Hypertension Research (2019) 42:257–272
https://doi.org/10.1038/s41440-018-0142-1

ARTICLE

A comprehensive study revealed SNP–SNP interactions and a sex-dependent relationship between polymorphisms of the *CYP2J2* gene and hypertension risk

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Received: 31 August 2017 / Revised: 18 June 2018 / Accepted: 12 July 2018 / Published online: 5 December 2018

Abstract

This study investigated whether common polymorphisms of cytochrome P450 2J2 (CYP2J2), a major enzyme that controls the biosynthesis of vasoactive epoxyeicosatrienoic acids, are collectively involved in the molecular basis of essential hypertension (EH). A total of 2314 unrelated Russian subjects from the Kursk (discovery sample: 913 EH patients and 645 controls) and Belgorod (replication sample: 345 EH patients and 411 controls) regions were recruited for this study. Eight single nucleotide polymorphisms (SNPs), including rs890293, rs11572182, rs10493270, rs1155002, rs2280275, rs7515289, rs11572325, and rs10889162, of *CYP2J2* were genotyped using the MassARRAY 4 system and TaqMan-based assays. Significant associations were identified among the SNPs rs890293 (OR = 2.17, 95%CI 1.30–3.65), rs2280275 (OR = 1.59, 95%CI 1.10–2.37) and rs11572325 (OR = 1.89, 95%CI 1.22–2.95) and the risk of EH in females from the Kursk population. Sixteen *CYP2J2* genotype combinations only showed significant associations with EH risk only in females. A common haplotype, T-T-G-C-C-C-T-A, increased the risk of EH in females. The bioinformatic analysis enabled identification of the SNPs that possess regulatory potential and/or are located within the binding sites for multiple transcription factors that play roles in the pathways involved in hypertension pathogenesis. Moreover, the polymorphisms rs890293, rs2280275, and rs11572325 were found to be significantly associated with hypertension risk in the Belgorod population. In conclusion, the rs2280275 and rs11572325 SNPs of *CYP2J2* may be considered novel genetic markers of hypertension, at least in Russian women. However, sex-specific associations between *CYP2J2* gene polymorphisms and hypertension require further investigation to clarify the specific genetic and/or environmental factors that are responsible for the increased disease susceptibility of women compared to that of men.

Keywords: essential hypertension · arachidonic acid metabolism · epoxyeicosatrienoic acids · CYP2J2 · single nucleotide polymorphism · sex dimorphism.

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41440-018-0142-1) contains supplementary material, which is available to authorized users.

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Introduction

Essential hypertension (EH) represents a multifactorial disorder with a high worldwide prevalence and related increased rates of morbidity, mortality, and disability from cardiovascular and cerebrovascular complications [1, 2]. The mechanisms of blood pressure regulation are complex and are determined by tight interactions between various genetic and environmental factors [3, 4]. Candidate gene and genome-wide association studies have identified numerous genetic variants that influence blood pressure and hypertension susceptibility across different populations; nevertheless, few genetic loci have been successfully validated in independent studies [5–8].

Despite the progress in the omics sciences in the previous decade, the molecular mechanisms of hypertension remain to be defined, such as the role of genes involved in the biosynthesis of vasoactive eicosanoids synthesized from arachidonic acid (AA) in disease pathogenesis. An increasing body of studies has provided compelling evidence that epoxyeicosatrienoic acids (EETs), vasoactive products of AA derived from the cytochrome P450 epoxygenase pathway, possess antihypertensive, anti-inflammatory and cardioprotective properties [9–11]. EETs regulate the vascular tone and fluid-electrolyte transport in cardiovascular and renal tissues, as well as protect cells against the oxidative stress induced by hypoxia and reoxygenation [10, 12, 13]. These biological functions made the metabolism of EETs an attractive pathway for investigating hypertension pathogenesis.

Cytochrome P-450 2J2 (CYP2J2), a major enzyme of the epoxygenase pathway in the heart and vasculature, metabolizes AA to all four regioisomeric cis-EETs, including 5,6-, 8,9-, 11,12- and 14,15-EETs [10, 13–15]. Taking into account the multifaceted cardiovascular and renal actions of EETs, single nucleotide polymorphisms (SNP) of the *CYP2J2* gene have become the targets for genetic association studies of cardiovascular disorders in various populations [16–27]. However, a limited number of studies has been conducted to date to investigate the contribution of *CYP2J2* gene polymorphisms to hypertension susceptibility [28–33]. Many of these association studies have shown inconsistent and inconclusive results, which may be due to different genetic factors underlying hypertension in diverse populations and different patterns of linkage disequilibrium between the investigated loci and the causal variant. Moreover, a substantial number of studies have been performed in populations with a small sample size and a limited number of *CYP2J2* polymorphisms. Thus, there is a clear need for a large and comprehensive research study with numerous SNPs investigated simultaneously so that a true estimate of the hypertension risk related to CYP2J2 gene polymorphisms can be obtained within a defined population. The present study was designed to investigate whether common polymorphisms of the *CYP2J2* gene are comprehensively involved in the molecular basis of EH.

Methods

Study participants

The study protocol was approved by the Ethical Review Committee of Kursk State Medical University and Belgorod State University, and written informed consent was obtained from all participants prior to recruitment. The discovery sample, including 1558 unrelated individuals (913 patients with EH and 645 age- and sex-matched healthy controls), was recruited from the Cardiology Clinics of Kursk Regional Clinical Hospital and the Neurology Clinics of Kursk Emergency Medicine Hospital. These study patients were enrolled during two study periods: the first period was between 2003 and 2006 and the second period was between 2007 and 2012 [34–37]. The control group included blood donors, healthy volunteers and hospital-based patients (all subjects were recruited from the surgical, traumatic, and infectious divisions of Kursk hospitals) with normal blood pressure and without cardiovascular or other chronic diseases. The control group was recruited over the same periods of time [34–38]. To ensure an ethnically homogeneous population, only Russian residents living in Central Russia for at least two generations have been included in the study. The replication population included DNA samples obtained from 756 unrelated Russian individuals (345 hypertensive patients and 411 controls) as a part of the biobank of Belgorod State University. Detailed information regarding this population is described elsewhere [36].

The diagnosis of EH in both population samples was established by qualified cardiologists according to the WHO criteria or if patients had a history of receiving an antihypertensive drug. EH in untreated patients was defined as a seated systolic and/or diastolic blood pressure above 140 and/or 90 mm Hg, respectively, during at least two separate measurements. All hypertensives included in the study had no clinical signs, symptoms, or laboratory findings suggestive of secondary hypertension. All study subjects completed a questionnaire concerning conventional demographic characteristics, which are summarized in Table 1.

Selection of SNPs and genetic analysis

Potentially functional SNPs were selected using a set of web-based SNP selection tools available at SNPinfo Web

Table 1 Baseline and clinical characteristics of the study populations

Baseline and clinical characteristics	Healthy controls	EH patients	*P*-values
Discovery sample (Kursk region): 645 normotensive controls and 913 EH patients			
Age mean ± S.D	58.7 ± 7.6	58.4 ± 8.9	0.49
Gender, *N* (%) (M – male, F- female)	M 365 (56.6) F 280 (43.4)	M 488 (53.5) F 425 (46.5)	0.24
Mean systolic blood pressure (mm Hg)	124.7 ± 8.6	162.3 ± 10.2	**<0.001**
Mean diastolic blood pressure (mm Hg)	76.8 ± 9.5	102.9 ± 10.7	**<0.001**
Body mass index (kg/m^2)	27.4 ± 8.3	28.2 ± 10.5	0.11
Positive family history of hypertension, *N* (%)	312 (49.8)	568 (62.8)	**0.004**
Replication sample (Belgorod region): 411 population controls and 345 EH patients			
Age mean ± S.D.	57.1 ± 9.8	58.2 ± 9.7	0.12
Gender, *N* (%) (M – male, F- female)	M 260 (63.3) F 154 (36.7)	M 203 (58.8) F 142 (41.2)	0.24
Mean systolic blood pressure (mm Hg)	NA	168.4 ± 9.5	–
Mean diastolic blood pressure (mm Hg)	NA	105.6 ± 9.3	–
Body mass index (kg/m^2)	25.6 ± 3.8	26.1 ± 4.6	0.12
Positive family history of hypertension, *N* (%)	NA	NA	–

Bolded is statistically significant *P*-value

NA not available

Server (https://snpinfo.niehs.nih.gov). The selection of SNPs was based on their predicted functional characteristics, minor allele frequency (MAF>5% in Europeans) and haplotype tagging properties. Initially, ten common SNPs were selected for the study. Two selected SNPs (rs2271800 and rs10889160) showed too low genotype call rates (<60%) and were therefore excluded from the study. Although the polymorphism −76G>T (rs890293) showed a call rate of 81.5%, the SNP was included in the study because this functional polymorphism in the promoter of the *CYP2J2* gene represents the most interesting genetic variant that has been intensively investigated in cardiovascular diseases in different populations of the world. In addition, this SNP was found to be associated with hypertension risk in our preliminary study conducted on a small population sample of the Kursk region [31]. The average call rate for the remaining seven SNPs was 99.4% (Supplementary Table 1). Thus, eight SNPs, including rs890293, rs11572182, rs10493270, rs1155002, rs2280275, rs7515289, rs11572325 and rs10889162, of the *CYP2J2* gene were included for statistical data analysis.

Total DNA was isolated from 5 ml peripheral blood samples obtained from all study subjects using standard procedures of SDS–proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. SNP genotyping was performed by a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) iPLEX platform (Agena Bioscience, Inc., San Diego, CA, USA) at the Core Facility "Medical Genomics" in the Research Institute of Medical Genetics (Tomsk, Russia). The primer sequences used for genotyping the *CYP2J2* polymorphisms are shown in Supplementary Table 1. Multiplex polymerase chain reaction was performed on the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Genotypes of *CYP2J2* in the discovery population (Kursk region) were analyzed via the MassARRAY 4 system (Agena Bioscience, Inc., San Diego, CA, USA). The replication cohort (Belgorod region) was used for genotyping the SNPs that showed significant associations with hypertension risk in the discovery population. Genotyping was performed using TaqMan-based real-time PCR assays (Applied Biosystems, USA) on the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, USA). Blind replicates were included for quality control. As quality controls, ~5% of the samples were randomly selected for repeated genotyping, and the analysis showed 100% reproducibility of the initial genotyping results.

The genotype and mRNA expression data for the *CYP2J2* gene obtained from the Genotype-Tissue Expression (GTEx) project were used to evaluate genotype–phenotype correlations [39, 40]. The GTEx project was created to establish a sample and data resource for studies on the relationship between genetic variation and gene expression in multiple human tissues. The expression levels were evaluated using the Affymetrix Expression Array at the GTEx Laboratory Data Analysis and Coordinating Center (Broad Institute) in samples of tibial arteries obtained from 285 postmortem individuals. The transcription levels were called via the RNA-SeQC tool, after filtering for unique mapping, proper pairing, and exon overlap. Genotyping data were obtained using the Illumina

OMNI 5M SNP Array (a description of the laboratory methods is available at the website https://www.gtexportal.org/home/documentationPage#staticTextLabMethods).

Statistical and bioinformatic data analyses

Statistical power was estimated using the Genetic Association Study (GAS) Power Calculator online (http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html) assuming an EH prevalence of 40% and the existing sample sizes of 913 hypertensive patients and 645 healthy controls in Kursk population. Analysis of the association between various SNPs and hypertension risk (multiplicative model) could detect the genotype relative risk (GRR) of 1.25-1.41 assuming 80% power and a 5% type I error ($\alpha = 0.05$). Allele frequencies were estimated by the gene counting method: the total number of copies of an allele is calculated across the studied group by taking twice the number of homozygotes for this allele plus the number of heterozygotes. The frequency of the allele is subsequently calculated as a proportion of the allele counts divided by double the total sample size. The chi-square test was used to identify significant departures from Hardy–Weinberg equilibrium. Categorical variables, such as sex and family history of hypertension, were compared using the chi-square test. SNPStats software [41] was applied to evaluate the frequencies of alleles, genotypes and haplotypes of *CYP2J2*. The strength of the association of alleles, genotypes and haplotypes with EH risk in gender-stratified groups was measured by multiple logistic regression analysis to calculate odds ratios (OR) with 95% confidence intervals (CI) and adjusted for age and body mass index (BMI). A *P*-value ≤ 0.05 was considered statistically significant. To control for multiple testing, false discovery rate (FDR) based *Q*-values were calculated for each SNP association using the method proposed by Benjamini and Hochberg [42] and implemented in the FDR calculator available online at http://www.sdmproject.com/utilities/?show=FDR.

The effects of the investigated SNPs on the *CYP2J2* mRNA levels (data from the GTEx project) were evaluated by *T*-statistics. The functionality of the SNPs was also assessed in silico by the SNP Function Prediction tool developed by Xu and Taylor [43] and available online (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). This tool utilizes the information of the TRANSFAC database (BIOBASE Corporation, Wolfenbuettel, Germany) on potential transcription factor recognition sites. Only transcription factor binding sites (TFBS) for which the core or matrix match score was impacted or that were eliminated or created by variant sequences are considered to be regulatory within a particular SNP. rSNPBase, a database of curated regulatory SNPs (http://rsnp.psych.ac.cn), was also used to analyze and interpret genotype-phenotype relationships [44].

Results

Associations between *CYP2J2* gene polymorphisms and EH

The allele and genotype frequencies of the *CYP2J2* gene polymorphisms in the discovery population stratified by gender are shown in Table 2. The observed genotype frequencies of all SNPs in the case and control groups were in Hardy–Weinberg equilibrium ($P > 0.05$). The allele and genotype frequencies for the investigated *CYP2J2* polymorphisms were in accordance with those reported in other European populations (SNP database, https://www.ncbi.nlm.nih.gov/snp). As shown in Table 2, allele −76T of SNP rs890293 showed an association with an increased risk of EH in females: the odds ratio adjusted for age and BMI ($_{adj}$OR) was 2.05 (95%CI 1.23–3.39, $P = 0.004$, $Q = 0.02$). A significant difference in the distribution of genotype −76G/T was identified between the hypertensive and normotensive females ($_{adj}$OR = 2.17, 95%CI 1.30–3.65, $P = 0.008$, $Q = 0.04$). Moreover, two other polymorphisms, rs2280275, and rs11572325, of *CYP2J2* were significantly associated with the risk of EH in females. In particular, allele C and genotype T/C of SNP rs2280275 were associated with an increased risk of EH: $_{adj}$OR = 1.50, 95%CI 1.11–2.05, $P = 0.01$, $Q = 0.03$ and $_{adj}$OR = 1.59, 95%CI 1.10–2.37, $P = 0.01$, $Q = 0.04$, respectively. The increased risk of hypertension in females was also associated with a carriage of allele T and genotype A/T for SNP rs11572325: $_{adj}$OR = 1.74, 95%CI, $P = 0.06$, $Q = 0.02$ and $_{adj}$OR = 1.89, 95%CI 1.22–2.95, $P = 0.02$, $Q = 0.05$, respectively. As shown in Table 2, these associations remained statistically significant after adjustment for multiple testing ($Q \leq 0.05$). Notably, no associations of the *CYP2J2* gene polymorphisms with the risk of EH were identified in males ($P > 0.05$). We subsequently performed an association analysis for the polymorphisms that showed significant associations with EH risk in the discovery population (SNPs rs890293, rs2280275, and rs11572325). Table 3 shows the frequencies of the genotypes and alleles for the three *CYP2J2* SNPs in Belgorod individuals. As shown in Table 3, these polymorphisms were found to be significantly ($P < 0.05$, $Q < 0.05$) associated with hypertension risk in the Belgorod population. To assess whether estrogens contribute to hypertension in females, we analyzed the associations of SNPs rs890293, rs2280275 and rs11572325 with EH risk separately in pre- and post-menopausal women (i.e., women younger or 45 years old, respectively) from the Kursk population (all hypertensive women from the Belgorod

Table 2 Genotype and allele frequencies for SNPs of the *CYP2J2* gene in hypertensive patients and healthy controls (discovery population)

Polymorphism	Genotype, allele	Males, *N* (%)[a]					Females, *N* (%)[a]				
		Healthy controls	EH patients	*P*-value	*Q*-value	$_{adj}$OR (95% CI)[b]	Healthy controls	EH patients	*P*-value	*Q*-value	$_{adj}$OR (95% CI)[b]
rs890293	G/G	253 (88.2)	330 (87.3)	0.48	0.88	1.00	223 (90.7)	292 (81.3)	**0.008**	**0.04**	1.00
	G/T	34 (11.8)	46 (12.2)			1.02 (0.63–1.67)	22 (8.9)	64 (17.8)			2.17 (1.30–3.65)
	T/T	0 (0.0)	2 (0.5)			–	1 (0.4)	3 (0.8)			1.62 (0.27-11.43)
	T	34 (5.9)	50 (6.6)	0.67	0.99	1.11 (0.71–1.76)	24 (4.9)	70 (9.7)	**0.004**	**0.02**	2.05 (1.23–3.39)
rs11572182	T/T	266 (72.9)	353 (72.3)	0.83	0.95	1.00	193 (68.9)	310 (72.9)	0.52	0.59	1.00
	T/G	91 (24.9)	127 (26.0)			1.07 (0.79–1.48)	79 (28.2)	105 (24.7)			0.82 (0.58–1.19)
	G/G	8 (2.2)	8 (1.6)			0.73 (0.29–1.92)	8 (2.9)	10 (2.4)			0.83 (0.35–2.01)
	G	107 (14.7)	143 (14.7)	0.95	0.99	1.01 (0.77–1.36)	95 (17.0)	125 (14.7)	0.30	0.40	0.81 (0.60–1.28)
rs10493270	G/G	294 (80.8)	397 (81.4)	0.97	0.97	1.00	219 (78.2)	337 (79.3)	0.87	0.87	1.00
	G/A	67 (18.4)	87 (17.8)			0.95 (0.67–1.38)	59 (21.1)	86 (20.2)			0.94 (0.63–1.40)
	A/A	3 (0.8)	4 (0.8)			0.95 (0.23–3.92)	2 (0.7)	2 (0.5)			0.64 (0.10–3.87)
	A	73 (10.0)	95 (9.7)	0.92	0.99	0.94 (0.69–1.36)	63 (11.3)	90 (10.6)	0.79	0.79	0.92 (0.62–1.38)
rs1155002	C/C	149 (41.0)	168 (39.9)	0.09	0.71	1.00	110 (39.4)	177 (44.5)	0.27	0.43	1.00
	C/T	181 (49.9)	193 (45.8)			0.83 (0.65–1.11)	124 (44.4)	172 (43.2)			1.30 (0.95–1.81)
	T/T	33 (9.1)	60 (14.3)			1.62 (1.01–2.42)	45 (16.1)	49 (12.3)			0.87 (0.56–1.39)
	T	247 (34.0)	313 (37.2)	0.22	0.99	1.12 (0.92–1.45)	214 (38.4)	270 (33.9)	0.12	0.21	0.82 (0.65–1.05)
rs2280275	T/T	258 (71.1)	346 (70.9)	0.66	0.88	1.00	214 (77.3)	286 (67.5)	**0.01**	**0.04**	1.00
	T/C	101 (27.8)	133 (27.3)			0.96 (0.70–1.35)	57 (20.6)	125 (29.5)			1.59 (1.10–2.37)
	C/C	4 (1.1)	9 (1.8)			1.59 (0.58–5.14)	6 (2.2)	13 (3.1)			1.35 (0.51–3.64)
	C	109 (15.0)	151 (15.5)	0.84	0.99	1.04 (0.81–1.37)	69 (12.5)	151 (17.8)	**0.01**	**0.03**	1.50 (1.11–2.05)
rs7515289	A/A	195 (53.4)	262 (53.7)	0.27	0.83	1.00	154 (55.0)	220 (51.9)	0.47	0.59	1.00
	A/C	153 (41.9)	191 (39.1)			0.88 (0.66–1.22)	100 (35.7)	170 (40.1)			1.21 (0.89–1.63)
	C/C	17 (4.7)	35 (7.2)			1.54 (0.89–2.94)	26 (9.3)	34 (8.0)			0.83 (0.49–1.46)
	C	187 (25.6)	261 (26.7)	0.65	0.99	1.05 (0.87–1.39)	152 (27.1)	238 (28.1)	0.73	0.79	1.03 (0.85–1.41)
rs11572325	A/A	296 (81.1)	398 (82.2)	0.61	0.88	1.00	242 (86.4)	326 (76.9)	**0.02**	**0.05**	1.00
	A/T	67 (18.4)	81 (16.7)			0.88 (0.61–1.29)	35 (12.5)	92 (21.7)			1.89 (1.22–2.95)
	T/T	2 (0.5)	5 (1.0)			1.65 (0.42–7.78)	3 (1.1)	6 (1.4)			1.22 (0.34–4.59)
	T	71 (9.7)	91 (9.4)	0.88		0.94 (0.66–1.44)	41 (7.3)	104 (12.3)	**0.006**	**0.02**	1.74 (1.19–2.58)
rs10889162	G/G	308 (85.6)	403 (85.9)	0.31	0.83	1.00	244 (87.8)	347 (82.4)	0.12	0.24	1.00
	G/A	52 (14.4)	63 (13.4)			0.93 (0.61–1.38)	31 (11.2)	71 (16.9)			1.61 (1.00–2.56)
	A/A	0 (0.0)	3 (0.6)			–	3 (1.1)	3 (0.7)			0.65 (0.13–3.24)
	A	52 (7.2)	69 (7.4)	0.99	0.99	1.01 (0.69–1.53)	37 (6.7)	77 (9.1)	0.13	0.21	1.41 (0.95–2.17)

Statistically significant *P*-values and *Q*-values are bolded

[a]Absolute number and percentage of individuals/chromosomes with particular genotype/allele

[b]Odds ratio with 95% confidence intervals adjusted for age and BMI

Table 3 Genotype and allele frequencies for the three SNPs of *CYP2J2* in Belgorod EH patients and controls

Polymorphism	Genotype, allele	Males, *N* (%)[a]					Females, *N* (%)[a]				
		Controls	EH patients	*P*-value	*Q*-value	$_{adj}$OR (95% CI)[b]	Controls	EH patients	*P*-value	*Q*-value	$_{adj}$OR (95% CI)[b]
rs890293	G/G	182 (87.5)	162 (87.6)	0.21	0.29	1.00	122 (91.7)	110 (83.3)	**0.037**	**0.037**	1.00
	G/T	26 (12.5)	21 (11.3)			0.91 (0.49–1.67)	10 (7.5%)	22 (16.7)			2.43 (1.10–5.39)
	T/T	0 (0.0)	2 (1.1)			NA (0.00–NA)	1 (0.8)	0 (0.0)			0.00 (0.00–NA)
	T	26 (6.3)	25 (6.8)	0.77	0.93	1.09 (0.62–1.92)	12 (4.5)	22 (8.3)	0.07	0.07	1.90 (0.91–3.99)
rs2280275	T/T	179 (68.8)	142 (70.0)	0.29	0.29	1.00	119 (78.8)	91 (64.1)	**0.016**	**0.037**	1.00
	T/C	79 (30.4)	56 (27.6)			0.89 (0.59–1.34)	28 (18.5)	47 (33.1)			2.19 (1.25–3.84)
	C/C	2 (0.8)	5 (2.5)			3.15 (0.60–16.48)	4 (2.6)	4 (2.8)			1.30 (0.30–5.53)
	C	83 (1.6)	66 (1.6)	0.90	0.93	1.02 (0.72–1.45)	36 (11.9)	55 (19.4)	**0.013**	**0.031**	1.74 (1.11–2.81)
rs11572325	A/A	213 (81.9)	170 (83.7)	0.056	0.17	1.00	131 (86.8)	107 (75.3)	**0.036**	**0.037**	1.00
	A/T	47 (18.1)	30 (14.8)			0.80 (0.48–1.32)	18 (11.9)	33 (23.2)			2.22 (1.19–4.07)
	T/T	0 (0.0)	3 (1.5)			NA (0.00–NA)	2 (1.3)	2 (1.4)			1.20 (0.15–8.94)
	T	47 (9.0)	36 (8.9)	0.93	0.93	0.98 (0.62–1.54)	22 (7.3)	37 (13.0)	**0.021**	**0.031**	1.90 (1.07–3.54)

Statistically significant *P*-values and *Q*-values are bolded

NA not available

[a]Absolute number and percentage of individuals/chromosomes with particular genotype/allele.

[b]Odds ratio with 95% confidence intervals adjusted for age and BMI

population were postmenopausal). The results are shown in Supplementary Table 2. Although the group of pre-menopause women was too small (85 EH patients and 56 controls), all three polymorphisms tended to be associated with the risk of EH in both the pre- and post-menopausal women, which suggests that sex hormones do not explain the relationship between the SNPs and disease risk in women.

Interactions between SNPs of *CYP2J2* and risk of EH

To investigate whether interactions between SNPs of *CYP2J2* contribute to the disease risk, we performed an association analysis of genotype combinations with EH risk in gender-stratified groups. Table 4 summarizes the significant associations identified between *CYP2J2* genotype combinations and the risk of EH. Twenty-seven genotype combinations showed significant associations with the risk of EH in females. Notably, eight of these associations were due to SNP–SNP interactions that involved polymorphism rs890293, whereas the SNP rs2280275 was identified in eight genotype combinations associated with hypertension risk in females. Moreover, six disease-associated genotype combinations in females included the rs11572325 genotypes. As shown in Table 4, only four *CYP2J2* genotype combinations were associated with the risk of EH in males.

Table 5 shows the estimated *CYP2J2* haplotypes and their associations with hypertension risk in females and males. Eight common *CYP2J2* haplotypes (frequency ≥ 1%) were identified in both males and females. Haplotypes H1 (G-T-G-C-T-A-A-G) and H2 (G-T-G-T-T-A-A-G) were found in approximately 60% of the studied individuals. A significant difference in the distribution of haplotypes was observed between the hypertensive and normotensive females ($P = 0.0052$), whereas no significant difference in the haplotype distribution was identified between the hypertensive and normotensive males ($P = 0.22$). Haplotype H5 (T-T-G-C-C-C-T-A) was found to be associated with an increased risk of EH in females (OR = 1.70, 95%CI 1.02–2.84, $P = 0.041$). It is important to note that the rare haplotypes (frequency≤1%, Supplementary Table 3) showed a significant association with hypertension susceptibility in both females ($_{adj}$OR = 4.17, 95%CI 1.21–14.23, $P = 0.024$) and males ($_{adj}$OR = 2.81, 95%CI 1.16–6.94, $P = 0.027$).

Figure 1 shows the pairwise linkage disequilibrium coefficients (D' and r) between the SNPs of the *CYP2J2* gene in females and males. All investigated SNPs were in linkage disequilibrium to each other with various degrees. In particular, the D'-values between SNPs rs890293 and rs1155002, rs890293 and rs11572325, and rs1155002 and rs10889162 were stronger in the males than in the females, whereas the D'-values between the polymorphisms

Table 4 Associations between *CYP2J2* genotype combinations and with the risk of essential hypertension

№	Genotype combinations	Healthy controls, *N* (%)[a]	EH patients, *N* (%)[a]	*P*-value	*Q*-value	$_{adj}$OR (95% CI)[b]
Females (425 cases, 280 controls)						
1	rs11572182TT/rs890293GT	16 (6.5)	51 (14.2)	0.006	0.018	2.31 (1.30–4.19)
2	rs10493270GG/rs890293GT	19 (7.7)	57 (15.9)	0.005	0.016	2.20 (1.25–3.94)
3	rs2280275AA/rs890293GG	184 (75.7)	233 (65.1)	0.008	0.018	0.62(0.44–0.89)
4	rs2280275AG/rs890293GT	16 (6.6)	54 (15.1)	0.004	0.016	2.44 (1.32–4.65)
5	rs2280275AG / rs11572182TT	31 (11.2)	82 (19.3)	0.008	0.018	1.87 (1.20–2.92)
6	rs2280275AA/rs10493270GG	163 (58.8)	213 (50.2)	0.042	0.045	0.70 (0.54–0.97)
7	rs2280275AG/rs10493270GG	47 (17.0)	110 (25.9)	0.008	0.018	1.68(1.14–2.78)
8	rs2280275AG/rs1155002CC	31 (11.2)	69 (17.4)	0.047	0.047	1.64 (1.03–2.60)
9	rs7515289AC/rs890293GT	16 (6.5)	48 (13.4)	0.019	0.027	2.14 (1.17–3.97)
10	rs7515289AC/rs11572182TT	33 (11.8)	81 (19.1)	0.025	0.032	1.74 (1.12–2.72)
11	rs7515289AC/rs2280275AG	43 (15.5)	108 (25.5)	0.003	0.016	1.83 (1.23–2.76)
12	rs11572325AA/rs890293GG	209 (85)	258 (72.1)	0.0004	0.011	0.47 (0.31–0.71)
13	rs11572325AT/rs890293GT	19 (7.7)	52 (14.5)	0.019	0.027	1.98 (1.14–3.58)
14	rs11572325AT/rs11572182TT	17 (6.1)	51 (12.0)	0.021	0.028	2.03 (1.16–3.67)
15	rs11572325AA/rs11572182GT	61 (21.8)	65 (15.3)	0.042	0.045	0.66 (0.45–0.97)
16	rs11572325AA/rs10493270GG	189 (67.5)	247 (58.3)	0.019	0.027	0.69 (0.51–0.94)
17	rs11572325AT/rs10493270GG	27 (9.6)	83 (19.6)	0.0009	0.012	2.24 (1.40–3.59)
18	rs11572325AT/rs1155002CT	13 (4.7)	39 (9.8)	0.026	0.032	2.17 (1.14–4.14)
19	rs11572325AA / rs2280275AA	214 (77.3)	281 (66.4)	0.005	0.016	0.59 (0.43–0.86)
20	rs11572325AT/rs2280275AG	31 (11.2)	83 (19.6)	0.005	0.016	1.90 (1.20–3.02)
21	rs11572325AT/rs7515289AC	27 (9.6)	71 (16.8)	0.013	0.023	1.86 (1.15–2.97)
22	rs10889162GG//rs890293GG	213 (87.3)	280 (78.7)	0.012	0.023	0.55 (0.35–0.86)
23	rs10889162GA/rs890293GT	22 (9.0)	56 (15.7)	0.028	0.032	1.84 (1.09–3.04)
24	rs10889162GG/rs2280275AA	211 (76.7)	278 (66.2)	0.005	0.016	0.62 (0.44–0.85)
25	rs10889162GA/rs2280275AG	24 (8.7)	60 (14.3)	0.044	0.045	1.70 (1.03–2.80)
26	rs10889162GG/rs11572325AA	229 (82.4)	312 (74.1)	0.017	0.027	0.63 (0.43–0.95)
27	rs10889162GA/rs11572325AT	19 (6.8)	57 (13.5)	0.009	0.018	2.02 (1.20–3.62)
Males (488 cases, 365 controls)						
1	rs1155002TT/rs10493270GG	33 (9.1)	60 (14.3)	0.040	0.045	1.64 (1.04–2.56)
2	rs2280275AA/rs1155002TT	33 (9.1)	60 (14.3)	0.042	0.045	1.64 (1.04–2.56)
3	rs11572325AA/rs1155002TT	33 (9.1)	59 (14.1)	0.045	0.045	1.63 (1.03–2.55)
4	rs10889162GG/rs1155002TT	33 (9.2)	59 (14.7)	0.038	0.045	1.66 (1.05–2.62)

Q-values at ≤ 0.05 mean statistically significant associations after adjustment for multiple tests by the FDR method

[a]Absolute number and percentage of individuals with particular genotype combination

[b]Odds ratio with 95% confidence intervals adjusted for age and BMI

rs11572325 and rs10493270 were stronger in the females than in the males (D'-values that differed between males and females are indicated in bolded cells in Fig. 1).

A comprehensive functional analysis of *CYP2J2* polymorphisms

To evaluate genotype–phenotype correlations, we used the genotype and mRNA expression data for the *CYP2J2* gene, which were obtained from the GTEx project. The relative expression levels of *CYP2J2* mRNA by different genotypes are shown in Fig. 2. The levels of *CYP2J2* mRNA were significantly correlated with SNPs rs1155002 ($P = 0.0002$) and rs10493270 ($P = 0.015$), as evaluated by *T*-statistics. No significant genotype-phenotype correlations were identified for the other investigated SNPs.

Functional prediction analysis performed in silico by the SNP Function Prediction tool revealed indicated the functionality of several polymorphisms (Table 5, Supplementary Tables 4–6). In particular, a regulatory potential was identified for SNPs rs890293, rs10493270, rs1155002, rs2280275 and rs7515289. The polymorphisms rs890293,

Table 5 Associations between haplotypes of *CYP2J2* and the risk of essential hypertension

Haplotypes	SNPs								Frequency		*P*-value	$_{adj}$OR (95%CI)[b]
	rs890293	rs11572182	rs10493270	rs1155002	rs2280275	rs7515289	rs11572325	rs10889162	Healthy controls	EH patients		
Females (705 subjects)												
H1	G	T	G	C	T	A	A	G	0.3761	0.3704	–	1.00
H2	G	T	G	T	T	A	A	G	0.2400	0.2333	0.971	1.01 (0.76–1.32)
H3	G	G	G	T	T	C	A	G	0.1373	0.0965	0.072	0.74 (0.52–1.08)
H4	G	T	A	C	T	A	A	G	0.1125	0.1059	0.812	0.94 (0.66–1.41)
H5	T	T	G	C	C	C	T	A	0.0427	0.0712	**0.041**	**1.70 (1.02 - 2.84)**
H6	G	G	G	C	C	C	T	G	0.0375	0.0368	0.341	1.35 (0.77–2.49)
H7	G	T	G	C	C	C	A	G	0.0305	0.0415	0.970	0.99 (0.58–1.75)
H8	G	T	G	C	C	C	A	A	0.0161	0.0106	0.491	0.71 (0.28–1.83)
Rare[a]	*	*	*	*	*	*	*	*	0.0073	0.0338	**0.023**	**4.13 (1.21–14.26)**
Global haplotype association *P*-value: **0.0052**												
Males (853 subjects)												
H1	G	T	G	C	T	A	A	G	0.4064	0.3755	-	1.00
H2	G	T	G	T	T	A	A	G	0.2358	0.2482	0.331	1.15 (0.89–1.45)
H3	G	G	G	T	T	C	A	G	0.1027	0.1077	0.457	1.12 (0.80 - 1.78)
H4	G	T	A	C	T	A	A	G	0.1003	0.0935	0.914	1.04 (0.76–1.82)
H5	T	T	G	C	C	C	T	A	0.0533	0.0488	0.972	1.02 (0.61–1.66)
H6	G	G	G	C	C	C	T	G	0.0398	0.0316	0.673	0.91 (0.54–1.72)
H7	G	T	G	C	C	C	A	G	0.0355	0.0468	0.248	1.38 (0.81–2.73)
H8	G	T	G	C	C	C	A	A	0.0150	0.0149	0.774	1.12 (0.50 - 2.81)
Rare[a]	*	*	*	*	*	*	*	*	0.0112	0.0330	**0.027**	**2.82 (1.14–6.65)**
Global haplotype association *P*-value: 0.234												

Statistically significant associations are bolded

[a]Rare haplotypes with frequency ≤ 0.01 are shown in Supplementary Table 2

[b]Odds ratio with 95% confidence intervals adjusted for age and BMI.

SNPs	rs890293	rs11572182	rs10493270	**rs1155002**	rs2280275	rs7515289	**rs11572325**	**rs10889162**
rs890293		0.9781	0.9939	0.6064	0.8743	0.9991	0.7875	0.8687
		-0.1221	-0.1006	-0.1313	0.5881	0.4685	0.6745	0.8461
rs11572182	0.9932		0.9961	0.5788	0.1476	0.9994	0.2890	0.9948
	-0.1069		-0.1494	0.3337	0.1471	0.6942	0.2278	-0.1274
rs10493270	0.9880	0.9950		0.9979	0.9957	0.9974	0.9948	0.9937
	-0.0848	-0.1364		-0.2597	-0.1499	-0.2154	-0.1176	-0.1033
rs1155002	0.8937	0.5257	0.9979		0.9987	0.1204	0.9549	0.6387
	-0.1730	0.2922	-0.2460		-0.3214	0.0999	-0.2413	-0.1419
rs2280275	0.9058	0.1571	0.9952	0.9987		0.993	0.9451	0.8696
	0.5539	0.1533	-0.1398	-0.3161		0.6922	0.7422	0.6006
rs7515289	0.9366	0.9939	0.9972	0.0729	0.9994		0.9425	0.9553
	0.4075	0.6902	-0.1968	0.0584	0.7111		0.5160	0.4599
rs11572325	0.8895	0.2868	0.7829	0.9632	0.9448	0.9389		0.7350
	0.7111	0.2248	-0.0841	-0.2331	0.7226	0.5109		0.6464
rs10889162	0.8975	0.9957	0.9936	0.8744	0.8884	0.9425	0.6630	
	0.8305	-0.1158	-0.0922	-0.1829	0.5870	0.4432	0.5728	

Fig. 1 Linkage disequilibrium between SNPs of *CYP2J2* in females and males. The upper part of the matrix contains (above the diagonal) the LD-values between SNPs in females. The lower triangle (below the diagonal) contains the LD-values between SNPs in males. Gray cells represent *D'*-values, whereas white cells represent *r*-values of LD. Bolded cells show *D'*-values that differ between males and females. All LD-values in the matrix are statistically significant

rs11572182, and rs10889162 of *CYP2J2* were found to be located within the binding sites for multiple transcription factors, as evaluated by the TRANSFAC and/or rSNPBase databases. SNPs rs10493270, rs1155002, rs2280275, rs7515289 and rs11572325 were identified to have the potential for the regulation of *CYP2J2* gene expression at the post-transcriptional level through an RNA-binding protein-mediated mechanism. Expression quantitative trait loci, or eQTLs, were found for polymorphisms rs10493270, rs1155002 and rs2280275. These data demonstrate the functionality for a majority of the investigated polymorphisms, including those associated with hypertension risk in our study.

Discussion

Polymorphisms of *CYP2J2* and their relationship with hypertension pathogenesis

To date, only seven studies have been conducted to assess associations between polymorphisms of the *CYP2J2* gene and hypertension susceptibility: four studies in Caucasians [20, 28, 31, 33], two studies in Asians [30, 32] and one study in blacks [29]. Five of these studies investigated the association of hypertension with the only one SNP rs890293 (−50 or −76G>T) located at the promoter of *CYP2J2* in small population samples (<200 EH patients). Only one study performed by Fava and colleagues [20] investigated the relationship among the rs890293 polymorphism, blood pressure and hypertension risk in a large urban-based sample of Swedes with hypertension ($N =$ 1858). The results from these studies have been conflicting: two studies performed in Russian [31] and Saudi Arabian [32] populations indicated a strong positive association between SNP rs890293 and hypertension risk; in contrast, one study in white Americans showed a negative association of this polymorphism and disease risk [28], whereas studies conducted in Swedish [20], Bulgarian [33] and African American [29] populations did not identify a relationship between the SNP and hypertension susceptibility. Another study performed on a sufficient number of cases and controls (415 hypertensives and 426 normotensive individuals) from the Chinese Han population investigated eight SNPs of *CYP2J2*; however, one half of them (including SNP rs890293) exhibited an MAF <3% [30]. This study indicated a relationship between SNP rs1155002 and systolic blood pressure and hypertension risk in females, a finding that was not supported by the results of our study. The present study confirmed the results of our previous study performed on an independent sample of 295 cases and 281 controls of Russian origin from the Kursk region [31]. This study reported a positive association of SNP rs890293 with hypertension risk.

The present study investigated eight common SNPs (minor allele frequency >5%) of *CYP2J2* and identified significant associations of three SNPs, including rs890293, rs2280275, and rs11572325, with the risk of EH exclusively in females. We found that the haplotype T-T-G-C-C-C-T-A is associated with an increased risk of EH in females. In addition, the rare *CYP2J2* haplotypes that contain the variant alleles at the three SNPs rs890293 (T), rs2280275 (C) and rs11572325 (T) prevailed in hypertensives compared with healthy individuals non-depending on their sex (Supplementary Table 3). Furthermore, we identified sixteen *CYP2J2* genotype combinations associated with EH risk in females, and the strengths of the associations were greater for genotype combinations than for the genotypes alone. Interestingly, these genotype combinations comprise the rs890293, rs2280275 and rs11572325 polymorphisms, each

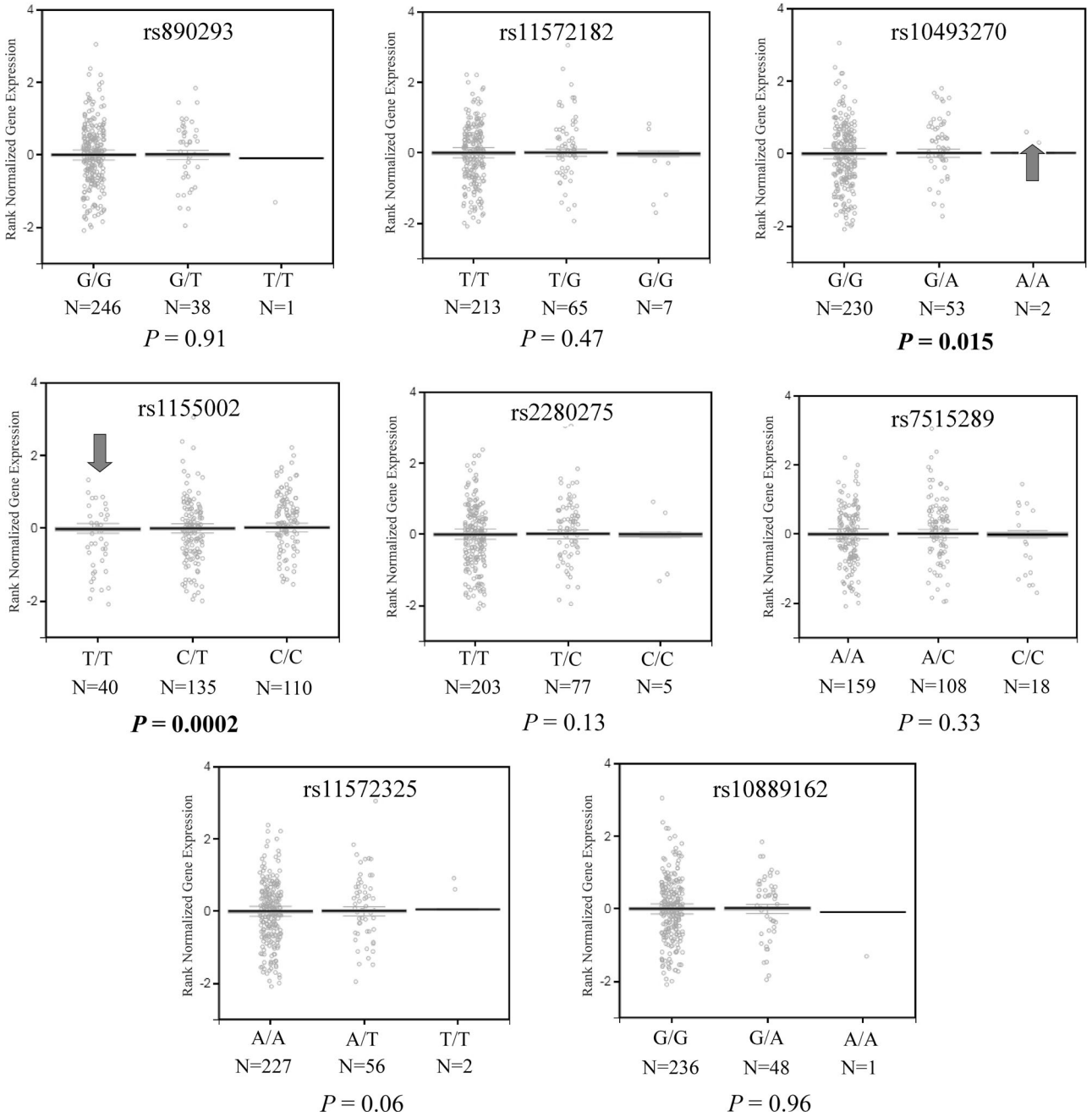

Fig. 2 Impact of polymorphisms on *CYP2J2* gene expression. The charts show the relative expression levels of the *CYP2J2* gene mRNA (rank normalized gene expression) by different genotypes in the samples of tibial arteries obtained from 285 postmortem individuals as a part of the GTEx project. GTEx (Genotype-Tissue Expression) is a project created to establish a sample and data resource for studies on the relationship between genetic variation and gene expression in multiple human tissues to identify expression quantitative trait loci, or eQTLs. The data used for the analyses described in the manuscript were obtained from the GTEx Portal (https://www.gtexportal.org), dbGaP accession number phs000424.v6.p1 on 07/14/2017

of which showed an association with disease risk. This finding suggests that these SNPs represent *trans*-acting modifiers that have the potential to modulate the expression and/or activity of the *CYP2J2* gene. To our knowledge, this investigation is the first study to identify associations between polymorphisms rs2280275 and rs11572325 of the *CYP2J2* gene and the risk of EH. Interestingly, SNPs rs2280275 and rs11572325 have been found to be related to the risk of coronary artery disease [23] and myocardial infarction [19], respectively. In particular, Zhu and co-workers reported that a carriage of allele T of rs2280275 is associated with an increased risk of coronary artery disease in men of the Uygur population from China, whereas no statistically significant association of this SNP was

identified in women [23]. Thus, this SNP showed the same trend in association with an increased risk of EH in our population but, in contrast, only in women. Zhu with co-workers suggested that the sex-specific association identified may be modified by estrogens, which are known to protect vascular cells against oxidative stress and may also protect the EETs against being hydrolyzed by soluble epoxide hydrolase [23]. We suppose that a sex-specific association between SNP rs2280275 and hypertension risk in our population could be modified by not established risk factors that influence the disease risk. The study of Marciante and co-workers [19] indicated that the polymorphism rs11572325 of *CYP2J2* is associated with an increased risk of myocardial infarction that does not depend on sex, age, hypertension and other confounding factors. SNPs rs2280275 and rs11572325 are located in intronic sequences of the gene, and they cannot directly influence the expression or activity of *CYP2J2*. However, the polymorphisms are in a positive linkage disequilibrium with the regulatory SNP rs890293 (Fig. 1) located in the promoter of the *CYP2J2* gene and interrupting a critical binding site for the transcription factor Sp1, thus resulting in decreased promoter activity in vitro and reduced levels of *CYP2J2* epoxygenase metabolites in vivo [45, 46]. Moreover, the polymorphisms rs890293, rs2280275 and rs11572325 collectively contribute to the activity and/or expression of *CYP2J2* and thus influence the biosynthesis of vasoactive EETs. The bioinformatic analysis (Table 6) enabled the identification of the RNA-binding protein ELAVL1, which has the potential to influence the stability of mRNA and potentially the expression of *CYP2J2* at the posttranscriptional level via interaction with the polymorphisms rs2280275 and rs11572325; moreover, the biological effects of the ELAVL1 protein have been demonstrated for some genes [47, 48]. It is plausible to assume that posttranslational modification of the *CYP2J2* transcript by ELAVL1 may represent an important mechanism implied in the vascular regulation of epoxygenase activity. However, before definitive conclusions may be made, experimental studies that investigate the effects of ELAVL1 on the activity or expression of *CYP2J2* are required. Finally, the mechanisms by which individuals with this genetic variant might be more susceptible to hypertension include: a) endothelial dysfunction; b) decreased ion channel-dependent vasodilatation; c) enhanced epithelial sodium channel activity; d) increased cortical renin release; e) decreased renal blood flow; and f) increased cardiac contractility and heart rate [10, 49].

Functionality of SNPs and expression of *CYP2J2*

Functional studies of the common polymorphisms of the *CYP2J2* gene in the literature are limited to only SNP rs890293. To evaluate the phenotypic effects of the studied SNPs, we used the *CYP2J2* genotype and mRNA expression data in peripheral (tibial) arteries obtained from the GTEx project. The levels of *CYP2J2* mRNA were correlated with the polymorphisms rs1155002 and rs10493270, whereas the SNPs associated with hypertension risk showed no relationship with the *CYP2J2* expression. The inconsistency of these data may be explained by differences in the samples and analytical methods used, as well as a variation in the *CYP2J2* transcript levels across tissues as analyzed by the GTEx project (these data are available at www.gtexportal.org).

Because of the limited data on the functional significance of the investigated SNPs, we performed an in silico analysis of the regulatory potential for all polymorphisms using various bioinformatic tools. The bioinformatic analysis indicated the functionality of three SNPs (rs890293, rs2280275, and rs11572325) associated with EH. In particular, the SNPs possess a regulatory potential (excluding rs11572325) as identified by the SNP Function Prediction tool. All three SNPs are identified as rSNP (regulatory polymorphisms) and are in LD with other rSNPs according to the rSNPbase data. In addition, the intronic polymorphisms rs2280275 and rs11572325 have the potential to be regulated at the posttranslational level through an RNA binding protein-mediated mechanism. We identified numerous transcription factors related to the polymorphism rs890293 of *CYP2J2*, which are worthwhile to note because they are involved in various biological processes in the cardiovascular system, may regulate blood pressure and may be related to hypertension pathogenesis (data obtained from the Gene Ontology database, www.geneontology.org). For example, the *CYP2J2* gene expression may be directly regulated by the PPARA (Peroxisome Proliferator Activated Receptor Alpha) signaling pathway (GO:0035357) through an interaction of the transcription factor TEAD2 (TEA domain transcription factor 2) with the promoter SNP rs890293. TEAD2 is known to be involved in the pathways that regulate vasculogenesis (GO:0001570) and embryonic heart tube morphogenesis (GO:0003143). Transcription factor NF1 (neurofibromin 1) is involved in many biological processes in the heart and vessels, such as the negative regulation of endothelial cell proliferation (GO:0001937), positive regulation of endothelial cell proliferation (GO:0001938), heart development (GO:0007507), regulation of angiogenesis (GO:0045765), negative regulation of angiogenesis (GO:0016525) and regulation of blood vessel endothelial cell migration (GO:0043535). Transcription factors, such as PAX6 (paired box 6) and E2F4 (E2F transcription factor 4), are important components of the pathways involved in the regulation of blood vessel development (GO:0001568) and blood circulation (GO:0008015), respectively. Two intronic polymorphisms

Table 6 Bioinformatic analysis for the regulatory potential of the studied single nucleotide polymorphisms of the *CYP2J2* gene

SNP ID	Allele	Location withi gene	SNP Function Prediction (FuncPred)[a]			Regulatory annotations on SNPs (rSNPBase)[b]							TFBS and Gene Sets associated with SNP	
			TFBS	miRNA	Regulatory potential	rSNP	LD-proxy of rSNP (r2 > 0.8)	Proximal regulation	Distal regulation	miRNA regulation	RNA binding protein mediated regulation	eQTL	TRANSFAC Database	rSNPBase
rs890293	G/T	5`UTR	Yes	No	0.1494	Yes	Yes	Yes	No	No	No	No	CACD, ETF (TEAD2), IK, MYOG/NF1, PAX6, SP1, VDR	Max, E2F6, Pol2, c-Myc, CHD2, TBP, MAZ, MXI1, UBTF, E2F4
rs11572182	T/G	5`UTR	Yes	No	No	Yes	Yes	Yes	No	No	No	No	LEF1, TCF1, PLZF (ZBTB16), PPARA, PPARG, SPZ1, TBP	–
rs10493270	G/A	intron	No	No	0.0674	Yes	No	No	No	No	Yes	Yes	–	ELAVL1[a]
rs1155002	C/T	intron	No	No	0.1567	Yes	Yes	No	No	No	Yes	Yes	–	ELAVL1[a]
rs2280275	T/C	intron	No	No	0.1126	Yes	Yes	No	No	No	Yes	Yes	–	ELAVL1[a]
rs7515289	A/C	intron	No	No	0.1456	Yes	Yes	Yes	No	No	Yes	No	–	ELAVL1[a]
rs11572325	A/T	intron	No	No	No	Yes	Yes	No	No	No	Yes	No	–	ELAVL1[a]
rs10889162	G/A	5`UTR	Yes	No	No	Yes	Yes	Yes	No	No	No	No	AIRE, CDPCR3_01, GRE_C, HAND1E47_01, MYOGNF1_01, RAX5, PAX6, PAX8	–

TFBS, transcription factor binding sites predicted by the TRANSFAC database on potential transcription factors, their genomic binding sites and DNA binding profiles (BIOBASE Corporation, Wolfenbuettel, Germany, http://gene-regulation.com) and available via the SNP Function Prediction tool (National Institute of Environmental Health Sciences (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.php)

The only TFBS whose core or matrix match score was impacted, or which were eliminated or created by variant sequences were included in this table to be regulatory within a particular SNP (all TFBS identified by TRANSFAC are listed in Supplementary Tables 3–5)

rSNPBase is a database of curated regulatory SNPs (http://rsnp.psych.ac.cn).

[a]Post-transcriptional regulation: associated with RNA binding protein. Gray cells indicate SNPs associated with hypertension susceptibility in the present study

rs2280275 and rs11572325 associated with hypertension risk are located within specific sites for ELAVL1, a mRNA-binding protein that binds to the 3'-UTR region of mRNAs and increases their stability [47]. Interestingly, this mRNA-stabilizing protein participates in biological processes, such as the regulation of blood vessel endothelial cell migration (GO:0043535) and the positive regulation of blood vessel endothelial cell migration (GO:0043536).

Nevertheless, it is unclear which of the three disease-associated SNPs possesses a phenotypic effect on the disease development because the promoter (rs890293) and intronic (rs2280275 and rs11572325) polymorphisms are in strong positive linkage disequilibrium to each other (Fig. 1). We suggest that the non-coding rs2280275 and rs11572325 variants, which are in close proximity to the rs890293 polymorphism, may act as cis-acting modifiers that influence the transcriptional activity of the *CYP2J2* gene, a well-known mechanism for the regulation of gene expression [50]. At the very least, this suggestion can be supported by our finding that the frequency of the haplotype T-T-G-C-C-C-T-A (the variant alleles for each SNP are underlined) was higher in hypertensive than normotensive females. However, it remains unclear whether the effects of these SNPs on disease risk are attributed to their synergism or the linkage disequilibrium between them.

Sex dimorphism and hypertension susceptibility

The main finding of our study is a sex-specific association between single nucleotide polymorphisms of the *CYP2J2* gene and the risk of EH. The SNPs rs890293, rs2280275 and rs11572325 alone and in combinations with each other and other *CYP2J2* SNPs were associated with an increased risk of EH exclusively in females, even when the associations were assessed separately in pre- and post-menopausal women. Rare haplotypes have been associated with hypertension susceptibility in both sexes; however, the strength of this association was stronger in females than in males. As indicated in the literature [30, 51], only one study performed in a Chinese population has identified a female-specific effect of the *CYP2J2* gene polymorphism (rs1155002) on hypertension susceptibility.

The observed intergroup difference in the frequency of rare haplotypes may be related to the sex-specific difference in the recombination rates between sexes. It is well recognized that the number of recombination rates per meiosis differs between females and males and seems to be tightly regulated [52]. Moreover, recombination events occur nearly 2-fold higher in female meiotic divisions than in male meiosis, whereas the crossover recombination rates in males are 5-fold lower near the centromere regions of chromosomes and 10-fold higher near telomeres than those in females [53]. As indicated in Fig. 2, the LD coefficients between the SNP rs890293 and rs2280275 or rs11572325 (polymorphisms associated with EH risk in our study) were weaker in females than in males. This finding suggests that the phenotypic effects of these SNPs on the disease risk may differ in females and males. Moreover, it may particularly explain why the SNPs were associated with EH risk only in females. Sex-specific differences in recombination events are thought to be related to the effect of sex [54] or may result from differences in the genetic regulation of female and male meiosis [55].

The observed sex-specific difference in the contribution of the *CYP2J2* polymorphisms to hypertension susceptibility may be attributed to the regulatory effects of sex hormones on gene expression or activity. Estrogens are well known to be protective against hypertension and cardiovascular disease [56, 57] and may protect the EETs against being hydrolyzed by soluble epoxide hydrolase [58, 59]. Nevertheless, estrogen was found to be responsible for significant sex-specific differences in autonomic function in patients with hypertension: the baroreceptor reflex sensitivity and heart rate variability are more impaired in hypertensive women than in men [60, 61]. Undoubtedly, we cannot exclude that the female-specific association between the polymorphisms of the *CYP2J2* gene and the hypertension risk may be explained by sex differences in the major hemodynamic parameters determined by well documented anatomical and physiological features of the cardiovascular system in women [62–65]. As the hemodynamic parameters were not measured in our hypertensive patients, we could not evaluate whether these parameters explain the sex difference in hypertension susceptibility.

It is established that sex hormones possess regulatory effects on gene expression. It has been reported that the hormone β-estradiol has the potential to increase the levels of *CYP2J2* mRNA in a concentration-dependent manner, while testosterone has been shown to decrease transcript levels of the gene in human cardiomyocytes [66], a finding that may explain the sex-specific association between *CYP2J2* SNPs and hypertension identified in our study. In contrast, an experimental study of Ma and co-workers [67] showed that the renal expression of the mouse ortholog CYP2J5 (analogue of CYP2J2 in humans) is down-regulated by estrogen and upregulated by androgen. However, Athirakul and colleagues [68] indicated that deficiency of CYP2J5 in female mice was associated with increased blood pressure, enhanced proximal tubular transport rates, and exaggerated afferent arteriolar responses to angiotensin II and endothelin I, thus illustrating a sex-specific role for CYP2J5 in the regulation of blood pressure, proximal tubular transport, and afferent arteriolar responsiveness via an estrogen-dependent mechanism. Thus, it is reasonable to

speculate that the expression of *CYP2J2* and the associated biosynthesis of EETs may influence blood pressure through interaction with estrogen.

Furthermore, the bioinformatic analysis enabled the identification of the transcription factor MYOG (myogenin) capable of affecting the expression of *CYP2J2* via interaction with the SNP rs890293 at the gene promoter. Interestingly, this transcription factor is related to a pathway that regulates the cellular response to estradiol stimuli (GO:0071392), thereby indicating that the association of the polymorphism rs890293 with hypertension risk in females is somehow connected to the actions of estrogen and its effects may down-regulate the expression of *CYP2J2* in the carriers of allele −50T, resulting in a decreased biosynthesis of EETs. In addition, the transcription factor binding site for TEAD2 located at the SNP rs890293 (Table 3) is related to the pathways that regulate many biological processes in females. In particular, we identified the following enriched GO terms of partners interacting with the transcription factor TEAD2: the positive regulation of female receptivity (GO:0045925), hormone-mediated signaling pathway (GO:0009755), intracellular steroid hormone receptor signaling pathway (GO:0030518) and progesterone receptor signaling pathway (GO:0050847). Undoubtedly, further systems biology studies are required to obtain a better understanding of the complexity of the interrelationship among CYP2J2, sex hormones, EETs and blood pressure regulation.

Study limitations

The results of our study should be interpreted in the context of several limitations. The low genotype call rate for several SNPs of *CYP2J2* did not allow their inclusion in the study or a more comprehensive analysis of the contribution of the selected SNPs to the risk of EH. The case/control ratio in our study samples was 1.4, which indicates that the matching of hypertensive patients and healthy controls was suboptimal. A gender-specific association analysis was performed in a post-hoc manner on a sample of relatively low size; thus, the findings on the relationship between SNPs and hypertension risk in females must be carefully interpreted. Moreover, the interactions of the studied SNPs of the *CYP2J2* gene with other potential confounding factors, such as sex hormones and/or disease-related environmental risk factors in hypertensive females, were unexplored in the present study, which does not enable conclusions to be drawn with respect to the sex-specific relationship between the polymorphisms and disease risk. The mechanisms by which estrogens exert their regulatory effects on hypertension susceptibility through the modulation of *CYP2J2* gene expression remained to be elucidated in further studies. Thus, the study results should be considered preliminary, and further investigation in independent cohorts of hypertensive patients and controls is required to replicate the sex-specific associations of the studied SNPs of the *CYP2J2* gene and hypertension susceptibility.

Conclusions

The current findings show that polymorphisms of the *CYP2J2* gene may represent important determinants of susceptibility to EH and that their effects on disease risk are joint and sex-specific. We found for the first time that the rs2280275 and rs11572325 SNPs of *CYP2J2* could be novel genetic markers of susceptibility to EH, at least in Russian females. As this study reports a preliminary association between new SNPs of the *CYP2J2* gene and hypertension risk in females, a replication study in an independent population is warranted. Further studies will provide insights into the mechanisms that underlie the sexual dimorphism in the relationship among *CYP2J2* polymorphisms, gene expression and hypertension pathophysiology with the purpose of determining the most beneficial therapeutic options for the management of EH in women and men, expanding opportunities for the clinical application of pharmacogenetics and personalized medicine [69, 70].

Acknowledgements The study was supported by the Russian Science Foundation (№15-15-10010).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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