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Gene polymorphisms of tissue plasminogen activator and plasminogen activator inhibitor-1 in Turkish patients with generalized aggressive periodontitis

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

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

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Abstract

Aim: Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) have important roles in proteolytic events in periodontitis. The aim of this study was to investigate *TPA* and *PAI-1* gene polymorphisms in relation to susceptibility to generalized aggressive periodontitis (G-AgP).

Methods: Genomic DNA was obtained from peripheral blood of 90 G-AgP patients and 154 periodontally healthy subjects. 4G/5G polymorphism in the promoter region of the *PAI-1* gene and Alu-repeat insertion/deletion (I/D) polymorphism in intron 8 of the *TPA* gene were genotyped by polymerase chain reaction and endonuclease digestion.

Results: The genotype distributions of *TPA* and *PAI-1* genes were similar between G-AgP and healthy subjects ($p>0.05$). The distribution of *TPA* genotypes in G-AgP patients was 33.4% D/D, 44.4% I/D, and 22.2% I/I and was 26.3% D/D, 40.4% I/D, and 33.3% I/I in healthy subjects. The D allele was 55.6% in G-AgP and 46.6% in healthy subjects. There was a significant difference among study groups in D allele frequencies ($p = 0.044$). The *PAI-1* genotype distribution in G-AgP was 29.1% 4G/4G, 43.0% 4G/5G, and 27.9% 5G/5G, while it was 35.7% 4G/4G, 43.8% 4G/5G, and 20.5% 5G/5G in healthy subjects.

Conclusion: These data suggest that the D polymorphic allele of *TPA* gene polymorphism could be associated with susceptibility to G-AgP in Turkish subjects.

Key words: aggressive periodontitis; pathogenesis; periodontal disease; *TPA* and *PAI-1* gene polymorphisms

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Conflict of interest and source of funding statement

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The fibrinolytic system, also called the plasminogen-activating system, plays an important role in controlling proteolytic events in the extracellular matrix (Vassalli et al. 1991). This system is also involved in fibrinolysis, in balancing the coagulation of blood as well as in cell migration and tissue remodeling (Vassalli et al. 1991, Collen 1999). The plasminogen-activating system is activated by plasminogen activators (PAs) that are serine proteases. They activate the conversion of the inactive proenzyme plasminogen into the active enzyme, plasmin. Plasmin could participate in tissue destruction by indirectly converting the latent pro-MMPs into active forms, besides its high proteolytic

capacity (Murphy et al. 1999). It can also degrade various components of extracellular matrix proteins. Thereby, the fibrinolytic system could have a significant role in connective tissue destruction associated with periodontitis in concert with other mediators in the pathogenesis of periodontal disease (Kinnby 2002). Two types of PA that are encoded with different genes and therefore immunologically distinguishable were identified: the tissues/blood vessel-type activator and urokinase-type activator (Collen 1999). Their activities are regulated by specific inhibitors called plasminogen activator inhibitor-1 (PAI-1) and PAI-2 (Vassalli et al. 1991, Kinnby et al. 1999). The presence of the PA system was previously demonstrated in high levels in inflamed gingival tissues and gingival crevicular fluid (GCF) of periodontitis patients and thereby plays a key role in local inflammatory reactions in gingival tissues (Brown et al. 1995, Kinnby et al. 1999, Xiao et al. 2000). The destructive potential of the PAs plays an important role in the spread of inflammatory reactions and thereby could contribute to the initiation and progression of periodontal disease (Kinnby 2002). It was demonstrated that elevated plasma PAI-1 activity is related to the reduced fibrinolytic activity in individuals with cardiovascular disease (Kohler & Grant 2000).

Recent reports have indicated that candidate genes may be associated with both qualitative and quantitative aspects of host responses to microbial infection, which could influence the clinical outcome and susceptibility to periodontal disease (Loos et al. 2005, Shapira et al. 2005). Recently, an Alu repeat insertion/deletion (I/D) polymorphism of the *TPA* gene was identified within the intron 8, between exons 8 and 9 of this gene, which was sequenced and mapped on chromosome 8p12–p11.2 (Degen et al. 1986, Ludwig et al. 1992). I/D polymorphism of the *TPA* gene has been reported to be associated with several cardiovascular diseases such as myocardial infarction, which are possibly linked with periodontal disease (van der Bom et al. 1997, Hooper et al. 2000, Bang et al. 2001). It was suggested that Alu-repeat I/D polymorphism could be a marker of a functional mutation in the *TPA* gene that regulates the interaction between tissue plasminogen activator (t-PA) and PAI-1 owing to the positive correlation between t-PA and PAI-1 levels (Ladenvall et al. 2003). The I/I genotype of Alu repeat I/D polymorphism in the *TPA* gene was shown to be associated with plasma PAI-1 antigen in patients with myocardial infarction (van der Bom et al. 1997, Sartori et al. 2003).

The *PAI-1* gene located on chromosome 7q21.3–22 contains a promoter, nine exons, and eight introns. One of the several identified polymorphisms of the *PAI-1* gene is single guanosine I/D 4G/5G polymorphism, which is located in the promoter region 675 base pairs upstream from the transcriptional start site (Dawson et al. 1993). There is evidence that *PAI-1* 4G/5G I/D polymorphism in the *PAI-1* promoter region has been shown to modulate PAI-1 plasma levels (Eriksson et al. 1995). Recently, the prevalence of 4G allele has been shown to be associated with several systemic diseases such as osteonecrosis, meningococcal septic shock, and various cardiovascular diseases like myocardial infarction (Ossei-Gerning et al. 1997, Westendorp et al. 1999, Hooper et al. 2000, Bang et al. 2001, Kim et al. 2003). Disregulation in the coagulation and fibrinolytic systems plays an important role in the pathogenesis of periodontal diseases like in cardiovascular diseases (Kinnby 2002). When the proposed mechanisms of linkages between periodontal disease and systemic diseases are considered, *TPA* and *PAI-1* gene polymorphisms might provide further evidence about the association between periodontal and systemic diseases.

Aggressive periodontitis (AgP) comprises a heterogeneous group of periodontal diseases that affects adolescents and young adults (Tonetti & Mombelli 1999). It has been reported that *tumour necrosis factor-α*, *interleukin-1 (IL-1)*, *IL-1 receptor antagonist, IL-10*, and *Fcγ receptor* gene polymorphisms are associated with AgP in distinct populations (Parkhill et al. 2000, Craandijk et al. 2002, Gonzales et al. 2002, Tai et al. 2002, Berdeli et al. 2006, Nibali et al. 2006). On the other hand, *TPA* and *PAI-1* gene polymorphisms have not been investigated in generalized AgP (G-AgP) so far. The present study was undertaken to evaluate the 4G/5G polymorphism in the promoter region of *PAI-1* gene and Alu-repeat I/D polymorphism in intron 8 of *TPA* gene in a Turkish population with G-AgP, and also to investigate the association of the *TPA* and *PAI-1* gene polymorphisms with clinical periodontal parameters in Turkish subjects.

Material and Methods

Study population

A total of 244 unrelated Caucasians of Turkish descent residing in the same geographic region were included in the present study. Ninety G-AgP patients and 154 subjects with healthy periodontal conditions were recruited from the Department of Periodontology, School of Dentistry, Ege University over a period of 4 years between 2002 and 2006. All of the patients and healthy controls were from the western region of Turkey and were of low to moderate socio-economical level. The study protocol was approved by the Ethics Committee of the Ege University School of Medicine. All participants gave written informed consent in accordance with the Helsinki declaration. Medical and dental histories were taken. All the study groups had at least 20 teeth in the mouth. None of the subjects had a history or current manifestation of serious systemic disease, 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. Smokers in both G-AgP and healthy groups were smoking more than 10 cigarettes/day for more than 5 years. G-AgP patients were diagnosed in accordance with the clinical criteria for G-AgP agreed by consensus at the World Workshop in Periodontics in 1999 as follows (Armitage 1999):

G-AgP group

The G-AgP group consisted of 53 females and 37 males between the ages of 16 and 39 (mean of 29.71 ± 5.7 years). These patients demonstrated a generalized pattern of severe destruction and clinical attachment loss of $\geqslant 5$ mm on eight or more teeth; at least three of these were other than central incisors or first molars.

Healthy group

The healthy group consisted of 93 females and 61 males who exhibited PPD < 3 mm and no clinical attachment loss, bleeding on probing, and radiographic evidence of bone loss (mean age 37.79 ± 9.9 years; range 17–70 years). These individuals were healthy volunteers from the staff and other patients in the Dental School.

Determination of periodontal status

Upon entering the study, all subjects received a full-mouth clinical periodontal examination including the measurement of probing depth (PD) and clinical attachment loss (CAL). Dichotomous measurement of supragingival plaque accumulation and bleeding on probing were also recorded. All measurements were performed at six sites per tooth for the whole mouth.

Genomic DNA preparation

Two millitres of whole-blood samples was collected into EDTA – anticoagulated tubes by the standard venipuncture method. Genomic DNA was extracted from whole-blood samples using a commercially available genomic DNA purification kit (Nucleospin Blood, Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. DNA concentration was determined by the PicoGreen dsDNA quantitation kit (Molecular Probes Inc., Eugene, OR, USA) according to the manufacturer's instructions and diluted as 100 ng/μl.

Genotyping of the TPA gene

Alu-repeat (I/D polymorphism in intron 8 of the *TPA* gene was genotyped by a polymerase chain reaction (PCR) (van den Eijnden-Schrauwen et al. 1995). Synthetic oligonucleotides obtained from TIB MOLBIOL Syntheselabor (Berlin, Germany) were used. Primer sequences were for upstream (5′-TCCGT AACAGGACAGCTCA-3′) and downstream (5′-ACCGTGGCTTCAGTCAT GGA-3′), respectively.

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-walled PCR strip tubes (Axygen Scientific Inc., Union City, 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), 2.5 mmol $MgCl_2$, 50 μmol/l each of the dGTP, dATp, dTTP and dCTP (Promega, Madison, WI, USA), 5 pmol each of forward and reverse primers and 1.0 U AmpliTaq Gold polymerase (PE Applied Biosystems). The cycling conditions comprised a hot start at 95°C for 10 min., followed by 35 amplification cycles at 95°C for 45 s, 58°C for 60 s, and 72°C for 60 s, and a final extension at 72°C for 7 min. Amplified products (I allele = 967 bp, D allele = 655 bp) were resolved on 1.5% agarose gels and visualized by ethidium bromide staining. Samples were classified according to three genotypes: I/I, I/D, and D/D.

Genotyping of the PAI-1 gene

4G/5G polymorphism, which is located in the promoter region 675 bp upstream from the transcriptional start site of the *PAI-1* gene was genotyped by PCR and endonuclease digestion (Ferrari et al. 2002). Amplification was carried out on a GeneAmp PCR System 9700 (PE Applied Biosystems) in a 25 μl reaction mixture in 0.2 ml thin-walled PCR strip tubes (Axygen Scientific) containing 100 ng genomic DNA solution, GeneAmp Gold Buffer (15 mmol/l Tris-HCl, pH 8.0, 50 mmol/l KCl; PE Applied Biosystems), 1.5 mmol $MgCl_2$, 50 μmol/l each of the dGTP, dATP, dTTP, and dCTP (Promega), 5 pmol each of forward and reverse primers, and 0.5 U AmpliTaq Gold polymerase (PE Applied Biosystems). The sequences of the forward and reverse primers were used; 5′-CCAACAGAGG ACTCTTGGTCT-3′ and 5′-CACAGAG AGAGTCTGGCCACGT-3′, respectively. The cycling conditions comprised a denaturation step at 95°C for 10 min., followed by 35 amplification cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 7 min. The 100 bp PCR products for *PAI-1* gene were analysed on a 2.0% agarose gel prestained with ethidium bromide. Genotyping was performed with using the *Bsl*I (MBI Fermentas, Vilnius, Lithuania) restriction enzyme as manufactured. Briefly, 10 μl of PCR product was mixed with 5 U *Bsl*I and appropriately buffered and incubated at 55°C during a 4-h period. The fragments, a single one of 99 bp for the 4G allele and two fragments of 77 and 22 bp, respectively, for the 5G allele, were separated on a 3% metaphor agarose gel (FMC BioProducts, BioConcept, Allschwill, Switzerland) stained with ethidium bromide, and visualized under ultraviolet light.

Statistical analysis

The minimal required sample size was estimated to detect a 15% difference between allele frequencies of study groups with a significance level of 0.05 and 80% power by using PAWE version 1.2, a freely available genetic power calculator (Gordon et al. 2002). χ^2 analysis was used to test for deviation of genotype frequencies from Hardy–Weinberg equilibrium. The distribution of *TPA* I/D and *PAI-1* 4G/5G genotypes and allele frequencies in G-AgP and periodontally healthy groups were also analysed by a χ^2 test. Allele frequencies were calculated from the observed numbers of genotypes. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were also assessed. Differences in clinical parameters between G-AgP patients with D and 4G-positive genotype and patients with D and 4G-negative genotype 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 sample size calculation revealed minimum 69 and 118 subjects for allelic test in the G-AgP and healthy groups, respectively, and 86 and 148 subjects for the genotypic test in the G-AgP and healthy groups, respectively. The clinical parameters of the patient groups are summarized in Table 1. The frequencies of *TPA* and *PAI-1* genotypes in the healthy group were found to be in accordance with those expected by the Hardy–Weinberg equilibrium ($p>0.05$, $\chi^2<5.99$).

Distribution of *TPA* genotype, allele frequency, and carriage of rare allele

The distribution of *TPA* I/D gene polymorphism among the study groups is presented in Table 2. There was no significant difference among the groups in the distribution of *TPA* I/D genotypes ($\chi^2 = 3.443$, $p = 0.179$). Among the G-AgP subjects, 33.4% were homozygous for the Alu deletion allele (DD), 44.4% were heterozygous carriers of the Alu insertion (ID), and 22.2% were homozygous for the I allele (II). The D allele was found in 55.6% of the G-AgP patients as compared with 46.1% in the healthy group. There was a significant difference among the study groups in D allele

frequencies ($\chi^2 = 4.060$, $p = 0.044$). The frequency of D allele carriage of the *TPA* gene was similar between G-AgP and healthy subjects ($\chi^2 = 3.220$, $p = 0.073$, OR: 1.768, 95% CI: 0.945–3.308; Table 2).

In a subgroup analysis when the smokers were excluded, identical genotype distribution was observed among non-smoker study groups and no significant difference was found between non-smoker G-AgP and non-smoker healthy subjects for overall allele frequencies ($\chi^2 = 3.408$, $p = 0.182$, $\chi^2 = 3.421$, $p = 0.064$, respectively; Table 2).

Distribution of *PAI-1* genotype, allele frequency, and carriage of rare allele

The distribution of *PAI-1* 4G/5G gene polymorphism among the study groups is presented in Table 3. There was no significant difference among the groups in the distribution of *PAI-1* 4G/5G genotypes ($\chi^2 = 1.573$, $p = 0.455$). The 4G allele was found in 50.6% of the G-AgP patients as compared with 56.8% in the healthy group. There was no significant difference among the study groups in allele frequencies ($\chi^2 = 1.797$, $p = 0.180$). The frequency of 4G allele carriage of the *PAI-1* gene was similar between G-AgP and healthy subjects ($\chi^2 = 1.218$, $p = 0.270$, OR: 0.710, 95% CI: 0.387–1.306; Table 3).

In a subgroup analysis when the smokers were excluded, there were no significant differences in the distribution *PAI-1* 4G/5G genotypes and allele frequencies between non-smoker G-AgP and non-smoker healthy subjects ($\chi^2 = 0.617$, $p = 0.735$, $\chi^2 = 0.619$, $p = 0.431$, respectively; Table 3).

Carriage of rare allele in relation to susceptibility to periodontitis

In order to investigate whether carriage of rare allele is associated with clinical

Table 1. Characteristics of the generalized aggressive periodontitis (G-AgP) and healthy groups

	All subjects		Non-smokers	
	G-AgP	Healthy	G-AgP	Healthy
No. of subjects	90	154	61	147
Mean age (years ± SD)	29.71 ± 5.7	37.79 ± 9.9	29.0 ± 6.2	37.82 ± 10.1
Age range	16–39	17–70	16–39	17–70
Male/female	37/53	61/93	27/34	56/91

Table 2. Genotype distributions, allele frequencies, and D allele carriage of *TPA* in generalized aggressive periodontitis (G-AgP) and healthy groups

	All subjects				Non-smokers			
	G-AgP ($n = 90$), *n* (%)	Healthy ($n = 154$), *n* (%)	*p* (χ^2 test)	OR (95% CI)	G-AgP ($n = 61$), *n* (%)	Healthy ($n = 147$), *n* (%)	*p* (χ^2 test)	OR (95% CI)
Genotype								
II	20 (22.2)	52 (33.6)			12 (18.9)	49 (33.3)	$p = 0.182$	
ID	40 (44.4)	62 (40.4)	$p = 0.179$		29 (48.3)	59 (40.4)		
DD	30 (33.4)	40 (26.0)			20 (32.8)	39 (26.3)		
Carriage of D allele	63 (77.8)	97 (66.4)	$p = 0.073$	1.768 (0.945–3.308)	44 (80.0)	94 (66.7)	$p = 0.066$	2.000 (0.947–4.224)
Allele frequency								
I	80 (44.4)	166 (53.9)			53 (43.4)	157 (53.4)		
D	100 (55.6)	142 (46.1)	$p = 0.044$	1.461 (1.010–2.115)	69 (49.5)	137 (46.6)	$p = 0.064$	1.492 (0.975–2.282)

TPA, tissue plasminogen activator; OR, odds ratio; CI, confidence interval; I, insertion; D, deletion.

Table 3. Genotype distributions, allele frequencies, and 4G allele carriage of *PAI-1* in generalized aggressive periodontitis (G-AgP) and healthy groups

	All subjects				Non-smokers			
	G-AgP ($n = 90$), *n* (%)	Healthy ($n = 154$), *n* (%)	*p* (χ^2 test)	OR (95% CI)	G-AgP ($n = 61$), *n* (%)	Healthy ($n = 147$), *n* (%)	*p* (χ^2 test)	OR (95% CI)
Genotype								
5G/5G	25 (27.9)	33 (21.6)			16 (25.4)	30 (20.5)		
4G/5G	39 (43.0)	67 (43.1)	$p = 0.455$		25 (42.4)	65 (43.8)	$p = 0.735$	
4G/4G	26 (29.1)	54 (35.3)			20 (32.2)	52 (35.7)		
Carriage of D allele	62 (72.1)	120 (78.4)	$p = 0.270$	0.710 (0.387–1.306)	44 (74.6)	116 (79.5)	$p = 0.445$	0.759 (0.373–1.544)
Allele frequency								
4G	91 (50.6)	175 (56.8)			65 (53.3)	169 (57.5)		
5G	89 (49.4)	133 (43.2)	$p = 0.180$	0.777 (0.537–1.124)	57 (46.7)	125 (42.5)	$p = 0.431$	0.843 (0.552–1.289)

OR, odds ratio; CI, confidence interval.

parameters, differences in clinical parameters between G-AgP patients with D-positive (DD and ID) and 4G-positive (4G/4G and 4G/5G) genotype and patients with D-negative (II) and 4G-negative (5G/5G) genotype were compared by the non-parametric Mann–Whitney *U*-test. No significant differences were found in clinical parameters between G-AgP patients with D- and 4G-positive genotype and patients with D- and 4G-negative genotypes ($p>0.05$). When only non-smoker G-AgP patients were taken into consideration, clinical periodontal parameters were found to be similar between G-AgP patients with an allele-positive genotype and an allele-negative genotype ($p>0.05$) (Table 4).

Table 4. Clinical parameters (mean ± SD) of generalized aggressive periodontitis (G-AgP) patients distributed by D-positive/negative and 4G-positive/negative genotype

	All G-AgP subjects ($n=90$)			Non-smoker G-AgP subjects ($n=62$)		
	DD, ID ($n=5$)	II ($n=85$)	p^*	DD, ID ($n=5$)	II ($n=57$)	p^*
Age	26.67 ± 5.9	28.56 ± 5.1	0.297	28.80 ± 6.3	27.82 ± 5.4	0.454
PD (mm)	4.03 ± 0.9	3.85 ± 0.9	0.457	3.75 ± 0.9	3.63 ± 0.9	0.563
CAL (mm)	4.68 ± 1.4	4.53 ± 1.3	0.691	4.20 ± 1.0	4.16 ± 1.2	0.825
BOP (%)	73.90 ± 18.6	68.54 ± 23.7	0.363	72.46 ± 20.1	63.45 ± 26.8	0.293
Plaque (%)	83.05 ± 20.6	75.05 ± 23.8	0.164	79.75 ± 23.04	67.84 ± 25.9	0.101
	4G/4G, 4G/5G ($n=7$)	5G ($n=83$)	p^{**}	4G/4G, 4G/5G ($n=5$)	5G ($n=57$)	p^{**}
Age	29.77 ± 5.7	28.96 ± 5.9	0.429	29.11 ± 6.1	27.60 ± 6.4	0.374
PD (mm)	3.98 ± 0.9	4.0 ± 1.1	0.981	3.78 ± 0.8	3.63 ± 0.9	0.595
CAL (mm)	4.67 ± 1.4	4.64 ± 1.4	0.874	4.29 ± 1.1	4.08 ± 0.9	0.583
BOP (%)	71.87 ± 19.9	74.73 ± 19.8	0.528	71.20 ± 21.0	72.04 ± 21.9	0.896
Plaque (%)	82.67 ± 21.4	79.07 ± 20.5	0.303	79.27 ± 23.4	76.23 ± 24.3	0.759

*No significant difference between D-positive and D-negative genotypes (Mann–Whitney test, $p>0.05$).

**No significant difference between 4G-positive and 4G-negative genotypes (Mann–Whitney test, $p>0.05$).

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

Multiple logistic regression model

Multiple logistic regression analysis was used to evaluate the association of the D- and 4G-positive genotype with periodontal disease susceptibility, while adjusting for significant confounders. In this model, D allele carriage, subject age, and smoking were found to be significant confounders ($p=0.025$, $p<0.0001$ and $p<0.0001$, respectively). On the other hand, gender and 4G allele carriage were not statistically significant ($p=0.851$ and $p=0.643$, respectively) (Table 5).

Table 5. Logistic regression analysis for susceptibility to generalized aggressive periodontitis

	OR	95% CI	*p*-value
Age	0.871	0.831–0.913	0.000
Gender	0.851	0.587–2.313	0.851
Smoking	13.588	4.654–39.670	0.000
D allele carriage	2.443	1.120–5.328	0.025
4G allele carriage	0.827	0.371–1.846	0.643

OR, odds ratio; CI, confidence interval.

Discussion

It has been suggested that periodontitis could exhibit similarities in causative genetic factors to cardiovascular diseases. The relationship between coronary heart disease and periodontal disease may be dependent on the risk factors that both diseases have in common (Kweider et al. 1993, Beck et al. 1998). The *TPA* gene polymorphism has been investigated previously in patients with cardiovascular diseases (van der Bom et al. 1997, Hooper et al. 2000, Bang et al. 2001). On the other hand, there is no information about *TPA* gene polymorphism in connection with periodontal disease. Our results indicate that the distribution of *TPA* genotype in G-AgP patients was similar to those of the periodontally healthy group. On the other hand, the D allele frequency of G-AgP patients (55.6%) was significantly higher compared with those of periodontally healthy subjects (46.1%). Hooper et al. (2000) have found the D allele to be 63% in patients with venous thromboembolism, and 56% in controls. They suggested that the *TPA* I/D polymorphism is significantly associated with venous thromboembolism in African Americans. Another case–control study from the Netherlands found homozygosity for the I allele of the *TPA* gene polymorphism to be associated with a two-fold increase in risk for myocardial infarction (van der Bom et al. 1997). Other studies from different populations failed to detect an association between *TPA* I/D allele frequencies or genotype distributions and cardiovascular disease (Ridker et al. 1997, Steeds et al. 1998, Wang et al. 2002). We were not able to compare our study with any other study because there was no previous study investigating *TPA* gene polymorphism in periodontal disease.

In the present study, G-AgP and healthy subjects had a similar 4G/5G *PAI-1* genotype. The 4G allele carriage rate of G-AgP patients was 72.1%, while healthy subjects had a carriage rate of 78.4%. The 4G allele frequency found in our healthy group was very similar to those reported previously in Turkish subjects and in other populations (Westendorp et al. 1999, Akar et al. 2000, Bang et al. 2001, Balta et al. 2002, Holla et al. 2002, Kim et al. 2003). On the other hand, Hooper et al. (2000) found these allele frequencies to be lower (25%) in African Americans compared with Caucasians.

Several studies have shown a significant increase in both the frequency and carriage rate of 4G allele in a variety of chronic inflammatory and infectious diseases (Akar et al. 2000, Bang et al. 2001, Holla et al. 2002, Kim et al. 2003). Westendorp et al. (1999) found that carriers of the 4G allele may produce increased amounts of PAI-1 in response to *N. meningitidis* infection, and patients whose relatives were 4G/4G genotype carriers had an increased risk of developing septic shock rather

than meningitis. There was a significant association between the 4G/5G genotype of *PAI-1* and atherothrombotic stroke (Bang et al. 2001). Others have shown a link between either myocardial infarction or venous thromboembolism and the 4G/5G genotype of *PAI-1* (Eriksson et al. 1995, Ossei-Gerning et al. 1997, Fu et al. 2001). Hooper et al. (2000) failed to find any association between the 4G/4G *PAI-1* genotype and either myocardial infarction or venous thromboembolism. Holla et al. (2002) investigated the role of the *PAI-1* gene polymorphism in chronic periodontitis in relation to smoking status. They found a strong association between the 4G allele and chronic periodontitis especially in non-smokers and suggested the 4G allele to be one of the risk factors for periodontitis. In the present study, although we found similar genotype distribution and allele frequency in the *PAI-1* gene in G-AgP and healthy subjects, our patient group showed a trend for lower 4G allele frequency (50.6%) compared with our healthy subjects (56.8%), which could suggest differences in polymorphic alleles in different populations.

Different factors could influence increased susceptibility to G-AgP, which is a multifactorial periodontal disease (Tonetti & Mombelli 1999). Smoking is known to be a well-established confounding factor for periodontitis (Papapanou 1996). Therefore, in the present study, *TPA* and *PAI-1* gene polymorphisms were evaluated in non-smoker subjects as well. There was no association between non-smoker G-AgP patients and *TPA* and *PAI-1* gene polymorphisms. In the present study, logistic regression analysis was performed to evaluate the effects of genotype on an increased severity to G-AgP while adjusting confounding factors such as smoking, age, and gender. In this model, there was a significant association between the D-positive genotype of *TPA* and the presence of G-AgP. Smoking and patient age was also found to be significant confounders to the increased susceptibility to G-AgP. As indicated, D allele carriers seem to be approximately three-fold more likely to develop G-AgP in the presence of smoking.

As a result, we could suggest that the D allele of the *TPA* gene could be associated with susceptibility to aggressive periodontitis. On the other hand, due to the complexity of the disease, this does not necessarily show that the investigated allele is causatively related to periodontal disease. In other words, it is not possible to set up a cause and effect relationship between a specific allele and the disease. This may be in linkage disequilibrium with other, functionally relevant alleles within the *TPA* gene or, the polymorphic allele may be in close proximity to the susceptibility gene associated with the disease rather than being causative (Shapira et al. 2005, Takashiba & Naruishi 2006). Also, population substructure and, consequently, spurious differences in allele frequencies between cases and controls were not excluded by the study. To the best of our knowledge, this is the first report investigating the association between *TPA* and *PAI-1* gene polymorphisms and G-AgP in a Turkish population. Further studies are needed to present the role of *TPA* and *PAI-1* gene polymorphisms in periodontal disease in different populations. Thus, the analysis of gene polymorphisms in a sample of Turkish individuals represents important evidence for the study of periodontal disease in Turkey.

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Address:
Gülnur Emingil
Department of Periodontology
School of Dentistry
Ege University
Bornova-35100, İzmir Turkey
E-mail: gemingil@yahoo.com

Clinical Relevance

Scientific rationale for study: *TPA* and *PAI-1* gene polymorphisms have been linked with cardiovascular diseases that are possibly associated with periodontitis. Therefore, this study was undertaken to evaluate whether periodontal diseases share a common susceptibility gene with systemic diseases.

Principal findings: D allele positivity was higher in G-AgP patients compared to healthy subjects.

Practical implications: The D polymorphic allele of the *TPA* gene seems to be associated with periodontitis. This might indicate that periodontal and systemic diseases that are possibly associated with periodontitis have also an intersection point on the genetic background.