ASSOCIATION BETWEEN ACE GENE INSERTION (I) / DELETION (D) POLYMORPHISM AND PRIMARY HYPERTENSION IN TURKISH PATIENTS OF TRAKYA REGION

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ABSTRACT
Hypertension is immensely common in Turkish subjects in Trakya region. The renin-angiotensin system (RAS) helps maintain blood pressure and salt homeostasis and appears important in the pathogenesis of hypertension. Angiotensin I-converting enzyme (ACE) is a key component of RAS. Insertion/Deletion (I/D) polymorphism of the ACE gene has been implicated in the pathogenesis of cardiovascular diseases. In addition to this, the association between ACE I/D polymorphism and hypertension is controversial, when numerous studies have addressed the role of ACE I/D polymorphism in the development of hypertension, there were different studies showed that no correlation has been found between ACE I/D polymorphism and in the development of hypertension. The objective of our study was to investigate the relation between the ACE gene I/D polymorphism and primary hypertension in Turkish subjects in Trakya region. We analyzed the ACE gene I/D polymorphism in 79 patients with primary hypertension as a primary hypertensive group and 38 age matched healthy individuals as a control group by using a polymerase chain reaction assay, and agarose gel electrophoresis system. The genotype distributions were not different between the patients and normal control groups in the men. But the frequency of ACE Deletion/Deletion (DD) genotype in patients with primary hypertension (35.5%) was significantly higher than in controls (21.4%) in the women. This result suggested that ACE DD genotype may be associated with primary hypertension in the women, not in the men, and showed the possibility of ACE DD genotype as a potent risk factor for primary hypertension for the women not for the men.

Introduction
Primary hypertension is of unknown etiology; its diverse hemodynamic and pathophysiologic derangements are unlikely to result from a single cause (22, 8, 2). Heredity is a predisposing factor (11), but the exact mechanism is unclear. Environmental factors (eg. high salt intake, obesity, stress) seem to act only in genetically susceptible persons (5). The renin-angiotensin system (RAS) may be the most important of the endocrine systems that affect the control of blood pressure (10, 17, 15). This system has been implicated in the pathological changes of organ damage through modulation of gene expression, proliferation, fibrosis, and inflammatory response (19, 9, 18). Angiotensin I-converting enzyme (ACE) is a component of RAS. ACE is a key enzyme in the generation of angiotensin (AT)-II (a potent vasoconstrictor and aldosterone-stimulating peptide) from AT-I (a vasoinactive decapeptide). The ACE gene consists of 26 exons and 25 introns and spans...
21 kb on chromosome 17q23 (7, 13). The polymorphism consists of the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence resulting in 3 genotypes (DD and II homozygote, and ID heterozygote) (21). An I/D (region in intron 16) polymorphism of the ACE gene correlates with circulating ACE plasma activity (21); higher plasma ACE activity is observed in subjects with ACE-D (16). An increase in plasma ACE activity may increase blood pressure through increased production of angiotensin II. Studies have demonstrated that ACE insertion (I) / deletion (D) polymorphism is not only associated with diabetes (4, 6), coronary heart disease (23), and diabetic nephropathy (24), but have also demonstrated that ACE I/D polymorphism is associated with hypertension (3, 12). In primary hypertension no early pathologic changes occur. Ultimately, generalized arteriolar sclerosis develops; it’s particularly apparent in the kidney (nephrosclerosis) and is characterized by medial hypertrophy and hyalinization. Nephrosclerosis is the hallmark of primary hypertension, left ventricular hypertrophy and, eventually, dilation develops gradually. This study was designed to investigate whether ACE I/D polymorphism is associated with primary hypertension in Turkish subjects in Trakya region.

Materials and Methods

Materials
All reagents for PCR Amplification and Gel Electrophoresis were purchased from Fermentas Life Sciences (ELIPS), Istanbul, Turkey. All other chemicals were bought from Sigma or Merck, Darmstadt, Germany, and were of the highest purity available.

Methods

Patients
Approval for the study was obtained from the Ethics Committee of Trakya University School of Medicine.

The study included 117 Turkish individuals from Trakya region. Untreated 79 patients with mild to moderate primary hypertension (48 males and 31 females); mean age 43.52±8.06 years, and 38 age-matched healthy individuals as a control group (24 males and 14 females); mean age 38.40±6.53 years. Patients who were found to have renal disease, secondary hypertension or already on anti-hypertensive treatment were excluded from the study.

Blood Pressure

Blood pressure was measured in the morning follow 12 hours fasting by using manual sphygmomanometer after resting for 5 minutes in seated position. Presence of hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg and/or diastolic blood pressure (DBP) ≥90 mm Hg.

DNA Extraction

DNA was extracted from whole blood containing ethylenediamine-tetraacetic acid (EDTA) as an anticoagulant by a standard salting-out procedure, followed by phenol/chloroform extraction and resuspended in a TE (10 mmol/L Tris / 1 mmol/L EDTA pH 7.6) buffer (1). DNA purity and quantity were assessed by absorbance values in spectrophotometer and checked by 0.5% agarose gel electrophoresis.

Determination of genotypes

Amplification of Genomic DNA by Polymerase Chain reaction (PCR)

To determine the ACE genotype of the patients and control groups, a genomic DNA fragments on intron 16 of the ACE gene was amplified by PCR in a 50 μl PCR reaction mixture containing 200 ng of DNA, deoxynucleotide triphosphates (0.2 mM of each), upstream and downstream oligonucleotide primers (20 pmol), 75 mM Tris-HCl (Ph 8.8), 20 mM (NH₄)₂SO₄, 0.01% Tween 20, and 2.5 U of Taq DNA polymerase (Fermentas Life Sciences). The PCR primers with the sequences reported by Rigat B et al. were used (20): ACE-1 24-Mer MBI (5’-CTGGAGACCACTCCC-ATCCTTCT-3’) and ACE-2 25-Mer MBI
Ethidium bromide-stained gel of representative PCR products of ACE gene I/D polymorphism shows the D allele (190 bp, lane 1, 2, 5), the I allele (490 bp, lane 6), and the ID genotype (190 bp, and 490 bp, lane 3, 4); lane 7 is a size marker (GeneRuler 50bp DNA Ladder-Fermentas Life Sciences).

(5’-GATGTGGCC-ATCACATTGGTCA-GAT-3’). The reaction contained 3 mM MgCl₂. In all PCR experiments several reactions containing no DNA were included to control the possibility of contamination. DNA amplification were performed with a Techne (TechGene) DNA Thermal Cycler with 5 min of denaturation at 94°C, followed by 30 cycles with denaturation for 1 min at 94°C, annealing for 1 min at 58°C, and extension for 2 min at 72°C. Followed by 5 min of extension at 72°C. To increase the specificity of DD genotyping, PCR amplifications were performed with an insertion of specific primer pair (5’-TTTGAGACGGA-GTCTCGCTC-3’ and 5’-GATGTGGCCATCACATTGGTCAGAT-3’ mixed in 25 μl reaction (200ng genomic DNA, 100 pmol of primers; 0.2 mM each of deoxynucleotide triphosphates and 2.5 mM of magnesium chloride; 1.25 U of Taq DNA polymerase (Fermentas Life Sciences); 75 mM Tris-HCl (Ph 8.8), 20 mM (NH₄)₂SO₄, 0.01% Tween 20). With 4 min of denaturation at 94°C, followed by 32 cycles of 30 s at 94°C, 30 s at 62°C (annealing), and 1 min at 74°C (extension). Only the (I) allele produces a 335 bp amplicon.

Detection of ACE Polymorphism by Electrophoresis

The reaction products were electrophorized on 2% agarose gels and stained with ethidium bromide. Under ultraviolet light two bands, insertion (I; 490bp) and deletion (D; 190 bp) were visible (Figure). The 335 bp fragment was identified on 3% agarose gels and stained with ethidium bromide. The reaction yields no product in the sample of DD genotype.

Statistical Analysis

Chi square tests according to pearson were performed to compare the frequency of ACE genotype between the groups. A P value <0.05 was considered significant.

Results and Discussion

At baseline the two groups were similar with regard to sex, age, and body mass index (Table 1). Primary hypertension patients had higher systolic and diastolic blood pressures.

Group 1, normal controls; group 2, patients group. Data are expressed as means ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2 shows the ACE genotype distributions of normal controls and patients with primary hypertension.

Several studies have shown a high prevalence of the DD genotype among patients with primary hypertension (6). On the other hand, researches have shown no differences in the allele frequencies and genotype distributions of ACE gene polymorphisms between the control and hypertension group (14). In our study, the overall frequencies of the genotypes II, ID, and DD were 31.9, 36.2, and 31.9, respectively in men, and 17.8, 51.1, and 31.1 respectively in women. The individual allele frequencies for I and D were 50.0% for each in men, while in women it was 43.3, 56.7
TABLE 1
Clinical characteristics of the control and primary hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=38)</th>
<th>Group 2 (n=80)</th>
</tr>
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<tbody>
<tr>
<td>Sex (M:F)</td>
<td>24:14</td>
<td>48:31</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.40±6.53</td>
<td>43.52±8.06</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.81±3.59</td>
<td>28.78±3.37</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117.11±9.98</td>
<td>155.59±11.31</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74.63±7.00</td>
<td>98.25±6.47</td>
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</table>

TABLE 2
Distributions of ACE genotype and allele frequencies in the controls and primary hypertension patients subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal control (n=38)</th>
<th>Primary hypertensive (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
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<tr>
<td>II</td>
<td>12 (31.6)</td>
<td>19 (24.1)</td>
</tr>
<tr>
<td>ID</td>
<td>15 (39.5)</td>
<td>34 (43.0)</td>
</tr>
<tr>
<td>DD</td>
<td>11 (28.9)</td>
<td>26 (32.9)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>39 (51.3)</td>
<td>72 (45.6)</td>
</tr>
<tr>
<td>D</td>
<td>37 (48.7)</td>
<td>86 (54.4)</td>
</tr>
</tbody>
</table>

Percentages are shown in parentheses.

respectively, and 47.4%, 52.6% respectively in the entire sample. The observed genotype frequencies are in agreement with frequencies predicted by Hardy-Weinberg equilibrium.

The frequencies of the genotypes II, DI, and DD were 29.2, 39.6 and 31.2 respectively in male patient group, whereas the frequencies of the genotypes II, DI, and DD were 37.5, 29.2 and 33.3 respectively in male control group. Statistical analyses have shown no statistically significant difference between the frequencies of the genotype of the two groups.

The frequencies of the genotypes II, DI, and DD were 16.1, 48.4 and 35.5 respectively in female patient group, whereas the frequencies of the genotypes II, DI, and DD were 21.4, 57.2 and 21.4 respectively in female control group. Analyses of this data have shown that the frequencies of the DD genotype in female patient group were statistically significantly higher than that in female control group.

This study have verified a relationship between the ACE DD genotype and the development of primary hypertension in female population of Trakya, but this relationship was not reflected to be true for the male population.

REFERENCES