Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 204–211 doi: 10.1111/j.1600-051X.2008.01379.x

Journal of Clinical Periodontology

Renin–angiotensin gene polymorphisms in relation to severe chronic periodontitis

Ali Gürkan[1], Gülnur Emingil[1], Buket Han Saygan[1], Gül Atilla[1], Timur Köse[2], Haluk Baylas[1] and Afig Berdeli[3]

[1]Department of Periodontology, School of Dentistry, Ege University, Izmir, Turkey; [2]Department of Biostatistics and Medical Informatics, School of Medicine, Ege University, Izmir, Turkey; [3]Molecular Medicine Laboratory, Department of Pediatrics, School of Medicine, Ege University, Izmir, Turkey

Gürkan A, Emingil G, Saygan BH, Atilla G, Köse T, Baylas H, Berdeli A. Renin–angiotensin gene polymorphisms in relation to severe chronic periodontitis. J Clin Periodontol 2009; 36: 204–211. doi: 10.1111/j.1600-051X.2008.01379.x.

Abstract

Aim: Evidence suggests that the ultimate product of the renin–angiotensin system (RAS), angiotensin II, exerts inflammatory actions. The present study aimed to evaluate the inter-relation between gene polymorphisms of the RAS components; angiotensin converting enzyme (ACE), angiotensinogen (AGT) and angiotensin II type-I receptor (AT1R), and severe chronic periodontitis (CP).

Material and Methods: DNA was obtained from peripheral blood of 90 CP patients and 126 periodontally healthy subjects, and the clinical parameters were recorded. ACE I/D, AGT M235T and AT1R A1166C polymorphisms were genotyped by the PCR–RFLP method. Chi-square, ANOVA and logistic regression methods were used in statistical analyses.

Results: The frequency of the ACE D allele was significantly lower in the CP group than the healthy group ($p_{corr} = 0.015$). CP subjects exhibited increased C allele carriage and C allele frequency of the AT1R gene ($p_{corr} = 0.03$ and $p_{corr} = 0.03$, respectively). All clinical parameters of CP patients were found to be similar in variant allele-carrying and non-carrying subjects ($p > 0.05$).

Conclusions: The present findings suggest that ACE I/D and AT1R polymorphisms might be associated with susceptibility to CP but not with disease severity. The D allele of ACE I/D might be associated with decreased, whereas the C variant of AT1R A1166C might be associated with an elevated risk for CP in Turkish population.

Key words: ACE, AGT, AT1R, periodontitis, polymorphism

Accepted for publication 21 November 2008

Angiotensin (Ang) II, the final and bioactive component of the renin–angiotensin system (RAS), contributes to inflammation and the related tissue destruction as a key cytokine (Ruiz-Ortega et al. 2001, Phillips & Kagiyama 2002, Suzuki et al. 2003, Das 2005). Ang II, which is proinflammatory in nature, regulates the expression of various cytokines, growth factors, chemokines and other molecules contributing to the development of inflammation (Ruiz-Ortega et al. 2001, Suzuki et al 2003). Therefore, an elevation in Ang II production may play a role in the onset and progression of inflammation. Although our current knowledge regarding the role of RAS in periodontal disease is limited to only one study (Hollá et al. 2001), the biological activities of the members of this system suggest their potential contribution to the pathogenesis of periodontitis.

An insertion (I) or a deletion (D) of a 287 bp *Alu* repetition sequence localized inside the intron 16 of the angiotensin-converting enzyme (ACE) gene was identified (Rigat et al. 1990). About 50% of the inter-individual variance in plasma ACE levels is determined by this polymorphism. The mean plasma ACE levels were reported to be twice that of II subjects in DD subjects whereas subjects, with the ID genotype had intermediate levels (Rigat et al. 1990). A point mutation has been described at codon 235 of the angiotensinogen (AGT) gene, resulting from a thymidine (T) to cytosine (C) replacement at position 704 in exon 2, leading to a methionine (Met) to threonine (Thr) substitution (Jeunemaitre et al. 1992). The AGT M235T polymorphism is in synergistic interaction with the ACE D allele (Ludwig et al. 1997) and in very tight linkage with the functional AGT A

Conflict of Interest and Source of Funding

The authors declare that they have no conflict of interests. The study was self-funded by the authors and their institution.

(– 6) polymorphism, which plays a critical role in transcriptional control (Inoue et al. 1997). Carriers of the 235T allele have higher levels of plasma AGT compared with non-carriers (Jeunemaitre et al. 1992). Ang II type 1 receptor (AT1R) gene harbours a polymorphism as a consequence of adenine (A) to cytosine (C) transversion at position 1166 located in the 3′ untranslated region (Bonnardeaux et al. 1994). The C allele of the AT1R A1166C polymorphism has been shown to increase sensitivity to Ang II (Spiering et al. 2000). The aforementioned polymorphisms are the most commonly studied ones among the RAS genes in relation to various diseases and have been linked with the onset and progression of various micro- and macrovascular diseases and rheumatoid arthritis (Inoue et al. 1997, Ludwig et al. 1997, Kennon et al. 1999, Fatini et al. 2000, Spiering et al. 2000, Tsai et al. 2004, Acartürk et al. 2005, Berdeli et al. 2005, Sekuri et al. 2005, Uppal et al. 2007), which is linked with periodontitis by means of molecular pathogenesis and inflammatory aetiology (Bartold et al. 2005, Davé & van Dyke 2008).

Periodontitis, which is initiated and perpetuated in response to microbial challenge, exhibits features of a complex genetic disease whose phenotype pattern is modulated by both genetic and environmental factors (Yoshie et al. 2007). Mounting data suggest a key role for the genetic features in individual susceptibility to periodontitis (Kinane et al. 2005, Yoshie et al. 2007). To date, there is only one study available on the association of the ACE I/D polymorphism with chronic periodontitis (CP) (Hollá et al. 2001). Nevertheless, the relation between polymorphisms of other RAS components and CP has not been determined previously. Considering the role of RAS in both vascular events and inflammation, whether genetic variances in this system have implications in susceptibility to CP was hypothesized. Therefore, the present study was undertaken to compare the genotype, variant allele carriage and allele frequencies of ACE I/D, AGT M235T and AT1R A1166C polymorphisms in CP patients and periodontally healthy subjects.

Material and Methods

Study population

A total of 216 unrelated Turkish subjects, including 90 CP patients and 126 periodontally healthy subjects were recruited from the Department of Periodontology, School of Dentistry, Ege University, over a period of 4 years between 2002 and 2006. All the study subjects were residing in the Aegean region of Turkey and belonged to a low to moderate socioeconomic level. The study protocol was approved by the Ethics Committee of the Ege University, School of Medicine. Participants eligible for the study were informed about the purpose of the study and gave written informed consent in accordance with the Helsinki declaration. Medical and dental histories were taken. All the study subjects had at least 20 teeth. None of the CP patients and healthy subjects had a history of or a current manifestation of systemic conditions that could modify the periodontal status including diseases that have been associated with investigated RAS polymorphisms (such as cardiovascular diseases, hypertension, renal diseases, diabetes and allergy), or had transmissible infectious diseases (HIV, hepatitis). Pregnancy was also an exclusion criterion for the study. Smoking status and history was determined by a self-administrated questionnaire regarding present or previous tobacco smoking habits, 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 CP and healthy groups were smoking more than ten cigarettes per day for more than 5 years. Subjects who had never smoked or had quit smoking at least five years ago were considered as non-smokers. CP patients were diagnosed in accordance with the clinical criteria agreed upon by consensus at the World Workshop in Periodontics in 1999 (Armitage 1999) as follows:

CP group: The CP group included 90 subjects having at least 20 teeth, had severe CP and exhibited at least four sites with clinical attachment loss (CAL) $\geqslant$5 mm in at least two separate quadrants. They also had bleeding on probing (BOP) at $>$80% of the proximal sites. The bone loss estimation was radiographically performed in each patient for assessment of the extent and severity of alveolar bone loss. Examinations were particularly focused on consistency of periodontal destruction with plaque accumulation in order to distinguish from aggressive forms of periodontitis. The characteristics of the CP group are outlined in Table 1.

Table 1. Demographic characteristics of the study subjects

	Chronic periodontitis	Healthy
Subjects (*n*)	90	126
Smokers (*n*)	37	6
Age (Mean SD)	48.2 ± 6.2	38.7 ± 9.7
Gender		
Male *n* (%)	59 (65.6)	55 (43.7)
Female *n* (%)	31 (34.4)	71 (56.3)

Healthy control group: This group included 126 subjects exhibiting probing depth (PD) $<$3 mm and CAL$\leqslant$ 2 mm at more than or equal to 90% of the measured tooth sites as well as no BOP at examination and no alveolar bone loss in radiography (i.e., distance between the cementoenamel junction and bone crest $\leqslant$3 mm at $>$95% of the proximal tooth sites). These individuals were periodontally healthy volunteers from the staff and other patients referring to the School of Dentistry. The characteristics of the healthy control group are given in Table 1.

Determination of periodontal status

The clinical periodontal parameters were assessed at six sites around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations) for the whole mouth excluding the third molars and included PD, CAL, BOP and supragingival plaque accumulation. The cementoenamel junction was considered as the reference point in measurements of CAL. PD and CAL measurements were performed using a manual Williams probe. BOP (deemed positive if it occurred within 15 s after periodontal probing) and supragingival plaque accumulation were recorded dichotomously.

Genotyping of ACE, AGT and AT1R polymorphisms

Genotyping of the ACE I/D gene was carried out as described previously (Tabel et al. 2005). Analysis of the AGT M235T polymorphism was performed according to Russ et al. (1993), while AT1R A1166C polymorphism was analysed according to Bonnardeaux

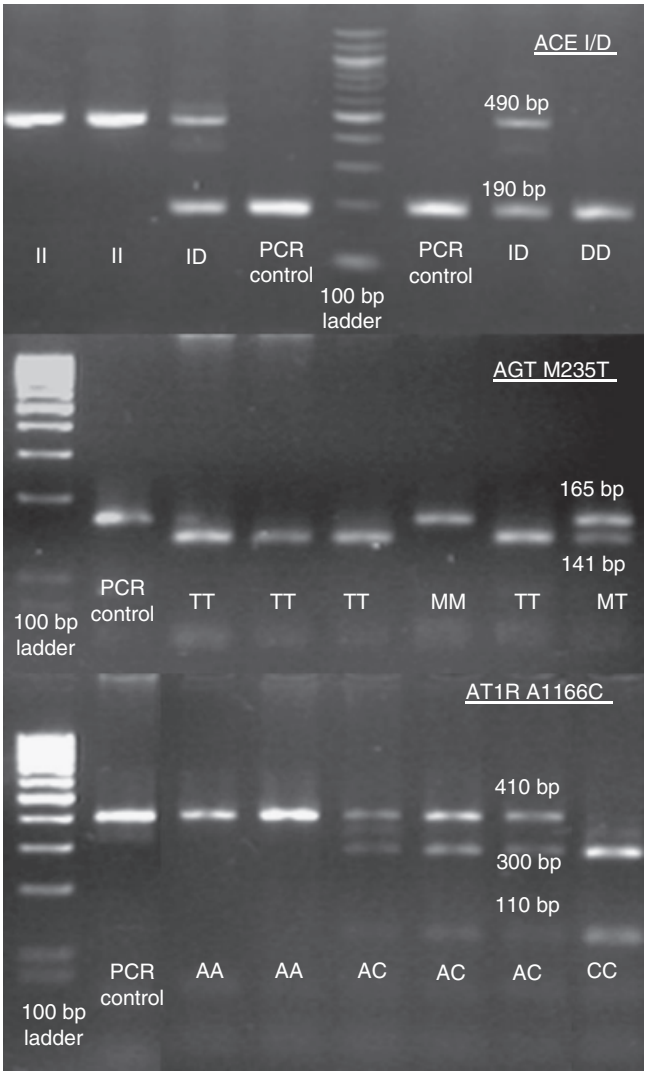

Fig. 1. Agarose gel electrophoresis image of angiotensin converting enzyme (ACE), angiotensinogen (AGT) and angiotensin II type-I receptor (AT1R) polymorphisms.

et al. (1994). Preparation of genomic DNA, DNA amplification, sense and antisense primers used, thermal cycling conditions, restriction enzymes, PCR products and digestion of PCR products were as described elsewhere (Tabel et al. 2005). Only a slight modification was made in the method for the digestion of PCR products in the AT1R polymorphism; undigested PCR product was 410 bp, whereas the digestion yielded in 300 bp and 110 bp fragments (Fig. 1).

Statistical analysis

The Chi-square (χ^2) test with 1 degree of freedom was used to evaluate the concordance of genotype frequencies with Hardy–Weinberg equilibrium in CP and healthy groups. The distribution of ACE, AGT and AT1R genotypes, variant allele carriage and allele frequencies in CP and healthy groups were also analysed by the χ^2 test. Allele frequencies were calculated from the observed numbers of genotypes. The odds-ratio (OR) and 95% confidence interval (95% CI) were also assessed. Furthermore, subjects were subgrouped according to their smoking status and analyses were repeated. Corrected p values were calculated for multiple testing using the Bonferroni method and 0.017 (0.05/3) was set as the threshold degree for significance because three separate comparisons were performed. The clinical parameters of subjects distributed by carriage of the variant allele in the CP group were compared by a randomized block design where smoking status was utilized as a block. The relationship between variant allele carriage and disease status was analysed by multiple logistic regression analysis while adjusting for age, gender and smoking status. All data analyses were performed using a statistical package (SPSS 15.0, SPSS Inc., Chicago, IL, USA). Post hoc power calculations were performed by a statistical software (PAWE version 1.2; Gordon et al. 2002) at the 5% significance level.

Results

Power was calculated as 0.80, 0.34 and 0.71 for ACE, AGT and AT1R, respectively. The distributions of CP and control subjects exhibiting ACE, AGT and AT1R genotypes were in accordance with H–W equilibrium ($p > 0.05$, $\chi^2 < 3.84$). Also, the distribution of ACE, AGT and AT1R genotypes subgrouped by gender in the healthy ($p = 0.51$, $\chi^2 = 1.33$; $p = 0.63$, $\chi^2 = 0.94$; $p = 0.64$, $\chi^2 = 0.88$, respectively) and CP groups ($p = 0.64$, $\chi^2 = 0.88$; $p = 0.43$, $\chi^2 = 1.69$, $p = 0.10$, $\chi^2 = 0.95$, respectively) were similar.

ACE I/D genotypes, allele frequency and carriage of the D allele

The distributions of ACE I/D genotypes among the study groups are presented in Table 2. The distribution of ACE genotypes and the frequency of the D allele carriage between CP and healthy subjects were different ($p = 0.03$ and $p = 0.03$, respectively), but did not reach significance following correction. The frequency of the D allele was significantly lower in the CP group when compared with the healthy group ($p = 0.005$), which persisted even after correction ($p_{corr} = 0.015$).

When the smokers were excluded, no significant difference was found in the distribution of ACE I/D genotypes and D allele carriage between CP and healthy groups ($p > 0.05$). The allele frequencies of non-smoker CP and non-smoker healthy subjects exhibited a slightly significant difference ($p = 0.04$), but this significance disappeared following correction (Table 2).

AGT M235T genotypes, allele frequency and carriage of the T allele

The distributions of AGT M235T genotypes among the study groups are

Table 2. Genotype distributions, allele frequencies and variant allele carriage of *RAS* gene polymorphisms in chronic periodontitis (CP) and healthy groups

	CP $n = 90$(%)	Healthy $n = 126$(%)	$p^*(\chi^2)$	p_{corr} †	OR (95% CI)	CP $n = 53$(%)	Healthy $n = 120$(%)	$p^*(\chi^2)$	p_{corr} †	OR (95% CI)
ACE I/D										
Genotype										
II	18 (20.0)	12 (9.5)			Referent	10 (18.9)	11 (9.2)			Referent
ID	35 (38.9)	43 (34.1)	0.03 (6.92)	NS	0.54 (0.23–1.28)	20 (37.7)	42 (35.0)	0.13 (4.02)	–	0.52 (0.19–1.44)
DD	37 (41.1)	71 (56.4)			0.35 (0.15–0.80)	23 (43.4)	67 (55.8)			0.38 (0.14–1.01)
D allele carriage	72 (80.0)	114 (90.5)	0.03 (4.82)	NS	0.36 (0.17–0.76)	43 (81.1)	109 (90.8)	0.07 (3.24)	–	0.43 (0.17–1.10)
Allele frequency										
I	71 (39.4)	67 (26.6)	**0.005** (7.98)	**0.015**	Referent	40 (37.7)	64 (26.7)	**0.04** (4.29)	NS	Referent
D	109 (60.6)	185 (73.4)			0.56 (0.37–0.84)	66 (62.3)	176 (73.3)			0.60 (0.37–0.98)
AGT M235T										
Genotype										
MM	17 (18.9)	20 (15.9)			Referent	9 (17.0)	18 (15.0)			Referent
MT	42 (46.7)	48 (38.1)	0.20 (2.92)	–	1.03 (0.48–2.22)	22 (41.5)	45 (37.5)	0.77 (0.53)	–	0.98 (0.38–2.53)
TT	31 (34.4)	58 (46.0)			0.63 (0.29–1.37)	22 (41.5)	57 (47.5)			0.77 (0.30–1.98)
T allele carriage	73 (81.1)	106 (84.1)	0.56 (0.33)	–	0.81 (0.40–1.65)	44 (83.0)	102 (85.0)	0.74 (0.11)	–	0.86 (0.36–2.37)
Allele frequency										
M	76 (42.2)	88 (34.9)	0.12 (2.38)	–	Referent	40 (37.7)	81 (33.8)	0.47 (0.51)	–	Referent
T	104 (57.8)	164 (65.1)			0.73 (0.50–1.09)	66 (62.3)	159 (66.2)			0.84 (0.52–1.35)
AT1R A1166C										
Genotype										
AA	42 (46.7)	81 (64.3)			Referent	25 (47.2)	76 (63.3)			Referent
AC	43 (47.8)	42 (33.3)	0.03 (7.07)	NS	1.97 (1.12–3.48)	25 (47.2)	41(34.2)	0.12 (4.33)	–	1.85 (0.95–3.63)
CC	5 (5.6)	3 (2.4)			3.21 (0.73–14.18)	3 (5.6)	3 (2.5)			3.04 (0.58–16.03)
C allele carriage	48 (53.3)	45 (35.7)	**0.01** (6.65)	**0.03**	2.06 (1.19–3.57)	28 (52.8)	44 (36.7)	**0.047** (3.95)	NS	1.94 (1.01–3.95)
Allele frequency										
A	127 (70.6)	204 (81.0)	**0.01** (6.33)	**0.03**	Referent	75 (70.8)	193 (80.4)	**0.047** (3.93)	NS	Referent
C	53 (29.4)	48 (19.0)			1.77 (1.13–2.78)	31 (29.2)	47 (19.6)			1.770 (1.01–2.87)

P values in bold face denote statistical significance.
*Comparisons in this column are not significant unless the corresponding *p* value is < 0.017.
†Corrected p (pcorr) are for comparisons of three genotypes in the CP group (n = 3 independent comparisons). All comparisons are with healthy controls.
OR, odds ratio; CI, confidence interval; NS, non-significant.

presented in Table 2. The distribution of AGT M235T genotypes among the study groups was similar ($p = 0.20$). The frequency of T allele carriage (MT and TT genotypes) was not significantly different in the healthy and CP groups ($p = 0.56$). The percentage of the T allele was higher in the healthy group than that of the CP group but the differences did not reach significance ($p = 0.12$).

In the non-smoker subgroup analysis, the distribution of AGT M235T genotypes and T allele carriage in the non-smoker CP group was found to be similar to the healthy group and no significant difference was found between non-smoker CP and non-smoker healthy subjects for allele frequencies (Table 2).

AT1R A1166C genotypes, allele frequency and carriage of the C allele

The distribution of AT1R A1166C genotypes among the study groups is presented in Table 2. The distribution of AT1R A1166C genotypes tended to be different between groups ($p = 0.03$); however, this difference was not significant after correction. The frequency of C allele carriage was approximately 18% higher in the CP group when compared with the healthy group ($p = 0.01$). The C allele frequency of the CP group was also significantly higher than that of the healthy group ($p = 0.01$). Overall, the C allele carriage and the C allele frequency exhibited elevated ORs in a similar manner (ORs: 2.06 and 1.77, respectively), and the differences between study groups remained after correction ($p_{corr} = 0.03$ and 0.03, respectively).

In subgroup analysis, a similar genotype distribution was observed in the non-smoker study groups ($p > 0.05$). The C allele carriage and C allele frequency of the AT1R A1166C polymorphism were higher in the CP group as compared with healthy group, and were marginally significant ($p = 0.047$ and $p = 0.047$, respectively), but these differences were not found to be significant following correction (Table 2).

Variant allele carriage in relation to the severity of chronic periodontitis

In order to investigate whether carriage of the variant allele is associated with the severity of chronic periodontitis, differences in the clinical parameters between

Table 3. Logistic regression models for the association between variant allele carriage and susceptibility to chronic periodontitis

	Adjusted odds ratio	95% confidence interval	*p*
ACE I/D			
Age	1.31	1.11–1.22	**<0.0001**
Gender			
Women	Referent		
Men	1.31	0.63–2.71	0.47
Smoking			
Nonsmokers	Referent		
Smokers	18.09	6.27–52.21	**<0.0001**
ACE D allele			
Non-carriers	Referent		
Carriers	0.47	0.18–1.27	0.14
AGT M235T			
Age	1.16	1.11–1.22	**<0.0001**
Gender			
Women	Referent		
Men	1.25	0.61–2.58	0.55
Smoking			
Nonsmokers	Referent		
Smokers	19.64	6.71–57.47	**<0.0001**
AGT T allele			
Non-carriers	Referent		
Carriers	1.34	0.51–3.51	0.55
AT1R A1166C			
Age	1.17	1.11–1.23	**<0.0001**
Gender			
Women	Referent		
Men	1.32	0.63–2.76	0.46
Smoking			
Nonsmokers	Referent		
Smokers	19.40	6.56–57.34	**<0.0001**
AT1R C allele			
Non-carriers	Referent		
Carriers	2.44	1.17–5.08	**0.02**

p values in bold face indicate statistical significance.
ACE, angiotensin converting enzyme; AGT, angiotensinogen; AT1R, angiotensin II type-I receptor polymorphisms.

variant allele carrier and non-carrier subjects in the CP group were compared while adjusting for smoking. No significant differences were found in the clinical parameters between the D allele-carrying and non-carrying genotypes of the ACE I/D polymorphism ($p > 0.05$). The clinical parameters of AGT M235T gene T allele carrier and non-carrier CP patients were similar ($p > 0.05$). The clinical parameters also did not show difference between C allele carriers and non-carriers in the CP group regarding the AT1R A1166C polymorphism ($p > 0.05$).

Multiple logistic regression models

Multiple logistic regression analysis was used to evaluate the association of the ACE, AGT and AT1R genotypes with susceptibility to CP, while adjusting for modifying factors such as subject age, gender and smoking. In all these models, subject age and smoking were found to be significantly associated with CP ($p < 0.0001$ and $p < 0.0001$). Moreover, C allele carriage of the AT1R gene was found to be associated with susceptibility to CP ($p = 0.02$, adjusted ORs: 2.44 in reference to non-carriers) (Table 3).

Discussion

The relationship between RAS of particular ACE gene polymorphisms and cardiovascular diseases has attracted considerable attention recently. However, the findings of these studies showed variations and the conclusions remained controversial (Carluccio et al. 2001). Previous studies showed the association of the DD genotype or the D allele of the ACE I/D polymorphism with coronary artery disease (CAD) (Acartürk et al. 2005, Berdeli et al. 2005, Sekuri et al. 2005), while others reported no interaction between the ACE I/D polymorphism and CAD (Araz et al. 2002, Akbulut et al. 2003) as well as ischaemic stroke (Tuncer et al. 2006) in Turkish population. To date, only one study has evaluated the ACE I/D polymorphism in relation to CP in a Czech population, which reported similar genotype and allele frequency distributions in subjects with CP and periodontally healthy subjects (Hollá et al. 2001). In the present study, differences between groups regarding ACE genotypes and the D allele carriage did not reach significance. Also, no relation between disease status and ACE genotypes was observed in logistic regression analysis. In addition, carriage of D allele did not significantly influence the severity of CP as was evident by the clinical parameters distributed by variant allele carriage status. Concerning these points, the findings from the subjects with severe CP were in line with a previous report investigating the relation of CP to ACE polymorphism (Holla et al. 2001). However, the D allele frequency of the ACE I/D polymorphism was higher in controls than in cases, suggesting a protective effect for the D variant of the ACE I/D gene in susceptibility to CP.

The present results are inconsistent with the theory that subjects with the D variant of the ACE I/D gene will have elevated levels of ACE and thus will produce more Ang II that will lead consequently to tissue destruction, because these genetic features were more frequent in periodontal healthy subjects. In contrast, an increase in plasma Ang II levels are controlled by a reduction in renin release (Danser 2003). In addition, it has been suggested that plasma Ang II levels is not influenced by ACE levels (Danser 2003). Expression of a particular gene may vary between different tissues or conditions; thus, variation in the genetic code of a molecule may be specific for a particular tissue (Kinane et al. 2005). Taken together, a difference between the local and the circulating ACE gene expressions might be assumed. Therefore, as the Ang II is also produced by local mechanisms, it could be speculated that the ACE I/D polymorphism might influence gingival tissue levels rather than plasma levels of Ang II. Moreover, this finding might support the possible anti-inflammatory effects of ACE, which acts by degrading bradykinin and substance P (Scholzen et al. 2003). Furthermore, it has been shown

that the presence of the ACE D allele is associated with decreased type I collagen degradation (Tziakas et al. 2007) and a decreased risk for myocardial infarctus (Andrikopoulos et al. 2004). The aforementioned points might help to clarify why a genetic determinant that is associated with higher circulating levels of ACE was observed more frequently in the healthy group when compared with the CP group.

In previous studies, the T allele of the AGT M235T gene has been proposed to be an independent risk determinant for CAD (Ludwig et al. 1997, Lanz et al. 2005). In contrast, reports are also available linking the AGT 235 MM genotype, the M allele or the M allele carriage with an increased risk for CAD in Turkish (Berdeli et al. 2005) as well as in other populations (Tsai et al. 2004, Nakase et al. 2007). In the present study, the TT genotype, T allele carriage and T allele frequency of the AGT M235T gene were lower in the CP group compared with the healthy group. However, the differences were found to be statistically similar. Nevertheless, these findings do not exclude the contribution of this genetic variant to the susceptibility to CP, because the statistical power of the present sample size was low for the AGT M235T polymorphism arising as a consequence of small percentage differences between allele frequencies in study groups.

The A1166C polymorphism is in a non-coding region of the AT1R gene; thus, transversion does not alter the amino-acid sequence of the receptor (Duncan et al. 2001). However, this variant is proposed to be a genetic marker in linkage disequilibrium with an unidentified gene activated in response to Ang II. Cardiovascular events and AT1R A1166C polymorphism have been found to be linked in Turkish (Agachan et al. 2003, Sekuri et al. 2005) and in Norwegian and Italian populations (Berge et al. 1997, Fatini et al. 2000). In the present study, the genotype distribution, variant allele carriage and allele frequency of the AT1R A1166C polymorphism showed an approximately two times increased risk for CP in C allele carriers and for the C allele. This effect was more significantly evident, about 2.5 times increased risk, when corrected for confounding factors in the regression analysis. The AT1R A1166C polymorphism was found to be closely related to fibrinolytic molecules namely tissue plasminogen activator and plasminogen activator inhibitor 1 (Asselbergs et al. 2007). The interplay between CP and cardiovascular events has been an issue of interest for many years; however, the exact mechanisms that underlie this association have not been elucidated. Chronic periodontal inflammation and eventually elevated circulating inflammatory molecules might be strong candidates for this association (Davé & van Dyke 2008). From this point of view, the present finding might be of particular importance, indicating a genetic commonality between cardiovascular conditions and CP, besides elevated circulating cytokine levels.

CP is a multifactorial disease, whose manifestation and progression is influenced by a variety of factors such as genetics, smoking, age and gender (Heitz-Mayfield 2005, Palmer et al. 2005). Therefore, in the present study, the investigated gene polymorphisms were evaluated in non-smoker subjects as well. Analysis of the genotype distribution, allele carriage and allele frequency of RAS genes yielded similar percentages in the non-smoker subgroup and in the whole group. It has been suggested that male gender is associated with the prevalence, extent and severity of periodontitis (Heitz-Mayfield 2005). Also, recent studies reported an association between RAS polymorphisms and gender (Reich et al. 2003, Röcken et al. 2007). Because there was a non-proportional distribution of genders in the study groups, the study population was also subgrouped in order to evaluate the interaction between RAS genotypes and gender. In addition, when evaluating whether the rare allele carriage of the investigated RAS polymorphisms was a risk factor for severe CP, subject age, sex and smoking were selected as correction factors. The incidence of periodontal disease in terms of attachment and alveolar bone loss is associated with ageing and is more frequent in younger subjects in comparison with older ones (Grossi et al. 1994, 1995). However, it has been proposed that the increased level of periodontal destruction observed with ageing is a result of cumulative destruction rather than increased rates of destruction (Genco 1996). Also, the influence of age was deemed as negligible as compared with plaque in periodontal tissue destruction (Abdellatif & Burt 1987). However, at least to remove any question mark arising from the mean age differences between groups, the mean age of the subjects should have been similar. Thus, the mean age difference between groups may be a limitation of the present study.

Within the limits of the present study, which is the first to address RAS gene polymorphisms in relation to CP, it is proposed that ACE I/D polymorphisms might be associated with decreased, while the AT1R A1166C polymorphism might be associated with increased susceptibility to CP in Turkish population. However, due to the complexity and multifactorial feature of the disease, a direct cause–effect relation is unlikely between investigated polymorphisms and CP. Different populations can exhibit different genotype frequencies, which may be influenced by racial and regional factors and may complicate interpretation of the results of genetic studies. Therefore, additional case–control studies are warranted regarding the relation of gene polymorphisms of RAS gene polymorphisms and CP in other populations in order to further determine its role in the susceptibility to periodontitis. Moreover, whether functional genetic variants in other loci of RAS genes and as yet unidentified polymorphisms in RAS molecules contribute to inter-individual differences in susceptibility to CP remains to be determined.

References

Abdellatif, H. M. & Burt, B. A. (1987) An epidemiological investigation into the relative importance of age and oral hygiene status as determinants of periodontitis. *Journal of Dental Research* **66**, 13–18.

Acartürk, E., Attila, G., Bozkurt, A., Akpinar, O., Matyar, S. & Seydaoglu, G. (2005) Insertion/deletion polymorphism of the angiotensin converting enzyme gene in coronary artery disease in southern Turkey. *Journal of Biochemistry and Molecular Biology* **38**, 486–490.

Agachan, B., Isbir, T., Yilmaz, H. & Akoglu, E. (2003) Angiotensin converting enzyme I/D, angiotensinogen T174M–M235T and angiotensin II type 1 receptor A1166C gene polymorphisms in Turkish hypertensive patients. *Experimental and Molecular Medicine* **35**, 545–549.

Akbulut, T., Bilsel, T., Terzi, S., Ciloglu, F., Unal Dayi, S., Sayar, N., Peker, I. & Yesilcimen, K. (2003) Relationship between ACE gene polymorphism and ischemic chronic heart failure in Turkish population. *European Journal of Medical Research* **8**, 247–253.

Andrikopoulos, G. K., Richter, D. J., Needham, E. W., Tzeis, S. E., Zairis, M. N., Gialafos, E. J., Vogiatzi, P. G., Papasteriadis, E. G., Kardaras, F. G., Foussas, S. G., Gialafos, J. E., Stefanadis, C. I., Toutouzas, P. K. &

Mattu, R. K. (2004) The paradoxical association of common polymorphisms of the renin–angiotensin system genes with risk of myocardial infarction. *European journal of the Cardiovascular Prevention and Rehabilitation* **11**, 477–483.

Araz, M., Aynacioglu, S., Okan, V., Akdemir, I. & Aktaran, S. (2002) Angiotensin-converting enzyme gene polymorphism and coronary heart disease in Turkish type 2 diabetic patients. *Acta Cardiologica* **57**, 265–269.

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–7.

Asselbergs, F. W., Williams, S. M., Hebert, P. R., Coffey, C. S., Hillege, H. L., Navis, G., Vaughan, D. E., van Gilst, W. H. & Moore, J. H. (2007) Epistatic effects of polymorphisms in genes from the renin–angiotensin, bradykinin, and fibrinolytic systems on plasma t-PA and PAI-1 levels. *Genomics* **89**, 362–369.

Bartold, P. M., Marshall, R. I. & Haynes, D. R. (2005) Periodontitis and rheumatoid arthritis: a review. *Journal of Periodontology* **76** (Suppl. 11), 2066–2074.

Berdeli, A., Sekuri, C., Sirri Cam, F., Ercan, E., Sagcan, A., Tengiz, I., Eser, E. & Akin, M. (2005) Association between the eNOS (Glu298Asp) and the RAS genes polymorphisms and premature coronary artery disease in a Turkish population. *Clinica Chimica Acta* **351**, 87–94.

Berge, K. E., Bakken, A., Bøhn, M., Erikssen, J. & Berg, K. (1997) A DNA polymorphism at the angiotensin II type 1 receptor (AT1R) locus and myocardial infarction. *Clinical Genetics* **52**, 71–76.

Bonnardeaux, A., Davies, E., Jeunemaitre, X., Féry, I., Charru, A., Clauser, E., Tiret, L., Cambien, F., Corvol, P. & Soubrier, F. (1994) Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension* **24**, 63–69.

Carluccio, M., Soccio, M. & De Caterina, R. (2001) Aspects of gene polymorphisms in cardiovascular disease: the renin–angiotensin system. *European Journal of Clinical Investigation* **31**, 476–488.

Danser, A. H. (2003) Local renin–angiotensin systems: the unanswered questions. *The International Journal of Biochemistry and Cell Biology* **35**, 759–768.

Das, U. N. (2005) Is angiotensin-II an endogenous pro-inflammatory molecule? *Medical Science Monitor* **11**, RA155–RA162.

Davé, S. & van Dyke, T. (2008) The link between periodontal disease and cardiovascular disease is probably inflammation. *Oral Diseases* **14**, 95–101.

Duncan, J. A., Scholey, J. W. & Miller, JA. (2001) Angiotensin II type 1 receptor gene polymorphisms in humans: physiology and pathophysiology of the genotypes. *Current Opinion in Nephrology and Hypertension* **10**, 111–116.

Fatini, C., Abbate, R., Pepe, G., Battaglini, B., Gensini, F., Ruggiano, G., Gensini, G. F. & Guazzelli, R. (2000) Searching for a better assessment of the individual coronary risk profile. The role of angiotensin-converting enzyme, angiotensin II type 1 receptor and angiotensinogen gene polymorphisms. *European Heart Journal* **21**, 633–638.

Genco, R. J. (1996) Current view of risk factors for periodontal diseases. *Journal of Periodontology* **67** (Suppl. 10), 1041–1049.

Gordon, D., Finch, S. J., Nothnagel, M. & Ott, J. (2002) Power and sample size calculations for case-control genetic association tests when errors present: application to single nucleotide polymorphisms. *Human Heredity* **54**, 22–33.

Grossi, S. G., Genco, R. J., Machtei, E. E., Ho, A. W., Koch, G., Dunford, R., Zambon, J. J. & Hausmann, E. (1995) Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *Journal of Periodontology* **66**, 23–29.

Grossi, S. G., Zambon, J. J., Ho, A. W., Koch, G., Dunford, R. G., Machtei, E. E., Norderyd, O. M. & Genco, R. J. (1994) Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *Journal of Periodontology* **65**, 260–267.

Heitz-Mayfield, L. J. A. (2005) Disease progression: identification of high-risk groups and individuals for periodontitis. *Journal of Clinical Periodontology* **32**, 196–209.

Hollá, L. I., Fassmann, A., Vaskù, A., Znojil, V., Vanek, J. & Vácha, J. (2001) Interactions of lymphotoxin alpha (TNF-beta), angiotensin-converting enzyme (ACE), and endothelin-1 (ET-1) gene polymorphisms in adult periodontitis. *Journal of Periodontology* **72**, 85–89.

Inoue, I., Nakajima, T., Williams, C. S., Quackenbush, J., Puryear, R., Powers, M., Cheng, T., Ludwig, E. H., Sharma, A. M., Hata, A., Jeunemaitre, X. & Lalouel, J. M. (1997) A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription in vitro. *The Journal of Clinical Investigation* **99**, 1786–1797.

Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y. V., Lifton, R. P., Williams, C. S., Charru, A., Hunt, S. C., Hopkins, P. N., Williams, R. R., Lalouel, J. M. & Corvol, P. (1992) Molecular basis of human hypertension: role of angiotensinogen. *Cell* **71**, 169–180.

Kennon, B., Petrie, J. R., Small, M. & Connell, J. M. (1999) Angiotensin-converting enzyme gene and diabetes mellitus. *Diabetic Medicine* **16**, 448–458.

Kinane, D. F., Shiba, H. & Hart, T. C. (2005) The genetic basis of periodontitis. *Periodontology 2000* **39**, 91–117.

Lanz, J. R., Pereira, A. C., Lemos, P. A., Martinez, E. & Krieger, J. E. (2005) Angiotensinogen M235T polymorphism is associated with coronary artery disease severity. *Clinica Chimica Acta* **362**, 176–181.

Ludwig, E. H., Borecki, I. B., Ellison, R. C., Folsom, A. R., Heiss, G., Higgins, M., Lalouel, J. M., Province, M. A. & Rao, D. C. (1997) Associations between candidate loci angiotensin-converting enzyme and angiotensinogen with coronary heart disease and myocardial infarction: the NHLBI Family Heart Study. *Annals of Epidemiology* **7**, 3–12.

Nakase, T., Mizuno, T., Harada, S., Yamada, K., Nishimura, T., Ozasa, K., Watanabe, Y. & Nagata, K. (2007) Angiotensinogen gene polymorphism as a risk factor for ischemic stroke. *Journal of Clinical Neuroscience* **14**, 943–947.

Palmer, R. M., Wilson, R. F., Hasan, A. S. & Scott, D. A. (2005) Mechanisms of action of environmental factors – tobacco smoking. *Journal of Clinical Periodontology* **32**, 180–195.

Phillips, M. I. & Kagiyama, S. (2002) Angiotensin II as a pro-inflammatory mediator. *Current Opinion in Investigational Drugs* **3**, 569–577.

Reich, H., Duncan, J. A., Weinstein, J., Cattran, D. C., Scholey, J. W. & Miller, J. A. (2003) Interactions between gender and the angiotensin type 1 receptor gene polymorphism. *Kidney International* **63**, 1443–1449.

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P. & Soubrier, F. (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *The Journal of Clinical Investigation* **86**, 1343–1346.

Röcken, C., Neumann, K., Carl-McGrath, S., Lage, H., Ebert, M. P., Dierkes, J., Jacobi, C. A., Kalmuk, S., Neuhaus, P. & Neumann, U. (2007) The gene polymorphism of the angiotensin I-converting enzyme correlates with tumor size and patient survival in colorectal cancer patients. *Neoplasia* **9**, 716–722.

Ruiz-Ortega, M., Lorenzo, O., Rupérez, M., Esteban, V., Suzuki, Y., Mezzano, S., Plaza, J.J & Egido, J. (2001) Role of the renin–angiotensin system in vascular diseases: expanding the field. *Hypertension* **38**, 1382–1387.

Russ, A. P., Maerz, W., Ruzicka, V., Stein, U. & Gross, W. (1993) Rapid detection of the hypertension-associated Met235Thr allele of the human angiotensinogen gene. *Human Molecular Genetics* **2**, 609–610.

Scholzen, T. E., Staander, S., Riemann, H., Brzoska, T. & Luger, T. A. (2003) Modulation of cutaneous inflammation by angiotensin-converting enzyme. *The Journal of Immunology* **170**, 3866–3873.

Sekuri, C., Cam, F. S., Ercan, E., Tengiz, I., Sagcan, A., Eser, E., Berdeli, A. & Akin, M. (2005) Renin–angiotensin system gene polymorphisms and premature coronary heart disease. *Journal of the Renin Angiotensin Aldosterone System* **6**, 38–42.

Spiering, W., Kroon, A. A., Fuss-Lejeune, M. M., Daemen, M. J. & de Leeuw, P. W. (2000) Angiotensin II sensitivity is associated with the angiotensin II type 1 receptor A(1166)C polymorphism in essential hypertensives on a high sodium diet. *Hypertension* **36**, 411–416.

Suzuki, Y., Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V. & Egido, J. (2003) Inflammation and angiotensin II. *The International Journal of Biochemistry and Cell Biology* **35**, 881–900.

Tabel, Y., Berdeli, A., Mir, S., Serdaroğlu, E. & Yilmaz, E. (2005) Effects of genetic poly-

morphisms of the renin–angiotensin system in children with nephrotic syndrome. *Journal of the Renin Angiotensin Aldosterone System* **6**, 138–144.

Tsai, C. T., Lai, L. P., Lin, J. L., Chiang, F. T., Hwang, J. J., Ritchie, M. D., Moore, J. H., Hsu, K. L., Tseng, C. D., Liau, C. S. & Tseng, Y. Z. (2004) Renin–angiotensin system gene polymorphisms and atrial fibrillation. *Circulation* **109**, 1640–1646.

Tuncer, N., Tuglular, S., Kiliç, G., Sazci, A., Us, O. & Kara, I. (2006) Evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of ischaemic stroke. *Journal of Clinical Neuroscience* **13**, 224–227.

Tziakas, D. N., Chalikias, G. K., Stakos, D. A., Papazoglou, D., Papanas, N., Papatheodorou, K., Chatzikyriakou, S. V., Kotsiou, S., Maltezos, E. & Boudoulas, H. (2007) Effect of angiotensin-converting enzyme insertion/deletion genotype on collagen type I synthesis and degradation in patients with atrial fibrillation and arterial hypertension. *Expert Opinion on Pharmacotherapy* **8**, 2225–2234.

Uppal, S. S., Haider, M. Z., Hayat, S. J., Abraham, M., Sukumaran, J. & Dhaunsi, G. S. (2007) Significant association of insertion/deletion polymorphism of the angiotensin-converting enzyme gene with rheumatoid arthritis. *Journal of Rheumatology* **34**, 2395–2399.

Yoshie, H., Kobayashi, T., Tai, H. & Galicia, J. C. (2007) The role of genetic polymorphisms in periodontitis. *Periodontology 2000* **43**, 102–132.

Address:
Dr. *Ali Gürkan*
Department of Periodontology
Ege University
School of Dentistry
Bornova 35100
Izmir
Turkey
E-mail: ali.gurkan@ege.edu.tr

Clinical Relevance

Scientific rationale for the study: Host response to periodontopathic microorganisms can be modulated by genetic factors. Accumulated evidence highlighted the role of RAS in inflammatory response; thus, the potential involvement of this molecular system in the pathogenesis of periodontitis can be suggested. The present study investigated common polymorphisms within the RAS genes in relation to severe CP.

Principal findings: The D allele frequency of ACE I/D polymorphism was lower in severe CP subjects. Conversely, subjects exhibiting the AT1R A1166C genetic variant were more frequent in the severe CP group.

Practical implications: Investigated polymorphic variations within ACE and AT1R genes might be associated with severe CP in a Turkish population.