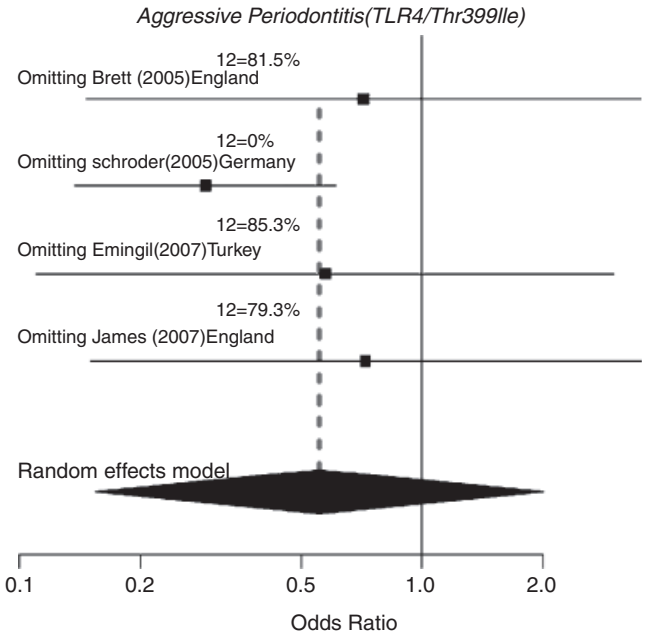

Fig. 2. Influential analysis (random-effects model). Pooled odds ratios and 95% confidence intervals for excluding each data set in the meta-analysis (up to July 2008).

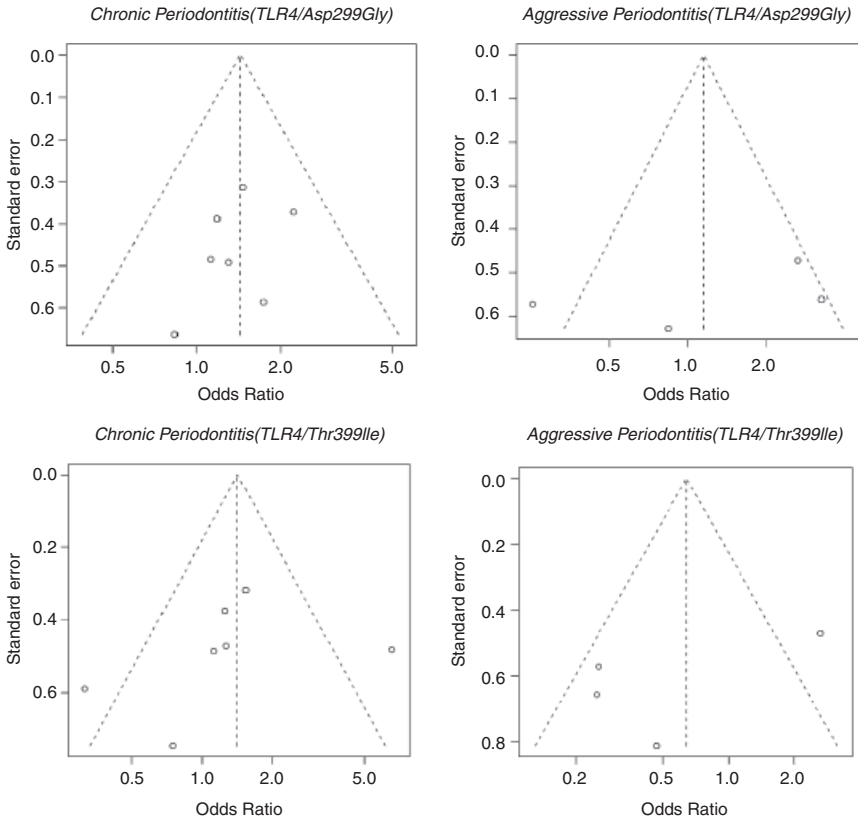

Fig. 3. Funnel plots of *TLR4* studies included in the meta-analysis. Points indicate odds ratios from the studies included in the meta-analysis (a: $p = 0.45$ and 0.34, b: $p = 0.50$ and 0.69, c: $p = 0.10$ and 0.34, d: $p = 1.0$ and 0.77; Begg & Mazumdar 1994 and Harbord et al. 2006, respectively).

Establishing the genetic association of a modest size requires larger sample sizes (Todd 1999, Ioannidis et al. 2001a, b). Conflicting results are often obtained in studies of the genetics of complex traits, including investigations of gene–gene and gene–environment interactions, and population stratification complicates these findings (Todd 1999, Brennan 2002). In our meta-analysis, many of the individual studies appeared to be underpowered. A sample size of 500–2000 case–control pairs is needed in order to detect a moderate genetic effect size (Brennan 2002). The large number of potential genetic risk factors and the relatively low frequency of polymorphic variants may contribute to the fact that initial observations may not be validated by subsequent studies.

Reporting bias is another limitation of the meta-analysis approach. Negative results do not tend to be published, and whereas positive ones make it to the press (Ioannidis et al. 2006). In addition, we could observe small study effects, which are caused by small studies that show a stronger effect and are more likely to be published (Sterne et al. 2000). In order to probe for possible small study effects and publication bias we performed a funnel plot analysis (Fig. 3). The results for two different tests on funnel plot asymmetry (Begg & Mazumdar 1994, Harbord et al. 2006) showed no clear indication of funnel plot asymmetry for both variants in chronic periodontitis (Asp299Gly, $p = 0.10$ and Thr399Ile, $p = 0.13$) or aggressive periodontitis (Asp299Gly, $p = 0.50$ and Thr399Ile, $p = 0.81$), suggesting that publication bias is unlikely to have had a marked effect on our findings.

Four studies (Table 1) of aggressive periodontal disease and *TLR4* polymorphisms, consisting of 295 cases and 456 controls, were included in our study. The genetic heterogeneity between studies for aggressive periodontitis was high (Asp299Gly, $I^2 = 78.2\%$, $p = 0.003$, and Thr399Ile, $I^2 = 78.3\%$, $p = 0.003$). Moreover, the trends of the four studies moved in opposite directions. While studies from Germany (Schröder et al. 2005) and England (Brett et al. 2005) showed an increased risk of aggressive periodontitis for 299Gly carriers, another study from England (James et al. 2007) showed a protective effect for aggressive periodontitis for the same allele. Interestingly, two studies from England showed opposite trends (5.5% and 16.3%, James et al. 2007 and 17.8% and 7.2%, Brett et al. 2005 for cases *versus* controls). Similarly, the *TLR4* Thr399Ile polymorphism was very heterogeneous and the association results showed opposite trends. While Ile399 carriers were at an increased risk of aggressive periodontitis in Germans

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(Schröder et al. 2005), other studies (Brett et al. 2005, Emingil et al. 2007, James et al. 2007) showed a protective effect for Ile 399 carriers. The lack of consistent directional effects in these studies could be due to the relatively small effect size of this polymorphism, which is not readily detectable in all samples due to potential population heterogeneity or differences in linkage disequilibrium patterns in different populations with a putative functional variant. In order to examine the relative contribution to heterogeneity of each study in our meta-analysis, we excluded each study from the analysis consecutively. The *TLR4* Thr399Ile data from the study by Schröder et al. (2005) contributed to heterogeneity in our meta-analysis. After exclusion of this study in the analysis, the test of heterogeneity for *TLR4* Thr399Ile was not significant, I^2 decreased from 78.3% to 0%, and the summary OR revealed that Ile299 carriers had a decreased risk of aggressive periodontitis OR = 0.29 (95% CI: 0.14–0.61; $p = 0.01$). Our analysis supported the findings of James et al. (2007), who reported a protective effect of *TLR4* variation in aggressive periodontitis.

The heterogeneous findings reported in the literature are peculiar, because both *TLR4* variants studied are reported to be in considerable linkage disequilibrium with each other in the various reports. One would expect that similar association results would be found for both variants in the published literature. Although these variants are in considerable linkage disequilibrium with each other, they are not in complete linkage disequilibrium in some populations. Therefore, differential allele transmission is present and these variations may explain these reported results.

Nevertheless, as with all meta-analyses, our meta-analysis has several limitations. First, our study was limited to published studies. We did not search for unpublished trials or original data. Reporting, publication, English language, database, citation, and multiple publication biases have potentially affected the pool of scientific evidence in meta-analysis (Egger & Smith 1998). This study could eliminate only the multiple publication bias and our funnel analysis did not show any publication bias or small study effect. Second, genetic structure or environmental factors such as smoking, systemic diseases, or age may also result in undetectable variability. Some of the studies did not report the systemic disease or the smoking status of the subjects. Differences in the ascertainment of the cases in the different studies could have affected the results of the present study. Two studies (Brett et al. 2005, James et al. 2007) used convenient samples of blood donors as controls. Thus, we could not account for these confounders. Next, SNP-based haplotype analysis was not available. Haplotype-based methods have been suggested to have greater statistical power than tests based on single-locus analyses when the genetic variants are in linkage disequilibrium with a causative diallelic locus (Schaid 2004). Ferwerda et al. (2007) showed that while the *TLR4* 299Gly/399Thr haplotype showed an increased, rather than a blunted, pro-inflammatory tumour necrosis factor (TNF)-α response in blood cell culture when challenged by LPS, the *TLR4* 299Gly/399Ile haplotype did not differ from the wild-type *TLR4* cytokine response. There was only one study (Schröder et al. 2005) that examined the haplotypes of the two polymorphisms. Despite these limitations, we believe that this meta-analysis provides valuable information on the effect of *TLR4* in periodontal disease.

At present, we do not know why *TLR4* 399Ile (*TLR4*+896 A>G) carriers were protected against aggressive periodontitis, while *TLR4* 299Gly (*TLR4* +1196 C>T) carriers were at risk for chronic periodontitis. It is possible that *TLR4* polymorphisms may play different roles in the pathogenesis of aggressive and chronic periodontitis (Greenstein & Hart 2002a, b). Similar to other genes, underlying genetic susceptibility might be different in chronic and aggressive forms of periodontitis (Walker & Karpinia 2002). The other likely explanation is that these two variants might have different functional effects on gene expression. The Asp299Gly mutation but not the Thr399Ile mutation, has been associated LPS with hyporesponsiveness (Arbour et al. 2000, Kinane et al. 2006a, b). Overexpression studies indicated that the Asp299Gly variant might have a greater functional impact than the Thr399Ile (Arbour et al. 2000). We can speculate that the *TLR4* 299Gly (*TLR4*+1196 C>T) acts as a loss-of-function variant, increasing the individual susceptibility to chronic periodontitis. On the other hand, the *TLR4* 399Ile (*TLR4*+896 A>G) may be a gain-of-function variant, protecting against aggressive periodontitis. Yet, we cannot exclude the possibility that these polymorphic variants may be markers of disease risk rather than causative mutations. More detailed functional analysis of these variants is needed to clarify their molecular mechanisms in periodontitis. In addition, these two variants are in considerable linkage disequilibrium in many populations and one could argue that it is unlikely they may have the opposite effects described. One possibility is that these effects may arise from protein–protein interactions between TLR4 and its binding targets at the cell surface [lymphocyte antigen 96 (LY96), monocyte differentiation antigen CD14, toll-like receptor adaptor molecule 2 (TICAM2), and myeloid differentiation primary response gene 88 (MYD88)].

In conclusion, our meta-analysis provides compelling evidence of an association between *TLR4* Asp299Gly and the risk of chronic periodontal disease, whereas the *TLR4* Thr299Ileu polymorphism appears to protect against aggressive periodontitis. Future studies should focus on the functional role of these two *TLR4* polymorphisms: Asp299Gly and Thr399Ile.

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Clinical Relevance

Scientific rationale for the study: Although *TLR4* is a good biological candidate gene for periodontal disease, studies present conflicting results.

Principal findings: Our meta-analysis indicates that *TLR4* harbours potentially important variable sites, which are associated with an increased risk of chronic periodontitis and a decreased risk of aggressive periodontitis.

Practical implications: Finding susceptibility loci for periodontal disease can help in designing new strategies for early disease detection and prevention and aid the development of new therapeutic approaches.

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