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Original Article:

Association of Angiotensinogen Gene M235T and T174M Polymorphisms with Coronary Heart Disease in Rostov Population.

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Abstract: Objectives: In this study, we examined the relationship between the AGT gene M235T and T174M polymorphisms and CHD risk in Rostov population. **Material and Methods:** We studied two groups of patients with clinical and coronary angiography evidence of CHD and age- and gender-matched controls, respectively. AGT gene M235T and T174M polymorphisms were analyzed by polymerase chain reaction. **Results:** The frequency distribution of AGT (M235T) genotypes among patients and controls (M235T: 28.5% and 16.2%; 235T: 22.4% and 7.3%, respectively) was statistically different (P = 0.03 and 0.001, respectively). CHD odds ratio associated with M235T heterozygotes and 235T homozygotes were 2.63 (1.1-6.1) and 4.88 (1.7-13.8), respectively. **Conclusion:** This study demonstrates the contribution of AGT gene M235T polymorphism, but not the AGT (T174M), to the presence of CHD risk in Rostov population. Genetic association studies involving very large sample size are needed to provide conclusive evidence on the effects of the AGT gene and other genes within the RAS system on risk of CHD.
Key Words: Coronary heart disease, Genetic polymorphism, Angiotensinogen gene, Risk factor.

Introduction:
Today the primary reasons of human mortality worldwide are noninfectious diseases among which the leading place is taken by the cardiovascular system diseases (CSD). For several decades CSD are the prevailing factor resulting in morbidity and mortality among the able-bodied population in industrially developed countries. In 2012 the CSD contribution to the overall mortality of the population was 1056 thousand people, that is equal 55.4% of the total number of deaths, in Russia. Furthermore, the mortality rate from these diseases in Russia significantly exceeds this index in Western Europe and North America countries.(1)
The coronary heart disease (CHD) is the most common abnormality among CSD, which occurs mainly due to atherosclerotic lesions of arteries. In multiple studies it was shown that CHD has both environmental and genetic etiology (2), nevertheless the concrete causes are still to be discovered.(3,4) Significant evidence demonstrate great contribution of genetic factors, at the same time received data about the various genes contribution to CHD development are controversial and require further studies in different populations.(5,6)
The genes coding for the proteins of the renin-angiotensin system (RAS) may assist in the development of CHD, due to their core role in the regulation of blood pressure and vascular remodeling.(7) Angiotensinogen is the integral component of RAS system. It is considered to be involved in the pathogenesis of hypertension and CHD.(8,9) Many studies have established and replicated the association of the two polymorphisms (M235T, T174M) in the *AGT* gene with CHD, and it was determined that their frequency differ in various populations. However, results from these reports are conflicting, and more studies are required to further confirm these findings.(8-11)
Thus the one of the key priorities of the present and future medicobiological studies are the disease causation understanding and enhancement of the opportunities in the

field of the CHD prevention, diagnostics and treatment in different populations. Therefore we designed case-control study to examine the relationship between the genetic markers in the renin-angiotensin system and the presence of CHD in individuals residing in Rostov-on-Don, Russia.

Materials and Methods

The total population of this study consisted of 209 Russian individuals, residents of Rostov-on-Don, was divided into two groups: 50 angiographically verified CHD patients and 159 healthy subjects (Table 1). Patients with CHD were recruited from patients admitted to the cardiology section of Rostov regional clinical hospital (Rostov-on-Don, Russia). Documented stable CHD was diagnosed by the clinical evaluation and coronary angiography or occurrence of myocardial infarction (MI) as defined by WHO criteria. The control subjects were randomly selected and were age matched with CHD patients. They had no history of CHD, MI or stroke. Informed consent was obtained from all participants and the design of the study was approved by the local ethics committee.

Table 1: Clinical characteristics of study participants

Clinical characteristics	CHD (n=50)	Control (n=159)
Age (mean ± standard deviation)	61.2 ± 2.9	55.5 ± 9.0
Male (men/women)	35/15	79/80
Ever smoked (%)	98	9
Type-2 diabetes (%)	38	0
Systolic blood pressure (mm Hg, mean ± standard deviation)	144.1 ± 1.82	137.1 ± 1.18
Diastolic blood pressure (mm Hg, mean ± standard deviation)	88.8 ± 0.97	81.7 ± 0.59
CHD = coronary heart disease.		

CHD risk factors were determined according to the criteria of the European society of cardiology: a hypertensive condition was diagnosed when systolic blood pressure values were = 139 mm Hg and/or diastolic blood pressure values = 89 mm Hg or when being medicated against hypertension; subjects were considered smokers when consuming more than five cigarettes per day or had stopped smoking at least 1 year before sample collection or non-smokers when never smoked.

Isolation of DNA and genetic polymorphism detection. DNA was extracted from leukocytes by standard procedures.(12) The identification of the AGT gene T174M and M235T polymorphisms was carried out by allele-specific PCR according to the protocol (Genetic Polymorphism Detection Kit by Allele-specific PCR in human genome «SNP-EXPRESS», Lytech Company, Moscow, Russia). The analysis is based on the amplification reaction with two pairs of the allele-specific primers. PCR products were separated on 3% agarose gel stained with ethidium bromide. Then the gel was visualized under UV transilluminator (GelDoc, BioRad).

Genotype frequencies in cases and controls were tested for Hardy–Weinberg equilibrium using Hardy-Weinberg equilibrium calculator on http://www.oege.org/software/hwe-mr-calc.shtml (13), and the deviation between the observed and expected frequencies was tested for significance using the Chi-square test (?2) using BIOSTAT. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated regarding the presence of CHD with respect to the existence of polymorphism. The difference was considered significant at $P < 0.05$.(14)

Results

The genotypes frequencies of the *AGT* gene were in agreement with Hardy–Weinberg equilibrium estimated by ?2 in both groups. The allele frequencies were q (235T) = 0.36 and 0.15, q (174?) = 0.12 and 0.16 for patients and controls, respectively. The CHD group showed significantly increased frequencies of the *AGT* MT and TT genotypes compared with controls ($P = 0.03$ and 0.001, respectively), whereas the analyses did not show a statistically significant difference for the *AGT* ?174? polymorphism between cases and controls (Table 2). Subjects with *AGT* gene M235T polymorphism MT and TT genotypes were significantly more likely to develop CHD (OR = 2.63, 95% CI = 1.1-6.1, $P = 0.03$ and OR = 4.88, 95% CI = 1.1-6.1, $P = 0.001$, respectively), than individuals with *AGT* gene ?174? polymorphism TM and MM genotypes (OR = 0.65 95% CI = 0.28-1.5,$P > 0.05$ and OR = 0.96 95% CI = 0.04-10.8, $P > 0.05$, respectively).

Table 2: *AGT* genotypes distribution in cases and controls

Gene polymorphism	Genotype	CHD n (%)	Control n (%)	OR (CI 95%)	*P*
AGT-M235T	MM	25 (49.1)	122 (76.5)	-	-
	MT	14 (28.5)	26 (16.2)	2.63 (1.1-6.1)	0.03
	TT	11 (22.4)	11 (7.3)	4.88 (1.1-6.1)	0.001
AGT-T174M	TT	39 (77.6)	112 (70.4)	-	-
	TM	10 (20.4)	44 (27.7)	0.65 (0.28-1.5)	> 0.05
	MM	1 (2.0)	3 (1.9)	0.96 (0.04-10.8)	> 0.05

CHD = coronary heart disease; *AGT* = angiotensinogen gene.

Discussion

Among multiple *AGT* gene variants the most studied in relation to CHD are M235T and T174M. The genotype and allele frequencies of both *AGT* polymorphisms vary in different populations (Table 3). The 235T allele frequency lies within the range from 0.39 to 0.91 among the various ethnic groups, whereas the 174M allele – from 0.08 to 0.14. In the present study 235T and T174M allele frequencies were 0.15 and 0.16 in our control population, respectively.

The results of our study provide evidence of an association between the *AGT* gene variants and the risk for CHD in the Rostov population. In agreement with several other studies that have detected the *AGT* gene polymorphisms role in the development of this disease in other ethnic groups, including Spanish population (15), Slovaks (16), Polish (17) and Tunisians (18) etc., we ascertained that the homozygosity for 235T was associated with increased risk for CHD in Rostov population. However, it was revealed that M235T variant as well as T174M do not contribute to the increased risk of CHD in the Indian population.(11)

The lack of association for the T174M variant with CHD in our study appears to be in conflict with those reports implicating it in the disease.(18-20) It is possible that these controversial results point to the practical relevance of the ethnic variability, and could be explained by several factors, such as inter-ethnical variation.(21)

Mechanism by which AGT-235T genotype could be contributing to the development of CHD is unknown. In most cases the association of the *AGT* gene variants with different cardiovascular diseases could be illustrated by direct influence of the *AGT* on the blood pressure level. Other factors involved in artery wall injury should be considered.

Table 3: The allele and genotype frequencies of the *AGT* M235T and T174M polymorphisms in various populations

Population	Sample size (n)	*AGT* M235T					*AGT* T174M					References
		Genotype frequency			Allele frequency		Genotype frequency			Allele frequency		
		MM	MT	TT	M235	235T	TT	TM	MM	T174	174M	
Japanese (Nagoya)	352	3.7%	31.8%	64.5%	0.20	0.80	82.7%	16.2%	1.1%	0.91	0.09	(22)
Japanese (Oita)	170	9.4%	25.3%	65.3%	0.22	0.78	80.0%	18.8%	1.2%	0.89	0.11	(20)
Chinese (Taiwan)	337/336*	1.2%	16.0%	82.8%	0.09	0.91	80.4%	19.0%	0.6%	0.90	0.10	(23)
Chinese (Shanghai)	90	7.8%	24.4%	67.8%	0.20	0.80	81.1%	18.9%	0%	0.91	0.09	(24)
Indian (India)	131	8.4%	30.5%	61.1%	0.24	0.76	77.9%	20.6%	1.5%	0.88	0.12	(11)
Bedouin (Gulf)	61	26.2%	42.6%	31.1%	0.47	0.53	77.0%	21.3%	1.6%	0.88	0.12	(25)
German (Berlin)	102	38.2%	45.1%	16.7%	0.61	0.39	78.4%	20.6%	1.0%	0.89	0.11	(26)
German (Giessen)	511	32.9%	48.3%	18.8%	0.57	0.43	76.1%	22.5%	1.4%	0.87	0.13	(27)
French and Irish	741	34.8%	50.2%	15.0%	0.60	0.40	78.0%	20.8%	1.2%	0.88	0.12	(28)
Austrian	732/731**	32.4%	48.8%	18.9%	0.57	0.43	74.1%	24.1%	1.8%	0.86	0.14	(29)
Danish	7975	34.9%	48.7%	16.4%	0.59	0.41	77.1%	21.4%	1.4%	0.88	0.12	(30)
Italian (Florence)	209	40.2%	41.1%	18.7%	0.61	0.39	76.6%	22.0%	1.4%	0.88	0.12	(31)
Russian (Moscow)	90	22.2%	46.7%	31.1%	0.46	0.54	75.6%	23.3%	1.1%	0.87	0.13	(32)
Russian (Tomsk)	122	-	-	-	-	-	84%	16%	0%	0.92	0.08	(33)
Russian (Tomsk)	150	30%	42%	25%	0.52	0.48	-	-	-	-	-	(34)

*- the sample size differ for allele and genotype frequencies calculations for M235T and T174M polymorphisms (337 and 336, respectively);
** - the sample size differ for allele and genotype frequencies calculations for M235T and T174M polymorphisms (732 and 731, respectively).

Conclusion:

In conclusion, the results indicated the significant associations of the *AGT* gene polymorphism M235T with CHD risk in the Rostov population. However, further gene-gene and gene-environment interactions investigations are required to study the associations. Furthermore genetic association studies involving very large sample size are needed to provide conclusive evidence on the effects of the *AGT* gene and other genes within the RAS system on risk of CHD.

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