Genetic background of left ventricular hypertrophy in Uzbek hypertensive men

Hipertansiyonlu Özbek erkeklerinde sol ventrikül hipertrofisinin genetik temeli

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Objectives: We evaluated the prevalences of ACE/ID, AGT/M235T, AT1R/A1166C, and CYP11B2/C344T genetic polymorphisms and their association with left ventricular hypertrophy (LVH) in Uzbek hypertensive men.

Study design: The study included 172 Uzbek men (mean age 47±10 years) with untreated essential hypertension (EH) of grade 1-2 and 60 normotensive subjects (mean age 41±10 years). All subjects underwent complete M-mode echocardiography to determine left ventricular mass (LVM) and LVM index (LVMI). Genomic DNA was extracted from peripheral blood and was analyzed using polymerase chain reaction-restriction fragment length polymorphism assays.

Results: Left ventricular hypertrophy was detected in 148 hypertensive patients (86.1%). The frequencies of the D-allele of the ACE gene and T-allele of the CYP11B2 gene were higher among hypertensive patients than in the controls. There was a significant association between the AGT/M235T polymorphism and LVH. The 235T-allele of the AGT gene, the D-allele of the ACE gene, and the 344T-allele of the CYP11B2 gene were identified as "damaging" alleles. All the patients had "damaging" alleles, the number being one in only seven patients (4.1%), two in 52 patients (30.2%), and three in 89 patients (51.7%). The severity of LVH significantly increased with the number of "damaging" alleles. Among paired carriage of "damaging" alleles, the combination of the D+235T-alleles was found as the most unfavorable pair associated with the degree of LVH.

Conclusion: There is an association between EH and ACE/ID and CYP11B2/C344T gene polymorphisms in Uzbek males, with higher frequencies of the D-allele of the ACE gene and T-allele of the CYP11B2 gene. Our findings provide evidence for the association of AGT/M235T polymorphism with LVH in Uzbek males, combination of the D+235T-alleles being the most unfavorable pair associated with LVH.

Key words: Hypertension/genetics; hypertrophy, left ventricular/epidemiology; polymorphism, genetic; Uzbekistan/epidemiology.

Amaç: Bu çalışmada hipertansif Özbek erkeklerinde ACE/ID, AGT/M235T, AT1R/A1166C ve CYP11B2/C344T genetik polimorfizmlerin sıklıkları ve bunların sol ventrikül hipertrofisiyle (SVH) ilişkisi araştırıldı.

Çalışma planı: Çalışmaya derece 1-2 esansiyel hipertansiyonu olan ve tedavi görmemiş 172 Özbek erkek (ort. yaş 47±10) ve normotansif 60 gönüllü (ort. yaş 41±10) alındı. Tüm olgular sol ventrikül (SV) kütlesi ve kütle indeksini belirlemek için M-mod ekokardiyografi ile değerlendirildi. Genomik DNA'nın elde edilmesi için periferik kan örnekleri alındı ve polimeraz zincir reaksiyonunda sınırlı parça uzunluk polimorfizm teknikleriyle analiz edildi.

Bulgular: Hipertansif hastaların 148'inde (%86.1) SVH saptandı. Kontrol grubuyla karşılaştırıldığında, hipertansif grupta ACE geninde D-aleli ve CYP11B2 geninde T-aleli sıklıkları anlamlı yükseklik gösterdi. Özbek hipertansif erkeklerinde AGT/M235T polimorfizmi ile SVH arasında anlamlı ilişki görüldü. AGT geninin 235T-aleli, ACE geninin D-aleli ve CYP11B2 geninin 344T-aleli hasarlı aleller olarak belirlendi ve tüm hipertansif hastalarda hasarlı alel görüldü. Bir hasarlı alel sadece yedi hastada (%4.1) görülürken, 52 hastada (%30.2) iki, 89 hastada (%51.7) üç hasarlı alel vardı. Sol ventrikül hipertrofisinin derecesi hasarlı alel sayısıyla artmaktaydı. İkili hasarlı alel birlikteliği açısından, SVH derecesini en olumsuz etkileyen ikili D+235T-alelleri olarak saptandı.

Sonuç: Özbek erkeklerinde esansiyel hipertansiyon ACE/ID ve CYP11B2/C344T gen polimorfizmleri ile ilişkili bulunurken, hipertansiyona ACE geninin D-aleli ve CYP11B2 geninin T-aleli sıklığı eşlik etmektedir. Bulgularımız Özbek erkeklerinde AGT/M235T polimorfizmi ile SVH arasındaki ilişkiye de kanıt sağlamakta, D+235T-alellerinin birlikteliğinin SVH'yi en olumsuz etkileyen ikili olduğunu göstermektedir.

Anahtar sözcükler: Hipertansiyon/genetik; hipertrofi, sol ventrikül/epidemiyoloji; polimorfizm, genetik; Özbekistan/epidemiyoloji.

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Hypertension is still one of the most common problems in cardiology and is responsible for high cardiovascular morbidity and mortality. Overall, 26.4% of the adult population worldwide in 2000 had hypertension.[1] In Uzbekistan, hypertension is present in more than 26.6% of the adults. Essential hypertension (EH) is a major determinant of left ventricular hypertrophy (LVH), which is a major and independent risk factor for cardiovascular morbidity and mortality,[2] even among normotensive subjects.[3] Left ventricular mass (LVM) is closely related to the complex interaction between genetic, environmental, and lifestyle factors.

The genetic basis of EH is complex. A large number of studies have been devoted to the search of genetic markers that predispose to unfavorable clinical course of EH with development of severe organ damage in connection with remodeling processes of the heart and vessels. The study of polymorphisms in gene candidates encoding protein structures and functions, hormones, enzymes, regulating neurohormonal systems, endothelium, transmembrane electrolyte channels, and cellular pumps is one of the main direction in the study of genetic nature of LVH in patients with EH. The renin-angiotensin-aldosterone system (RAAS) genes have been most extensively studied as hypertension candidate genes. However, the results vary depending on population or study.[4-9] There are multiple causes of such divergence. The most important reason is that hypertension is polygenic in nature. Genes of the RAAS components take up the leading place among gene candidates whose structural polymorphisms are associated with cardiovascular remodeling processes. Among the great number of gene candidates encoding the RAAS components, the genes of ACE, AGT, AT1R, and aldosteron-synthase (CYP11B2) are of great interest. It is important to note that occurrence of different alleles of gene candidates may vary depending on population or ethnicity.[10] Unfortunately, there is no study on the polymorphism of hypertension candidate genes in the Uzbek population, except for some works carried out in our center.

The objective of the present study was to evaluate the prevalences of gene polymorphisms (ACE/ID, AGT/M235T, AT1R/A1166C, and CYP11B2/C344T) and their association with LVH in Uzbek patients with EH.

PATIENTS AND METHODS

The study included 172 ethnic Uzbek men (mean age 47±10 years) with untreated EH of grade 1-2 (WHO, 2003) and 60 normotensive subjects (mean age 41±10 years). The diagnosis of EH was based on the criteria proposed in the 2003 WHO/ISH Statement on the Management of Hypertension.

Exclusion criteria were symptomatic hypertension, clinical evidence for cerebrovascular or coronary heart diseases, cardiac arrhythmia, heart failure, renal impairment, diabetes mellitus, metabolic and other background diseases.

All patients gave informed consent, and the Ethics Committee of the Republican Specialized Center of Cardiology approved the study.

Echocardiography. Central hemodynamic parameters and LVM were estimated using M-mode echocardiography.[11,12] Left ventricular hypertrophy was determined on the basis of calculation of LVM by the Penn method and of LVM index (LVMI) – value of LVM indexed to body surface area (g/m^2).[13] Left ventricular mass index >125 g/m^2 was considered the cutoff level for LVH.

Gene polymorphism analysis. Genomic DNA was extracted from peripheral blood using the Diatom DNA Prep 200 Kit according to the manufacturer's protocol. Polymerase chain reaction-restriction fragment length polymorphism-based techniques and visualization were performed according to previously described methods to determine the I/D polymorphism of the ACE gene, the M235T polymorphism of the AGT gene,[14] the C344T polymorphism of the CYP11B2 gene,[15] the A1166C polymorphism of the AT1R gene.[16]

Statistical analysis. Continuous variables were expressed as mean±standand deviation and categorical variables as percentages. Differences in continuous variables between cases and controls were analyzed using the unpaired Student's t-test. Deviations from the Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed using the chi-square test with 1 degree of freedom (df), whereas differences in genotype distributions between cases and controls were assessed by the chi-square test with 2 df. The association between alleles or genotypes and LVH was tested using odds ratio (OR) with 95% confidence interval. The significance level for all the analyses was set at $p=0.05$. Statistical analyses were performed using Microsoft Office Excel 2007 and Statistica v6.0 (StatSoft, USA).

RESULTS

Essential hypertension was grade 1 in 82 patients (47.7%) and grade 2 in 90 patients (52.3%). The mean

Table 1. Allele and genotype frequencies of gene polymorphisms and associations with essential hypertension

		Hypertensives (n=172)		Controls (n=60)					
Genes	Alleles	n	%	n	%	χ^2	*p*	OR	95% CI
ACE	I	81	47.1	41	68.3	16.10	**0.00006**	0.41	0.27 - 0.64
	D	91	52.9	19	31.7			2.42	1.56 - 3.76
AGT	M	86	50.0	26	43.3	1.58	0.21	1.31	0.86 - 1.99
	T	86	50.0	34	56.7			0.76	0.50 - 1.15
AT1R	A	146	84.9	51	85.0	0.04	0.85	1.06	0.60 - 1.87
	C	26	15.1	9	15.0			0.95	0.53 - 1.68
CYP11B2	C	67	39.0	29	48.3	4.05	**0.04**	0.65	0.43 - 0.99
	T	105	61.1	31	51.7			1.53	1.01 - 2.33
	Genotypes								
ACE	II	40	23.3	34	56.7	13.76	**0.0002**	0.23	0.12 - 0.43
	ID	82	47.7	14	23.3			2.99	1.53 - 5.84
	DD	50	29.1	12	20.0			1.64	0.80 - 3.34
AGT	MM	27	15.7	8	13.3	2.36	0.12	1.21	0.52 - 2.83
	MT	118	68.6	36	60.0			1.46	0.79 - 2.68
	TT	27	15.7	16	26.7			0.51	0.25 - 1.04
AT1R	AA	122	70.9	41	68.3	0.04	0.84	1.13	0.60 - 2.14
	AC	48	27.9	19	31.7			0.84	0.44 - 1.58
	CC	2	1.2	0	0.0			1.77	0.08 - 37.48
CYP11B2	CC	26	15.1	14	23.3	4.03	**0.04**	0.59	0.28 - 1.21
	CT	81	47.1	31	51.7			0.83	0.46 - 1.50
	TT	65	37.8	15	25.0			1.82	0.94 - 3.53

body mass index was 26.2±3 kg/m^2. Hypertensive patients were divided into two groups depending on the presence (n=148, 86.1%) or absence (n=24, 14%) of LVH. The two groups did not significantly differ with regard to BMI and blood pressure.

Allele and genotype frequencies and genetic association. The allele and genotype frequencies and their associations with EH and LVH are presented in Table 1 and 2. The distributions of all alleles and genotypes were within the Hardy-Weinberg equilibrium. Odds ratios were calculated with a multiplicative heritage model for alleles and with an additive heritage model for genotypes (Table 1, 2).

Investigation of the ID polymorphism of the ACE gene revealed that the proportion of individuals with the D-allele and the ID-genotype in the hypertensive group was higher than in controls. The T-allele and TT-genotype of the C344T-polymorphism of the CYP11B2 gene occurred more often in hypertensive patients than in controls. The genotypes and alleles of the other polymorphic genes did not differ significantly between hypertensive and normotensive subjects (Table 1).

We also analyzed the relationship between gene polymorphisms and LVMI as the marker of LVH. The ORs estimated for alleles and genotypes are presented in Table 2. The genetic models disclosed that the TT-genotype of the AGT gene was associated with an increased risk for LVH.

The 235T-allele of the AGT gene, the D-allele of the ACE gene, and the 344T-allele of the CYP11B2 gene were identified as "damaging" alleles.

Carriage of "damaging" alleles was found in all the patients. Of interest, one "damaging" allele was noted only in seven (4.1%) patients. "Mono"-type carriage of the D-allele of the ACE gene was noted in three (2.3%) of 132 patients having this marker (overall frequency 1.7%); 344T-allele of the CYP11B2 gene in two (1.4%) of 144 patients having this marker (overall frequency 1.2%); 235T-allele of the AGT gene in two (1.4%) of 144 patients having this marker (overall frequency 1.2%). Frequencies of "mono"-type carriage of different "damaging" alleles did not differ.

The carriage of two "damaging" alleles was noted in 52 patients (30.2%). The combinations were as follows: 344T-allele of the CYP11B2 gene with 235T-allele of the AGT gene (n=22, 42.3%); D-allele of the ACE gene with 235T-allele of the AGT gene (n=17, 32.7%) and 344T-allele of the CYP11B2 gene (n=13, 25%).

Table 2. Allele and genotype frequencies of gene polymorphisms and associations with left ventricular hypertrophy

		Hypertensives (n=172)		Controls (n=60)					
Genes	Alleles	n	%	n	%	χ^2	*p*	OR	95% CI
ACE	I	81	47.1	29	48.3	0.02	0.9	0.96	0.52 - 1.77
	D	91	53.0	31	51.7			1.04	0.56 - 1.91
AGT	M	82	47.7	36	60.0	2.7	0.1	0.60	0.32 - 1.11
	T	90	52.3	24	40.0			1.68	0.90 - 3.12
AT1R	A	145	84.3	54	90.0	0.86	0.35	0.63	0.24 - 1.68
	C	27	15.7	6	10.0			1.58	0.60 - 4.21
CYP11B2	C	69	40.1	26	43.3	0.22	0.64	0.86	0.47 - 1.60
	T	103	59.9	34	56.7			1.16	0.62 - 2.14
	Genotypes								
AACE	II	38	22.1	12	20.0	0.02	0.9	1.09	0.38 - 3.17
	ID	85	49.4	32	53.3			0.82	0.34 - 1.93
	DD	49	28.5	16	26.7			1.19	0.45 - 3.23
AGT	MM	25	14.5	12	20.0	4.15	**0.04**	0.66	0.22 - 1.96
	MT	113	65.7	48	80.0			0.50	0.18 - 1.42
	TT	34	19.8	0	0.0			12.1	0.71 - 204.74
AT1R	AA	121	70.4	48	80.0	0.94	0.33	0.62	0.22 - 1.77
	AC	49	28.5	12	20.0			1.51	0.53 - 4.29
	CC	2	1.2	0	0.0			0.84	0.04 - 17.95
CYP11B2	CC	30	17.4	10	16.7	0.21	0.65	1.07	0.34 - 3.38
	CT	78	45.4	32	53.3			0.70	0.29 - 1.66
	TT	64	37.2	18	30.0			1.44	0.56 - 3.68

The carriage of three "damaging" alleles was noted in 89 patients (51.7%), the most common combination being 344T-allele of the CYP11B2 gene, 235T-allele of the AGT gene, and D-allele of the ACE gene seen in 64 patients (71.9%, overall 37.2%). The prevalence of this combination was significantly higher than all other triple combinations (p=0.000).

The severity of LVH significantly increased with the number of "damaging" alleles making a combination, showing that the greater number of "damaging" alleles as genetic markers, the higher tendency to LVM (Fig. 1).

Comparisons to evaluate paired contributions of the "damaging" alleles to the development of arterial hypertension and cardiovascular remodeling showed that the carriage of D+235T-alleles was the most unfavorable combination associated with the degree of LVH (Table 3).

DISCUSSION

A number of candidate genes responsible for hypertension have been studied in different ethnic populations, but the results of these studies are inconsistent and often controversial. Our findings showed that Uzbek patients with EH exhibited a significantly greater frequency of the heterozygous ID-genotype and D-allele of the ACE gene, while in the healthy subjects

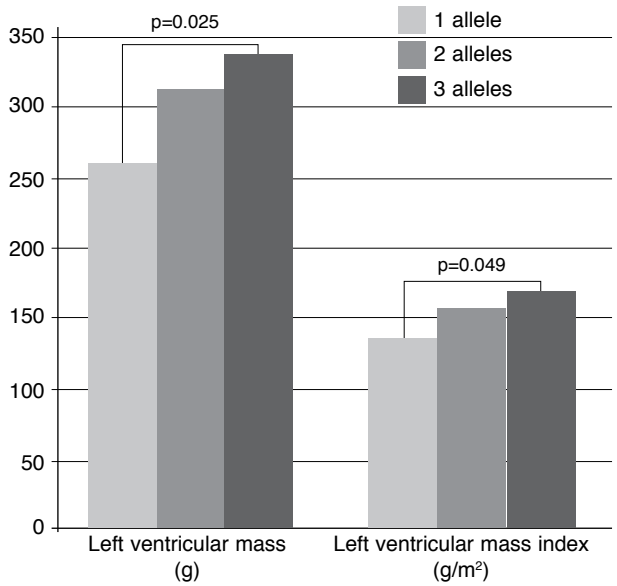

Figure 1. The relationship between the number of "damaging" alleles and left ventricular mass and mass index.

Table 3. Associations between the parameters of left ventricular hypertrophy and paired combinations of "damaging" alleles of the renin-angiotensin-aldosterone system genes

	344T+235T (I) (n=22)	*p* (I-II)	D+344T (II) (n=13)	*p* (II-III)	D+235T (III) (n=17)	*p* (I-III)	*f/p* (I,II,III)
Systolic blood pressure (mmHg)	159.5±13.6	N S	158.3±15.3	N S	156.2±10.5	N S	0.299 / 0.743
Diastolic blood pressure (mmHg)	103.5±9.3	N.S	101.7±8.4	N.S	97.1±5.9	**0.019**	3.031 / 0.058
Relative wall thickness	47.9±4.7	N.S	46.8±6.3	N.S	49.6±8.7	N.S	0.684 / 0.509
Left ventricular mass (LVM) (g)	301.4±69.0	N.S	287.2±72.8	**0.049**	336.4±69.3	N.S	2.343 / 0.107
Left ventricular mass index (g/m^2)	153.7±34.2	N.S	142.8±34.7	**0.048**	169.1±34.5	N.S	2.236 / 0.118
End-diastolic volume/LVM	0.42±0.06	N S	0.45±0.09	N S	0.41±0.09	N S	1.000 / 0.375

p: Difference between two groups using the Student's t-test; f/p: Single-factor analysis of variance.

the II-genotype was predominant with a significantly higher frequency of the I-allele.

The allele frequencies and genotype distributions of the ID-polymorphism of the ACE and A1166C-polymorphism of the AT1R genes observed in our study are very similar to those reported for other Caucasian populations,[17-21] with the exception of a higher frequency of the DD-genotype in control subjects in a Norwegian study.[22] An association between the D-allele of the ACE gene and EH has not yet been confirmed,[23,24] although O'Donnell et al.[25] found a limited association in hypertensive males in the Framingham Heart Study. There are many reports on the association of the ACE gene with LVH.[26] Association of the D-allele of the ACE gene with increased LVM has been noted in a number of studies; however, the results of these studies on association of the I/D-polymorphism of the ACE gene with LVH are controversial. A Japanese study found no relationship between the I/D polymorphism of the ACE gene and large LVM in women with hypertension, whereas such association was found in Japanese hypertensive men who had concentric LVH.[27,28]

In our study, LVH showed an association only with the AGT M235T gene polymorphism. Relationship between the 235T allele of the AGT gene and hypertension was first demonstrated by Jeunemaitre et al.[29] in 1992. However, this relationship is different in various ethnic groups. Some studies indicated an association between LVH and the 235T allele.[30]

Data on the relationship between the A1166C polymorphism of the AT1R gene and risk for hypertension and its complications are also conflicting. Some authors showed an association of the 1166C allele with hypertension and LVH,[31-33] but we did not find such relationship in our study.

At present, it is not clear whether there is a relationship between the 344T allele or 344C allele of the CYP11B2 gene and LVH. Stella et al.[34] found a positive correlation between the 344T allele of the CYP11B2 gene and LVMI, whereas such a relation was not found in our study. Kupari et al.[15] reported an association of the 344CC genotype and 344C allele of the CYP11B2 gene with LVM, end-diastolic and end-systolic diameters in a Finnish healthy young sample in comparison with the 344TT homozygous group.

The results of our study show a high prevalence of combined carriage of "damaging" alleles, compared with the isolated carriage of one "damaging" allele. The accumulation of "damaging" alleles may contribute to LVH. The possibility of synergism of combined carriage of "damaging" alleles can be expected in cases of combined carriage of D+235T alleles, which proved to be the most unfavorable combination associated with LVH, with possible priority of the 235T allele in these processes.

In conclusion, there is an association between EH and ACE/ID and CYP11B2/C344T gene polymorphisms in Uzbek males. The frequencies of the D-allele of the ACE gene and T-allele of the CYP11B2 gene were higher among male patients with EH than in the control subjects. The results of this research provide evidence for the association of AGT/M235T polymorphism with LVH in Uzbek males with EH, but combination of the D+235T-alleles was the most unfavorable combination associated with LVH.

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