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Angiotensin converting enzyme I/D, angiotensinogen T174M-M235T and angiotensin II type 1 receptor A1166C gene polymorphisms in Turkish hypertensive patients

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Abbreviations: ACE, Angiotensin converting enzyme; AGN, Angiotensinogen; ATR1, Angiotensin II type 1 receptor; BMI, Body mass index; DBP, Diastolic blood pressure; PCR, Polymerase Chain Reaction; RAS, Renin Angiotensin System; RFLP, Restriction Fragment Length Polymorphism; SBP, Systolic blood pressure

Abstract

Essential hypertension is a multifactorial disease in which genetic and enviromental factors play an important role. These factors differ in each population. As there are no existing data for the Turkish population, we investigated four Renin Angiotensin System (RAS) gene polymorphisms, the angiotensin converting enzyme (ACE), angiotensinogen (AGN) M235T/T174M and angiotensin II type 1 receptor A1166C polymorphism in 109 hypertensive and 86 normotensive Turkish subjects. Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP), and agarose gel electrophoresis tecniques were used to determine these polymorphism. The frequencies of person that carry ACE D allel (DD+ID) was significantly higher in hypertensive group (99.1%) than controls (80%) ($P < 0.000$). M235T TT genotype was also found significantly higher in hypertensives than control group (20% vs 2.7%; $P < 0.001$). The frequency of AGN 174M allele was higher in the hypertensive group than control subjects (8.76% vs 4.81%). Frequency of ATR1 C allele (AC+CC genotypes) was found higher hypertensives than controls (39.4% vs 25.9%; $P = 0.054$). Our results suggest that an interaction exists between the RAS genes and hypertension in Turkish population.

Keywords: A1166C; Angiotensin converting enzyme; Angiotensinogen; hypertension M325T; polymorphism; T174M

Introduction

Essential hypertension is a multifactorial and polygenic disorder, and several genes are thought to play a role in its pathogenesis. The renin-angiotensin system (RAS) is one of the important factors that regulates the blood pressure, as well as fluid and electrolyte balance, and may have an important role in the pathogenesis of hypertension (Malik *et al.*, 1997).

The angiotensin converting enzyme (ACE) I/D polymorphism, identified in 1990 by Rigat *et al.*, is partially associated with the plasma ACE level. Although the ACE DD genotype increases the plasma ACE concentration and the risk for numerous cardiovascular-renal diseased states, such as myocardial infarction (Cambien *et al.*, 1992), cardiomyopathy (Marian *et al.*, 1993), IgA nephropathy (Harden *et al.*, 1995), and diabetic nephropathy (Marre *et al.*, 1994), the findings from case-control studies have not been consistently positive.

Several molecular variants of the angiotensinogen (AGN) gene have been detected. A polymorphism in exon 2, in which methionine at position 235 is replaced by threonine (M235T variant) has been associated with essential hypertension in both whites (Jeunemaitre *et al.*, 1992) and Japanese (Hata *et al.*, 1994, Nishiuma *et al.*, 1995). However, other studies have failed to show an association of the M235T variant with essential hypertension in whites at British (Caulfield *et al.*, 1994), Australian (Bennett *et al.*, 1993) and Japanese (Morise *et al.*, 1995) population. Another polymorphism in exon 2, in which threonine at position 174 is replaced by methionine (T174M variant), has also been associated with essential hypertension in whites (Jeunemaitre *et al.*, 1992) and Japanese (Morise *et al.*, 1995). However, Caulfield *et al.* (Caulfield *et al.*, 1994) failed to detect an associ-

ation of the T174M variant with essential hypertension in whites in the British population. Thus the association between angiotensinogen gene polymorphisms and essential hypertension remains controversial.

Of the two subtypes of angiotensin II receptors (ATR1 and ATR2), ATR1 appears to mediate the physiologic effects of angiotensin II important for blood pressure regulation, and thus polymorphism in this gene has the potential to affect blood pressure regulation. As in studies of other renin-angiotensin system genes, association studies of ATR1 polymorphism present a mixed picture, including significant associations (Bonnardeaux *et al.*, 1994; Wang *et al.*, 1997; Kainulainen *et al.*, 1999), negative findings (Schmidt *et al.*, 1997), and inconclusive results (Szombathy *et al.*, 1998; Tiret *et al.*, 1998).

Interethnic differences in vascular disease demography indicate the need to examine the relationship between three RAS gene polymorphisms and hypertension in Turkey. Investigation of this important ethnic group may help to establish a genetic basis to account for the observed ethnic differences in the incidence and pathogenesis of vascular disease. We aimed to investigate the relation between polymorphism of RAS genes and hypertension in Turkish population.

Materials and Methods

Subjects

The hypertensive group consisted of 109 subjects from the hypertension outpatient clinic of Marmara University Hospital. Hypertension was defined as systolic blood pressure (SBP) $>$ 140 mmHg or diastolic blood pressure (DBP) $>$ 90 mmHg or those currently receiving one or more antihypertensive drugs. Secondary forms of hypertension were excluded by clinical and laboratory examination. The 86 normotensive controls had negative family history off hypertension and SBP $<$ 140 mmHg and DBP $<$ 90 mmHg on three occasions spanning two months. Demographic and blood chemistry data were obtained from questionaries and laboratory examinations.

Genotyping protocols

Genomic DNA was extracted from peripheral blood leukocytes usind salting out method as described by Miller *et al.* (Miller *et al.*, 1988). Polymerase chain reaction based protocols were used to identify the ACE gene I/D (Rigat *et al.*, 1992; Lindpaintner *et al.*, 1995), AGN M235T (Russ *et al.*, 1993), AGN T174M (Caulfield *et al.*, 1994) and ATR1 A1166C (Bonnardeaux *et al.*, 1994) polymorphisms as described previously.

Statistical analysis using SPSS Version 10.0 included the χ^2 test for genotype and allele frequencies comparison. Clinical laboratory data are expressed as means ± SD. Mean values were compared between patients with hypertensives and control subjects by the unpaired Student's t test. A level of $P < 0.05$ was considered statistically significant.

Results

The clinical and biochemical parameters of the controls and hypertensive subjects are shown in Table 1. Between the two groups, a statistically significant difference was observed in the BMI, BP level and LDL-Cholesterol.

Table 2 show the distribution of the genotype and allele frequencies of ACE, AGN and ATR1 gene polymorphisms of controls and hypertensive subjects among males and females. Hovewer, pooling ACE DD and ID individidual and compared II genotypic group, the frequencies of person that carry ACE D allel (DD+ID) was significantly higher in hypertensives groups (99.1%) than controls (80%) (χ^2:20.66; P = 0.000). When the sexes were examined separately, this difference was higly significant in women (P = 0.010, Fisher exact test). AGN M235T TT genotypes was found significantly higher in hypertensive than control groups (20% vs 2.7%; (χ^2:11.52, P=0.001), in male subjects this ratio was 22.6% in hypertensive and 2.3% in controls (P = 0.007, Fisher exact test);

Table 1. Clinical and biochemical charecteristics of the controls and hypertensive subjects.

	Control n = 86	Hypertensives n = 109
Female/Male	35/51	77/32
Age (year)	51.64±11.46	51.73±9.72
BMI (kg/m^2)	25.52±3.56	27.65±4.45**
SBP (mmHg)	123.95±14.20	156.46±23.52**
DBP (mmHg)	74.94±11.61	98.61±13.37**
Total Cholesterol (mg/dl)	186.08±36.44	198.48±52.15
Triglyceride (mg/dl)	145.90±50.36	148.15±70.92
HDL-Cholesterol (mg/dl)	40.57±12.40	38.85±12.71
LDL-Cholesterol (mg/dl)	114.81±34.30	129.77±49.50*
VLDL-Cholesterol (mg/dl)	29.83±12.11	30.45±15.17

BMI, Body mass index *: $P<0.05$; **: $P<0.001$

Table 2. Genotype and allele prevalences of polymorphism of the renin-angiotensin system among normotensive and hypertensive subjects.

	Male		Female		Total	
ACE genotypes	Controls	Hypertensives	Controls	Hypertensives	Controls	Hypertensives
DD	18 (35.3%)	13 (40.6%)	18 (52.9%)	36 (46.8%)	36 (42.4%)	49 (45.0%)
II	12 (23.5%)	-	5 (14.7%)	1 (1.3%)	17 (20.0%)	1 (0.9%)
ID	21 (41.2%)	19 (59.4%)	11 (32.4%)	40 (51.9%)	32 (37.6%)	59 (54.1%)
ACE alleles						
D	57 (55.8%)	45 (70.31%)	47 (69.11%)	112 (72.72%)	104 (61.17%)	157 (72.01%)
I	45 (44.11%)	19 (29.68%)	21 (30.88%)	42 (27.27%)	66 (38.82%)	61 (27.98%)
AGNM235T genotypes						
MM	12 (27.3%)	8 (25.8%)	11 (36.7%)	24 (34.8%)	23 (31.1%)	32 (32.0%)
TT	1 (2.3%)	7 (22.6%)	1 (3.3%)	13 (18.8%)	2 (2.7%)	20 (20.0%)
MT	31 (70.5%)	16 (51.6%)	18 (60.0%)	32 (46.4%)	49 (66.2%)	48 (48.0%)
M235T alleles						
M	55 (31.25%)	32 (51.61%)	40 (66.66%)	80 (57.97%)	95 (64.1%)	112 (56.0%)
T	33 (18.75%)	30 (48.38%)	20 (33.33%)	58 (42.02%)	51 (34.45%)	88 (44.0%)
AGNT174M genotypes						
TT	45 (90.0%)	22 (78.6%)	30 (90.9%)	58 (84.1%)	75 (90.4%)	80 (82.5%)
MM	-	-	-	-	-	-
TM	5 (10.0%)	6 (21.4%)	3 (9.1%)	11 (15.9%)	8 (9.6%)	17 (17.5%)
T174M alleles						
T	95 (95.0%)	50 (89.28%)	63 (95.45%)	127 (92.02%)	158 (95.18%)	177 (91.23%)
M	5 (5.0%)	6 (10.71%)	3 (4.54%)	11 (7.97%)	8 (4.81%)	17 (8.76%)
ATR1 genotypes						
AA	37 (77.1%)	18 (60.0%)	23 (69.7%)	45 (60.8%)	60 (74.1%)	63 (60.6%)
CC	1 (2.1%)	1 (3.3%)	-	5 (6.8%)	1 (1.2%)	6 (5.8%)
AC	10 (20.8%)	11 (36.7%)	10 (30.3%)	24 (32.4%)	20 (24.7%)	35 (33.7%)
ATR1 alleles						
A	84 (87.5%)	47 (78.33 %)	56 (84.84%)	114 (77.02%)	140 (86.4%)	161 (77.40%)
C	12 (12.5%)	13 (21.66 %)	10 (15.15%)	34 (22.97%)	22 (13.58%)	47 (22.59%)

Values in parantheses are percentages, comparison was performed by χ^2 test

in female subjects 18.8% vs 3.3% ($P = 0.035$, Fisher exact test) The frequency of AGN 174 M allele was slightly increased in hypertensive patients as compared to the control subjects. The estimated relative risk of hypertension with carrier M allele as compared with control subjects were 1.992 (OR: 1.992, 95% CI 0.812-4.883). Frequency of ATR1 C allele carriers (AC+CC genotypes) was found higher hypertensives than controls (39.4% vs 25.9%)($P = 0.054$). In males this ratio was 40% in hypertensives and 22.9% in controls (OR:2.242; 95% CI: 0.831-6.055) and in women (39.2%) versus control (30.3%) (OR 1.482; 95% CI: 0.617-3.563).

Discussion

In this study, we aimed to investigate the association between ACE (I/D) and angiotensinogene (T174M/M-235T) and ATR1 A1166C gene polymorphisms in Turkish hypertensive patients.

The frequency of the ACE deletion (D) allele has been found to be higher in hypertensive patients than control subjects (χ^2: 20.66, $P = 0.000$). When we compared ACE allele among the controls in the present study and with that in populations, reported by Abbud (Abbud *et al*., 1998), Bedir (Bedir *et al*., 1999) and O'Donnel (O'Donnell *et al*., 1998), it was found to be

similar.

Evidences of genetic linkage between the angiotensinogen gene and essential hypertension has been described; both M235T and T174M variants were strongly associated with high blood pressure (Jeunemaitre *et al.*, 1992). However, the frequency of the *T* allele of M235T polymorphism markedly varies among ethnic groups. The frequency of the *T* allele varies between 0.35 and 0.49 in whites (Jeunemaitre *et al.*, 1992; Caulfield *et al.*, 1994) and between 0.60 and 0.79 in Japanese (Morise *et al.*, 1995; Nishiuma *et al.*, 1995), thus the frequency in the Japanese population is 1.2 to 2.3 times that in the white population. In our study, the frequency of the *T* allele was 0.34 in the control subjects, a value similar to those reported for whites by Jeunemaitre *et al.* (Jeunemaitre *et al.*, 1992) (0.35) and Caulfield *et al.* (Caulfield *et al.*, 1994) (0.49) but significantly different than those obtained for black and Japanese populations by Rotimi *et al.* (Rotimi *et al.*, 1994) (0.95), Nishiuma *et al.* (Nishiuma *et al.*, 1995) (0.62) and Ishigami *et al.* (Ishigami *et al.*, 1994) (0.65), Hata *et al.* (Hata *et al.*, 1994) (0.75) and Morise *et al.* (Morise *et al.*, 1995) (0.79).

In this study we observed a higher frequency of AGT 174 M allele in the hypertensive group (8.76%) than in control subjects (4.81%) in Turkish population. The estimated relative risk of hypertension with carrier M allele as compared with control subjects was 1.992 with 95% CI 0.812-4.883. We found T174M genotype frequencies similar to other populations and found no homozigous T174M MM genotype like Forrester *et al.* (Forrester *et al.*, 1996), and Rotimi *et al.* (Rotimi *et al.*, 1994).

The ATR1 A1166C polymorphism is located at the 5' end of the 3' untranslated region of the gene (Bonnardeaux *et al.*, 1994). It has been shown that the frequency of the ATR1 C allele is increased in patients with severe hypertension (Bonnardeaux *et al.*, 1994). In our study, there was a relationship observed between the ATR1 A1166C polymorphism and hypertension.

In conclusion, we found association between the ACE, AGN and ATR1 gene polymorphism and hypertension in Turkish population, in agreement with recent reports performed in different white populations. Further candidate gene analysis can be expected to elucidate the genetic background of essential hypertension in future.

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