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GENETIC ASPECTS OF ENDOTHELIUM DYSFUNCTION IN UZBEK HYPERTENSIVE PATIENTS

The objective of the study was to evaluate the prevalence of ACE/ID, AGT/M235T, AT1R/A1166C, CYP11B2/C344T, B2BKR/+9-9, ecNOS/4a4b, GNB3/C825T, and ARB2/Gln27Glu gene polymorphisms and their association with endothelium dysfunction (ED) in Uzbek hypertensive patients. The study found an association between ED and AGT M235T gene polymorphism. Genetic models disclosed that the T-allele, MT-, and TT-genotypes of the AGT gene were associated with an increased risk of ED. However, multivariate logistic regression analysis confirmed the negative association between the MM genotype of the AGT gene and positive association between IMT and ED. An association between essential hypertension (EH) in Uzbek males with ACE/ID and GNB3/C825T gene polymorphisms has been demonstrated. There is an association of AGT/M235T gene polymorphism and ED in Uzbek hypertensive patients.

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Introduction

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 in 2000 had hypertension (Kearney et. al., 2005). In Uzbekistan, hypertension is present in more than 26.6% of adults. Essential hypertension (EH), a polygenic and multifactor disorder, induces structural and functional changes in arteries that thicken and become less compliant over time, enhancing greatly the risk of atherosclerosis. Genetic elements contribute to 30-50% of the blood pressure variability in human EH. The underlying pathological defect in EH is due, at least in part, to endothelial dysfunction (ED) (Panza, 1997). ED is now recognized as an early, perhaps initial, stage in the pathogenesis of cardiovascular diseases and has been found in patients with EH (Linder et. al., Panza, 1990). Unfortunately, studies of genetic polymorphisms of candidate genes involved in the development of EH and ED in the Uzbek population were not previously conducted, except for small studies piloted in our center.

The objective of the present study was to evaluate the prevalence of gene polymorphisms (ACE/ID, AGT/M235T, AT1R/A1166C, CYP11B2/C344T, B2BKR/+9-9, ecNOS/4a4b, GNB3/C825T, and ARB2/Gln27Glu) and their association with ED in Uzbek patients with EH.

Materials and methods

The study included 174 ethnic Uzbek men in the mean age of 46.85±9.57 with untreated EH of stage I–II (WHO, 2003) and 60 normotensive subjects in the mean age of 40.8±10.3. The diagnosis of EH was based on the 2003 WHO/ISH Statement on the management of hypertension criteria. The subjects, included into the study had not received statins, acetylsalicylic acid and other medication affecting on the endothelium functional condition and haemostasis. Exclusion criteria were symptomatic hypertension, clinical evidence of cerebrovascular or coronary heart diseases, cardiac arrhythmia, heart

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failure, renal impairment, diabetes mellitus, metabolic and other background diseases, alcohol intake greater than 30 g of pure ethanol per day, and smoking. Research purposefully does not join women for an exception of effects of estrogenic modulations of endothelium function. All patients gave informed consent, and the Ethics Committee of the Republican Specialized Center of Cardiology approved the study.

The blood plasma samples were analyzed by Daytona Autoanalyser (Randox Laboratories, Ltd., UK) to determine total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) levels. Low-density lipoprotein cholesterol (LDL-C) level was calculated by the Friedewald formula. The lipid profiles were determined accordingly with ATP III Guidelines (2004). Microalbuminuria was defined as an albumin urinary excretion (AUE) level between 20 and 200 mg/L and determined on Daytona Autoanalyser by immunoturbidimetric assay.

A 7.5 MHz high-resolution ultrasound (EnVisorC®, Philips, Nederlands) was used to measure carotid artery intima-media thickness (IMT). IMT was defined as maximum thickness of IMT at the region of interest detected in both left and right carotid artery including common carotid artery (Stein et. al., 2008).

Assessment of flow-mediated dilation (FMD) of the brachial artery has been used as a method of determining endothelial function. The diameter of the brachial artery was measured from two dimensional ultrasound images, with a 7.5 MHz linear array transducer and a standard EnVisorC® system. In each study, scans were taken at rest and during reactive hyperemia. The FMD was estimated as the percent change in the diameter relative to the baseline diameter (BD) at rest. Level of FMD ≥10% was taken as the norm threshold based on the Celermajer works (Celermajer et. al., 1992; Corretti et. al., 2002).

Genomic DNA was extracted from peripheral blood using the Diatom ™ DNA Prep 200 Kit according to the manufacturer's protocol. Nomenclatures of the studied genes are presented in Table 1.

Polymerase chain reaction restriction fragment length polymorphism-based (PCR-RFLP) techniques and visualization were employed and performed according to previously described methodologies to determine the insertion/deletion (I/D) polymorphism of the ACE gene (Cambien et. al. 1992), the M235T polymorphism of the AGT gene (Russ et. al., 1993), the C344T polymorphism of the CYP11B2 gene (Kupari et.al., 1998), the A1166C polymorphism of the AT1R gene (Bonnardeaux et. al., 1994), the Gln27Glu polymorphism of the ARB2 gene (Bengtsson et. al., 2001), the 4a/4b polymorphism of the ecNOS gene (Zateyshchikov et. al., 2000), the +9/-9 polymorphism of the B2BKR gene (Dhamrait et. al., 2003), and the C825T polymorphism of the GNB3 gene (Benjafield et. al., 1998).

Statistical analysis

Continuous variables are expressed as mean ± SD deviation and categorical variables as percentages. Differences in continuous variables between cases and controls were examined using the unpaired Student t-test. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by x^2-test with 1 degree of freedom (df), whereas differences in genotype distributions between cases and controls were assessed by the χ^2-test with 2 df. The association between alleles or genotypes and ED was tested using Odds Ratios (ORs) with 95% confidence intervals. In order to estimate adjusted ORs, a multivariate logistic regression model was employed with endothelium status as the dichotomous dependent variable. The significance level for all the analyses was set at α=0.05. Statistical analysis was performed using a Microsoft Office Excel 2007 and Statistica v6.0 (StatSoft, USA).

Results

1 and 2 grades of EH were identified in 47.7% and 52.3% of cases. The average of BMI was 26.2±3 kg/m². The number of patients with LDL-C >120 mg/dL was 35% with the

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average value of 129.37±34.09 mg/dL. Hypertensive patients were divided into two groups depending on the presence (136 patients) or absence (38 patients) of ED, defined by ΔD% (<10% and >10%). In contrast hypertensive patients with ED showed significantly higher values of SBP (160.15±14.88 mm Hg vs. 154.11±11.87 mm Hg; p=0.022). However there was no difference in DBP (101.55±8.7 mm Hg vs. 98.88±5.7 mm Hg; p>0.05). Groups did not significantly differ in the level of BMI and LDL-C.

The distribution of all alleles and genotypes were within the Hardy-Weinberg equilibrium. Investigation of ID polymorphism of the ACE gene in hypertensive patients revealed that the proportion of individuals with the D-allele (53.2%; OR: 2.45 [95% CI: 1.58-3.8]) and the ID-genotype (47.7%; OR: 3.0 [95% CI: 1.54-5.85]) was higher than in the controls. The C-allele (64.1%; OR: 2.41 [95% CI: 1.58-3.68]) and CC-genotype (37.4%; OR: 72.38 [95% CI: 4.40-1190.34]) of C825T polymorphism of the GNB3 gene occurred more often in hypertensive patients than in controls. The genotypes and alleles of the other gene polymorphisms did not differ significantly between hypertensive and normotensive subjects.

We analyzed the relationship between gene polymorphisms and ΔFMD as the markers of ED. Taking into account the scarcity of 4a/4a-genotype carriers of the ecNOS gene as well as the CC-genotype of the AT1R gene, the groups 4a/4a+4a/4b and AC+CC-genotypes were formed for ease of analysis. The groups were studied (ΔFMD>10% and ΔFMD<10%) for the determination of gene polymorphisms association with ED. ORs were calculated with a multiplicative heritage model for alleles and with an additive heritage model for genotypes. These genetic models disclosed that the T-allele and MT-, TT-genotypes of the AGT gene were associated with an increased risk for ED: OR 3.18 (95% CI: 1.80-5.63); OR 2.24 (95% CI: 1.08-4.66); OR 15.94 (95% CI: 0.95-268.79) accordingly.

TABLE 1. NOMENCLATURES OF THE STUDIED GENES

Gene	Symbol	Chromo-some	Alleles	Polymorphism	Character of polymorphism
Angiotensinogen	AGT	1q42-43	M, T	M235T	Substitution of Methionin/Threonin at 235 nucleotide position
Angiotensin-I-converting enzyme	ACE	17q23	I, D	I/D	Insertion/deletion Alu-repeat of 287 bp at16 intron
Angiotensin II Type 1 receptor	AT1R	3q21-q25	A, C	A1166C	Substitution of Adenine/Cytosine at 1166 nucleotide position
Aldosterone synthase	CYP11B2	8q22	C, T	C344T	Substitution of Cytosine/Thymidine at 344 nucleotide position
Bradykinin B_2-receptor	B2BKR	14q32.1-q32.2	+9, -9	+9/-9	Presence (+9) or absence (-9) of nine bp repeat sequence in exon 1
Endothelial constitutive NO-synthase	ecNOS	7q35-36	4a, 4b	4a/4b	Variable tandem 27 bp repeat at 4 intron
β_2-adrenoreceptor	ARB2	5q31-32	Gln, Glu	Gln27Glu	Substitution of Glutamine/Glutamic acid at 27 nucleotide position
G-protein β_3-subunit	GNB3	12p13	C, T	C825T	Substitution of Cytosine/Thymine at 825 nucleotide position in exon 10

However, multivariate logistic regression analysis (dependent variable: ΔFMD; independent variables: AGT genotype status, age, BMI, SBP, DBP, TC, HDL-C, IMT, AUE, AI, BD) confirmed the negative association between the MM genotype of the AGT gene and positive association between IMT and ED (Table 2). We further analyzed the relationship between polymorphic markers of the studied genes and the quantitative characteristics of ED markers (Table 3). Carriage of the TT-genotype, T-allele of the AGT gene, C-allele of the AT1R gene, 4a-allele in the 4a/4a+4a/4b group of ecNOS gene and the +9/+9- genotype of the B2BKR gene showed a significant higher level of FMD disturbances.

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TABLE 2. MULTIVARIATE ANALYSIS FOR THE ENDOTHELIAL DYSFUNCTION BY AGTMM, AGE, BMI, SBP, DBP, DBASELINE, CIMT, MAU, TC, HDL-C, AI.

	Estimate	SE	Wald statistics	p
AGT_{MM}	-2.114	0.692	9.340	0.002
Age	-0.028	0.037	0.555	0.456
BMI	-0.133	0.089	2.256	0.133
SBP	0.017	0.040	0.192	0.661
DBP	0.055	0.060	0.812	0.368
$D_{baseline}$	0.549	0.554	0.981	0.322
CIMT	5.082	2.134	5.673	0.017
MAU	0.005	0.032	0.024	0.877
TC	-0.016	0.019	0.682	0.409
HDL-C	0.163	0.110	2.213	0.137
AI	0.638	0.514	1.542	0.214

Note: AGT = angiotensinogen gene; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; Dbaseline = brachial artery baseline diameter; CIMT = carotid intima-media thickness; MAU = Microalbuminuria; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; AI = atherogenity index; SE = standart error.

TABLE 3. ASSOCIATION OF GENE POLYMORPHISMS WITH FMD (ΔD%) IN HYPERTENSIVE PATIENTS

Gene	Genotypes			Alleles	
ACE	II	ID	DD	I	D
	4.6±5.7	3.4±7.5	3.7±5.4	3.69±7.58	3.57±7.08
	F=0.456; p=0.634			p>0.05	
AT1R	AA	AC+CC		A	C
	4.15±7.57	1.69±9.52		3.89±7.80	0.94±9.91
	p>0.05			p=0.016	
AGT	MM	MT	TT	M	T
	8.13±7.83	3.34±4.63	1.74±3.76	7.6±6.3	1.8±6.5
	F=20.331; p_{MM-TT}=0.000			p=0.000	
CYP11B2	TT	TC	CC	T	C
	3.15±8.88	3.25±7.6	4.81±8.43	3.18±8.38	3.89±7.91
	F=0.448; p=0.64			p>0.05	
B2BKR	+9/+9	+9/-9	-9/-9	+9	-9
	0.49±5.18	8.9±7.6	7.8±6.7	3.44±7.3	7.87±7.16
	F=8.515; $p_{(-9/-9)-(+9/+9)}$=0.000; $p_{(+9/-9)-(+9/+9)}$=0.000			p=0.000	
ecNOS	4a/4a+4a/4b		4b/4b	4a	4b
	0.6±7.1		4.2±5.8	0.45±8.56	4.02±8.31
	p=0.001			p=0.008	
GNB3	CC	CT	TT	C	T
	4.62±5.66	4.02±5.16	4.46±3.22	3.32±5.95	3.32±5.67
	F=0.263; p=0.769			p>0.05	
β_2-AR	Gln27Gln	Gln27Glu	Glu27Glu	Gln	Glu
	4.43±4.79	5.11±5.01	5.82±7.05	4.62±4.8	5.29±5.3
	F=0.644; p=0.526			p>0.05	

Note: Comparison was performed by Fisher test and/or t-test

Discussion

Although a number of candidate genes responsible for hypertension have been studied in different ethnic populations, the results until now are inconsistent and often controversial. Thus, our investigation showed that in ethnic Uzbek patients with EH there is a significantly greater frequency of the intermediate ID genotype and D-allele of the ACE gene, while in the healthy subjects the II genotype was predominant with a significantly

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higher frequency of the I allele. The allele frequency and genotype distribution of the ID ACE and A1166C AT1R polymorphisms observed in our study are very similar to those already reported in other Caucasian populations (Tiret et. al., 1994; Jeunemaitre et. al., 1992; Schunkert et. al., 1994; Samani et. al., 1996; Alvarez, 1998), with the exception of a reported higher frequency of the DD-genotype in control subjects in a Norwegian study (Bohn et. al., 1993). An association between the ACE gene D allele and EH has not yet been confirmed (Berge et. al., 1994; Jeunmaitre et. al., 1992) although O'Donnel et al. (1998) found an association limited to hypertensive males in the Framingham Heart Study. In patients with EH there is a significantly higher frequency of the CC genotype of the CNB3 gene in the absence of normotensive subjects. A significantly higher frequency of the GNB3 T allele has also been reported in three independent studies in subjects with EH using unselected normotensive control subjects of European origin (Benjafield et. al., 1998; Siffert et.al., 1998; Beige et. al., 1999), however other studies have reported different findings (Brand et. al., 1999; Shioji et. al., 2004). In individuals of African descent, the T allele of GNB3 was reported to be a susceptibility factor for the development of hypertension (Dong et. al., 1999). In a Japanese population, Izawa et al. (2003) demonstrated an association between hypertension and the C825T polymorphism of CNB3 gene in male subjects. In summary, it may be concluded that the distribution of alleles and genotypes of the analyzed genes (excluding GNB3) in Uzbek EH patients and healthy males corresponds to the distribution previously shown in Caucasian individuals. Some studies demonstrated an association between genetic markers and ED however these were invasive (Perticone et. al., 1998; Rossi et. al., 2001-2003; Turner et. al., 1999). Moreover, some showed no relation. We found no relationship between the ACE/ID, AT1R/A1166C, GNB3/C825T, CYP11B2/C344T, ARB2/Gln27Glu, BKRB2/+9-9, ecNOS/4a4b gene polymorphisms and the presence of ED. Perticone et al. (1998) demonstrated an association of the ACE gene polymorphism with hypertension, but failed to do so with ED, a result similar to that of Rossi et al. (2001). The latter group, in a subsequent study (Rossi et. al., 2003), showed an association between the ecNOS gene and both endothelial function and hypertension however a number of similar studies were negative (Zee et. al., 1992; Demirel et. al., 2005). The present study found an association only between AGT/M235T gene polymorphisms and ED. Our data is in agreement with results of some previous studies (Jeunemaitre et. al., 1992; Demirel et. al., 2005) concerning AGT/M235T gene polymorphism. In the present study we did not find a clear association of ED with other gene polymorphisms, but carriers of C-allele of AT1R gene, -9-allele of B2BKR gene and 4a-allele of ecNOS gene have more disorders of FMD. Only the TT genotype of AGT gene was significantly associated with the risk of ED. The IMT can also be associated with ED.

Consequently, there is an association between EH in Uzbek males with ACE/ID and GNB3/C825T gene polymorphisms. The frequency of occurrence of the D-allele ACE and C-allele GNB3 is higher among male patients with EH than control subjects. Results of the research testify to the association of AGT/M235T polymorphism and ED in Uzbek males with EH.

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