Angiotensinogen M235T polymorphism and left ventricular indices in treated hypertensive patients with normal coronary arteries

Antihipertansif tedavi alan normal koroner arterli hastalarda anjiyotensinojen M235T polimorfizminin sol ventrikül parametreleri ile ilişkisi

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ABSTRACT

Objective: Hypertension and left ventricular hypertrophy (LVH) are important causes of morbidity and mortality in the population. Angiotensinogen (AGT) M235T polymorphism has been associated with LVH, left ventricular dimensions, coronary artery disease and antihypertensive drug response in previous studies. We examined relationship between AGT M235T polymorphism and echocardiographic left ventricular indices in a Turkish population of treated hypertensive patients with normal coronary arteries.

Methods: In this cross-sectional study a Turkish population of 92 hypertensive patients treated in our outpatient clinic were enrolled. All patients had normal coronary angiographic examinations. Genotypes for AGT M235T were determined from peripheral leukocytes. Left ventricular dimensions, mass and function indices, after adjustment for clinical covariates were analyzed by multiple regression analysis according to genotypes.

Results: Genotype frequencies for AGT M235T were MM-24.7%, MT-52.8% and TT-22.5%. Left ventricular end systolic (LVES) dimensions for AGT M235T MM, MT, TT genotypes were 17.9±4.2 mm, 19.4±6.2 mm, and 16.4±2.9 mm, respectively (p=0.08). Angiotensinogen M235T TT genotype showed a trend towards a lower LVES dimension but results were not statistically significant. Left ventricular ejection fractions for AGT M235T MM, MT, TT subgroups were 61.3±15.0%, 59.4±14.0%, and 67.8±8.5%, respectively (p=0.07). Angiotensinogen M235T TT genotype showed a tendency towards lower left ventricular mass index but results were not statistically significant. None of the AGT M235T genotypes predicted left ventricular dilatation, mass or function in treated hypertensive patients with normal coronary arteries.

Conclusion: Angiotensinogen M235T polymorphism was not useful to predict left ventricular mass, function, hypertrophy or dilatation in a small population of treated Turkish hypertensive patients with normal coronary arteries. *(Anadolu Kardiyol Derg 2007; 7: 257-61)*

Key words: Angiotensinogen, genotypes, hypertension, left ventricular hypertrophy, coronary arteries

ÖZET

Amaç: Hipertansiyon ve sol ventrikül hipertrofisi (SVH) toplumda önemli bir morbidite ve mortalite sebebidir. Daha önce yapılan çalışmalarda anjiyotensinojen (AGT) M235T hipertrofisi SVH, sol ventrikül çapları, koroner arter hastalığı ve antihipertansif tedaviye yanıt ile ilişkili bulunmuştur. Çalışmamızda antihipertansif tedavi alan normal koroner arterli hastalarda AGT M235T polimorfizmi ile ekokardiyografik sol ventrikül parametreleri arasındaki ilişkiyi inceledik.

Yöntemler: Bu kros-seksiyonel çalışmaya 92 hasta dahil edildi ve periferik lökositlerden AGT M235T genotiplemesi yapıldı. Çalışmaya alınan tüm hastaların daha önce yapılan koroner anjiografileri normal idi. Sol ventrikül çapları, kitlesi ve fonksiyonu ile ilgili indeksler genotiplere göre çoklu regresyon analizleri ile incelendi.

Bulgular: Genotip dağılımı MM-%24.7, MT-%52.8 ve TT-%22.5 şeklinde idi. Sol ventrikül sistol sonu çapları AGT M235T MM, MT, TT tipleri için sırası ile 17.9±4.2 mm, 19.4±6.2 mm, 16.4±2.9 mm ve p=0.08 idi. Sol ventrikül ejeksiyon fraksiyonları AGT M235T MM, MT, TT grupları için sırası ile %61.3±15.0, %59.4±14.0, %67.8±8.5 ve p=0.07 idi. Anjiyotensin M235T TT genotipi daha düşük sol ventrikül kitle indeksi göstermeye eğilimli idi fakat sonuç istatistik anlamlı bulunmadı.

Sonuç: Tedavi edilmiş hipertansif normal koroner arterli hastalarda AGT M235T genotiplerinden hiçbiri sol ventrikül dilatasyonu, fonksiyonu veya kitlesi ile ilişkili bulunmamıştır. *(Anadolu Kardiyol Derg 2007; 7: 257-61)*

Anahtar kelimeler: Anjiyotensinojen, hipertansiyon, genotip, sol ventrikül hipertrofisi, koroner arterler

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Introduction

Hypertension is a common problem and is a major risk factor for morbidity and mortality in general population (1). Hypertension is a complex disease and is influenced by genetic and environmental factors. The cause of hypertension is not determined in 90% of patients. Genetic causes account for 20-60% of blood pressure variability in general population and many genes interact in pathogenesis (2). Molecular genetics of some rare hypertensive diseases like glucocorticoid-suppressible hyperaldosteronism (3), Liddle's syndrome (4), and apparent mineralocorticoid excess (5) have been identified. Although there are many studies published about association of single nucleotide polymorphisms (SNP) and hypertension, results are divergent. Parameters such as ethnicity, body weight, antihypertensive therapy, modulation of genes by environmental factors all contribute to divergent results.

Left ventricular hypertrophy (LVH) is a multifactorial entity and hypertension is one of the most common causes. Left ventricular hypertrophy is the most powerful independent risk factor for morbidity and mortality in hypertensive patients (6-8). Familial studies have shown that LVH in hypertension shows a genetic predisposition but exact genes responsible for hypertrophy are unknown (9-12). Left ventricular hypertrophy regresses with antihypertensive therapy and regression of LVH improves prognosis (13, 14). Recent studies have shown that antihypertensive response and LVH regression are related to different renin angiotensin system (RAS) polymorphisms (15-17).

Angiotensinogen (AGT) is a key component of RAS and Jeunetmaire et al (18) demonstrated a linkage between AGT gene and essential hypertension in two populations. A threonine to methionine substitution at position 235 (M235T) of AGT gene has been associated with higher blood pressure and higher plasma angiotensinogen levels in populations with different ethnic backgrounds (18-23). There are also studies concerning association of AGT polymorphism with coronary artery disease, myocardial infarction, and hypertrophic cardiomyopathy (24-27). A molecular variant in the proximal promoter of the AGT gene, an adenine instead of guanine, six nucleotides upstream from the site of transcription initiation, A(-6)G, has been reported. The A(-6)G is in a very tight linkage disequilibrium with M235T, the abnormal manifestations associated with AGT (M235T) polymorphism could simply reflect the modifications in the promoter activity induced by nucleotide substitution at the -6 position (28, 29).

The aim of the study was to detect whether there is an association between AGT M235T polymorphism and echocardiographic left ventricular indices in a Turkish population of treated hypertensive patients with normal coronary arteries.

Methods

Study Population

Overall, 92 hypertensive patients whose coronary angiography showed normal coronary arteries from Istanbul School of Medicine Cardiology Department were enrolled in the study. All of the patients were on one or more antihypertensive drugs. Patients had mild to moderate hypertension. Hypertension control was assessed by following cardiologist in the outpatient clinic and all patients were reported to have a well-controlled hypertension.

Patients who had rheumatic valvular disease, myocardial infarction and secondary hypertension were not enrolled into the study. Ethics committee approval and informed consent was obtained from all patients.

Anthropometric measurements and clinical parameters were determined by a questionnaire based study.

Echocardiography

All patients were studied by M-mode echocardiography to determine left ventricular size (left ventricular end diastolic diameter- LVEDD, left ventricular end systolic diameter-LVESD, interventricular septum-IVST and posterior wall thickness - PWT) by three expert sonographers with two recorders (Vingmed System V and Vivid III, General Electric). Pulsed Doppler velocimetry was used to determine peak early (E) and late atrial (A) diastolic transmitral velocities. Penn convention criteria were applied for measurement of left ventricular (LV) dimension and calculation of LV mass (LVM) (30). Left ventricular mass index (LVMI) was calculated by indexation of LVM to body surface area. Left ventricular ejection fraction (LVEF) was calculated according to the modified Simpson formula (31).

Coronary Angiography

All coronary angiographies were carried out in a single catheterization laboratory by 4 expert cardiologists. Coronary anatomy was normal in all patients.

Genotyping

Detection of AGT M235T polymorphism

Sufficient DNA for analysis was available from 89 of the patients. Samples of DNA were purified from peripheral blood leucocytes with the use of a standard protocol, 80 ng genomic DNA was subjected to 30 rounds of specific amplification of exon 2 of the angiotensinogen gene in 20 µL of a buffer that contained 50 mmol/L KCL, 5 mmol/L Tris-HCL, 0.01% gelatin, 1.5 mmol/L MgCl2, 125 µmol/L NTPs, 10 pmol 5'-CCGTTTGTGCAGGGCCTG and reverse primer 5'-TGCTGTCCACACTGGACCCC, and 0.5 U Taq polymerase at 94°C for 1 minute, 61°C for 1 minute, 72°C for 1 minute. The specific mismatches incorporated into the antisense primer create a Tth111 I site if the T235 variant is present; subsequent digestion with this enzyme at 65°C thus results in diagnostic fragments that are visualized by ethidium bromide staining and UV transillumination after electrophoresis on a horizontal submarine 3.5% agarose gel (Fig. 1).

The design of the study was cross-sectional and data were collected during a 6-month period.

Statistical Analysis

Data were analyzed using SPSS for Windows release 7.5.1 software (Chicago, Il, USA). According to M235T allele status, continuous data were compared with the use of ANOVA and classified values with Kruskal-Wallis (asymmetrical data distribution) tests, respectively. Effects of M235T allele status on LVMI, LVEDDI (LVEDD indexed to body surface area), LVESDI (LVESD indexed to body surface area), LVEF, fractional shortening (FS), diastolic E and A velocities were examined with multiple linear regression analysis after adjustment for age, gender, body mass index, atrial fibrillation (AF), diabetes mellitus (DM) and

hypertension treatment status (HTTx). Left ventricular hypertrophy was defined as a LVMI of >134g/m^2 in men or >110 g/m^2 in women. Left ventricular hypertrophy (absence or presence) was analyzed as a categorical variable by logistic regression analysis. Continuous data are summarized as mean±SD or as median. All tests were two sided, and a p value of less than 0.05 was considered to indicate statistical significance.

Results

Genotype Frequencies

Characteristics of the study population of 89 patients are shown in Table 1. Genotype frequencies for AGT M235T were MM 24.7%, MT 52.8% and TT 22.5%. There was no deviation from Hardy-Weinberg equilibrium for AGT M235T polymorphism distribution. All patients in the study received antihypertensive therapy and none of the patients used alcohol. Antihypertensive therapy depended on the cardiologist opinion following the patient. Clinicians reported that all patients' hypertension was well controlled. Duration of hypertension, age, sex, smoking status, presence of AF or presence of DM were not statistically different across AGT M235T genotypes.

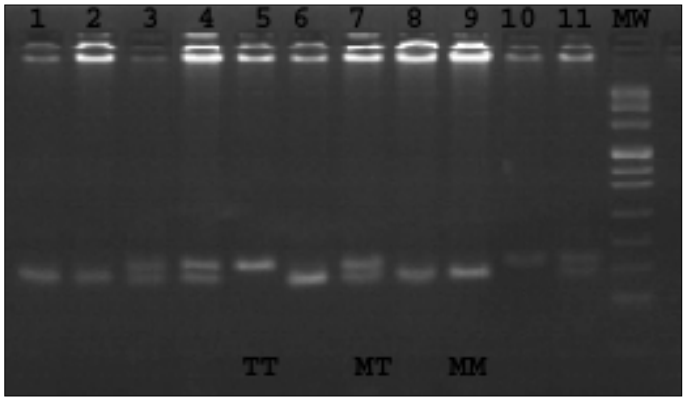

Figure 1. M235T variants of angiotensinogen gene. Lane MW contains DNA molecular weight marker. Lane TT contains band at 303 bp, lane MT contains bands at 266 bp and at 303 bp, lane MM contains band at 266 bp.

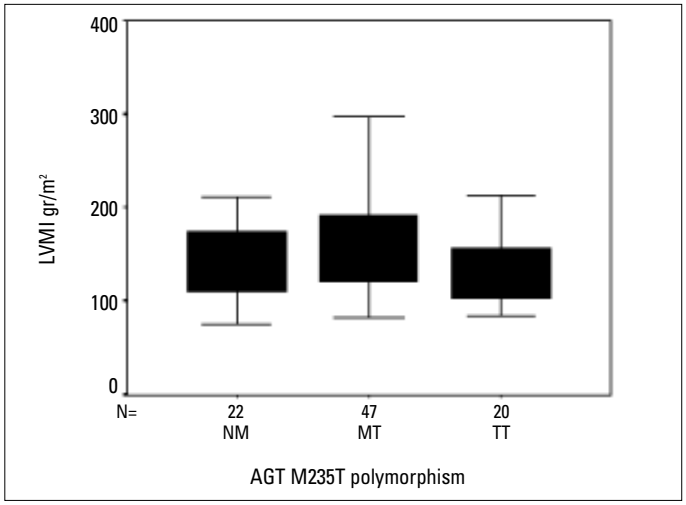

Figure 2. LVMI displayed as a function of AGT M235T genotype. LVMI did not differ significantly (p=0.09) across AGT M235T polymorphisms

AGT- angiotensinogen, LVMI- left ventricular mass index

Echocardiographic Findings

Echocardiographic left ventricular indices are listed in Table 2. Left ventricular end- systolic and end-diastolic dimensions indexed to body surface area, IVST, PWT, LVMI, transmitral peak velocities E and A, and left atrial sizes showed no statistically significant differences across AGT M235T genotypes. Left ventricular end systolic dimensions for AGT M235T MM, MT, TT 17.9±4.2 mm, 19.4±6.2 mm, and 16.4±2.9 mm, respectively (p=0.08). The AGT M235T TT genotype showed a trend towards a lower LVESD dimension but results were not statistically significant. The AGT M235T TT genotype showed a tendency towards lower LVMI but results were not statistically significant (Fig. 2). Left ventricular ejection fraction percentages for AGT M235T MM, MT, TT were 61.3±15.0%, 59.4±14.0%, and 67.8±8.5%, respectively (p=0.07), while LVEF percentages tended to be higher in M235T AGT TT genotype but results were not statistically significant. Left ventricular hypertrophy was present in 76.4% of the study

Table 1. Anthropometric and biochemical data

Parameters	Angiotensinogen polymorphism		M235T genotype	p
	MM	MT	TT	
Sex M/F	7/15	22/25	4/16	0.09
Age, years	57.4±11.4	59.3±9.7	60.9±10.2	0.56
DM, %	4.5	7.9	4.5	0.86
Smoking, %	5.6	12.4	3.4	0.73
Alcohol, %	0	0	0	1
AF, %	3.4	4.5	1.1	0.61
HT duration, years	8.2	9.3	6.3	0.23
HT Tx, %	100	100	100	1

AF- atrial fibrillation, DM- diabetes mellitus. HT age- duration of hypertension, HT Tx- treatment for hypertension

Table 2. Echocardiographic measurements and AGT M235T genotypes

Variables	Angiotensinogen polymorphism		M235T genotype	p*
	MM	MT	TT	
LVEDD, mm/m^2	26.9±3.8	28.38±5.3	26.64±4.4	0.29
LVESD, mm/m^2	17.9±4.2	19.4±6.2	16.4±2.9	0.08
IVST, mm	12.2±1.4	12.4±1.8	12.3±2	0.89
PWT, mm	10.7±1.2	11±1.7	10.9±1.6	0.82
LV EF, %	61.3±15	59.4±14	67.8±8.5	0.07
LV FS, %	33.4±9.8	32.3±10	37.9±6.6	0.08
LVMI, g/m^2	142.4±38.6	162.5±60.6	136.1±35.1	0.09
LVH, %	77.3	78.7	70	0.73
E, cm/s	62.9±12.9	71.4±26.6	71.2±24.4	0.42
A, cm/s	74.9±18.3	80.4±26.6	90.9±22.3	0.14
LA size, mm	36.6±6.2	38.7±5.6	38.8±7.9	0.40

Values are expressed as mean ± SD and percentages
*significance by ANOVA, Kruskal-Wallis and Chi-square tests
A- late diastolic transmitral peak velocity, AGT- angiotensinogen, E- early diastolic transmitral peak velocity, IVST- interventricular septal thickness, LA- left atrium size, LVEDD- left ventricular end-diastolic dimension, LVEF- left ventricular ejection fraction, LVESD- left ventricular end-systolic dimension, LVFS- left ventricular fractional shortening, LVH- left ventricular hypertrophy, LVMI- left ventricular mass index, PWT- posterior wall thickness

population. Left ventricular hypertrophy percentages for AGT M235T MM, MT, TT were 77.3%, 78.7% and 70%, respectively (p=0.73).

Multivariate analysis after adjustment for age, sex, presence of AF, presence of DM, smoking, alcohol use or duration of hypertension showed no relation between left ventricular size, Doppler indices and AGT M235T genotype status. In logistic regression analysis, incidence of LVH was not different across AGT M235T genotypes.

Discussion

We found no association between AGT M235T polymorphism and LVMI or LVH in treated hypertensive patient population. Our patients had mild to moderate hypertension and were receiving different antihypertensive drugs including calcium channel blockers, beta-blockers, angiotensin converting enzyme inhibitors or angiotensin II receptor blockers. Hypertension was well-controlled in all patients.

Our study patients carrying TT allele tended to show lower LVMI and LVESD but results were not statistically significant (p>0.05). Although patients were receiving different hypertensive therapies, patients with TT allele showed a higher tendency for LVH regression. Previously Kurland et (17) reported that hypertensive patients carrying T allele of the M235T responded with the greatest reduction in LVMI when treated with the angiotensin II receptor blocker irbesartan. In the same study, change in LVM was independent of blood pressure response. We are not able to do a subgroup analysis due to small sample size but a larger study in our population is needed to see whether any drug subgroup responds with a better LVM regression or blood pressure control is the single predictor of LVM regression in TT genotype.

In a meta-analysis of 45267 subjects, M235T genotype was associated with a stepwise increase in angiotensinogen levels in white subjects and a significant but moderate increase in risk of hypertension in both white and Asian subjects. Genotype did not predict plasma angiotensinogen levels in Asian and black subjects or hypertension in black subjects (25). In a previous study performed on Turkish hypertensive patients, Agachan et al (32) reported that M235T TT genotype was found significantly higher in hypertensive subjects than in control group (20% vs 2.7%; p=0.001). The AGT M235T polymorphism distribution in hypertensive subjects was MM 32%, MT 48%, TT 20% and distribution in normotensive subjects was MM 31.1%, MT 66.2%, TT 2.7%. The AGT M235T polymorphism distribution in our study was MM 24.7%, MT 52.8%, TT 22.5% and results are in agreement with previous Turkish study.

In previous studies, AGT M235T TT variant was associated with increased risk of coronary artery disease and increased risk of myocardial infarction (33, 34). In our study, all patients had normal coronary arteries and none of the patients had electrocardiographic evidence of myocardial infarction. Coronary artery disease and resulting myocardial ischemia is ruled out so that changes observed in LV may be more easily attributed to hypertension duration, antihypertensive drugs and genetic polymorphisms. In previous studies coronary artery disease was not ruled out directly by coronary angiography.

Studies of SNPs and disease relationships in different populations and cardiovascular disease subgroups will be helpful in the future.

In conclusion, we could not find an association between AGT M235T polymorphism and left ventricular mass, dilatation and function in our study group of Turkish hypertensive patients with normal coronary arteries and receiving different antihypertensive drugs. A larger study with higher power is needed to see whether only hypertensive therapy independent of drug class explains lack of associations between AGT M235T polymorphism and LV hypertrophy and dilatation in Turkish hypertensive population.

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