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Relationship between *EPHX2* gene polymorphisms and essential hypertension in Uygur, Kazakh, and Han

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ABSTRACT. We investigated the association between rs751141 polymorphisms in the *EPHX2* gene and essential hypertension in Uygur, Kazakh, and Han subjects in Xinjiang, China. A total of 302 essential hypertensive patients in Uygur, 267 in Kazakh, and 368 in Han, as well as 323 normotensive controls in Uygur, 284 in Kazakh, and 348 in Han were enrolled in this study. The TaqMan assay was used to detect the rs751141 G/A gene polymorphism in *EPHX2*. The rs751141 G/A genotype frequencies for the GA+AA genotypes were 40.2% in essential hypertensive subjects and 52.0% in control subjects in the Han population. The frequencies were significantly different between the 2 Han groups ($P < 0.01$). The rs751141G/A gene polymorphism showed no significant difference between essential hypertensive patients and normotensive controls in Kazakh and Uygur (all $P > 0.05$). Essential hypertension in Xinjiang was associated with the rs751141 G/A allele gene polymorphism in *EPHX2* in Han subjects but not in Kazakh and Uygur subjects. The rs751141 allele gene polymorphism may be an independent protective factor against essential hypertension in the Han population.

Key words: Epoxy compounds; Essential hypertension; Single nucleotide polymorphisms

INTRODUCTION

Hypertension is an important public health issue worldwide because of its high prevalence and concomitant effect on increasing disease risk (Slama et al., 2002; Calhoun et al., 2002). Essential hypertension (EH) is a predisposing risk factor for stroke, myocardial infarction, congestive heart failure, and arterial aneurysm, as well as the leading cause of chronic renal failure (Pierdomenico et al., 2009; Hackam et al., 2010). Approximately 90-95% of hypertension cases, affecting more than 1 billion adults worldwide, is the EH subtype (Lloyd-Jones et al., 2009). Epoxidation eicosatrienoic acid (EETs) and arachidonic acid through cytochrome P450 oxidase metabolism of biologically active substances in the cardiovascular system plays an important biological role, which is related to vasodilatation, anti-inflammatory, antiplatelet, and antiproliferative effects as well as an association with hypertension (Jung et al., 2005; Ai et al., 2007). However, because of the very short half-life of EETs, soluble epoxide hydrolase (EPHX2) is rapidly hydrolyzed into dihydroxy derivatives and loses its biological activity (Abdu et al., 2011). Previous studies have examined the association between *EPHX2* polymorphisms and cardiovascular disease. Studies have found that human *EPHX2* loci have multiple single nucleotide polymorphisms (SNPs) and that the rs751141G/A polymorphism is closely related to coronary atherosclerotic disease and stroke (Przybyla-Zawislak et al., 2003). Based on these results, we hypothesized that rs751141 may also have a role in the pathogenesis of hypertension. Therefore, we genotyped 1 SNP in *EPHX2* (rs751141G/A) using the TaqMan allelic discrimination assay to explore the association between the polymorphisms and EH among Uygur, Kazakh, and Han individuals in the Xinjiang area of China.

MATERIAL AND METHODS

Ethics approval

This study was approved by the Ethics Committee of The First Affiliated Hospital of Shihezi University and adhered to the standards of the Declaration of Helsinki. All participants provided written informed consent.

Subjects

The study population included 937 subjects (302 Uygur, 267 Kazakh, and 368 Han subjects) who lived in the Xinjiang Uygur Autonomous Region of China. They were enrolled in an epidemiologic survey on hypertension prevalence that was launched in 2008 and had been diagnosed with EH. The diagnosis of hypertension was established if the patient was on antihypertensive medication or the mean of 3 measurements showed a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg. Individuals who had secondary hypertension, had multiple organ failure, were pregnant or lactating, or had been taking birth control pills for a long period of time were excluded from the study.

The control subjects included 955 individuals (323 Uygur, 284 Kazakh, and 348 Han subjects), also selected from the epidemiologic survey launched in 2008. Individuals who had prehypertension or had taken any antihypertensive medication were excluded from the study.

Biochemical analyses

Fasting venous blood samples (5 mL) were collected into tubes containing ethylene diaminetetraacetic acid. The samples were centrifuged into the plasma, serum, and blood cells (including leukocytes). Genomic DNA was extracted from peripheral leukocytes using the standard phenol-chloroform method and stored at -80°C for future analysis. Plasma glucose, total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels were measured using standard methods using an Olympus AU2700 automatic biochemical analyzer (Olympus Co. Ltd., Tokyo, Japan) after 12-h overnight fasting. The low-density lipoprotein cholesterol concentration was estimated using the Friedewald formula.

Genotyping of *EPHX2*

We identified 1 SNP (rs751141) using the HaploView 4.2 software and the International HapMap Project phases I and II database (http://www.hapmap.org). In this case-control study, genotyping was confirmed using the TaqMan SNP allelic discrimination assay (Shanghai Gene Core Bio Technologies Co., Ltd., Shanghai, China). The TaqMan Universal PCR Master Mix (2X) was obtained from Applied Biosystems (Foster City, CA, USA). Fluorescent probes and polymerase chain reaction (PCR) amplification primers for the 2 alleles were designed and synthesized by Shanghai Gene Core Bio Technologies (Shanghai, China). All fluorescent probes were TaqMan TAMRA probes consisting of a TAMRA modifying group, a 5' reporter dye, and a 3' nonfluorescent quencher. Table 1 shows the list of probe and primer sequences.

Table 1. Sequences of probes and primers.

rs ID	Primer (5'→3')	Probe (5'→3')	Allele
rs751141	F: 5'-CGGGAGGAGCAGATGACTCT-3'	5' FAM-CATAGCTAGGACCCGGTAACCTGCCT-TAMRA-3'	G
	R: 5'-TGGAGTGTGCCTGTTTGTTTTC-3'	5' HEX-CCATAGCTAGGACCTGGTAACCTGCCT-TAMRA-3'	A

F = forward primer; FAM and HEX = fluorescence dyes; R = reverse primer; TAMRA = TAMRA-quenched probe.

PCR amplification was done using an ABI 7900HT thermal cycler (Applied Biosystems) with a PCR temperature profile consisting of incubation at 50°C for 2 min, denaturation at 95°C for 10 min, 50 cycles of denaturation at 95°C for 15 s, and annealing at 60°C for 60 s. Each PCR amplification reaction contained 5 mL 1X Universal Master Mix (Applied Biosystems) with 0.2 mM each of the 2 probes, 1 mM of each primer, and 1 ng/mL genomic DNA. The genotypes of the 2 alleles were differentiated using the SDS 2.1 software (Applied Biosystems), and 10% of all genotyping reactions were repeated in independent PCRs to evaluate the consistency and ensure intraplate and interplate reproducibility. No discrepancies were detected between the repeated samples. In addition, all paired case and control DNA samples were run in the same batches.

Statistical analysis

All continuous variables are reported as means ± standard deviation. Differences be-

tween EH and control groups were analyzed using an independent samples *t*-test. Differences in the frequencies of smoking, hyperlipidemia, coronary artery disease, diabetes, and stroke were analyzed using χ^2 or Fisher's exact tests when appropriate. Hardy-Weinberg equilibrium was assessed by χ^2 analysis, which was also used to compare genotype and allele frequencies between the EH and control groups. All analyses were conducted separately by race. $P < 0.05$ was considered to be statistically significant. The SPSS 17.0 software for Windows (SPSS Institute, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Subject characteristics

Table 2 shows the demographic characteristics for the case and control groups. There was no difference in age and gender between the 2 groups. In all 3 ethnicities, the systolic blood pressure and diastolic blood pressure in the case group were the highest. The prevalence of coronary artery disease and hyperlipidemia was higher in hypertensive subjects than in controls in the Uygur population. The hypertensive group included a higher proportion of smokers in the Han population, but not in the other 2 populations. Significantly higher serum total cholesterol, triglyceride, and low-density lipoprotein levels were identified in the case group for all 3 ethnicities. However, Han fasting glucose concentrations in patients with hypertension high blood pressure, patients suffering from Kazakh, and Uyghur high-density lipoprotein levels were higher. The genotype distributions of the SNPs (rs751141) were in Hardy-Weinberg equilibrium in all control groups. The minor allele frequencies in the controls published in the NCBI database (Table 3) for the Han population were very similar, indicating that the selected group was representative of the population.

Table 2. Baseline characteristics of 3 ethnic samples.

Characteristics	Kazak		Uygur		Han	
	Controls (N = 284)	Hypertension (N = 267)	Controls (N = 323)	Hypertension (N = 302)	Controls (N = 348)	Hypertension (N = 368)
Age (yrs)	50.6 ± 14.5	50.0 ± 13.3	55.9 ± 8.7	54.4 ± 8.0	48.1 ± 9.9	49.1 ± 10.7
Men (%)	41.1	45.3	51.1	53.6	49.4	52.7
BMI (kg/m^2)	24.2 ± 3.8	26.6 ± 4.3*	27.0 ± 4.5	27.6 ± 3.4	23.56 ± 3.18	25.48 ± 2.83*
SBP (mm Hg)	118 ± 11	150 ± 22*	122 ± 8	156 ± 13*	116.7 ± 10.3	147.0 ± 13.7*
DBP (mm Hg)	76 ± 8	94 ± 12*	78 ± 6	94 ± 43*	76.1 ± 7.1	96.9 ± 10.2*
Stroke (%)	0.6	1.8	2.8	5.0	0.8	1.3
CAD (%)	12.9	17	0.0	34.4*	2.3	5.4
Hyperlipidemia (%)	5.2	12.5*	30.7	43.0*	2.6	2.5
DM (%)	2.6	2.7	1.4	1.6	3.1	3.3
Smokers (%)	8.4	6.4	21.4	21.0	24.6	34.7*
Total cholesterol (mg/dL)	171.15 ± 39.00	193.82 ± 40.54*	164.09 ± 29.73	191.51 ± 28.57*	166.41 ± 32.43	177.99 ± 35.14*
Triglycerides (mg/dL)	101.77 ± 85.84	123.01 ± 90.27*	115.93 ± 110.62	162.83 ± 110.62*	109.73 ± 146.02	160.18 ± 149.56*
Fasting glucose (mM)	4.80 ± 1.31	4.79 ± 1.35	4.85 ± 1.04	4.83 ± 1.26	4.66 ± 1.07	4.99 ± 1.58*
LDL cholesterol (mg/dL)	100.23 ± 37.54	117.65 ± 38.31*	115.33 ± 36.76	131.58 ± 38.31*	110.68 ± 32.12	126.55 ± 47.21*
HDL cholesterol (mg/dL)	49.54 ± 13.54	59.60 ± 13.93*	41.02 ± 8.90	50.70 ± 14.32*	55.34 ± 12.01	53.41 ± 13.93

BMI = body mass index; CAD = coronary artery disease; DBP = diastolic blood pressure; DM = diabetes mellitus; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SBP = systolic blood pressure; yrs = years. Continuous variables are given as means ± SD. *$P < 0.05$ *vs* control, using *t*-test for continuous variables and χ^2-test for categorical variables.

Table 3. Genetic diversity in different ethnic population on rs751141.

	MAF from NCBI		
SNP	CEU	YRI	CHB
rs751141	0.05	0.083	0.233

CEU = population of Western European ancestry; CHB = Han Chinese in Beijing; YRI = Yoruba in Ibadan, Nigeria; MAF = minor allele frequency; SNP = single-nucleotide polymorphism.

Association analysis

Table 4 shows the distribution of genotypes and alleles of SNPs in the *EPHX2* gene. The genotype distributions for rs751141 agreed well with the predicted Hardy-Weinberg equilibrium values (data not shown). For the Han population, the dominant model (AA *vs* GG+GA) in the distribution of rs751141 genotypes showed a significant difference between EH and control subjects (both $P < 0.001$). There was no significant difference between EH and control subjects in the Uygur and Kazakh populations, where the dominant model in the distribution of rs751141 genotypes was AA *vs* GG+GA (both $P > 0.05$).

Table 4. Association between rs751141 (C/T) variants and EH.

SNP	Ethnicity	Population	N	MAF	$P_{allelic}$	MM (N, %)	MM (N, %)	MM (N, %)	$P_{domiant}$
rs751141	Han	Control	348	0.32	<0.001	167 (48.0)	136 (39.1)	45 (12.9)	0.001
		EH	368	0.23		220 (59.8)	126 (34.2)	22 (6.0)	
	Uygur	Control	323	0.28	0.122	171 (52.9)	123 (38.1)	29 (9.0)	0.154
		EH	302	0.24		177 (58.6)	104 (34.4)	21 (7.0)	
	Kazak	Control	284	0.31	0.058	137 (48.2)	119 (41.9)	28 (9.9)	0.062
		EH	267	0.26		150 (56.2)	97 (36.3)	20 (7.5)	

SNP = single nucleotide polymorphism; EH = essential hypertension; N = number of subjects; MAF = minor allele frequency; $P_{allelic}$ = value of allele was determined by a two-sided χ^2 test; M = major allele; m = minor allele; $P_{domiant}$ = value was computed with multivariate logistic regression analysis by adjusting for gender, age, and body mass index.

DISCUSSION

EETs are powerful endogenous bioactive lipid epoxy compounds. They are widely expressed in the heart, liver, kidney, and other tissues to regulate homeostasis and play an important role in many pathophysiologic processes. Studies have confirmed that EETs have vasodilator, lowering blood pressure, anti-inflammatory, anti-platelet, and promote fibrinolysis, inhibition of vascular smooth muscle cell proliferation, and shift shape, promote angiogenesis, regulation of lipid metabolism, and insulin resistance effects (Newman et al., 2005; Yang et al., 2009; Nithipatikom and Gross, 2010; Skepner et al., 2011). EETs in smooth muscle cells can be activated by the Ca^{2+} and K^+ ion channels to promote formation of the open state and relaxation of vascular contraction, and are considered endogenous anti-hypertensive substances. In an experiment examining angiotensin II-induced hypertension in rats, the EET concentration level showed an important association with blood pressure (Imig et al., 2002). Because of EPHX2 is the main enzyme to make EET metabolism and inactivation, its activity level determines the concentration of EETs *in vivo* (Spector et al. 2004).

The EPHX2 is mainly involved in EPHX2 gene transcription, and functionally similar enzymes can catalyze the stereoselective hydrolysis of the epoxy compound to form an opti-

cally active epoxy with a bi-functional enzyme catalytic domain, C-terminal region containing the typical α/β-hydrolyses structure, and catalytic activity of EH. Epidemiological and animal studies revealed its anti-inflammatory, cholesterol-lowering, and cardiovascular disease prevention effects. The N-terminal region possesses lipid phosphates activity, may regulate cholesterol levels, and stabilizes the activity of EPHX2 (Newman et al., 2003; Eldrup et al., 2010). The *EPHX2* human gene locus contains multiple SNPs, and the expression of different EPHX2 genotype products have different activities, with the non-synonymous amino acid change SNPArg287Gln (R287Q, dbSNP database ref: rs751141G/A) as a hot spot. Studies have shown that 287Gln (287Q) mutations can make sEPHX2's salt bridge which is between the 287th arginine and the 252th glutamic acid disappear, leading to EPHX2 spatial structure change, destruction of its biological function, so that the activity of EPHX2 decreased from 25 to 58%.

The association analysis results suggested that for a large number of people, *EPHX2* rs751141G/A is closely associated with cardiovascular genetic polymorphisms. Wei et al. (2007) studied 1337 cases of African-Americans and 1645 cases of Caucasians and found that the 860A variant genotypes of *EPHX2* led to an increased risk of coronary heart disease. Zhang et al. (2007) reported that among Chinese people, the G860A (rs751141G/A) polymorphism can reduce the risk of ischemic stroke associated with the 860A+ allele. However, these conclusions have not been confirmed.

The Uygur and Kazakh populations in Xinjiang, China are relatively large and rarely intermarry with other populations, leading to regional and genetic isolation; this genetic homology along with common environmental factors affecting blood pressure (such as their living environment, eating habits, lifestyle, mental stress) show a consistent pattern of inheritance. In this study, we found that genetic polymorphisms of the *EPHX2* gene are associated with EH in a Han population in Xinjiang, China. After multivariate adjustment, the associations between *EPHX2* gene polymorphisms and EH did not change. However, we found no associations between *EPHX2* gene polymorphisms and EH in the Uygur and Kazakh populations. This may be because *EPHX2* loci contain unidentified functional SNPs, and in the different races the functional activity of SNPs on *EPHX2* has different effects on the cardiovascular system. In addition, primary hypertension caused by genetic, environmental, and other factors influence each other, and the interaction between genes may cause variations in gene expression, ultimately resulting in more complicated disease phenotypes. Nevertheless, further studies are needed to confirm our results.

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