Association between RAS gene polymorphisms (ACE I/D, AGT M235T) and Henoch-Schönlein purpura in a Turkish population

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Abstract. Henoch-Schönlein purpura (HSP) is a small-vessel vasculitis of autoimmune hypersensitivity, and renin-angiotensin system (RAS) regulates vascular homeostasis and inflammation with activation of cytokine release. Thus, we aimed to investigate the association between HSP and *ACE I/D* and *AGT M235T* polymorphisms. Genotyping was determined by allele specific PCR and PCR-RFLP. We obtained a significant difference in genotype distribution ($p = 0.003$) and allele frequencies ($p < 0.001$) of *ACE I/D* polymorphism between patients and controls, while no significant association was detected in genotype distribution ($p > 0.05$) and allele frequencies ($p > 0.05$) of the *AGT M235T* polymorphism. Risk assessment showed significant risk for HSP in the subjects both with the ID + DD genotype ($p = 0.019$, OR: 2.288, 95% CI: 1.136–4.609) and D allele (OR: D vs. I: 2.0528, 95% CI: 1.3632–3.0912, $p = 0.001$) while no significant risk was obtained for HSP in the subjects both with the MT + TT genotype ($p = 0.312$, OR: 1.3905, 95% CI: 0.7326–2.6391) and T allele (OR: T vs. M: 1.065, 95% CI: 0.729–1.557, $p = 0.743$). Furthermore, when patients were stratified by the presence of certain systemic complications of HSP, no significant association was detected with *ACE I/D*, and *AGT M235T* polymorphisms. Our findings suggest that *ACE I/D* polymorphism is significantly associated with HSP susceptibility.

Keywords: Henoch-Schönlein purpura (HSP), *ACE I/D*, *AGT M235T*, Single Nucleotide Polymorphism (SNP), organ involvements, Genotype-phenotype correlation

1. Introduction

Henoch-Schönlein purpura (HSP) is a rare, non-thrombocytopenic, leukocytoclastic, IgA mediated small-vessel vasculitis of autoimmune hypersensitivity [1]. HSP typically involves small vessels, and main-

ly affects skin, joints, gastrointestinal (GI) tract, and kidneys some of which progress to renal insufficiency especially in the adulthood. Rare complications of HSP include central and peripheral nervous system and pulmonary complications [2–4]. Immune and inflammatory response activation is a common feature of systemic vasculitis, and HSP is the result of a complex series of inflammatory and immunologic processes. Previously, important roles of pro-inflammatory cytokines and the renin-angiotensin system (RAS) components were shown on HSP pathogenesis [5,6]. RAS

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is a multifactorial hormonal system and major mediator in the regulation of sodium homeostasis, blood pressure, body fluid balance, and inflammation [7,8]. Cloned and identified genes encoding RAS include renin [8], *angiotensin converting enzyme gene-ACE* [9], *angiotensinogen gene-AGT* [10], *angiotensin I, angiotensin II, angiotensin II type 1 (AT1R) and type 2 (AT2R) receptor gene* [11]. *ACE* gene is chromosomally located at 17q23.3 and comprises 26 exons spanning 26 kb of genomic DNA [12], and is mostly expressed in vascular endothelium, lung, kidney, heart and testes [13]. *AGT* gene is located on chromosome 1q42.2 comprising 5 exons, and express pre-angiotensinogen or angiotensinogen precursor protein in the liver. *AGT* is cleaved by renin and inactive angiotensin I is produced. *ACE* catalyzes the conversion of angiotensin I into a physiologically active major effector peptide molecule angiotensin II, and inactivation of bradykinin [14]. Further, angiotensin II shows its physiological function by binding to *AT1R* and *AT2R* [8].

Functional effects of RAS gene polymorphisms on transcription and circulating enzyme levels have been mostly identified in the pathogenesis of cardiovascular and renal diseases, later, effects of this system components were associated with susceptibility or severity of autoimmune inflammatory conditions [15, 20]. So far, functionally relevant polymorphisms of this family have included 287 bp Alu repetitive sequence insertion/deletion (*I/D*) polymorphism in intron 16 of *ACE* [16], *T174M* and *M235T* polymorphisms of *AGT* [10], and *A1166C* polymorphism of *AGT1R* gene [11]. Effects of *ACE I/D* polymorphism were investigated in ischemic heart disease [17], SLE [18], malignant vascular injury [19], IgA nephropathy [21] and renal injury [22] in different ethnicities. Several SNPs (*A-5466C*, *T-3892C*, *A-240T*, *CJ237T*, *G2215A* and *A2350G*) of the *ACE* gene were also previously related to the risk of certain autoimmune diseases such as essential hypertension [23], left ventricular hypertrophy [24], IgA nephropathy [25], and diabetic nephropathy [26]. *DD* genotype of *ACE I/D* polymorphism was previously associated with higher tissue and plasma *ACE* levels and increased *ACE* activity in the vessel wall. Furthermore, *T* allele of the *AGT M235T* polymorphism was associated with a higher plasma angiotensinogen level [27]. Higher *ACE* levels lead to an increased conversion of angiotensin I to angiotensin II resulting in vascular inflammation including monocyte adhesion, activation with cytokine release, T-lymphocyte metabolism, and vascular impairment involving neointimal proliferation, vasospasm, platelet

activation, myocyte proliferation. Notably, inflammatory cells in particular monocytes/macrophages/T lymphocytes present at inflammatory sites produce high levels of all components of RAS, and vascular RAS regulates vascular homeostasis and inflammation with activation of cytokine release [28–30]. Furthermore, role of angiotensin II has been shown in renal tubular and mesangial cell proliferation in renal disease [31]. Therefore, RAS gene polymorphisms seem to be particularly biologically and clinically relevant to HSP susceptibility which occur due to vasculitis of the small blood vessels, and endothelial cell activation [32]. In this study, we examined the association between HSP susceptibility in Turkish patients and the biologically important *ACE I/D* and *AGT M235T* polymorphisms of these key components of RAS.

2. Subjects and methods

Present case-control study comprised 139 clinically diagnosed HSP (77 males/62 females; ages range from 2 to 17 years; female/male ratio was 1.2:1) patients at the department of Pediatric Nephrology in Ege University Faculty of Medicine. The ethnically matched healthy control individuals (ages range from 18 to 45 years; with no renal, vasculitic, or allergic diseases and no vasculitis/rheumatologic and other kidney disease history in family) included 72 healthy individuals for the *ACE* gene mutation analysis while fifteen more healthy individuals whose blood samples were later obtained were added up to this group ($n = 87$) for the *AGT* gene mutation analysis. Control group was chosen from adults because of the lower risk of HSP occurrence older than 20 years old. Criteria defined by the American Rheumatology College (ACR) were used for diagnosis and classification of HSP [33]. The procedures were in accordance with the ethical standard for human experimentations established by the Declaration of Helsinki of 1975, revised 1983. The study was approved by the Ethic Committee of Ege University, and detailed consent forms were signed by patients/parents.

2.1. Genomic DNA isolation

Genomic DNA was isolated from peripheral blood leukocytes using a QIAamp DNA Blood Isolation kit (Qiagen GmbH, Hilden, Germany). A Thermo Scientific NanoDrop spectrophotometer (Wilmington, USA) was used to determine the extracted DNA concentration. The quality assessment of the extracted DNA was determined by 2% agarose gel electrophoresis.

Fig. 1. Agarose gel electrophoresis image of *ACE* I/D polymorphism. Fig. 2. Agarose gel electrophoresis image of *AGT* M235T polymorphism.

2.2. Genotyping of *ACE* I/D and *AGT* M235T polymorphisms

The *ACE* (NM_000789.3/NP_000780.1) gene I/D polymorphism was determined by allele specific Polymerase Chain Reaction (PCR) assay [34]. The 25 μ l PCR reaction mixture contained: one microliter (100 ng) of genomic DNA was added to amplification buffer containing 20 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each of dATP, 2'-deoxycytidine 5'-triphosphate, dGTP, 2'-deoxythymidine 5'-triphosphate, 10 pmol of each primer, and 1.0 U of platinum Taq DNA polymerase (Invitrogen, Paisley, UK). The DNA was amplified by cycling at 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min (GeneAmp 9700). After 35 cycles, the reaction was extended for an additional 10 min at 72°C. The oligonucleotide sequences of the primers were as follows;

F 5'-CTGGAGAGCCACTCCCATCCTTTCT-3'
R 5'-GACGTGGCCATCACATTCGTCAGAT-3'.

The PCR products were separated by 2% agarose gel electrophoresis, and 490 bp with insertion (I al-

lele) and 190 bp with deletion (D allele) were visualized with ethidium bromide staining in the UVP BioDoc-It System Bioluminescence Imaging Systems (Fig. 1) (Upland, Calif., USA).

The *AGT* gene (NM_000029.3/NP_000020.1) M235T polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The 25 μ l PCR reaction mixture contained: one microliter (100 ng) of genomic DNA was added to amplification buffer containing 20 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each of dATP, 2'-deoxycytidine 5'-triphosphate, dGTP, 2'-deoxythymidine 5'-triphosphate, 10 pmol of each primer, and 1.0 U of platinum Taq DNA polymerase (Invitrogen, Paisley, UK). The DNA was amplified by cycling at 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min (GeneAmp 9700). After 35 cycles, the reaction was extended for an additional 10 min at 72°C. The oligonucleotide sequences of the primers were as follows;

F 5'-CCGTTTGTGCAGGGCCTGGCTCTCT-3'
R 5'-CAGGGTGCTGTCCACACTGGACCCC-3'.

Table 1
Demographic characteristics and clinical manifestations of a series of patients with HSP

	n (%)
Children ages range from 2 to 17 years	139 (100)
Male/Female	77/62
Age at disease onset, months: mean $\pm$ SD	8.6 $\pm$ 3.1/ ranges between 18–45
Duration of follow-up mean $\pm$ SD	8.2 $\pm$ 7.5 months
Cutaneous involvement; palpable purpura and/or maculopapular rash	139 (100)
Joint involvement; arthralgia and/or arthritis	79 (56.8)
Gastrointestinal manifestations	64 (43.8)
Hypertension	6 (4.3)
Central nervous system (CNS) involvement	1 (0.7)
Renal manifestations	64 (46)
Proteinuria (present/absent) (mean $\pm$ SD)	29/110 (49.70 $\pm$ 4.21) ranges between 0.9–127 mg/m ² /h
Proteinuria (non-nephrotic/nephrotic)	13/16
Hematuria (present/absent)	59 (20 macroscopic; 39 microscopic)/80
Nephrotic syndrome	5 (12%)
Renal insufficiency	3 (4%)

Table 2
The allele and genotype frequencies of the *ACE* I/D Polymorphism both in patients and healthy controls

ACE I/D Polymorphism	Clinically diagnosed HSP patients group (n:139)	Healthy control group (n:72)	χ^2	p value	OR (95% CI)
Genotypes					
II	20 (14.4%)	20 (27.8%)	11.313	0.003	ID+DD vs. II
ID	58 (41.7%)	36 (50.0%)			OR: 2.288
DD	61 (43.9%)	16 (22.2%)			95% CI: 1.136–4.609
Alleles (2N)					
I	98 (35.3%)	76 (52.8%)	12.025	0.001	D vs. I
D	180 (64.7%)	68 (47.2%)			OR: 2.0528,
					95% CI:1.3632 to 3.0912

The PCR product was digested with restriction enzyme Tth111 I (New England Biolabs, UK) to identify the M > T polymorphism at 37°C for 16 h. Digested DNA fragment products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The presence of uncut 165 bp fragment band indicated MM, 141 (cleaved) + 24 bp fragment band indicated TT, and 165 + 141 + 24 fragment band indicated MT heterozygous genotype (Fig. 2).

2.3. Statistical analysis

All statistical analyses were performed with the SPSS 13.0 statistical program. Hardy-Weinberg equilibrium was assessed by chi-square goodness-of-fit test. Comparison of the genotypic and allelic frequencies between the groups was performed using the Fisher exact test or Chi-square test when appropriate. Logistic regression analysis was used to identify odds ratios (OR) and 95% confidence intervals (CI) to determine whether association exists between genotypes and the risk of developing HSP in patients as compared to the control samples. A two tailed p value < 0.05 was considered as statistically significant.

3. Results

The present study group included 139 clinically diagnosed HSP (77 males/62 females; ages range from 2 to 17 years; female/male ratio was 1.2:1) patients and ethnically matched healthy control individuals with no renal, vasculitic, or allergic diseases and no vasculitis/rheumatologic and other kidney disease history in family included 72 (49 females/23 males; ages range from 18 to 45 years) for *ACE* gene, and 87 (57 females/30 males; ages range from 18 to 45 years) for *AGT* gene. The demographic and clinical characteristics of the subjects have been summarised in Table 1. Mean disease onset of the patients who had been on follow-up for at least 6 months was 8.6 $\pm$ 3.1 years (Table 1). Patients had been followed up for a mean of 8.2 $\pm$ 7.5 months and for at least 6 months for kidney involvement. Skin, joint, GIS, and renal involvements and presence of hematuria, proteinuria, hypertension, and central nervous system at the time of diagnosis and follow-up were shown in Table 1. End stage renal disease was not determined in any patients, hence, hematuria, proteinuria, renal function disorder, and factors effecting those were investigated at follow-up. No

Table 3

The distribution of the genotype and allele frequencies of *ACE I/D* Polymorphism in association with main clinical features of 139 children with HSP

ACE I/D Polymorphism	Renal n (%)		Joint n (%)		GIS n (%)		Controls (n:72)
	Absence	Presence	Absence	Presence	Absence	Presence	
Genotypes							
II	6 (30%)	14 (70%)	8 (40%)	12 (60%)	6 (30%)	14 (70%)	20 (27.8%)
ID	35 (60.3%)	23 (39.7%)	26 (44.8%)	32 (55.2%)	35 (60.3%)	23 (39.7%)	36 (50%)
DD	34 (55.7%)	27 (44.3%)	26 (42.6%)	35 (57.4%)	34 (55.7%)	27 (44.3%)	16 (22.2%)
χ^2	5.651		0.154		5.651		
p value	0.059		0.926		0.059		
Alleles (2N)							
I	47 (48%)	51 (52%)	42 (41.6%)	59 (58.4%)	47 (47.5%)	52 (52.5%)	76 (52.8%)
D	103 (57.2%)	77 (42.8%)	78 (43.3%)	102 (56.7%)	103 (57.5%)	76 (42.5%)	68 (47.2%)
χ^2	2.192		0.081		2.600		
p value	0.139		0.776		0.107		
OR (95% CI) I vs D	0.689 (0.420–1.129)		0.931 (0.568–1.525)		0.667 (0.407–1.092)		

Table 4

The allele and genotype frequencies of the *AGT M/T* polymorphism both in patients and healthy controls

AGT M/T Polymorphism	Clinically diagnosed HSP patients group (n:139)	Healthy control group (n: 87)	χ^2	p value	OR (95% CI)
Genotype					
MM	37 (26.6%)	18 (20.7%)	4.891	0.087	MT/TT vs. MM OR: 0.7192, 95% CI: 0.3789–1.365
MT	59 (42.4%)	50 (57.5%)			
TT	43 (30.9%)	19 (21.8%)			
Allele					
M	133 (47.8%)	86 (49.4%)	0.107	0.743	T vs. M: OR: 1.065, 95% CI: 0.729–1.557
T	145 (52.2%)	88 (50.6%)			

relation between gender and organ involvements was observed in our patient group ($p > 0.05$).

Genotype analysis of the *ACE I/D* polymorphism did not reveal a significant deviation from Hardy-Weinberg equilibrium in any group. The allele and genotype frequencies of the *ACE I/D* polymorphism both in patients and healthy controls were reported in Table 2. Three genotypes (II, ID, and DD) of *ACE I/D* polymorphism were found in the patients and controls. Distribution of *ACE I/D* polymorphism genotypes and alleles in patients and controls were as follows; in the patients group, 14.4% of them were genotype II, 41.7% were genotype ID, and 43.9% were genotype DD whereas in the healthy control group, 27.8% of the individuals were genotype II, 50% were genotype ID, and 22.2% were genotype DD. I allele frequency was 35.3 and 52.8% in the patients group and the healthy control group, respectively, whereas D allele frequency was 64.7 and 47.2% in the patients and the healthy control group, respectively (Table 2).

The overall distribution of the *ACE I/D* polymorphism genotypes and alleles differed significantly between the patients group and healthy controls ($p = 0.003$, and $p = 0.001$, respectively). This was reflected in increases in the frequency of the D allele, and the

homozygote DD genotype in patients group (64.7%, and 43.9%).

Patients with ID+DD genotypes had a higher risk of developing HSP compared to patients with II genotype (ID+DD vs. II; χ^2 : 5.535, $p = 0.019$, OR: 2.288, 95% CI: 1.136–4.609).

Furthermore, the DD genotype was associated with a 2.7-fold increased risk for HSP compared to patients with II+ID genotype (χ^2 : 9.604, $p = 0.002$, OR = 2.737, 95% CI = 1.431–5.237).

The frequency of the D allele in patients group (64.7%) was significantly higher than in healthy controls (47.2%). Risk assessment showed that individuals possessing an *ACE* D allele had an increased risk of HSP (odds ratio: D vs. I: 2.0528, 95% CI: 1.3632 to 3.0912, $p = 0.001$). The presence of D allele carries a 2.05-fold increased risk for HSP relative to the I allele (95% CI: 1.3632 to 3.0912, $p = 0.001$).

In order to evaluate the exact effect of *ACE I/D* polymorphism on various complications of the disease, we performed genotype-phenotype correlations, but no significant association was found between the absence and presence of different organ involvements and the *ACE I/D* polymorphism. No statistically significant differences between HSP patients and HSP nephritis,

Table 5

The distribution of the genotype and allele frequencies of *AGT M/T* polymorphism in association with main clinical features of 139 children with HSP

	Renal n (%)		Joint n (%)		GIS n (%)		Controls (n:87)
	Absence	Presence	Absence	Presence	Absence	Presence	
Genotypes							
MM	15 (40.5%)	22 (59.5%)	13 (35.1%)	24 (64.9%)	18 (48.6%)	19 (51.4%)	18 (20.7%)
MT	35 (59.3%)	24 (40.7%)	28 (47.5%)	31 (52.5%)	34 (57.6%)	25 (42.4%)	50 (57.5%)
TT	25 (58.1%)	18 (41.9%)	19 (44.2%)	24 (55.8%)	23 (53.5%)	20 (46.5%)	19 (21.8%)
χ^2	3.667		1.434		0.743		
p value	0.160		0.488		0.690		
Alleles (2N)							
M	65 (48.9%)	68 (51.1%)	54 (40.6%)	79 (59.4%)	70 (52.6%)	63 (47.4%)	86 (49.4%)
T	85 (58.6%)	60 (41.4%)	66 (45.5%)	79 (54.5%)	80 (55.2%)	65 (44.8%)	88 (50.6%)
χ^2	2.654		0.683		0.180		
p value	0.103		0.408		0.671		
OR (95% CI) M vs T	0.675 (0.420–1.084)		0.818 (0.508–1.317)		0.903 (0.563–1.448)		

with or without renal, gastrointestinal manifestations, and arthritis, hypertension or CNS were observed ($p > 0.05$) (Table 3). No significant correlation was detected between *ACE I/D* polymorphism and epidemiological (age and sex) features ($p > 0.05$). Presence of D allele did not show a risk factor for clinical characteristics ($p > 0.05$). The distribution of the genotype and allele frequencies of *ACE I/D* polymorphism in association with clinical characteristics of the HSP patients were given in Table 3.

Genotype analysis of the *AGT M/T* polymorphism did not reveal a significant deviation from Hardy-Weinberg equilibrium in any group. The allele and genotype frequencies of the *AGT M/T* polymorphism both in patients and healthy controls were reported in Table 2. Three genotypes (MM, MT, and TT) of *AGT M/T* polymorphism were found in the patients and controls. Distribution of *AGT M/T* polymorphism genotypes and alleles in patients and controls were as follows; in the patients group, 26.6% of them were genotype MM, 42.4% were genotype MT, and 30.9% were genotype TT whereas in the healthy control group, 20.7% of the individuals were genotype MM, 57.5% were genotype MT, and 21.8% were genotype TT. M allele frequency was 47.8% and 49.4% in the patients group and the healthy control group, respectively, whereas T allele frequency was 52.2% and 50.6% in the patients and the healthy control group, respectively (Table 4).

The overall distribution of the *AGT M/T* polymorphism genotypes and alleles did not differ significantly between the patients group and healthy controls ($p = 0.087$, and $p = 0.743$, respectively). The frequencies of homozygote TT genotype (30.9%) and T allele (52.2%) were higher in patients than in controls, however the result was insignificant. Patients with MT/TT genotypes

did not have a higher risk of developing HSP compared to patients with MM genotype (χ^2 : 1.022, $p = 0.312$, OR: 0.7192, 95% CI: 0.3789–1.365). Furthermore, patients with TT genotype (χ^2 : 2.224, $p = 0.136$, OR: 0.6238, 95% CI: 0.3346 to 1.163) and T allele (odds ratio: T vs. M: 1.065, 95% CI: 0.729 to 1.557, $p = 0.743$) did not have a higher risk of developing HSP compared to patients with MM+MT genotype.

In order to evaluate the exact effect of polymorphism on various complications of the disease, we performed genotype-phenotype correlations, but no significant association was found between the absence and presence of different organ involvements and the *AGT M/T* polymorphism. No statistically significant differences between HSP patients and HSP nephritis, with or without renal, gastrointestinal manifestations, and arthritis, hypertension or CNS were observed ($p > 0.05$). No significant correlation was detected between *AGT M/T* polymorphism and epidemiological (age and sex) features ($p > 0.05$). Presence of T allele did not show a risk factor for clinical characteristics ($p > 0.05$). The distribution of the genotype and allele frequencies of *AGT M/T* polymorphism in association with clinical characteristics of the HSP patients were given in Table 4.

4. Discussion

Present study investigated the genetic association between HSP and RAS polymorphisms involving I/D polymorphism of the *ACE* gene, and *M235T* polymorphism of the *AGT* gene. HSP patients who were homozygous for the deletion allele (DD) presented a significantly higher prevalence of HSP compared to patients homozygous for the insertion allele (II) or het-

erozygous (ID). Conversely, no association was found between HSP and the *M235T* polymorphism of the *AGT* gene. The association between DD genotype/D allele and HSP was consistent as shown by a high odds ratio, but the genotype-phenotype correlation analysis showed no significant association between *ACE I/D*, *AGT M235T*, and main clinical manifestations of HSP.

To date, a number of gene loci have been reported to contribute to the susceptibility and pathogenesis of HSP, such as the human leukocyte antigen (*HLA*) complex. *HLA* Class III region genotype C4B was associated with HSP in Caucasians [35], Korean [36], and Japanese [37] patients. Also, *HLA* Class II genes were indicated as genetic risk factors for HSP nephritis in Japanese patients (genotype DQA1*0301) [37], and in Italian patients (DRB1*01 or DRB1*11) [38]. *HLA-DRB1* 11/14 in Turkish children [39] and *HLA-DRB1**13 genotype in Turkish patients in another study of the same group were related to the susceptibility of HSP [40]. *HLA-DRB1**01 [41] and *HLA-B*35 [42] were associated to susceptibility of HSP in patients from Northwest Spain. *HLA* class I alleles -*HLA* A2, A11, and B35- were considered as risk factors for developing HSP [43]. Also, *HLA-A**11(*1101) and -*B**15(*1501) were associated with susceptibility to HSP in Mongolian children and *HLA-A**26(*2601), *HLA-B**35(*3503) and *HLA-B**52 were associated with susceptibility to HSP in Han children [44]. Role of chemotactic inflammatory markers such as *IL-1RA*, *IL-6*, *IL-8*, *IL-1β* [45–49], *eNOS* which is the major mediator in the regulation of vascular homeostasis, and *MIF* [50–52] were also previously demonstrated in HSP and relevant vascular disorders. As it has been previously stated, HSP is known to occur via vascular injuries by inflammatory reaction and immunologic activation. Therefore, we hypothesized that vascular development of HSP might have been related to the D and T alleles of the respective RAS genes. Accordingly, we obtained a relationship between *ACE* and HSP, however genetic association between *AGT M235T* and HSP pathogenesis was not detected. Moreover, possession of the polymorphic alleles of either *ACE I/D* or *AGT M235T* polymorphisms did not seem to have an impact on the development of gastric, renal, and articular manifestations of the disease, meaning that absence or presence of various organ involvement were found unrelated to any genotypes and/or alleles in the analysed polymorphisms. In contrast, Zhou et al. [53] and Liu et al. [54] significantly associated RAS gene polymorphisms (*ACE-I/D* and *AGT M235T*) with susceptibility to HSP, HSPN, organ involvement, and disease

severity, although conflicting data exist [55,56]. Similarly, Ozkaya et al. evidenced the relationship between *AGT M235T* and HSPN [57]. Different from Ozkaya et al.'s study conducted on Turkish patients, current work involved the association of genotypes and alleles with other clinical manifestations except for the renal involvement. However, even though there was a trend toward higher DD genotype frequency in patients with renal involvement across the healthy controls (44.3 vs. 22.2%), results were not statistically significant ($p > 0.05$). This was also the case when comparing patients with gastric (44.3 vs. 22.2%) and articular (57.4 vs. 22.2%) involvements across the healthy controls ($p > 0.05$). The same was evident for *AGT M235T* polymorphism which was found unrelated to the HSP susceptibility due to insignificant difference in the allele frequencies and genotype distributions among cases and controls. The lack of association between *AGT M235T* and HSP may be due to a loss of power which was 0.01, whereas it was 0.95 for the *ACE I/D* polymorphism that already showed significant association. Regarding the current and the recent [57] polymorphism studies conducted on Turkish patients, the discrepancy in the association of *AGT M235T* with HSP may be due to relatively small number of patients enrolled in the current study in terms of genetic epidemiology studies. Moreover, patients enrolled in the current study were mostly from Western region of Turkey while Ozkaya et al examined patients mostly from Northern region of Turkey. In case-control polymorphism studies, distribution of polymorphic alleles may have been affected by racial and regional factors, population heterogeneity due to selection of subjects from different regions, and possible unidentified polymorphisms in RAS which contribute to inter-individual differences in susceptibility to disease.

In previous reports, D allele was associated with increased *ACE* activity in the vessel wall leading to vascular inflammation [27–31]. The presence of D allele was found to carry a 2.05-fold increased risk for HSP relative to the I allele (95% CI: 1.3632 to 3.0912, $p = 0.001$) whereas there was not a significant risk for HSP in the subjects both with the TT genotype ($p = 0.087$, MT/TT vs. MM, OR:0.7192, 95% CI: 0.3789–1.365), and the T allele ($p = 0.743$, T vs. M, OR: 1.065, 95% CI: 0.729–1.557). Thus, this may be attributed to the dysregulation of vascular homeostasis and inflammation in “D” allele carriers leading to vasculitis of the small blood vessels, and endothelial cell activation, however further studies are required to estimate circulating *ACE* enzyme levels in patients with HSP.

In the present study, we also compared genotype frequencies of the healthy population with the previously published data on other populations. The frequency of the *ACE I/D* DD genotype (22.2%) in our control Turkish population was lower than that reported in German (26.8%) and Italian (42%) populations [55,58], but higher than Taiwan Chinese population (18%) [59]. Our data was in striking contrast to the results of previously reported on Italian population [55], but similar to the results of other studies on Chinese [59], German [58] and South Indian [60] populations. Also, previously reported DD genotype frequencies of Turkish population by different study groups were 11.6% [57], 30% [61], 58% [62], and 60% [63] all of which were different from that reported in the current study (22.2%). Regarding *AGT M/T* polymorphism, the frequency of TT genotype (21.8%) in our control Turkish population was lower than that reported in South Indian (44.8%) population [60], but higher than German population (4.6%) [58]. Other studies conducted on Turkish population identified TT genotype frequency as 48% [63], and 7.9% [57] which were in striking contrast to the results reported on the current Turkish population (21.8%). The inconsistency in the distribution of *ACE I/D* and *AGT M/T* polymorphism genotypes between these studies may be due to various sampling sizes and ethnic differences or different ancestries in different regions of the same country populations.

In conclusion, our findings suggest that *AGT M235T* polymorphism does not seem to contribute to the susceptibility and/or certain systemic manifestations of HSP, while *ACE I/D* polymorphism confers a significantly increased risk for susceptibility to HSP in the present Turkish population. Nonetheless, due to ethnic variations among restricted populations, polymorphisms in the respective genes have been frequently associated inconsistently with vascular pathologies, and association studies between RAS and HSP/HSPN have been limited and conflicting, thus underlying genetic pathways of HSP still remain elusive. Search for possible candidate genes and different pathogenic mechanisms in the development of HSP in patients with various genetic backgrounds are required to fully eliminate the potential role of these polymorphisms in the pathogenesis of HSP.

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