Association of regulator of G protein signaling (*RGS5*) gene variants and essential hypertension in Mongolian and Han populations

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ABSTRACT. Genetic variants of the *RGS5* gene are believed to be risk factors for hypertension and cardiovascular diseases. In this study, we investigated the association between *RGS5* gene variants and hypertension in the Mongolian and Han populations. Peripheral blood was obtained from 429 unrelated Mongolian herdsmen and 416 Han farmers [including essential hypertension (EH) patients and controls]. Nine tagSNPs within the *RGS5* genes were retrieved from HapMap, and the samples were individually genotyped using the polymerase chain reaction/ligase detection reaction assay. The distribution of the allele frequency of rs12035879 differed significantly between hypertensive subjects and controls in the Han population, while the distribution of the allele and genotype frequencies of rs16849802 differed significantly between hypertensive subjects and controls in the Mongolian population. We

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observed an association between rs16849802 and EH in the Mongolian population. The frequency of haplotype GAA was significantly higher in the EH group than in controls in the Mongolian population. However, the EH group and controls did not differ significantly in all 6 haplotypes in the Han population. The rs16849802 and haplotype GAA independently increased the risk of EH in Mongolian patients, and may be used as a risk factor for the prediction of high blood pressure.

Key words: Essential hypertension; Mongolian population; RGS5; Single nucleotide polymorphism

INTRODUCTION

Essential hypertension (EH) is a major risk factor for coronary heart disease, stroke, and renal disease. EH is a major worldwide public health burden (Kearney et al., 2005) that has also been steadily increasing in China for the past several years. The Chinese National Nutrition and Health Survey of 2002 showed the prevalence of EH among adults (over 18 years old) in China to be at 18.8%. However, the etiological basis of high blood pressure (BP) remains poorly understood. Research efforts to uncover the mechanisms underlying its onset are complicated because hypertension is affected by varying combinations of genetic and environmental factors (Hamet et al., 1998; Williams et al., 2004; Klimentidis et al., 2012). Genes play a major role in determining the blood pressure and EH susceptibility of an individual (Lifton, 1996; Rapp, 2000). Recently, the quantitative trait locus (QTL) region of the q arm of chromosome 1 has been observed in hypertensive animal and human populations (Kotchen et al., 2002; Ehret et al., 2009; Stoll et al., 2000). Clustered within the genomic region containing this QTL are genes encoding a large number of heterotrimeric G-protein signaling inhibitors, including RGS5.

Signal transduction through G-protein-coupled receptors (GPCRs) is a central mechanism in the regulation of cellular functions, and plays a major role in the development of human disease including hypertension (Li et al., 2007). Regulators of G-protein signaling belong to a diverse protein family originally discovered for their ability to accelerate signal termination in response to GPCR stimulation, thereby reducing the amplitude and duration of GPCR effects. RGS5, a member of the RGS family, is a potent GTPase-activating protein for Gα(q) and Gα(i), both of which are expressed strongly in pericytes and are present in vascular smooth muscle cells. Previous studies have shown that RGS5 plays a role in blood pressure regulation (Abramow-Newerly et al., 2006; Mezbah et al., 2011). A recent genome-wide linkage and candidate gene-based association study identified the human *RGS5* gene as contributing to the increase in blood pressure in the population (Chang et al., 2007), while a constructed RGS5 knockout mouse model displayed lower BP (Cho et al., 2008). Additionally, a haplotype-based analysis conducted by Xiao et al. (2009) showed evidence of the association of *RGS5* gene polymorphisms with essential hypertension in a Chinese population.

Xilin Gol League of the Inner Mongolia Autonomous Region, a gathering of individuals with Mongolian ancestry, and a primary residence of the Mongolian and Han population, is located in northwestern China. The prevalence of hypertension is higher in the Mongolian population in China. However, very few studies have attempted to evaluate the association of *RGS5* gene populations with hypertension in this population subset. Therefore, the aim of this study was to investigate the association between RGS5 polymorphisms and hypertension in Mongolian and Han sample populations.

MATERIAL AND METHODS

Study population

The study population, aged 20-70 years, was recruited from the Duolun and Dongwuqi regions of Xilin Gol League in Inner Mongolia. A total of 429 unrelated Mongolian herdsmen and 416 Han farmers were enrolled; these included 212 Mongolian EH patients, 217 Mongolian normotensives (controls), 200 Han EH patients, and 216 Han controls. Each individual belonged to a family that had been living in the locality for at least three generations without a history of mixed marriage. Hypertension was characterized in individuals with a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg, or in those undergoing antihypertension treatment. The normotensive group was selected based on the following criteria: SBP < 140 mmHg and DBP < 90 mmHg, and no previous diagnosis of EH. Individuals with a history of secondary hypertension, stroke, coronary heart disease, diabetes, kidney failure, thyroid gland disease, or excessive alcohol intake were excluded from this study.

Phenotype measurements

The subjects were seated in a quiet room and prevented from smoking, exercising, or drinking alcohol, tea, or coffee for at least 1 h before the physical examination. The demographic information of each individual was collected by an interview. Their weights and heights were measured by standard methods, as follows: the body weights and heights were measured with the subjects wearing only light indoor clothing and no shoes; the body mass index (BMI) was calculated by dividing the weight (kg) by height squared (m^2); the blood pressure was measured three times, with a 2 min interval between each measurement: SBP was recorded to the nearest 2 mmHg at the appearance of the first Korotkoff sound (phase I), and DBP was recorded to the nearest 2 mmHg at the disappearance of the fifth Korotkoff sound (phase V) (SBP and DBP were calculated as the means of three consecutive physician-obtained measurements); blood samples were collected after an overnight fast, and the total plasma cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured within 8 h in a local hospital. Informed consent was obtained from all subjects.

Selection of single nucleotide polymorphisms (SNPs)

Tagging SNPs were selected from the Chinese HapMap database (http://www.hapmap.org) based on pairwise $r^2 \geq 0.8$ and minor allele frequency (MAF) ≥ 0.05. In this study, 9 tagSNPs (rs10917696, rs12035879, rs2456899, rs16849802, rs2255642, rs7534573, rs1056514, rs6704267, and rs7355070) of RGS5 were chosen.

Genotyping

Genomic DNA was extracted from leukocytes in samples of peripheral blood using a commercial blood DNA extraction kit (TIANamp Blood DNA kit; Tiangen Biotech, Beijing, China) and was stored at -20°C. All genotyping was performed using the polymerase chain reaction (PCR)/ligase detection reaction assay. Primers were synthesized by Shanghai Hayu Biological Engineering Ltd. (Shanghai, China); the primers are detailed in Table 1. Each set of ligase detection

reaction probes comprised one common probe and two discriminating probes for the two types of allele, as shown in Table 2.

Table 1. Primers for amplification of single nucleotide polymorphisms in the RGS5 gene.

Gene	Upstream primer (5'→3')	Downstream primer (3'→5')	PCR length (bp)
rs10917696	CCTGTTGTACTGTTTTGTTC	GACAGCTGTCAACATAGAAC	98
rs12035879	CTTTACTGTTGTATGAGCAG	GTTGTGAAGTCTATTAAATT	106
rs2456899	GAGAAGGAACGATTAGGCTG	CAGCAGGGTGATGTGAGAAC	100
rs16849802	CATTTCTGGCTGTATGAGAG	CATATGTCTACATTGCCCCC	87
rs2255642	CTTATTTCTGCGGGAACCTC	TCTCTTTTGCATCCCACCTC	92
rs7534573	CTTGTGTTTAGAAACAGAGC	GCTAAGGAATATATAGTAG	100
rs1056514	CTAGGTTTTTGCCAAACTGC	TCATTTCTCTGAAATATAAC	98
rs6704267	TCCCATTTCACCCTATCCTG	CTGACACACATTTATTGCAAG	88
rs7355070	TATGACCTGCAATTGCTTGG	CCCTGACCCCTTTATTAATC	91

Table 2. Probes for the detection of single nucleotide polymorphisms in RGS5.

Probe name	Sequence (5'→3')	LDR length (bp)
rs10917696_modify	P-TGCTCTATGTTA(CT)GTTCTATGTTGATTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs10917696_C	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTGTTTTTCTCTATTTCCAATTGTTG	154
rs10917696_T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTGTTTTTCTCTATTTCCAATTGTTA	156
rs12035879_modify	P-ATTAGTCATTCCCTGAA(CT)AATGAACTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs12035879_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTGTTGTATGAGCAGAAATGCAATAAT	174
rs12035879_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTGTTGTATGAGCAGAAATGCAATAAC	176
rs2456899_modify	P-TAGTATCTTTCTGAGGGAAATGTTCTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs2456899_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTAGGCTGAAATTGTCTGCCTTGTCCT	178
rs2456899_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTAGGCTGAAATTGTCTGCCTTGTCCC	180
rs16849802_modify	P-CCTTTACTCTCTCATACAGCCAGAATTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs16849802_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTGCCCCCAAGTTAATAAAGTGCCAAT	154
rs16849802_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTGCCCCCAAGTTAATAAAGTGCCAAC	156
rs2255642_modify	P-GCACAGATTTAGACTGGGAGTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTT-FAM	
rs2255642_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT GGAACCTCTATTAAATGTTAGGT	121
rs2255642_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT GGAACCTCTATTAAATGTTAGGC	123
rs7534573_modify	P-TTAATACTACTACTACTATATATTCTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs7534573_C	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTCTGAAGTCATATCATAACACG	150
rs7534573_T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTCTGAAGTCATATCATAACACA	152
rs1056514R_modify	P-AAAAGCTAGTTATATTTCAGTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTT-FAM	
rs1056514R_T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATAACAAA TTTGAGAATCGTTGT	109
rs1056514R_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATAACAAATTTGAGAATCGTTGA	111
rs6704267_modify	P-GATGCTGTAAGGGTACACCATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs6704267_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACACATTTATTGCAAGGTGGATT	113
rs6704267_C	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACACATTTATTGCAAGGTGGATG	115
rs7355070_modify	P-TGAGTGGTCTAACTTTTGCTCCAAGTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs7355070_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATTAATCTTTATCTCAAT	150
rs7355070_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATTAATCTTTATCTCAAC	152

LDR, linkage disequilibrium region.

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The target DNA sequences were amplified using a multiplex PCR method. PCR of DNA extracted from each individual was carried out in a final volume of 20 µL, containing 1X PCR buffer, 3.0 mM $MgCl_2$, 2.0 mM deoxynucleotide triphosphate, 2 µL primers, 0.2 µL Qiagen HotStarTaq Polymerase (Qiagen, Shenzhen, China), 4 µL 1X Q-solution, and 50 ng genomic DNA. Thermal cycling was performed for rs10917696, rs12035879, rs2456899, rs16849802, rs7534573, rs1056514, rs6704267, and rs7355070 in a Gene Amp PCR system 9600 (Gene Amp, Norwalk, CT, USA) with an initial denaturation at 95°C for 2 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 90 s, and extension at 65°C for 1 s, and a final extension at 65°C for 1 min. The SNP at rs2255642 was amplified by an additional cycling step: an initial denaturation at 95°C for 2 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 90 s, and extension at 65°C for 30 s, and a final extension at 65°C for 1 min.

The ligation reaction for each sample was carried out in a reaction mixture (final volume 10 µL) containing 1X NEB Taq DNA ligase buffer, 2 pmol each probe mix, 0.05 µL Taq DNA ligase (Biowing, Jiangsu, China), and 4 µL multi-PCR product. The ligase detection reaction was performed for 40 cycles at 95°C for 2 min, 94°C for 15 s, and 50°C for 2 s. The fluorescent products of ligase detection reaction were differentiated by ABI PRISM 3730 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The Statistical Program for Social Sciences (SPSS version 11.0) software platform (IBM, Armonk, NY, USA) was used to perform all statistical analyses. The Hardy-Weinberg equilibrium was determined in each group using the chi-square test. Allele and genotype frequencies between groups were determined using the SHEsis software (Shi and He, 2005). The SHEsis software was also used to calculate the coefficient D' of linkage disequilibrium (LD) and to construct haplotypes. Haplotypes with frequencies <3% in the whole sample were considered to be rare. A strong LD was characterized when D' > 0.8.

RESULTS

Clinical characteristics of EH and control subjects

The characteristics of the EH patients and control subjects are summarized in Table 3. Significant differences in age, SBP, DBP, and BMI, and TC, TG, HDL-C, and LDL-C levels were observed between the EH patients and controls ($P < 0.05$). We observed no significant difference between the groups with respect to the gender.

Single-locus association study of tagSNPs and EH

Genotype frequencies of all 9 tagSNPs of RGS5 satisfied the Hardy-Weinberg equilibrium in EH subjects as well as controls in the Mongolian and Han populations ($P > 0.05$). The genotype and allele frequency distributions are shown in Table 4; the frequency of distribution of the A-allele of SNP rs12035879 differed significantly between hypertensive patients and controls in the Han population, while the allele and genotypes frequencies of rs16849802 differed significantly between the hypertensive subjects and controls in the Mongolian population ($P < 0.05$).

Table 3. Characteristics of Mongolian and Han individuals participating in this study.

	Mongolian		P	Han		P
	HT (N = 212)	NT (N = 217)		HT (N = 200)	NT (N = 216)	
SBP (mmHg)	155.47 ± 13.94	120.45 ± 12.22	0.00**	154.74 ± 15.92	120.45 ± 10.93	0.00**
DBP (mmHg)	92.34 ± 10.60	73.39 ± 8.59	0.00**	91.33 ± 10.32	78.87 ± 8.98	0.00**
BMI (kg/m^2)	27.78 ± 3.97	24.96 ± 3.71	0.00**	26.37 ± 3.27	24.52 ± 3.50	0.00**
TC (mM)	5.00 ± 1.05	4.66 ± 0.86	0.00**	4.99 ± 1.02	4.65 ± 0.88	0.046*
TG (mM)	2.02 ± 1.32	1.50 ± 1.17	0.00**	1.94 ± 1.12	1.60 ± 1.00	0.002**
HDL (mM)	1.25 ± 0.33	1.32 ± 0.32	0.00**	1.24 ± 0.32	1.28 ± 0.30	0.00**
LDL (mM)	3.21 ± 0.73	2.95 ± 0.70	0.00**	3.12 ± 0.79	2.88 ± 0.70	0.00**

HT, hypertensive; NT, normotensive; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol. All data are reported as means ± standard deviation. *P < 0.05, **P < 0.01.

Table 4. Frequency distribution of RGS5 genotypes and alleles.

Gene	Allele/Genotype	Han				Mongolian			
		HT [N (%)]	NT [N (%)]	χ^2	P	HT [N (%)]	NT [N (%)]	χ^2	P
rs2255642	AA	24 (0.120)	23 (0.106)	0.97	0.62	20 (0.095)	26 (0.120)	3.25	0.20
	AG	84 (0.420)	101 (0.468)			111 (0.526)	95 (0.440)		
	GG	92 (0.460)	92 (0.426)			80 (0.379)	95 (0.440)		
	A	132 (0.330)	147 (0.340)	0.10	0.75	151 (0.358)	147 (0.340)	0.29	0.59
	G	268 (0.670)	285 (0.660)			271 (0.642)	285 (0.660)		
rs7534573	CC	12 (0.060)	16 (0.074)	0.34	0.84	7 (0.033)	9 (0.042)	0.60	0.74
	CT	76 (0.382)	83 (0.384)			87 (0.412)	82 (0.380)		
	TT	111 (0.558)	117 (0.542)			117 (0.555)	125 (0.579)		
	C	100 (0.251)	115 (0.266)	0.24	0.62	101 (0.239)	100 (0.231)	0.07	0.79
	T	298 (0.749)	317 (0.734)			321 (0.761)	332 (0.769)		
rs1056514	AA	166 (0.843)	171 (0.803)	1.21	0.55	168 (0.796)	175 (0.806)	1.27	0.53
	AT	29 (0.147)	40 (0.188)			40 (0.190)	36 (0.166)		
	TT	2 (0.010)	2 (0.009)			3 (0.014)	6 (0.028)		
	A	361 (0.916)	382 (0.897)	0.92	0.34	376 (0.891)	386 (0.889)	0.01	0.94
	T	33 (0.084)	44 (0.103)			46 (0.109)	48 (0.111)		
rs6704267	AA	53 (0.269)	66 (0.310)	3.94	0.14	61 (0.289)	73 (0.336)	1.21	0.55
	AC	92 (0.467)	108 (0.507)			108 (0.512)	106 (0.488)		
	CC	52 (0.264)	39 (0.183)			42 (0.199)	38 (0.175)		
	A	198 (0.503)	240 (0.563)	3.05	0.08	230 (0.545)	252 (0.581)	1.10	0.29
	C	196 (0.497)	186 (0.437)			192 (0.455)	182 (0.419)		
rs7355070	AA	16 (0.080)	18 (0.085)	0.25	0.88	15 (0.071)	15 (0.069)	0.70	0.70
	AG	88 (0.440)	98 (0.460)			94 (0.445)	88 (0.407)		
	GG	96 (0.480)	97 (0.455)			102 (0.483)	113 (0.523)		
	A	120 (0.300)	134 (0.315)	0.21	0.65	124 (0.294)	118 (0.273)	0.45	0.50
	G	280 (0.700)	292 (0.685)			298 (0.706)	314 (0.727)		
rs10917696	CC	0 (0.000)	1 (0.005)	1.00	0.61	1 (0.005)	2 (0.009)		
	CT	30 (0.150)	30 (0.141)			30 (0.142)	35 (0.162)		
	TT	170 (0.850)	182 (0.854)			180 (0.853)	179 (0.829)		
	C	30 (0.075)	32 (0.075)	1.00	1.00	32 (0.076)	39 (0.090)	0.58	0.44
	T	370 (0.925)	394 (0.925)			390 (0.924)	393 (0.910)		
rs12035879	AA	40 (0.200)	25 (0.117)	5.62	0.06	32 (0.152)	31 (0.144)	1.07	0.59
	AG	100 (0.500)	112 (0.526)			101 (0.479)	114 (0.528)		
	GG	60 (0.300)	76 (0.357)			78 (0.370)	71 (0.329)		
	A	180 (0.450)	162 (0.380)	4.13	0.04*	165 (0.391)	176 (0.407)	0.23	0.62
	G	220 (0.550)	264 (0.620)			257 (0.609)	256 (0.593)		
rs2456899	AA	156 (0.784)	152 (0.704)	3.50	0.17	160 (0.758)	148 (0.685)	3.19	0.20
	AG	40 (0.201)	60 (0.278)			48 (0.227)	62 (0.287)		
	GG	3 (0.015)	4 (0.019)			3 (0.014)	6 (0.028)		
	A	352 (0.884)	364 (0.843)	3.10	0.08	368 (0.872)	358 (0.829)	3.15	0.08
	G	46 (0.116)	68 (0.157)			54 (0.128)	74 (0.171)		
rs16849802	AA	1 (0.005)	1 (0.005)	0.01	1.0				
	AG	20 (0.101)	22 (0.102)			20 (0.095)	8 (0.037)	5.81	0.02*
	GG	178 (0.894)	193 (0.894)			191 (0.905)	208 (0.963)		
	A	22 (0.055)	24 (0.056)	0.00	1.00	20 (0.047)	8 (0.019)	5.61	0.02*
	G	376 (0.945)	408 (0.944)			402 (0.953)	424 (0.981)		

HT = hypertensive; NT = normotensive. *P < 0.05.

Haplotypes of tagSNPs

Linkage disequilibrium plots of the *RGS5* genes in the study population are shown in Figures 1 and 2. The LD was measured among the tagSNPs separately in both groups by the Lewontin standardized disequilibrium coefficient D' (Slatkin, 2008). Adjacent SNPs in strong LD (D' > 0.8), rs2255642, rs1056642, and rs2456899 and rs2255642, rs2456899, and 16849802 in the Han and Mongolian populations, respectively, were chosen to structure the haplotypes for subsequent analyses. The frequency of haplotype GAA was significantly higher in the EH group (5%) than in the control group (1%) in the Mongolian population (Table 5). The haplotype frequency did not differ significantly between the patients and controls in the Han population (Table 6).

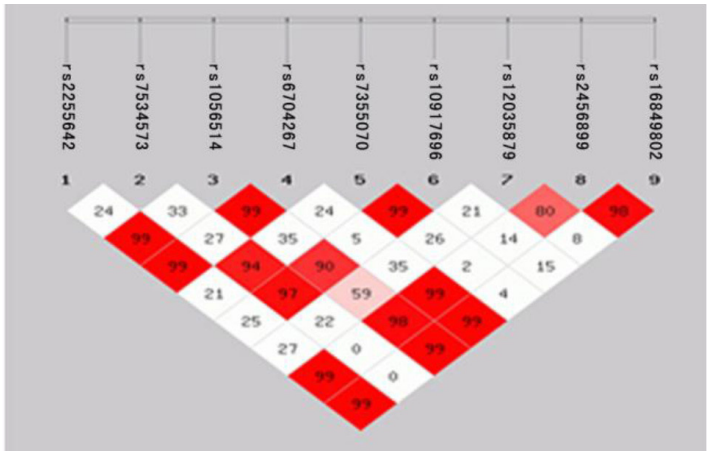

Figure 1. Linkage disequilibrium plot for the RGS5 gene in the Han population.

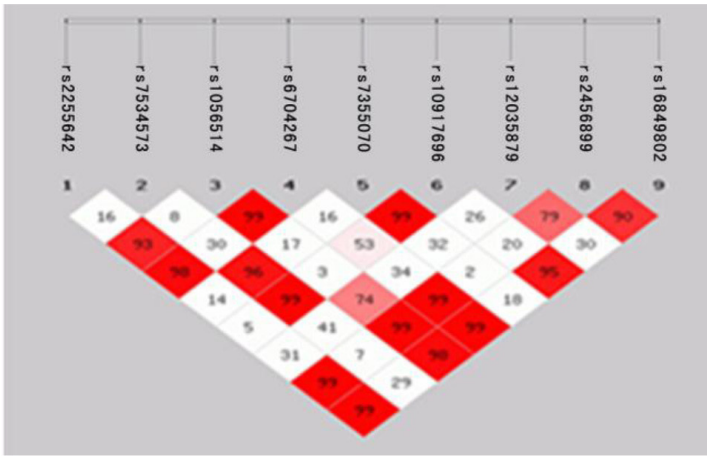

Figure 2. Linkage disequilibrium plot for the RGS5 gene in the Mongolian population.

Table 5. RGS5 haplotype frequency distribution in the Mongolian population.

Haplotype	HT [N (%)]	NT [N (%)]	χ^2	OR	95%CI	P
A A G	150.98 (0.36)	147.00 (0.34)	0.29	1.08	0.82-1.43	0.59
G A A	20.00 (0.05)	6.14 (0.01)	7.93	3.45	1.38-8.61	0.00*
G A G	195.02 (0.46)	204.86 (0.47)	0.12	0.95	0.73-1.25	0.73
G G A	0.00 (0.00)	1.86 (0.00)				
G G G	53.98 (0.13)	72.14 (0.17)	2.58	0.73	0.50-1	0.11
A G G	0.02 (0.00)	0.00 (0.00)				
Globe χ^2	10.14					
Fisher P	0.02*					

HT = hypertensive; NT = normotensive; OR = odds ratio; CI = confidence interval; χ^2 = Pearson chi-square, *P < 0.05.

Table 6. RGS5 haplotype frequency distribution in the Han population.

Haplotype	HT [N (%)]	NT [N (%)]	χ^2	OR	95%CI	P
A A A	92.98 (0.24)	100.98 (0.24)	0.00	1.00	0.73-1.38	1.00
A A G	0.02 (0.00)	0.02 (0.00)				
A T A	32.99 (0.01)	44.00 (0.10)	0.87	0.80	0.50-1.28	0.35
G A A	220.02 (0.56)	214.02 (0.50)	2.84	1.27	0.96-1.67	0.09
G A G	45.98 (0.12)	66.98 (0.16)	2.74	0.71	0.48-1.07	0.10
A T G	0.01 (0.00)	0.00 (0.00)				
Globe χ^2	4.48					
Fisher	0.21					

HT = hypertensive; NT = normotensive; OR = odds ratio; CI = confidence interval; χ^2 = Pearson chi-square, *P < 0.05.

DISCUSSION

Regulators of G-protein signaling (RGSs) are a family of proteins that promote GTPase activity of G-protein-coupled receptors. RGS5 is one of the intracellular regulators of G protein signaling (RGS) proteins. It is expressed in the heart, lungs, and kidneys, as well as in highly specialized cell types, such as vascular smooth muscle cells and cardiac myocytes. RGS5 has several potential roles in blood pressure regulation. Firstly, RGS5 inhibits G-protein signaling by inactivating Gα(q) and Gα(i), which mediate vasoconstrictors, such as angiotensin II and endothelin-1 (Hollinger and Hepler, 2002). Secondly, RGS5 is down-regulated during morphogenesis of the developing vasculature, and up-regulated in tumor-associated blood vessels, suggesting a role in angiogenesis (Furuya et al., 2004). Moreover, RGS5 might play a role in sensing hemodynamic change and remodeling arteries (Li et al., 2004). Recently, two independent studies demonstrated that RGS5-deficient mice were hypotensive, relative to the wild-type controls (Cho et al., 2008; Nisancioglu et al., 2008), further lending credence to its potential role in BP regulation.

Chang et al. (2007) studied 1,010 European American (EA) and 816 African American (AA) samples by variance-component linkage analysis, and observed that a 13 SNPs in *RGS5* were assigned to two LD blocks. Therefore, the eight SNPs were significantly associated with BP in at least one of the six studied sample groups. Mezbah et al. (2011), in a case-control study conducted in a well-characterized cohort of 968 African-Americans, genotyped 87 SNPs across the *RGS5* genes, and identified a significant association between the SNP rs2815272 and hypertension, SBP, and DBP; additionally, the SNPs at rs2815287 and rs2841997 were associated with both SBP and DBP. However, a literature search did not yield any recent reports assessing the contribution of *RGS5* polymorphisms to susceptibility of Mongolian individuals to hypertension. Therefore, we investigated the relationship between *RGS5* polymorphisms and hypertension in Mongolian and Han populations.

TagSNPs sufficiently capture most of the haplotype structure of the gene (Zhang et al., 2004); in this study, we performed analyses based on both SNPs and haplotypes. The distribution of allele frequencies of rs12035879 differed significantly between the EH patients and control subjects in the Han population; moreover, we observed significant differences in the genotype and allele distributions of rs16849802 in Mongolian EH patients and controls. These results hinted at a correlation between rs16849802 and incidence of hypertension in Mongolian individuals, and that the genetics of hypertension may be ethnicity-specific, that is, the same SNP may have different effects in different ethnic groups. Adjacent SNPs in strong LD (D' > 0.8) were selected (rs2255642, rs2456899, and rs16849802) in the Mongolian population in order to structure the haplotype. The frequency of haplotype GAA was significantly higher in the EH group (5%) than in the control group (1%) in the Mongolian population. The haplotype GAA had an independent effect of increasing the risk of EH in Mongolian patients, and may be used as a risk factor for the prediction of high blood pressure. We observed no significant differences in the haplotype frequency between Han patients and controls; this indicated that the SNPs at rs2255642, rs1056514, and rs2456899 had no cumulative effect in the Han population. Hypertension is a polygenic disease (Marteau et al., 2005), in which separate minor genes may have a weak or mildly noticeable impact on the blood pressure when multiple minor genes reach a certain threshold by epistasis; however, other interactions may play a role in the genetic architecture of blood pressure regulation, and candidate gene studies have limited scope to test for epistasis. Additionally, genome-wide studies have low power for both main effects and interactions. This indicates the need for elucidation of gene-gene interactions in the future; additionally, we discovered that the same gene may have different effects in different ethnic groups.

In conclusion, polymorphic variants in the *RGS5* gene on chromosome 1q were systematically investigated for their association with hypertension in the Mongolian and Han population. The results of this study extend the previous reports of association between the *RGS5* gene and hypertension. Based on the physiological role of the *RGS5* gene in blood pressure homeostasis, follow-up functional studies of these variants may help in interpreting their underlying pathophysiological implications.

Conflicts of interest

The authors declare no conflict of interest.

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