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A common polymorphism G-50T in cytochrome P450 2J2 gene is associated with increased risk of essential hypertension in a Russian population

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Abstract. The present study was designed to test whether common polymorphism G-50T within the promoter of human *CYP2J2* gene is associated with increased risk of essential hypertension in a Russian population. We studied 576 unrelated subjects, including 295 patients with hypertension and 281 healthy subjects. Genotyping for polymorphism G-50T of the *CYP2J2* gene was performed by polymerase chain reaction and restriction fragment length polymorphism techniques. The frequency of a −50T variant allele of *CYP2J2* gene was significantly higher in patients with hypertension versus healthy controls (OR 4.03 95%CI 1.80–9.04 $p = 0.0004$). The association of a −50GT genotype with hypertension remained significant after adjustment for age, gender and family history of hypertension by multivariate logistic regression (OR 4.78 95%CI 1.87–12.27 $p = 0.001$). It has been found that OR for −50GT genotype × gender interaction (OR 4.48 95%CI 1.93–10.39 $p = 0.00048$) was slightly higher than OR for −50GT genotype (OR 4.43 95%CI 1.91–10.29 $p = 0.00052$), suggesting a weak effect of gender on the risk of hypertension in the heterozygous carriers of −50GT genotype. A family history of hypertension has no effect on the association between a −50GT genotype and hypertension. In present study we demonstrate for the first time that a *CYP2J2*7* allele of the *CYP2J2* gene is clearly associated with an increased risk of essential hypertension. Furthermore, this study highlights the importance of P-450 epoxygenase pathway of arachidonic acid metabolism in the pathogenesis of hypertensive disease.

Keywords: Essential hypertension, arachidonic acid metabolism, cytochrome P450 *CYP2J2*, single nucleotide polymorphism, disease susceptibility, case-control studies

1. Introduction

In recent years, increasing interest has been focused on the vasoactive products of arachidonic acid (AA) derived from cytochrome P450 epoxygenase pathway, and impressive progress has been achieved in the understanding of their role both in vascular homeostasis and development of essential hypertension (EH) [4,14,22, 28,33,36,38]. The cytochrome P-450 2J2 (*CYP2J2*) is known to be one of the major enzymes of epoxygenase pathway of AA in extrahepatic tissues, which produces series of regioisomeric *cis*-epoxyeicosatrienoic acids (EETs) such as 5,6-, 8,9-, 11,12- and 14,15-EETs [7, 17,31,32].

1.1. Cardiovascular and renal actions of cytochrome P450-derived EETs

It is important to note, that EETs have been proposed to regulate vascular tone and fluid-electrolyte

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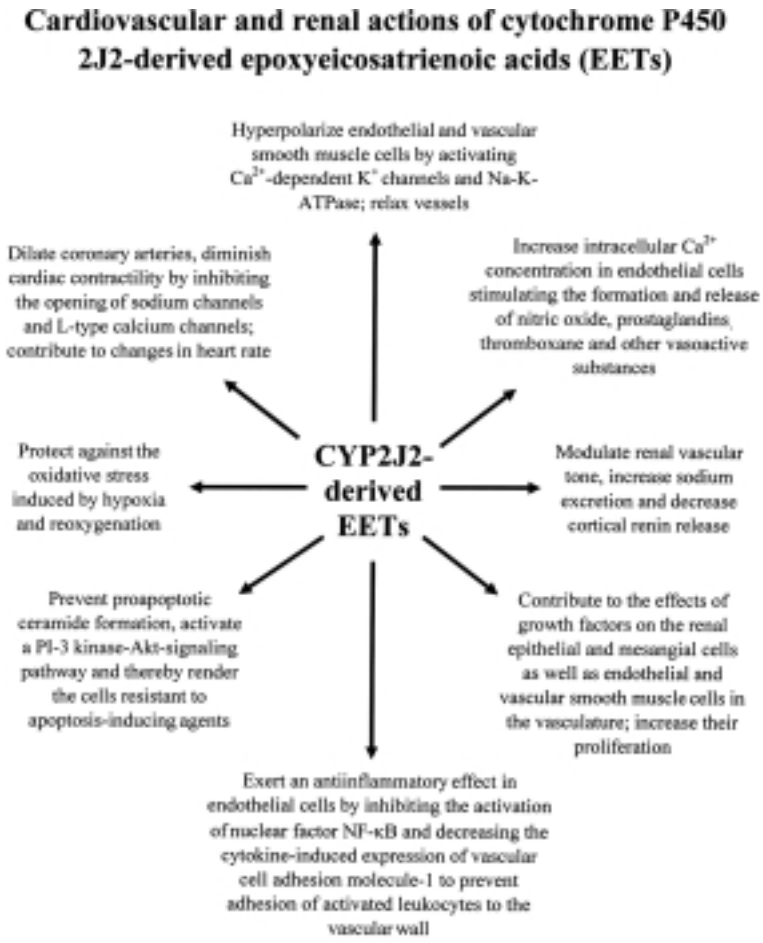

Fig. 1.

transport in cardiovascular and renal tissues, suggesting a crucial role of epoxygenase-derived eicosanoids in blood pressure regulation [7,16,32,36]. To demonstrate the involvement of these *CYP2J2*-derived eicosanoids in the pathogenesis of EH, a number of literature data were summarized in Figure. In the vascular cells, EETs activate Ca^{2+}-dependent K^{+}-channels and Na-K-ATPase resulting to the hyperpolarization of cell membranes and vasorelaxation [14,32]. Furthermore, EETs increase intracellular Ca^{2+} concentration in endothelial cells stimulating the formation and release of nitric oxide, prostaglandins (PGI, PGE_2, and $PGF_{2\alpha}$), thromboxane and other vasoactive substances [32]. In the kidney, EETs regulate renal hemodynamics, increase sodium excretion and decrease cortical renin release [21,32,37]. The endothelial cells transfected with *CYP2J2* are protected against the oxidative stress induced by hypoxia and reoxygenation [3]. 14,15-EET, a product of *CYP2J2* epoxygenase, activates a PI-3 kinase-Akt-signaling pathway and thereby renders the cells resistant to apoptosis-inducing agents such as hydrogen peroxide [18]. EETs have been suggested to increase the proliferation of various cell types, including vascular smooth muscle cells and endothelial cells demonstrating a potential role of cytochrome P450 eicosanoids to promote vascular remodeling [13,29].

1.2. A functional role of polymorphism G-50T of the CYP2J2 gene

Recent data demonstrate that human *CYP2J2* gene is highly polymorphic [25,30,34], and it has been proposed that genetic polymorphisms within the gene might contribute to expression and/or activity of the enzyme and, in turn, affect the biosynthesis of EETs. A number of single nucleotide polymorphisms (SNP) have been identified within human *CYP2J2* gene, however, only one common SNP (G-50T substitution with-

in the proximal promoter of the gene) was found to be functionally important [25]. The G-50T substitution interrupts a critical binding site for Sp1 transcription factor and thereby results in decreased *CYP2J2* promoter activity *in vitro* and reduced levels of *CYP2J2* epoxygenase metabolites *in vivo* [25,30].

1.3. Genetic studies of CYP2J2 G-50T polymorphism and human hypertension

Taking into account a number of cardiovascular and renal actions of cytochrome P450-derived EETs it is reasonable to hypothesize that the G-50T polymorphism of *CYP2J2* gene would contribute to pathogenesis of EH. However, only two studies have recently been done to search for association between G-50T polymorphism of *CYP2J2* gene and risk of EH [1,26]. The first study [1] showed no association of *CYP2J2* G-50T polymorphism with risk of EH in relatively small sample ($n = 108$) of African-American hypertensives. The second study, involving Caucasian hypertensive patients [26] has revealed that -50T variant allele was associated with the decreased risk of EH in males. Thus, these studies from 2 racial groups have yielded controversial results and show that the pathogenetic relevance of *CYP2J2* C-50T polymorphism should be clarified by independent studies in different populations. The present study was designed to investigate whether G-50T polymorphism of *CYP2J2* gene is associated with risk of essential hypertension in a Russian population.

2. Materials and methods

2.1. Study subjects

A total of 576 unrelated individuals, including 295 patients with EH and 281 healthy controls were recruited in this study. All study participants were of Russian origin from Central Russia. The Ethical Review Committee of the Kursk State Medical University has approved the study protocol. Written informed consent was obtained from all participants before the study. Hypertensive patients were recruited from the Cardiology Clinics of both Kursk Regional Clinical Hospital and Kursk Emergency Medicine Hospital between 2003 and 2006.

Demographic data were obtained on each subject from the official medical record at the time of enrollment and included the current age, sex and family history of essential hypertension. Subjects were defined as hypertensive according to World Health Organization criteria or if they were receiving any antihypertensive medication. Untreated hypertensive patients had established hypertension defined by a seated systolic and/or diastolic blood pressure greater than 140 and/or 90 mm Hg, respectively, on at least 2 separate measurements. Hypertension was diagnosed before the age of 60 years. All patients had no clinical signs, symptoms, and laboratory findings suggestive of secondary hypertension. Taking into account potentially confounding factors like coronary atherosclerosis and diabetes mellitus that might affect on the association between polymorphism G-50T of the *CYP2J2* and hypertension, all hypertensive and normotensive subjects with a history of diabetes mellitus and coronary artery disease were excluded from the study after physician examination, fasting lipid and glycemic profiles. Healthy individuals were categorized into a control group if they had a systolic blood pressure less than 130 mm Hg and/or a diastolic blood pressure less than 85 mm Hg on at least 3 separate measurements. Control subjects were on neither antihypertensives nor any other medication that could affect blood pressure values.

2.2. DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes by SDS/proteinase K treatment, phenol/chloroform extraction, and ethanol precipitation using a standard procedure. The G-50T polymorphism of *CYP2J2* gene was determined using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis (RFLP). To amplify a 242-base pair (bp) fragment of the *CYP2J2* gene, we used primers reported by Spiecker M. [30]. Amplification was performed in a final volume equal 25 μL of reaction mixture containing 1.5 U of thermostable *Taq* DNA polymerase (Lytech, Russia), about 1 μg DNA, 0.25 μM each primer, 250 μM of dNTPs, 2 mM of $MgCl_2$, 1x PCR-buffer of the following composition: 67mM Tris-HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 0.01% Tween-20. After PCR, 242-bp amplicon was digested with 5 U of the endonuclease *Alu*I (Sibenzyme, Russia) in a 10-μL reaction mixture at 37°C overnight according to the manufacturer's instructions. Digested products were resolved through ethidium bromide-stained 2% agarose gels and visualized under UV light on the GDS-8000 Computer Detection System (UVP Inc, USA). *Alu*I endonuclease cut PCR product into fragments sized 143 bp and 99 bp in the presence of *CYP2J2* -50T variant

Table 1
Baseline characteristics of study population

Baseline characteristics	Hypertensive patients n (%)	Healthy controls n (%)	p-values
Age mean $\pm$ S.D.	47.6 $\pm$ 10.1	46.1 $\pm$ 11.6	0.10
Gender	101 M (34.0%)	103 M (36.7%)	0.51
	196 F (66.0%)	178 F (63.3%)	
Family history of hypertension*	Yes 199 (78.3%)	Yes 98 (45.2%)	< 0.00001
	No 55 (21.7%)	No 119 (54.8%)	

S.D. – standard deviation; M – male, F – female.
*Family history data were obtained from 254 hypertensive and 217 normotensive probands.

allele, whereas *CYP2J2* $-$50G wild-type allele shows a single uncleaved 242-bp fragment. A "no template" control (water) and a positive control for the *CYP2J2*7* allele (DNA sample with a $-$50GT genotype of the *CYP2J2* gene) was used in each RFLP assay. The genotyping results were scored by two independent investigators who did not know whether the sample was from a case patient or a control. In addition, 20% of the samples from each study group were randomly selected to perform the repeated RFLP assays, and the results were 100% concordant. All heterozygous genotypes $-$50GT were confirmed by direct sequencing of the PCR products of the *CYP2J2* gene on ABI PRISM 310 Genetic Analyzer, with BigDye Terminator Kit 1.1 (Applied Biosystems; Foster City, CA, USA).

2.3. Statistical data analysis

Based on available data reported by two studies [25, 30] we estimated that at least a 10% difference for the *CYP2J2* alleles or genotypes could be present in patients. Based on this assumption, we calculated that it was necessary to study 199 subjects in each group in order to have 0.80 power and an alpha value of 0.05. Allele frequencies were estimated by the gene counting method, and Hardy-Weinberg equilibrium was tested by chi-square statistics. Frequencies of *CYP2J2* alleles and genotypes were compared between EH patients and controls by the use of Pearson's chi-square test with Yates' correction. The magnitude of the association of the *CYP2J2* G-50T polymorphism was estimated by odds ratio (OR) with approximate 95% confidence intervals (CI) calculated by Woolf's method [2]. A two-sided $p < 0.05$ was considered as statistically significant. Confounding influences of age, gender and family history of EH were assessed in a multiple logistic regression model. All analyses were performed using a STATISTICA version 6.0 for Windows software package (StatSoft Inc, USA).

3. Results

Demographics of the study subjects are listen in Table 1. There was no difference in a gender and age between groups of patients and controls ($p > 0.05$). As expected, a percentage of positive family history of hypertension was significantly greater in hypertensive versus normotensive subjects ($p < 0.00001$).

CYP2J2 genotype frequencies were in agreement with Hardy-Weinberg equilibrium in all groups ($p > 0.05$). Frequencies of alleles and genotypes of *CYP2J2* G-50T polymorphism in hypertensive patients and normotensive controls are shown in Table 2. Notably, frequency of $-$50T allele (*CYP2J2*7* allele) was somewhat lower in our Russian control sample than in other populations as reported by three independent studies [1,26,30]. No subjects with homozygous mutant genotype $-$50TT of *CYP2J2* gene were found in the study groups. The frequency of a $-$50T mutant allele of *CYP2J2* gene was significantly higher in EH patients versus healthy subjects (OR 4.03 95%CI 1.80–9.04 $p = 0.0004$). The heterozygous $-$50GT genotype was found in 10.2% of hypertensive patients whereas it was observed in only 2.5% of normotensive controls. The difference in a $-$50GT genotype between patients and controls remained significant (OR 4.78 95%CI 1.87–12.27 $p = 0.001$) after adjustment for confounding variables such as age, gender and family history of EH by unconditional multivariate logistic regression.

Taken into account a gender difference in susceptibility to complex human diseases we compared the frequency of *CYP2J2* variant between hypertensives and normotensives stratified by gender (Table 3). As can be seen in Table 3, the significant associations of a $-$50T allele with increased risk of hypertension have been found in both males (OR 3.86 95%CI 1.16–12.82 $p = 0.03$) and females (OR 3.89 95%CI 1.37–11.01 $p = 0.01$). A significant association of $-$50GT genotype with hypertension was observed in females (OR adjusted for confounding variables was 5.16 with 95%CI

Table 2
Distribution of alleles and genotypes of *CYP2J2* G-50T polymorphism in patients with essential hypertension and healthy subjects

Frequencies of alleles and genotypes of the *CYP2J2* G-50T polymorphism	Hypertensive patients ($n = 297$) n (%)[1]	Healthy controls ($n = 281$) n (%)[1]	χ^2 (p)[2]	OR (95% CI)[3]
Allele frequencies				
−50G allele	0.949	0.988	12.44	4.03
−50T allele	0.051	0.012	(0.0004)	(1.80-9.04)
Genotype frequencies				
−50GG genotype	265 (89.8)	274 (97.5)	12.87	4.78*
−50GT genotype	30 (10.2)	7 (2.5)	(0.0003)	(1.87–12.27)
−50TT genotype	0 (0.0)	0 (0.0)		

[1]Absolute number and percentage of individuals with particular genotype.
[2]Chi-square statistics with Yates' correction and p-values ($df = 1$).
[3]Odds ratio with 95% confidence intervals.
*Odds ratio adjusted for age, gender and family history of EH.

Table 3
Distribution of alleles and genotypes of *CYP2J2* G-50T polymorphism in patients with essential hypertension and healthy subjects stratified by gender

Frequencies of alleles and genotypes of the *CYP2J2* G-50T polymorphism	Hypertensive patients n (%)[1]	Healthy controls n (%)[1]	χ^2 (p)[2]	OR (95% CI)[3]
Male: hypertensive ($n = 100$), *normotensive* ($n = 103$)				
Allele frequencies				
−50G allele	0.940	0.985	4.68	3.86
−50T allele	0.060	0.015	(0.03)	(1.16–12.82)
Genotype frequencies				
−50GG genotype	88 (88.0)	100 (97.1)	4.87	3.78*
−50GT genotype	12 (12.0)	3 (2.9)	(0.03)	(0.90–15.90)
−50TT genotype	0 (0.0)	0 (0.0)		
Female: hypertensive ($n = 195$), *normotensive* ($n = 178$)				
Allele frequencies				
−50G allele	0.954	0.989	6.76	3.89
−50T allele	0.046	0.011	(0.01)	(1.37–11.01)
Genotype frequencies				
−50GG genotype	177 (90.8)	174 (97.8)	6.97	5.16*
−50GT genotype	18 (9.2)	4 (2.2)	(0.01)	(1.35–19.63)
−50TT genotype	0 (0.0)	0 (0.0)		

[1]Absolute number and percentage of individuals with particular genotype.
[2]Chi-square statistics with Yates' correction and p-values ($df = 1$).
[3]Odds ratio with 95% confidence intervals.
*Odds ratio adjusted for age and family history of EH.

1.35–19.63 $p = 0.016$). The association of −50GT genotype with increased risk of hypertension has been also found in males (unadjusted OR 4.55 95%CI 1.23–16.76 $p = 0.02$), but this association did not reach statistical significance (OR 3.78 95%CI 0.90–15.90 $p = 0.08$) after adjusting for age and family history of EH. To determine if there is a −50GT genotype × hypertension × gender interaction, a logistic regression analysis has been performed on a multiplicative scale. It has been found that OR for −50GT genotype × gender interaction (OR 4.48 95%CI 1.93–10.39 $p = 0.00048$) was slightly higher than OR for −50GT genotype (OR 4.43 95%CI 1.91–10.29 $p = 0.00052$), suggesting a weak effect of gender on the risk of hypertension in the heterozygous carriers of −50GT genotype.

We also examined *CYP2J2* allele and genotype frequencies in individuals with/without a family history of hypertension. Table 4 shows *CYP2J2* allele and genotype frequencies for hypertensive and normotensive subjects stratified by family history of EH. As shown in Table 4, among study subjects with or without a family history of hypertension, a −50T allele frequency was significantly higher in patients with hypertension versus healthy controls ($p < 0.05$). Adjusted odds ratios of

Table 4
Distribution of alleles and genotypes of *CYP2J2* G-50T polymorphism in patients with essential hypertension and healthy subjects stratified by family history

Frequencies of alleles and genotypes of the *CYP2J2* G-50T polymorphism	Hypertensive patients n (%)[1]	Healthy controls n (%)[1]	χ^2 (p)[2]	OR (95% CI)[3]
Positive family history: hypertensive ($n = 197$), *normotensive* ($n = 98$)				
Allele frequencies				
−50G allele	0.954	0.990	4.01	3.82
−50T allele	0.046	0.010	(0.05)	(1.01–14.48)
Genotype frequencies				
−50GG genotype	179 (90.9)	96 (98.0)	4.15	4.93*
−50GT genotype	18 (9.1)	2 (2.0)	(0.04)	(1.06–23.01)
−50TT genotype	0 (0.0)	0 (0.0)		
Negative family history: hypertensive ($n = 55$), *normotensive* ($n = 119$)				
Allele frequencies				
−50G allele	0.927	0.979	4.25	3.52
−50T allele	0.073	0.021	(0.04)	(1.17–10.55)
Genotype frequencies				
−50GG genotype	47 (85.5)	114 (95.8)	4.42	3.90*
−50GT genotype	8 (14.5)	5 (4.2)	(0.04)	(1.19-12.81)
−50TT genotype	0 (0.0)	0 (0.0)		

[1]Absolute number and percentage of individuals with particular genotype.
[2]Chi-square statistics with Yates' correction and p-values ($df = 1$).
[3]Odds ratio with 95% confidence intervals.
*Odds ratio adjusted for age and gender.

having hypertension attributable to carrying a *CYP2J2* −50GT genotype was 3.90 (95%CI 1.19–12.81 $p = 0.02$) for subjects with negative family history of EH and 4.93 (95%CI1.06–23.01 $p = 0.04$) for subjects with positive family history of the disease (Table 4). Multiplicative analysis of −50GT genotype × hypertension × family history interaction has revealed no effect of a family history of EH on the risk of hypertension in individuals possessing a *CYP2J2* −50GT genotype (OR for −50GT genotype OR 4.43 $p = 0.00052$; OR for −50GT genotype × family history interaction was 4.23 $p = 0.0017$).

4. Discussion

In the present study, we investigated an association between polymorphism G-50T of the *CYP2J2* gene and essential hypertension in an ethnically homogeneous Russian population. This is a first study reporting on the allele and genotype frequencies of polymorphism G-50T of the *CYP2J2* gene in a Russian population. We found for the first time that the −50T variant allele (*CYP2J2*7* allele) and −50GT genotype of *CYP2J2* epoxygenase −50T gene is clearly associated with an increased risk of hypertension. Odds ratio for this association was slightly higher in females than in males suggesting a weak effect of gender on the hypertension susceptibility attributed to polymorphism G-50T of the *CYP2J2* gene. A gender effect on the genetic susceptibility to complex diseases is a common finding in genetic association studies [20,24]. On the one hand, a gender difference in the genetic susceptibility to complex diseases can arise from specific gene-gene and/or gene-environment interactions within different ethnic backgrounds, on the other hand, it might be particularly explained for studies with cytochrome P450 genes, including *CYP2J2* gene, that have been found to play a crucial role in the metabolism of sex hormones [5,8].

On the whole, the results of our study appear to be at variance with data reported by King LM, et al. [26] who have observed a significant association between G-50T variant of *CYP2J2* gene and the decreased risk of EH in Caucasian males and Caucasians without a family history of hypertension, suggesting a protective affect of the mutation. These findings are somewhat surprising given that G-50T substitution leads to a reduced transcription of the *CYP2J2* gene [30]. The authors explained these data by concluding that *CYP2J2*-derived EETs have also been found to cause vasoconstriction in certain vascular beds and under certain experimental conditions [9,23,35], suggesting that these eicosanoids seem to have the prohypertensive actions. By contrast, given that *CYP2J2*-derived EETs are potent vasodilators, persons with diminished capacity to synthesize EETs due to the downregulation of *CYP2J2* expression (i.e. carriers of G-50T variant allele) might be more susceptible to hypertension. As can be seen above

(Fig. 1), the mechanisms by which plausible deficiency of *CYP2J2*-derived EETs might cause EH include: a) dysfunction in ion channel-dependent vasorelaxation, b) insufficiency to stimulate the formation and release of natural vasodilators, c) increased cardiac contractility and heart rate, d) increased cortical renin release and decreased sodium excretion in kidneys, e) promoted both apoptotic and oxidative vascular injury. These examples demonstrate a number of potential mechanisms by which G-50T mutation of *CYP2J2* gene might contribute to development of essential hypertension. Taking into account a majority of single copy of the mutant *CYP2J2*7* allele carriers identified in our study were found to have hypertension the G-50T mutation in proximal promoter of *CYP2J2* gene seems to have a dominant effect on the disease development.

In addition, numerous reasons might be responsible for discrepancy of our results with results reported by Dreisbach A.W., et al. [1] and King LM, et al. [26]. These reasons might include: 1) a difference in the genetic backgrounds between populations studied; 2) confounding by population stratification within ethnically heterogeneous American populations 3) chance or artifact, and possibly 4) due to yet unspecified environmental or genetic factors in populations studied. Furthermore, the discrepancy between gender-related associations of *CYP2J2*7* allele with hypertension in Caucasian populations might be due to a greater percentage of males in hypertensive group than in control group in the study of King LM, et al. [26].

The finding of low frequency of the *CYP2J2*7* allele in our control population is another issue that should be also discussed. The cohort presented in the paper is a part of our large Russian population sample including greater than 1000 participants with various pathologies like hypertension, asthma, peptic ulcer disease etc. Taking into account an important role of *CYP2J2* gene polymorphism in the development of different diseases (to date a number of diseases were found to be associated with *CYP2J2* gene polymorphism), we have recruited only healthy subjects having no either chronic diseases or positive family history of diseases mentioned above. In particular, to collect the appropriate normotensive controls we have used more strict criteria of inclusion of normotensives into our control group (a systolic blood pressure < 130 mm Hg and/or a diastolic blood pressure < 85 mm Hg) than those reported in related studies (a systolic blood pressure < 140 mm Hg and/or a diastolic blood pressure < 90 mm Hg). Taken together it seems to contribute into the low frequency of *CYP2J2*7* allele in our control population. Unfortunately, we cannot to compare our data with other Russian populations because the present study is a first report on the frequencies of G-50T polymorphism of the *CYP2J2* gene in a Russian population.

Certainly, the results of our study require further investigation in other population-based studies. Further work is clearly needed to address issues discussed above, but these studies will need to be substantially larger than the association studies published to date. Furthermore, functional genomics studies in combination with association studies are required to substantiate a relevance of polymorphism G-50T of the *CYP2J2* gene in the pathogenesis of essential hypertension. However, it is reasonable to expect an irregular replication of original data by further studies in different populations [15,19]. It is not surprising that the difficulty in understanding complex diseases like hypertension arises from the complexity and variability of the clinical phenotypes, the genetic heterogeneity of disease etiology, as well as from the racial/ethnic difference in gene-gene and gene-environment interactions that have been formed evolutionarily within each human population [6,10–12,27]. A limitation of our study was that we did not investigate common SNPs that have not been previously shown to be functionally significant (i.e. silent C173T SNP in exon 1, T18778G SNP in intron 5, C33291T, T33370A and A33465G in 3-prime untranslated region of the gene [30]) or in linkage disequilibrium with G-50T polymorphism in the *CYP2J2* gene. Nevertheless, the association of frequent, biologically plausible G-50T SNP in the *CYP2J2* gene with EH that has been found in the present study should be seen as a hypothesis-generating observation, and highlights the importance of P-450 epoxygenase pathway of arachidonic acid metabolism in the pathogenesis of hypertensive disease.

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