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Polymorphisms of TLR4 but not CD14 are associated with a decreased risk of aggressive periodontitis

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Abstract

Objective: To study whether there is an association between the frequency of functional polymorphisms in the toll-like receptor 4 (TLR4) and cluster differentiation 14 (CD14) genes and periodontitis.

Methodology: Genotyping for the TLR4 single-nucleotide polymorphisms (SNPs) Asp299Gly, Thr399Ile and the CD14 SNPs − 159 and − 1359 was completed for subjects with periodontal disease compared with control subjects. Two disease populations were investigated: 73 subjects with aggressive periodontitis (AgP; 28 males, 45 females) and 95 males with chronic periodontitis (CP). The TLR4 and CD14 polymorphisms were determined using SNaPshot primer extension with capillary electrophoresis. Comparison of allele and genotype frequencies for each polymorphism was by Fisher's exact test or χ^2 analysis.

Results: The TLR4 Asp299Gly genotype was present in a significantly ($p = 0.026$) lower proportion of AgP subjects (5.5%) compared with control subjects (16.3%). The unadjusted odds ratio for the Asp299Gly genotype to be associated with AgP was 0.30, 95% confidence interval 0.10–0.91. No differences were found in the prevalence of the TLR4 Asp299Gly genotype in men with CP (18.9%) compared with an age-matched control group with no evidence of periodontitis (17%). In addition, there was no difference in the distribution of the CD14 polymorphisms in either the AgP or CP populations studied compared with controls.

Conclusion: It is concluded that in West European Caucasians, the Asp299Gly TLR4 gene polymorphism is associated with a decreased risk of AgP but not CP. Promoter polymorphisms of the CD14 gene, however, did not influence susceptibility to inflammatory periodontitis in the population cohorts studied.

Key words: aggressive periodontitis; CD14; gene polymorphisms; toll-like receptor

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Periodontal diseases are opportunistic chronic infections characterized by inflammation and destruction of the supporting tissues of the affected teeth. Behavioural factors, such as smoking and stress, are well recognized as risk factors for the development and progression of periodontitis (Borrell & Papapanou 2005). However, a strong body of evidence now exists to suggest that the more aggressive forms of periodontitis can be influenced by genetic as well as environmental factors (Kinane et al. 2005, Loos et al. 2005, Shapira et al. 2005).

Conflict of interest and source of funding statement

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Clusters of bacterial species have been identified that seem to be associated with the progression of periodontitis and these are predominantly Gram-negative anaerobic bacteria that have lipopolysaccharide (LPS) as a component of their cell wall (Socransky & Haffajee 2005). Bacterial LPS activates the innate immune system via selective binding of highly conserved pathogen-associated molecular patterns. Immune cells are directly stimulated by

LPS through a receptor complex of the toll-like receptor 4 (TLR4) and cluster differentiation 14 (CD14; Aderem & Ulevitch 2000).

TLR4 is a member of the TLR superfamily and is a transmembrane receptor with a leucine-rich extracellular domain and an intra-cellular domain with high similarity to the interleukin-1 (IL-1) receptor (Akira & Takeda 2004). TLR–ligand complexes activate signal transduction pathways in both the innate and adaptive immune systems, leading to the release of inflammatory mediators (Akira & Takeda 2004). TLR4 operates in synergy with CD14, a leucine-rich 55 kDa glycoprotein. CD14 is either incorporated into the plasma membrane of macrophages, monocytes and neutrophils via a glycosylphosphatidylinositol anchor or enzymatically cleaved from the anchor as soluble CD14 (sCD14) in serum (Ulevitch & Tobias 1995).

Two recently identified variants of the human TLR4 gene that differ in the extracellular domain of the gene have been associated with increased risk of Gram-negative infections (reviewed in Schroder & Schumann 2005). One form of the TLR4 gene has an A->G substitution at nucleotide 896 downstream of the transcription start codon, which results in an aspartic acid to glycine substitution at position 299 of the amino acid sequence. This frequently co-segregates with a second single-nucleotide change that results in a threonine being replaced by isoleucine at amino acid 399. The relative effect of these two amino acid substitutions is not fully understood but there is some evidence that the Asp299Gly alteration may be more biologically significant (Arbour et al. 2000).

A number of single-nucleotide polymorphisms (SNPs) have also been identified in the promoter region of the CD14 gene. One polymorphism at -159 in the promoter has been associated with an increased susceptibility to inflammatory diseases (Klein et al. 2002, Obana et al. 2002, LeVan et al. 2005). It has been shown that homozygous carriers of the T allele at -159 have elevated levels of sCD14 (Obana et al. 2002). It is believed that an increased level of sCD14 is causally related to an increased risk of disease. In addition, other polymorphisms including the G->T at -1359 have also been associated with an increased risk of developing asthma or rhinitis (Buckova et al. 2003).

Previous studies have investigated the role that polymorphisms in genes such as TLR4 and CD14 might be playing in determining the susceptibility to periodontitis (Holla et al. 2002, Brett et al. 2005, Donati et al. 2005, Laine et al. 2005). The objectives of the present study are to determine the frequency of functional polymorphisms in the TLR4 and CD14 genes in a cohort of West European ethnicity and to test the hypothesis that these gene variants influence susceptibility to both aggressive and chronic periodontitis (AgP and CP).

Material and Methods

The study protocol was approved by the Local Research Ethics Committees in the Faculty of Medicine, Queens University, Belfast, and the East London and City Health Authority, London. Informed consent was obtained from all those recruited into the study.

Study populations

AgP

Seventy-three subjects with AgP were identified from referrals to the Periodontal Department, School of Dentistry, Queen's University, Belfast, and the Periodontal Consultant Clinic at the Royal London Hospital. Consecutive cases that met the inclusion criteria were recruited into the study in 2002–2003. Inclusion criteria for this group were clinical signs of generalized periodontitis with at least eight teeth exhibiting loss of periodontal attachment of $\geqslant 5$ mm. The age ranged between 20 and 39 years at the time of initial diagnosis. Twelve of those classified with AgP were over 35 years old at the time of their initial presentation but on further investigation it was clear that they had evidence of advanced periodontal disease before they were aged 35 years. All subjects in the AgP group were ethnically of Western European origin. A control group of blood donors from Northern Ireland was considered to be ethnically matched to the study group.

CP

A total of 95 men aged between 60 and 70 years with CP and age-matched controls ($n = 94$) were identified from those participating in the PRIME (Prospective Epidemiological Study of Myocardial Infarction) study in Northern Ireland. The 2200 men recruited into PRIME were selected from industry, the civil service and from the General Medical Practitioner lists so that the sample broadly matched the social class structure of the background population in Northern Ireland (Yarnell 1998). The current study of CP was completed as a nested case–control study within PRIME. CP was defined as $\geqslant 2$ teeth with $\geqslant 6$ mm clinical attachment loss and $\geqslant 1$ inter-proximal site with $\geqslant 5$ mm of probing pocket depth (Machtei et al. 1992). Control subjects were men with no pocketing or loss of periodontal attachment > 3 mm.

Genotyping

Genomic DNA was isolated from peripheral blood leucocytes using standard phenol chloroform extraction techniques (DNeasy, Qiagen, Hilden, Germany).

Detection of genetic polymorphisms

The TLR4 SNPs Asp299Gly and Thr399ILe and the two CD14 SNPs at -159 and -1359 were each typed using the SNaPshot (PE Applied Biosystems, Forster City, CA, USA) ddNTP primer extension method and capillary electrophoresis as previously described (Donn et al. 2002). In brief, polymerase chain reactions (10 μl final volume) contained 10 ng of genomic DNA, 0.05 μl of each primer (50 pmol/μl), 0.5 μl dNTPs (2 mM, Bioline London, UK), 0.3 μl of $MgCl_2$ (50 mM, Bioline), 1 μl NH_4 buffer (10 $\times$, Bioline) and 0.04 μl of Taq polymerase (5 U/μl, Bioline) using the primers and probes outlined in Table 1. PCR was performed using the reaction conditions of 95°C for 5 min., followed by 10 cycles of 94°C for 20 s, 55°C for 30 s and 72°C for 30 s with a further 20 cycles of 89°C for 20 s, 55°C for 30 s and 72°C for 30 s. A final extension phase was carried out at 72°C for 10 min. The fluorescent peaks obtained were detected using the Genescan 3.7 analysis software package and the Genotyper 3.7 software package was used to assign allele names.

Statistical Analysis

Deviation from Hardy–Weinberg equilibrium was tested using χ^2 goodness of fit. Comparison of allele and genotype frequencies for each polymorphism was by Fisher's exact test or χ^2 analysis, and

significance was set at $p < 0.05$. Where data were available for confounding variables, associations were adjusted using logistic regression analysis.

Results

No significant deviation from Hardy–Weinberg equilibrium was observed for any of the SNPs in the populations studied.

AgP

The mean age of the 73 (28 males, 45 females) subjects with AgP was 31.9 (SD 4.4), range 20–39 years. Almost half, 34 (47%), currently smoked while 12 (16%) had smoked in the past and 27 (37%) had never smoked. The 43 subjects recruited in Belfast were younger 30.6 (SD 4.2) years than the 30 recruited in London 34.2 (SD 4.6), $p = 0.002$. The subjects had an average 14.8 (SD 10.8), range 4–78 sites with $\geqslant 6$ mm loss of periodontal attachment, with no difference in severity between the Belfast and London subjects.

The TLR4 Asp299Gly genotype was present in 5.5% of the AgP subjects (Table 2), which was significantly ($p = 0.026$) lower than in population controls (16.3%). In all cases and controls, there was co-segregation of the Asp299Gly and Thr399Ile polymorphisms. The unadjusted odds ratio for the Asp299Gly genotype to be associated with AgP was 0.30, 95% confidence interval 0.10–0.91. The two females with AgP who had the Asp299Gly SNP were current smokers, while the two males included one current and one former smoker. These individuals had smoked 20 or more cigarettes per day for at least 10 years. The Asp299Gly polymorphism was not present in any of the 27 AgP subjects who had never smoked. There was no difference in the distribution of either the CD14 C(−159)T or the CD14 G(−1359)T polymorphisms between those with AgP and controls (Table 2).

CP

The men with CP had fewer teeth and were more likely to be current or past smokers than the controls (Table 3). Those with CP had on average 8.1 (SD 5.8) pockets $\geqslant 5$ mm and 16.4 (SD 9.9) sites with $\geqslant 6$ mm loss of attachment, while the controls had no sites with pocketing or loss of attachment > 3 mm.

There was no difference in the prevalence of the Asp299Gly genotype in men with CP (18.9%) compared with an age-matched group with no evidence of periodontitis (17%; Table 4). There was co-segregation of the Asp299Gly and the Thr399Ile polymorphism in 34 men, while a further five men had the Thr399Ile polymorphism alone. The distribution of the Thr399Ile polymorphism was not related to CP (Table 4). There were no differences in the distribution of either the CD14 C(−159)T or the CD14 G(−1359)T polymorphisms between men with CP and age-matched men with no evidence of periodontitis (Table 4). These associations were adjusted for ever smoking and systemic factors including diabetes and obesity. This did not change the outcomes and in all the logistic regression analyses only smoking emerged as a significant ($p < 0.05$) factor associated with CP (data not shown).

Table 1. Primers and probes used for polymerase chain reactions

SNP	Forward primer 5′–3′	Reverse primer 5′–3′	Forward probe 5′–3′	Reverse probe 5′–3′
TLR4 299	CCATTGAAG AATTCCGAT TAGCAT	GGGAAAATG AAGAAACAT TTGTCAA		AATTAAATA AGTCAATAA TA
TLR4 399	TCTCAGTAG AAATGGCTT GAG	CTCACTCAT TTGTTTCAA ATTG		AGCTCAGAT CTAAATACT TTAGGCTG
CD14 −159	TTAGGCTCC CGAGTCAAC AG	GATCACCTC CCCACCTCT C	CCTGCAGAA TCCTTCCTG TTACGG	
CD14 −1359	GGCTCCTGT AATCCCAGC TA	CCCTAGACC TCTGGGGAA AG	GATCACGCC ACTGCACTC CA	

Table 2. Association between TLR4 Asp299Gly, CD14 −159 and CD14 −1359 polymorphisms and aggressive periodontitis

		Allele distribution				Genotype distribution		
		cases, *N* (%)	controls, *N* (%)	*p*		cases, *N* (%)	controls, *N* (%)	*p*
TLR4Asp299Gly	A	142 (97.3)	226 (91.9)	0.031	AA	69 (94.5)	103 (83.7)	0.026
	G	4 (2.7)	20 (8.1)		AG	4 (5.5)	20 (16.3)	
CD14 −159	C	70 (47.9)	117 (47.6)	0.76	CC	20 (27.4)	27 (22)	0.38
	T	76 (52.1)	129 (52.4)		CT	30 (41.0)	63 (51.2)	
					TT	23 (31.5)	33 (26.8)	
CD14 −1359	G	116 (79.5)	200 (81.3)	0.70	GG	46 (63.0)	85 (69.1)	0.38*
	T	30 (20.5)	46 (18.7)		GT	24 (32.9)	30 (24.4)	
					TT	3 (4.1)	8 (6.5)	

*Fisher's exact test applied to all 2 × 2 tables and also where observed frequencies were low ($n < 5$); otherwise, χ^2 analysis was used.

Table 3. Men with chronic periodontitis and matched controls with no evidence of periodontitis

	Chronic periodontitis	Controls	*p*
Age (mean, SD)	65.3 (3.0)	64.7 (2.8)	0.18
Teeth (mean, SD)	21.2 (4.1)	22.8 (3.4)	0.004
Smoking			
Current	24 (25%)	5 (5%)	
Past	42 (44%)	42 (45 %)	0.0002
Never	29 (31%)	47 (50%)	
Coronary heart disease	18 (19%)	11 (12%)	0.17
Diabetes	7 (7%)	4 (4%)	0.54
Obese (BMI > 30)	22 (23%)	17 (18%)	0.38

The *t*-test was used for continuous variables and χ^2 or Fisher's exact probability test for categorical data.

Table 4. Association between TLR4 Asp299Gly, TLR4 Thr399Ile, CD14 −159 and CD14 −1359 polymorphisms and chronic periodontitis

		Allele distribution				Genotype distribution		
		cases, *N* (%)	controls, *N* (%)	*p*		cases, *N* (%)	controls, *N* (%)	*P*
TLR4Asp	A	171 (90.0)	172 (91.5)	0.72	AA	77 (81.1)	78 (83.0)	
299Gly	G	19 (10.0)	16 (8.5)		AG	17 (17.9)	16 (17.0)	0.59*
					GG	1 (1.1)	0	
TLR4Thr 399Ile	C	166 (88.3)	166 (90.2)	0.62	CC	73 (77.7)	74 (80.4)	
	T	22 (11.7)	18 (9.8)		CT	20 (21.3)	18 (19.6)	0.58*
					TT	1 (1.1)	0	
CD14	C	96 (50.5)	89 (47.8)	0.61	CC	24 (25.3)	21 (22.6)	
−159	T	94 (49.5)	97 (52.2)		CT	48 (50.5)	47 (50.5)	0.87
					TT	23 (24.2)	25 (26.9)	
CD14	G	147 (78.2)	152 (80.9)	0.70	GG	58 (61.7)	61 (64.9)	
−1359	T	41 (21.8)	36 (19.1)		GT	31 (33.0)	30 (31.9)	0.74*
					TT	5 (5.3)	3 (3.2)	

*Fisher's exact test applied to all 2 × 2 tables and also where observed frequencies were low ($n<5$); otherwise, χ^2 analysis was used.

Discussion

This study is the first to demonstrate a significant association between the carriage of the TLR4 Asp299Gly polymorphism and AgP in young Caucasian adults, of exclusively Western European origin, living in the United Kingdom. Subjects with AgP carried this allele significantly less frequently than a population-based control group. The carriage of this allele was in some way protective against the development of AgP in this study cohort. Two previously published studies have reported no significant association of the Asp299Gly SNP with AgP (Brett et al. 2005, Schroder et al. 2005). Interestingly, in the study of Brett et al. (2005) there was a lower prevalence of the Thr399Ile SNP in AgP; however, this only reached statistical significance when all periodontitis subjects, a combination of both aggressive and chronic, were compared with controls. In the current study, there was complete co-segregation of the Asp299 Gly and the Thr399Ile SNPs in both AgP cases and controls.

The ability to mount a prominent inflammatory response is an important facet of the innate immune response. TLR4 is considered to be the most important mammalian sensor of LPS. TLR4 has a transmembrane domain and represents the link between the LPS–CD14 complex and intra-cellular signalling, leading to the production of pro-inflammatory cytokines (Akira & Takeda 2004). Uncontrolled excessive TLR signalling can have serious consequences for example the induction of endotoxic shock (Akira & Takeda 2004). It is therefore not surprising that organisms have evolved mechanisms to modulate their TLR-mediated responses. TLR4 Asp299Gly, which affects the composition and structure of the extracellular domain of the receptor, is associated with hyporesponsiveness to inhaled LPS (Arbour et al. 2000). Carriers of Asp299Gly have lower levels of circulating cytokines than those with the wild-type allele (Kiechl et al. 2002). Recently, gingival epithelial cells from subjects with Asp299Gly have been shown to produce lower levels of pro-inflammatory and chemokine cytokines after challenge by the periodontal pathogen *Porphyromonas gingivalis* (Kinane et al. 2006). Increased production of IL-1α, associated with polymorphism of the IL-1 gene, has been implicated in the pathogenesis of periodontal damage due to bystander damage (Shirodaria et al. 2000). In the context of the current study, it is hypothesized that the TLR SNP is associated with lower levels of pro-inflammatory cytokine production in response to exposure to LPS, resulting in less bystander damage. This polymorphism would therefore have some benefit within the population by providing protection against AgP. Recent research has proposed that the Asp299Gly polymorphism in the TLR4 gene also confers protection in other conditions associated with inflammation including acute coronary events (Ameziane et al. 2003) and progressive atherosclerosis (Arbour et al. 2000).

In the present study, strict clinical criteria were applied for the case definition of AgP in line with those outlined by Armitage (2004). Subjects were all Western European, aged 40 or under at initial diagnosis and had severe periodontal disease affecting at least eight teeth. No attempt was made to subclassify the subjects into localized and generalized AgP. There were four subjects in the AgP group with the Asp299Gly SNP; however, each had a significant level of current or previous smoking that could have contributed to the expression of AgP. The increased prevalence of periodontitis in young smokers was highlighted by Haber et al. (1993), who reported that in the 19–30-year olds that they investigated, current smokers were almost four times more likely to have periodontitis than never smokers. Studies in Northern Irish populations have confirmed the importance of smoking as a factor in severe loss of periodontal attachment in young adults (Linden & Mullally 1994) and in generalized AgP (Mullally et al. 1999).

The study of the TLR4 Asp299Gly SNP and AgP was affected by the decision to use blood donors as controls. Details of the gender of the blood donors and of environmental factors such as smoking, which could have been included in the statistical analysis, were not available. Nevertheless, blood donors have been used as controls in many similar studies. Age matching is not a major consideration as the genotype will be independent of age. It was assumed that none or at most one of the control blood donors had AgP because the condition is very rare, with a reported prevalence of 0.01–0.02% in Caucasians from developed countries (Albandar & Tinoco 2002). There are regional differences in SNP allele frequencies within the British population

and the importance of matching cases and controls by specific geographical regions is discussed by Clayton et al. (2005). There is also considerable variability in the prevalence of the TLR4 Asp 299Gly SNP in different populations worldwide (Schroder & Schumann 2005). There may be differences between the populations in Northern Ireland and Southern England; however, because of the similarity in the frequency of the Asp299Gly SNP in the AgP cases in Belfast and London, we felt justified in combining the two groups. It is a limitation that the blood donor control group were drawn from the Belfast region. The study sizes were such that each had 80% power to detect as statistically significant ($p<0.05$) an odds ratio associated with each additional occurrence of the minor allele ranging from 1.7 for the more common minor alleles to 2.6 for the least common minor alleles. The analysis was not corrected for multiple comparisons, which is a limitation given the level of significance ($p=0.026$). The data were re-analysed in CLUMP, using a permutation test, and this confirmed that the results in relation to TLR4 Asp 299Gly were statistically significant ($p<0.05$).

In the current study, no association between TLR4 Asp299Gly and CP was found in men from Northern Ireland. This agrees with the results of several other investigations that reported no significant association between the Asp299Gly SNP and CP (Folwaczny et al. 2004b, Brett et al. 2005, Laine et al. 2005). Conflicting findings were reported by Schroder et al. (2005), who identified a strong ($p=0.0001$) association between Asp299Gly and CP with an almost five-fold increase in the prevalence of the SNP in those with periodontitis compared with controls. There were substantial differences in the clinical phenotypes of CP in the various studies. The current study investigated only males and this may also explain some of the differences between this investigation and that of Schroder et al. (2005).

There was no association between CD14 C −159 T and AgP in the current study. No other studies have explicitly addressed this SNP in relation to AgP but have included cases in the age range that could have identified AgP. However, only Yamazaki et al. (2003) carried out a sub-analysis that compared what they classified as a ''young disease'' group with an ''older advanced group''. They reported an increase in the frequency of the CD14 TT genotype in the young group and concluded that the SNP was not essential for periodontitis but that when present, it could contribute to the early expression of the disease (Yamazaki et al. 2003). All the subjects were Japanese and ethnic differences in genotype distribution may have to be considered when comparisons are made with the results presented here from UK subjects.

The current study did not find any significant differences in the distribution of the CD14 C −159 T polymorphism between males with CP and a matched control group who were resistant to periodontitis. There is also no evidence of any significant differences in genotype distribution in sub-analyses of ever *versus* never smokers or of mild/moderate *versus* severe periodontitis (data not shown). Several other studies have investigated the relationship of CD14 C −159 T with CP. Laine et al. (2005) reported a significantly higher frequency of the CD14 −159 TT genotype in cases with periodontitis than controls. It has been argued that the TT genotype is relevant because it is associated with increased serum levels of sCD14 (Baldini et al. 1999) and elevated levels of sCD14 in turn have been associated with periodontitis (Hayashi et al. 1999). In contrast, the study of Donati et al. (2005) reported a significant increase in carriage of the C rather than the T allele in cases of severe periodontitis. Other studies of European populations with periodontal disease have reported no significant difference in allele or genotype frequencies between the entire groups of cases and controls (Holla et al. 2002, Folwaczny et al. 2004a). These studies did, however, report associations from sub-analyses within the case groups. For example, Holla et al. (2002) reported a reduced frequency of CD14 −159 TT genotype in a subgroup with moderate compared with severe chronic periodontal disease. Folwaczny et al. (2004a) found an increase in the CD14 −159 C allele in females with CP but not in males. Why these studies should have produced such conflicting results is not clear. However, the outcomes of some of the analyses must be interpreted with caution because of the small size of the subgroups. Differences in ethnicity and the classification of disease phenotype may also explain some of the differences identified.

The data here suggest no association between the CD14 G −1359T polymorphism and AgP or CP. This finding confirms one previous study that also investigated this SNP and found no difference in allele or genotype distribution between healthy controls and CP (Holla et al. 2002). Holla et al. (2002) did perform a sub analysis that suggested a difference in CD14 G −1359T genotype distribution between those with moderate compared with severe periodontitis (Holla et al. 2002). The moderate disease group, however, was very small.

It is concluded from the results of the present study that the Asp299Gly TLR4 gene polymorphic form is associated with a decreased risk of AgP but not CP in West European Caucasians. This conclusion in relation to TLR4 and AgP should be interpreted cautiously, given the level of significance, the small size of the study and the limitations resulting from the use of blood donors as controls.

It is also concluded that in the same population groups, promoter polymorphisms of the CD14 gene do not influence susceptibility to inflammatory periodontitis. These findings are in contrast to some other reports and this may be the result of differences in the populations studied, subject selection or disease characterization. In general, the small size of many studies and the variability in outcomes point to the need for large multicentre collaborative studies, adequately powered to reduce the effects of sampling variation, to define reliably the association between functional polymorphisms of TLR4 and CD14 and both AgP and CP. These larger studies could also more effectively address the relevance of gene–environment interactions in periodontitis.

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Clinical Relevance

Scientific rationale for the study: It is not clear why some individuals are particularly susceptible to exposure to LPS and respond by developing periodontitis. This investigation focused on examining whether variability in genes associated with the LPS–receptor complex might explain some of the susceptibility to both aggressive and chronic forms of periodontitis.
Principal findings: In the West European Caucasians studied, the Asp299Gly TLR4 gene polymorphism was associated with a decreased risk of AgP but not CP. The carriage of this allele seemed protective against the development of AgP; however, the conclusion should be interpreted cautiously due to the size of the study. Promoter polymorphisms of the CD14 gene, however, did not influence susceptibility to AgP or CP.
Practical implications: Genetic studies are helping to unravel the pathogenesis of periodontitis. In future, genetic investigations may be useful in defining individual susceptibility, particularly to aggressive forms of periodontitis.