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TLR2 Arg753Gly, TLR4 Asp299Gly and Thr399Ile gene polymorphisms are not associated with chronic periodontitis in a Turkish population

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Abstract

Aim: Toll-like receptor (TLR) gene polymorphisms could affect the host's ability to respond to microbial pathogens. In this case–control study, the association of TLR2 and TLR4 gene polymorphisms with chronic periodontitis (CP) was investigated.

Materials and Methods: Genomic DNA was obtained from the peripheral blood of 83 patients with CP and 106 periodontally healthy subjects. The TLR2 Arg753Gly, Arg677Trp and TLR4 Asp299Gly, Thr399Ile gene polymorphisms were genotyped by the polymerase chain reaction-restriction fragment length polymorphism method. The data were analysed by a χ^2 test, logistic regression analysis and the Mann–Whitney U test.

Results: The 753Gln allele was found in 6.1% of the CP patients as compared with 6.6% in the control group ($p > 0.05$). The frequency of the 299Gly and 399Ile allele was 2.4% and 1.8% in CP patients. For the healthy subjects, the frequency was 2.8% for the 299Gly and 2.5% for the 399Ile allele ($p > 0.05$). None of the CP patients or healthy subjects showed homozygosity for the TLR2 and TLR4 alleles. Percentage of sites with bleeding on probing and plaque were significantly higher in 299Gly-positive patients compared with 299Gly-negative patients ($p < 0.05$).

Conclusion: These results showed that the TLR2 and TLR4 gene polymorphisms studied are not associated with susceptibility to CP in Turkish patients.

Key words: chronic periodontitis; gene polymorphisms; pathogenesis; periodontal disease; TLR

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The innate immune system is involved in the recognition of conserved pathogen associated molecular patterns present on pathogens and considered as first-line evidence (Aderem & Ulevitch 2000, Gordon 2002). The recognition of these molecules is facilitated by a group of receptors called toll-like receptors (TLRs) (Gordon 2002, Takeda et al. 2003). TLRs activate the nuclear factor-κB pathway (NF-κB), which results in the synthesis and release of proinflammatory cytokines, thereby augmenting the local inflammatory responses (Akira et al. 2001, Schnare et al. 2001). Among the 10 human TLRs identified so far, TLR2 and TLR4 are the most defined members (Takeda et al. 2003). TLR2 is mostly involved in the recognition of a variety of different bacterial cell components such as peptidoglycan and lipoproteins (Lien et al. 1999). TLR4 has been shown to specifically recognize lipopolysaccharide (LPS) of Gram-negative bacteria which

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work in cooperation with several protein components such as LPS-binding protein (LBP) and CD14 (Yoshimura et al. 2002). This leads to the activation of the inflammatory cells via the NF-κB pathway. Activation of the cytokine network following the initial host-microbial interaction is of importance in the pathophysiology of periodontal disease (Dixon et al. 2004). The potential biological and clinical significance of these receptors has been reported in several inflammatory, infectious and autoimmune disorders (Kiechl et al. 2002, Schröder & Schumann 2005). In this regard, the expression of the TLR2 and TLR4 was previously shown in gingival tissue of patients with periodontitis, and it was specifically associated with the severity of the disease (Mori et al. 2003, Ren et al. 2005).

It has been shown that human single nucleotide gene polymorphism, at position 753 arginine to glutamine in the human TLR2 gene (Arg753Gly), diminishes the ability of TLR2 to respond to bacterial cell wall components (Lorenz et al. 2002, Schröder et al. 2003). Two common gene polymorphisms in the extracellular domain of the TLR4 receptor have also been defined. One of these, a common A–G substitution at nucleotide 896, results in replacement of an aspartic acid (Asp) with glycine (Gly) at amino acid 299 (Asp299Gly). The other one is the replacement of threonine (Thr) with isoleucine (Ile) at amino acid 399 (Thr399Ile) in the extracellular domain of the TLR4 receptor (Arbour et al. 2000, Schmitt et al. 2002). Asp299Gly and Thr399Ile gene polymorphisms of human TLR4 have been shown to be associated with functional changes that predispose people to be less responsive to LPS and have an increased risk of severe infection susceptibility to pathogenic bacterial infections (Arbour et al. 2000, Agnese et al. 2002, Schmitt et al. 2002). It has been suggested that the carriage of these gene polymorphisms is related to a risk of atherosclerosis and other chronic inflammatory diseases (Kiechl et al. 2002, Schröder & Schumann 2005, Rohde et al. 2006). Recent findings have shown that these gene polymorphisms are also functional for a variety of bacterial and fungal pathogens other than LPS (Van der Graaf et al. 2006, Wu et al. 2006).

Periodontitis is a chronic multifactorial disease with a strong genetic component (Kinane & Hart 2003). Gene polymorphisms that modulate host immune responses to the microbial challenge have been associated with different clinical forms of periodontitis (Takashiba & Naruishi 2006). Several studies have shown that allelic variations in genes encoding molecules of the host defence, system such as cytokines could affect the susceptibility and progression of periodontal disease (Loos et al. 2005). Because TLR2 and TLR4 provide a critical link between factors produced by pathogens and the initiation of host defence, TLR2 and TLR4 gene polymorphisms may be important factors in susceptibility to bacterial infections and may thereby influence the inflammatory process.

The aim of the present study was to evaluate the TLR2 Arg753Gly and Arg677Trp, and TLR4 Asp299Gly and Thr399Ile gene polymorphisms in a Turkish population with CP and also to investigate the association of the TLR2 and TLR4 gene polymorphisms with clinical periodontal parameters in Turkish subjects.

Materials and Methods

Study population

A total of 189 unrelated Caucasians of Turkish descent residing in the same geographic region who had a similar socio-economic level were included in the present study. Eighty-three CP patients and 106 subjects with healthy periodontal conditions were recruited from the Ege University School of Dentistry Department of Periodontology over a period of 4 years between 2002 and 2006. The study protocol was approved by the Ethics Committee of the Ege University Faculty of Medicine. All participants gave written informed consent in accordance with the Helsinki Declaration. Medical and dental histories were taken. None of the subjects had a history or current manifestation of serious systemic diseases, which could impair immune response. Patients with medical disorders (such as diabetes mellitus, immunological disorders, hepatitis and HIV infections) and pregnant females were excluded from the study. Smoking status and history was determined by a self-administtered questionnaire regarding present or previous tobacco smoking habit, the duration (years) and the dose of the exposure (cigarettes/day) as well as the eventual date of cessation. Smoking status was classified as non-smokers or current smokers based on current smoking habits. Smokers in both the CP and healthy groups had been smoking more than 10 cigarettes/day for more than 5 years. Subjects who had never smoked or had stopped smoking $\geqslant$5 year ago were considered non-smokers. CP patients were classified as follows (Armitage 1999):

CP group

The CP group included 28 females and 55 males ranging in age from 35 to 63, with a mean of 48.3 ± 6.4 years. They had severe alveolar bone and clinical attachment loss (CAL) of $\geqslant$5 mm in multiple sites of all four quadrants of the mouth. A diagnosis of CP was made if the CAL was commensurate with the number of local factors of the patient.

Healthy group

The healthy group consisted of 66 females and 40 males who exhibited probing depth (PD) $<$3 mm and no CAL, together with $<$10% of sites with bleeding on probing (BOP) and radiographic evidence of bone loss (mean age 43.4 ± 7.2 years; range 35–70 years). These individuals were healthy volunteers from the staff and other patients in the Dental School.

Determination of periodontal status

The following clinical parameters were assessed to determine the clinical periodontal status in patients and healthy controls: PD and CAL measurements were performed using a manual Williams probe. Dichotomous measurements of supragingival plaque accumulation and BOP were also recorded. All measurements were performed at six sites per tooth for the whole mouth.

Genomic DNA preparation

Two ml of whole blood samples were collected into ethylenediaminetetraacetic acid – anticoagulated tubes by the standard venipuncture method. Genomic DNA was extracted from whole blood samples using the QIAmp blood DNA mini-kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Polymerase chain reaction (PCR) and enzyme digest

TLR2 Arg753Gln and Arg677Trp gene polymorphisms were genotyped by the method of Schröder et al. (2003). Designed primers spanned a region of 340 bp including both polymorphisms, using the following primers: forward 5-GCCTACT GGGTGGAGAACCT-3′ and reverse 5-G GCCACTCCAGGTAGGTCTT-3. For investigation of Arg753Gln gene polymorphism only an additional forward primer was designed, yielding a 264 bp product: 5-GGGACTTCATT-CCTGGC AAGT-3. Amplification was carried out on a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA, USA) in a 25 μl reaction mixture in 0.2 ml thin-wall PCR strip tubes (Axygen Scientific, Inc., CA, USA) containing 1 μl genomic DNA solution, GeneAmp Gold Buffer (15 mmol/l Tris-HCl, pH 8.0, 50 mmol/l KCl; PE Applied Biosystems), 2.0 mmol $MgCl_2$, 50 μmol/l each of the dGTP, dATp, dTTP and dCTP (Promega Inc., Madison, WI, USA); 25 pmol each forward and reverse primers and 1.0 U AmpliTaq Gold polymerase (PE Applied Biosystems). Three microlitre of the PCR product were incubated for 2 h with 0.5 U *Aci*I enzyme (NewEngland Biolabs, Beverly, MA, USA), in a total volume of 10 μl at 36°C.

Determination of the TLR4 gene polymorphisms was accomplished with PCR and restriction fragment length polymorphism by the method of Lorenz et al. (2002). The primers for TLR4 Asp299Gly were forward 5′-GATTAG CATACTTAGACTACTACCTCGA-3′ and reverse 5′-GATCAACTTCTGAAA AAGCATTCCCACC-3′. The primers for TLR4 Thr399Ile were forward 5′-GGTTGCTGTTCTCAAAGTGATTTT GGGACAA-3′ and reverse 5′-CCTG AAGACTGGAGAGTGAGTTAAATG CT-3′. Amplification conditions for TLR4 gene polymorphisms were described above for TLR2 polymorphisms, except for $MgCl_2$ concentrations (4.0 mM $MgCl_2$ for Asp299Gly and 3 mM for Thr399Ile). The cycling conditions comprised a hot start at 95°C for 10 min., followed by 35 amplification cycles at 95°C for 30 s, 62° C for 30 s (Asp299Gly) or 60°C (Thr399Ile) and 72°C for 25 s, followed by one elongation step at 72°C for 5 min.

The digest reaction was set up using 4 μl PCR product, appropriate restriction enzyme *NcoI* (TLR4 Asp299Gly) and *HinfI* (TLR4 Thr399Ile), 1 μl 10 × enzyme buffer (New England Biolabs). It was incubated overnight at 37°C and electrophoresed in a 3% NuSieve (FMC Bioproducts, Rockland, ME, USA) gel to identify the TLR4 alleles on the basis of the respective allele size. After digestion, the wild-type TLR4 allele sizes of 249 bp for the 299 residue and 406 bp for the 399 residue will not change; fragment sizes for carriers of the polymorphic allele will decrease to 23 bp for the 299 residue and 29 bp for the 399 residue. To confirm our PCR-RFLP results, PCR products for all genotypes were sequenced on an automated DNA sequencer (ABI PRISM 310 Genetic Analyzer, PE Applied Biosystems).

Statistical analysis

χ^2 analysis was used to test for deviation of genotype frequencies from Hardy–Weinberg equilibrium. The distribution of TLR2 Arg753Gly, Arg677Trp and TLR4 Asp299Gly and Thr399Ile genotypes and allele frequencies in CP and periodontally healthy groups was also analysed by the χ^2 test. Allele frequencies were calculated from the observed numbers of genotypes. Odds ratios (ORs) and 95% confidence intervals (95% CI) were also assessed. Differences in clinical parameters between subjects with 753Gln, 299Gly and 399Ile allele positive and 753Gly, 299Gly and 399Ile negative genotypes in the CP group were compared by the non-parametric Mann–Whitney *U* test. *p* values <0.05 were considered to be statistically significant. In a subgroup analysis, smokers were excluded and statistical analysis was performed in non-smokers as well. The relationship between genotype and disease status was analysed by multiple logistic regression analysis while adjusting for potential confounding factors including age, gender and smoking status. All data analysis was performed using a statistical package (SPSS Inc., version 14.0, Chicago, IL, USA).

Results

The clinical characteristics of the study groups are summarized in Table 1. The frequencies of TLR2 Arg753Gln, TLR4 Asp299Gly and Thr399Ile genotypes in the control group were found to be in accordance with those expected by the Hardy–Weinberg equilibrium ($p>0.05$, $\chi^2<5.99$). In the present study, 54 CP patients and 101 periodontally healthy controls were non-smokers.

Distribution of TLR2 Arg753Gln and Arg677Trp genotype and allele frequency

The distribution of TLR2 Arg753Gln gene polymorphism within all CP and healthy subjects is presented in Table 2. The distributions of TLR2 Arg753Gln genotypes were not different among the groups ($\chi^2=0.043$, $p=0.84$). The 753Gln allele was found in 6.1% of the CP patients as compared with 6.6% in the healthy group. There was no significant difference among the study groups in allele frequencies ($\chi^2=0.040$, $p=0.84$). None of the study subjects in the CP and periodontally healthy group showed homozygosity for the TLR2 mutant allele. The Arg677Trp allele was not found in any of the CP or healthy subjects.

In a subgroup analysis when the smokers were excluded, the distribution and allele frequencies of TLR2 Arg753Gln genotypes of the non-smoker group were similar to the smoker and non-smoker groups. There were no significant differences in the distribution of TLR2 Arg753Gln genotypes and allele frequencies between non-smoker CP and non-smoker healthy subjects ($\chi^2=0.024$, $p=0.88$, $\chi^2=0.022$, $p=0.88$, respectively) (Table 2).

Distribution of TLR4 Asp299Gly and Thr399Ile genotypes and allele frequencies

The distributions of TLR4 Asp299Gly and Thr399Ile gene polymorphisms among the study groups are presented

Table 1. Characteristics of chronic periodontitis (CP) and healthy groups

	CP group		Healthy group	
	all subjects	non-smokers	all subjects	non-smokers
No. of subjects	83	54	106	101
Mean age (years ± SD)	48.35 ± 6.4	49.46 ± 6.3	43.42 ± 7.2	43.5 ± 7.3
Age range	35–63	35–61	32–70	32–70
Male/female	55/28	35/19	40/66	37/64

Table 2. Genotype distributions and allele frequencies of the Arg753Gln polymorphism in the TLR2 gene of chronic periodontitis (CP) and healthy groups

TLR2 Arg753Gln	All subjects				Non-smokers			
	CP $n = 83$ (%)	Healthy $n = 106$ (%)	p (χ^2 test)	OR (95% CI)	CP $n = 54$ (%)	Healthy $n = 101$ (%)	p (χ^2 test)	OR (95% CI)
Genotype								
Arg753Arg	72 (86.7)	92 (86.8)			47 (87.0)	87 (86.1)		
Arg753Gln	11 (13.3)	14 (13.2)	0.84	0.913 (0.383–2.174)	7 (13.0)	14 (13.9)	0.88	0.926 (0.349–2.452)
Gln753Gln	0	0			0	0		
Gln allele carriage rate	11 (13.3)	14 (13.2)			7 (13.0)	14 (13.9)		
Allele frequency								
Arg	155 (93.9)	198 (93.4)	0.84	0.918 (0.397–2.124)	101 (93.5)	188 (93.1)	0.88	0.931 (0.364–2.380)
Gln	11 (6.1)	14 (6.6)			7 (6.5)	14 (6.9)		

OR, odds ratio; CI, confidence interval.

Table 3. Genotype distributions and allele frequencies of the Asp299Gly and Thr399Ile polymorphisms in the TLR4 gene of chronic periodontitis (CP) and healthy groups

	All subjects				Non-smokers			
	CP $n = 83$ (%)	Healthy $n = 106$ (%)	p (χ^2 test)	OR (95% CI)	CP $n = 54$ (%)	Healthy $n = 101$ (%)	p (χ^2 test)	OR (95% CI)
TLR4 Asp299Gly								
Genotype								
Asp/Asp	79 (95.2)	100 (94.3)			50 (92.6)	95 (94.1)		
Asp/Gly	4 (4.8)	6 (5.7)	0.80	0.844 (0.230–3.094)	4 (7.4)	6 (5.9)	0.72	1.267 (0.342–4.698)
Gly/Gly	0	0			0	0		
Gly allele carriage rate	4 (4.8)	6 (5.7)			4 (7.4)	6 (5.9)		
Allele frequency								
Asp	162 (97.6)	206 (97.2)			104 (96.3)	196 (97.0)		
Gly	4 (2.4)	6 (2.8)	0.80	0.848 (0.235–3.054)	4 (3.7)	6 (3.0)	0.73	1.256 (0.347–4.552)
TLR4 Thr399Ile								
Genotype								
Thr/Thr	80 (96.4)	101 (95.3)			51 (94.4)	96 (95.0)		
Thr/Ile	3 (3.6)	5 (4.7)	0.71	0.758 (0.176–3.265)	3 (5.6)	5 (5.0)	0.87	1.129 (0.259–4.917)
Ile/Ile	0	0			0	0		
Ile allele carriage rate	3 (3.6)	5 (4.7)			3 (5.6)	5 (5.0)		
Allele frequency								
Thr	163 (98.2)	207 (97.6)			105 (97.2)	197 (97.5)		
Ile	3 (1.8)	5 (2.4)	0.87	0.762 (0.179–3.235)	3 (2.8)	5 (2.5)	0.62	1.126 (0.264–4.803)

OR, odds ratio; CI, confidence interval.

in Table 3. The distributions of TLR4 Asp299Gly and Thr399Ile genotypes were similar between the CP group and periodontally healthy group ($\chi^2 = 0.066$, $p = 0.80$, $\chi^2 = 0.140$, $p = 0.71$, respectively). Also, there was no significant difference among the study groups in allele frequencies ($\chi^2 = 0.064$, $p = 0.80$, $\chi^2 = 0.026$, $p = 0.87$, respectively). 2.4% of the CP patients and 2.8% of the periodontally healthy subjects were identified to have the 299Gly polymorphic allele. For the 399Ile polymorphic allele, 1.8% of the CP patients and 2.4% of the periodontally healthy subjects had the 299Gly allele (Table 3).

Again, even in the group of non-smokers, the genotype distributions and allele frequencies of the TLR4 Asp299Gly gene of the CP group were similar to those of non-smoker healthy subjects ($\chi^2 = 0.125$, $p = 0.72$, and $\chi^2 = 0.121$, $p = 0.73$, respectively). The Thr399Ile genotype distribution and allele frequencies of the CP group were not different from those of non-smoker healthy subjects ($\chi^2 = 0.026$, $p = 0.87$, and $\chi^2 = 0.253$, $p = 0.62$, respectively) (Table 3).

753Gln, 299Gly and 399Ile allele positivity in relation to the susceptibility to periodontitis

In order to investigate whether 753Gln, 299Gly and 399Ile allele positivity is associated with clinical parameters, differences in clinical parameters between 753Gln-, 299Gly- and 399Ile- positive subjects and 753Gln-, 299Gly- and 399Ile- negative genotypes in the CP groups were compared by the non-parametric Mann–Whitney U test. No significant differences were found in clinical parameters between the 753Gln-positive and 753Gln-negative CP patients ($p > 0.05$). When only non-smoker CP patients were taken into consideration, percentage of sites with BOP was found to be significantly higher in 753Gln negative patients compared with 753Gln- positive patients ($p = 0.019$). Other clinical periodontal parameters were found to be similar between these allele-positive and- negative non-smoker CP patients ($p > 0.05$) (Table 4).

In the CP group, percentage of sites with BOP and plaque were found to be significantly higher in 299Gly- positive

Table 4. Clinical parameters (mean ± SD) of chronic periodontitis (CP) group distributed by subjects with TLR2 753Gln positive and TLR2 753Gln negative genotype

	All subjects			Non-smokers		
	positive Gln $n = 11$	negative Gln $n = 72$	p	positive Gln $n = 7$	negative Gln $n = 47$	p
Age	47.50 ± 3.5	48.60 ± 6.7	0.527	48.00 ± 4.0	49.68 ± 6.6	0.362
PD (mm)	4.00 ± 0.9	4.22 ± 0.9	0.865	3.75 ± 1.1	4.05 ± 0.8	0.880
CAL (mm)	5.15 ± 1.4	5.60 ± 1.1	0.585	4.85 ± 1.6	5.38 ± 1.1	0.528
BOP (%)	70.76 ± 17.6	79.66 ± 21.4	0.080	67.08 ± 16.1	82.84 ± 20.0	0.019*
Plaque (%)	86.50 ± 19.3	87.90 ± 15.1	0.797	84.71 ± 21.4	88.48 ± 13.8	0.900

*Significant difference between TLR2 753Gln positive and TLR2 753Gln negative genotypes (Mann–Whitney test, $p < 0.05$).

PD, probing depth; CAL, clinical attachment loss; BOP, bleeding on probing.

Table 5. Clinical parameters (mean ± SD) of chronic periodontitis (CP) group distributed by subjects with TLR4 299Gly and TLR4 399Ile positive, and TLR4 299Gly and TLR4 399Ile negative genotype

	All subjects			Non-smokers		
	positive Gly $n = 4$	negative Gly $n = 79$	p	positive Gly $n = 4$	negative Gly $n = 50$	p
Age	49.00 ± 6.2	48.32 ± 6.5	0.689	49.00 ± 6.2	49.50 ± 6.4	0.937
PD (mm)	3.73 ± 0.5	4.21 ± 0.9	0.223	3.73 ± 0.5	4.04 ± 0.9	0.433
CAL (mm)	5.86 ± 1.5	5.51 ± 1.2	0.586	5.86 ± 1.5	5.26 ± 1.1	0.414
BOP (%)	100 ± 0	77.15 ± 21.1	0.002*	100 ± 0	79.26 ± 20.2	0.005*
Plaque (%)	100 ± 0	86.51 ± 16.4	0.035*	100 ± 0	87.03 ± 14.9	0.047*
	positive Ile, $n = 3$	negative Ile, $n = 80$	p	positive Ile, $n = 3$	negative Ile, $n = 51$	p
Age	48.00 ± 6.9	48.36 ± 6.5	0.973	48.00 ± 6.9	49.55 ± 6.3	0.831
PD (mm)	3.61 ± 0.5	4.21 ± 0.9	0.155	3.61 ± 0.5	4.04 ± 0.9	0.271
CAL (mm)	4.58 ± 0.7	5.56 ± 1.2	0.103	4.58 ± 0.7	5.35 ± 1.17	0.184
BOP (%)	96.00 ± 6.9	77.58 ± 21.3	0.078	96.00 ± 6.9	79.90 ± 20.3	0.127
Plaque (%)	97.33 ± 4.6	86.77 ± 16.4	0.250	97.33 ± 4.6	87.45 ± 14.9	0.306

*Significant difference between TLR4 299Gly positive and TLR4 299Gly negative genotypes (Mann–Whitney test, $p < 0.05$).

PD, probing depth; CAL, clinical attachment loss; BOP, bleeding on probing.

patients compared with 299Gly- negative patients ($p = 0.002$ and $p = 0.035$, respectively). When only non-smoker CP patients were taken into consideration, percentage of sites with BOP and plaque were also significantly higher in 299Gly- positive patients compared with 299Gly- negative patients ($p = 0.005$ and $p = 0.047$, respectively). No significant differences were found in other clinical parameters between 299Gly- positive and 299Gly- negative CP patients ($p > 0.05$) (Table 5).

There was no significant difference in PD, CAL and in sites with BOP and plaque (%) between the 399Ile-positive and 399Ile-negative CP patients ($p > 0.05$). When only non-smoker CP patients were taken into consideration, these clinical periodontal parameters were found to be similar between allele-positive and- negative CP patients ($p > 0.05$) (Table 5).

Multiple logistic regression model

Multiple logistic regression analysis was used to evaluate the association of the TLR2 753Gln, TLR4 299Gln and TLR4 399Ile allele carriage with periodontal disease susceptibility, while adjusting for significant confounders. In this model, subject age, gender and smoking were found to be significant confounders ($p < 0.05$). On the other hand, the TLR2 753Gln, TLR4 299Gly and TLR4 399Ile allele positivities were not statistically significant ($p > 0.05$) (Table 6).

Discussion

The primary role of the inflammatory response is to protect the host against bacterial invasion as the first line defence (Sanz & Quirynen 2005, Shapira et al. 2005). TLR2 and TLR4 gene polymorphisms could provide information about altered host immune response to microbial infection (Schröder & Schumann 2005). The present study evaluated TLR2 Arg753Gly, TLR4 Asp299Gly and Thr399Ile gene polymorphisms in patients with CP and healthy controls. Results of the present study revealed that the distribution of TLR2 and TLR4 genotypes and allelic frequencies of CP patients were similar to those of periodontally healthy group. There is no data regarding TLR2 and TLR4 genotype in periodontitis in Turkish people up to date. Thus, our data could provide useful reference and representation of a Turkish population for studies to come.

There are only a limited number of studies investigating TLR2 gene polymorphisms in several chronic inflammatory diseases including periodontal disease (Folwaczny et al. 2004, Schröder et al. 2005). Folwaczny et al. (2004) have shown Asp753Gln allele to be 2.9% in CP patients, while 4.1% in healthy controls. Schröder et al. (2005) have also not found any difference between CP (4.31%) and healthy groups (7.76%) in the TLR2 Arg753Gln gene polymorphism. In the present study, CP (6.1%) and healthy control subjects (6.6%) had similar TLR2 allele. We observed a trend towards a higher frequency of the TLR2 gene polymorphism in CP group than the reports of Folwaczny et al. (2004) while similar frequency to those of Schröder et al. (2005) which could be attributable to ethnical differences between countries (Folwaczny et al. 2004, Schröder et al. 2005).

CP could have similarities in causative genetic factors to other chronic inflammatory and autoimmune disease (Flemmig 1999, Kinane & Hart 2003). Berdeli et al. (2005) have shown that the TLR2 753Gln allele frequency was significantly higher in the acute rheumatic fever (ARF) patients (45.9%) than those of healthy subjects (5.2%). In this study, 56 out of the 61 Turkish ARF patients were heterozygous for Arg753Gln, while no homozygous patients were present. Their data have indicated that Arg753Gln gene polymorphism is present in 10.34% of

Table 6. Results of logistic regression analysis for the association between TLR2 753Gln, TLR4 299Gly and TLR4 399Ile positive genotypes and susceptibility to chronic periodontitis (CP)

	Odds ratio	95% confidence interval	*p*
Age	1.12	1.06–1.18	<0.0001
Gender			
Female	Reference		
Male	2.24	1.11–4.54	0.025
Smoking			
Non-smokers	Reference		
Smokers	15.29	4.81–48.57	<0.0001
TLR2 Arg753Gln genotype			
753Gln negative genotype	Reference		
753Gln positive genotype	1.24	0.46–3.35	0.68
TLR4 Asp299Gly genotype			
299Gly negative genotype	Reference		
299Gly positive genotype	1.59	0.26–9.64	0.62
TLR4 Thr399Ile genotype			
399Ile negative genotype	Reference		
399Ile positive genotype	0.99	0.13–7.53	0.99

systemically healthy Turkish subjects. In the present study, Arg753Gln gene polymorphism was found to be 12.3% for periodontally healthy subjects, which is similar to those of previous study. The prevalence of Arg753Gln gene polymorphism in healthy Turkish subjects is slightly higher than those previously reported for German population (9.4%) (Schröder et al. 2003).

In the present study, CP patients had a TLR4 299Gly allele frequency (2.4%) similar to those of healthy control subjects (2.8%). That for the TLR4 399Ile allele was 1.8% in CP and 2.4% in the healthy control subjects. In contrast, in a study by Schröder et al. (2005), the frequency of TLR4 Asp299Gly and Thr399Ile gene polymorphisms was found to be higher in patients with CP (18.97%) than that of healthy subjects (5.17%). They suggested that these gene polymorphisms are significantly associated with CP patients. Folwaczny et al. (2004) have reported that the TLR4 299Gly allele frequency to be 4.1%, and that for the TLR4 399Ile allele to be 4.5% in the periodontitis group as compared with 3.3% for TLR2 299Gly allele and 3.7% for the TLR4 399Ile allele in the healthy population. They could not show any significant difference in Asp299Gly and Thr399Ile gene polymorphisms between CP and healthy subjects in a German population (Folwaczny et al. 2004). In a study comparing TLR4 gene polymorphisms in CP and healthy subjects, it was found that 11% of the CP patients and 7% of the healthy subjects were heterozygous for the Asp299Gly gene, while 7% of the CP and 18% of the healthy subjects were heterozygous for the Thr399Ile gene. No significant difference could be found in the distribution of TLR4 gene polymorphisms between CP and healthy subjects (Brett et al. 2005). In the present study, the lower frequencies of TLR4 299Asp and 399Ile alleles compared with other populations are considered to reflect some ethnic differences in the relative frequencies of the TLR4 genotype in Turkish subjects.

Different factors could influence increased susceptibility to periodontitis, which is a multifactorial periodontal disease (Shapira et al. 2005). Smoking is known to be a well-established risk factor for periodontitis that influences host inflammatory immune response by suppressing the activity of several mediators (Kinane & Chestnutt 2000, Palmer et al. 2005). Therefore, in the present study, TLR2 and TLR4 gene polymorphisms were evaluated in non-smoker subjects as well. Even in the group of non-smokers, there was no association between CP and TLR2 and TLR4 gene polymorphisms.

CP is a multifactorial disease whose manifestation and progression is influenced by a variety of factors such as genetic factors, smoking, age and gender (Albandar 2002, Heitz-Mayfield 2005). It has been suggested that increasing age and being male is associated with the prevalence, extent and severity of periodontitis (Heitz-Mayfield 2005). In the present study, logistic regression analysis was performed to evaluate the genotype effects on an increased severity of CP while adjusting these confounding factors. In this model, smoking as well as patient age and gender was found to be significant confounders to the increased susceptibility to CP. On the other hand, the TLR2 753Gln, TLR4 299Gln and TLR4 399Ile allele positivity was not associated with susceptibility to CP.

It was previously shown that BOP, which is a significant sign of periodontal inflammation and an early indicator of disease progression in periodontal tissues, was found to increase in IL-1 genotype-positive subjects (Lang et al. 2000). Our data have shown that the percentage of BOP was significantly higher in subjects carrying the TLR4 299Gly allele and lower in subjects carrying the TLR2 753Gln allele in the CP group, which could be attributable to significantly higher plaque levels in patients carrying these alleles. Therefore, a higher percentage of bleeding in the allele positive group does not necessarily show that the investigated allele is causatively related to periodontal disease. These TLR2 and TLR4 alleles may be in linkage disequilibrium with other, functionally relevant alleles within the TLR genes (Kinane et al. 2005, Takashiba & Naruishi 2006).

As a result, TLR2 Arg753Gly, TLR4 Asp299Gly and Thr399Ile gene polymorphisms are not associated with the clinical outcome of CP in the present study. Therefore, based on the findings reported here it may be suggested that TLR2 Arg753Gly, TLR4 Asp299Gly and Thr399Ile gene polymorphisms studied are not related to CP in Turkish population. More extensive studies in larger groups of patients and also in other ethnic populations should be undertaken in order to analyze the putative relevance of the TLR gene polymorphisms in the pathogenesis of periodontitis. To our best knowledge, this is the first study investigating TLR2 and TLR4 gene polymorphisms in CP in a Turkish population.

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Clinical Relevance

Scientific rationale for the study: TLRs for recognization of bacterial components are host molecules that are important in the innate immunity and the initiation of inflammatory responses. This study was undertaken to evaluate whether TLR gene polymorphisms are associated with CP.

Principal findings: No significant difference between CP and healthy subjects in the distribution of TLR2 and TLR4 genotypes and allele frequencies could be found.

Practical implications: TLR2 and TLR4 gene polymorphisms studied are not related to susceptibility to CP.