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Association of variants in renal salt reabsorption-related gene *SLC12A3* with essential hypertension in a Mongolian population

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ABSTRACT. Mounting evidence has implicated the *SLC12A3* gene in essential hypertension. Here, we examined the potential associations of common variants of the *SLC12A3* gene with blood pressure traits in Mongolians in China. Genomic DNA was extracted from 508 unrelated Mongolian patients with essential hypertension and 246 normotensive Mongolian subjects for genotyping. The genotype distributions of all selected polymorphisms were consistent with Hardy-Weinberg equilibrium. The presence of the G allele in the rs7187932 polymorphism was found to be associated with an increased risk of hypertension (OR: 1.30; 95%CI = 1.00-1.38; P = 0.048), whereas the rs2399594 G allele was associated with a reduced risk for hypertension (OR: 0.76; 95%CI = 0.60-0.97; P = 0.030). No significant difference was observed for other alleles. Haplotype analysis revealed an association of the rs2399594 and rs711746 GG haplotype with a reduced risk for hypertension (OR: 0.76; 95%CI = 0.60-0.97; P = 0.029). No significant association was

observed between other haplotypes and hypertension. These results suggest that the *SLC12A3* gene is a susceptibility gene for hypertension in the Mongolian population.

Key words: Hypertension; *SLC12A3*; TagSNP; Mongolian; Haplotype

INTRODUCTION

Hypertension is one of the most common cardiovascular diseases threatening human health. As the age of onset of hypertension has become increasingly younger, hypertension has become a focus of research worldwide. The causes of hypertension are heterogeneous, and include genetic factors, environmental factors, and lifestyles. Furthermore, over 160 candidate genes have been implicated in the regulation of blood pressure (BP) (Sõber et al., 2009).

Renal sodium reabsorption plays an important role in the development of hypertension. On average, the kidneys filter over 170 L plasma containing 23 moles of salt per day. Therefore, maintenance of salt homeostasis on a typical 100 mEq sodium diet requires that the kidneys reabsorb 99.5% of the filtered salt. This feat is accomplished by an integrated system of ion channels, exchangers, and transporters (Lifton et al., 2001). Of the reabsorbed salt, 7% is reclaimed by the Na-Cl cotransporter in the distal convoluted tubule (DCT). The mechanism whereby increased renal sodium reabsorption initiates BP elevation probably consists of increased water reabsorption as a result of increased net renal salt reabsorption in order to maintain plasma sodium concentrations at or near 140 mM; the resultant increased intravascular volume augments venous blood return to the heart, thereby raising cardiac output and leading directly to hypertension (Lifton et al., 2001).

The human *SLC12A3* gene is located in 16q13 and includes 26 exons spanning a length of 55 kb (Mastroianni et al., 1996; Simon et al., 1996). *SLC12A3* encodes the TSC protein, a transmembrane protein of 1021 amino acid residues with 12 transmembrane domains (Gamba, 2009). TSC is primarily expressed in the DCT of the kidney, and functions as a thiazide-sensitive Na-Cl cotransporter. Accumulating evidence shows that TSC plays an important role in BP regulation. The activation of TSC leads to increased kidney sodium reabsorption, which initiates the occurrence of hypertension. A number of association studies in animals and humans have shown that the *SLC12A3* gene is a susceptibility factor for hypertension. Melander et al. (2000) found that a G to A mutation in exon 23 of the *SLC12A3* gene (Arg904Gln) led to an increased risk for hypertension in a Southern Swedish population. This result was later confirmed by Matsuo et al. (2004), who demonstrated that the Arg904Gln polymorphism of *SLC12A3* is a genetic predisposing factor for hypertension in young Japanese women. In addition, Ji et al. (2008) and Acuña et al. (2011) reported that *SLC12A3* mutations are associated with reduced hypertension risk in the Framingham Heart Study. In addition, their studies suggested that lower BP is due to a reduced activity of this cotransporter. Therefore, with an increase of TSC activity, greater amounts of sodium and chloride might be transported from the kidneys to the plasma, resulting in an increased efficiency of kidney sodium reabsorption.

Matayoshi et al. (2004) indicated that the BP in patients with essential hypertension who were homozygous for the major alleles of the *SLC12A3* C1784T mutation was reduced by thiazide diuretics; however, the BP levels in those patients homozygous for the minor allele or in heterozygotes were not changed by thiazide diuretics. Thus, they suggested that Japanese patients with essential hypertension carrying the C1784T mutation of *SLC12A3* were

susceptible to the antihypertensive effect of thiazide diuretics. Keszei et al. (2007) found that a C>T mutation in exon 24 of *SLC12A3*, resulting in a substitution of Arg for Cys at the amino acid position 919 of TSC (Arg919Cys), increased Na^+ transport function due to a change in the intrinsic activity of the mutant transporter, suggesting that the Arg919Cys variant might contribute to the development of primary hypertension. However, they failed to demonstrate a correlation between the Arg904Gln variant and hypertension in a Canadian population. On the other hand, Wang et al. (2008) and our earlier study (Chang et al., 2011a) reported that the Arg904Gln polymorphism of the *SLC12A3* gene had no significant association with hypertension in Uyghur, Kazak, and Mongolian populations, which are minority populations in China. Furthermore, a case-control study performed by Aoi et al. (2008), following examination of three *SLC12A3* mutations including T180K, A569V, and L849H, verified that the *SLC12A3* gene was not involved in essential hypertension in a Japanese population.

In the earlier stage of our research, we also studied the association of the *SLC12A3* gene with hypertension in 385 unrelated Mongolians (158 Mongolian hypertension patients *vs* 227 Mongolian normotensives) and 523 Han (284 Han hypertension patients and 239 Han normotensives) in the Inner Mongolia region of China (Chang et al., 2011b). Our result showed that rs7204044 was a genetic factor for hypertension in the Mongolian population in Inner Mongolia. However, due to the limited sample size, the effects of common variants of the *SLC12A3* gene on the occurrence of essential hypertension in Mongolians in the Inner Mongolian region remained elusive. In the current study, we increased the sample size and conducted a linkage disequilibrium (LD) and haplotype analysis in Mongolian patients with hypertension *vs* healthy subjects of the same ethnic background to examine the possible associations of *SLC12A3* gene variants with hypertension.

MATERIAL AND METHODS

Subjects

Cross-sectional surveys were carried out on four separate occasions in three areas of Xilin Gol League in the Inner Mongolia Autonomous Region of China, including Dongwuzhumuxin county, Xianghuang county, and the city of Xilinhot. According to the World Health Organization (WHO) criteria, subjects with systolic BP (SBP) ≥ 140 mmHg and/or diastolic BP (DBP) ≥ 90 mmHg, or taking at least one antihypertensive medicine, were defined as essentially hypertensive. Subjects with secondary hypertension, severe cardiovascular disease, or kidney disease were excluded. All subjects were of Mongolian ethnic origin. The study protocol was approved by the Ethical Committee of Affiliated Hospital of Inner Mongolia Medical University and written informed consent was obtained from all participants.

Subject evaluation

Subject name, age, gender, ethnicity, height, weight, body mass index (BMI), waistline, hipline, and history of drinking and tobacco use were recorded. Smoking was defined as smoking at least one cigarette per day for at least one year, and drinking was defined as consuming 50 mL or more alcohol per day for at least one year. Body weight and height were measured with subjects wearing only light indoor clothing and no shoes. BMI was calculated by dividing the body weight (kg) by height squared (m^2). Blood samples were collected after an overnight

fast, and total plasma cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured within 8 h at a local hospital, using routine methods. Initially, 908 subjects were involved in our study.

Genotyping

Tag single nucleotide polymorphisms (tagSNPs) representing haplotypes of the *SLC12A3* gene were identified from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) based on pairwise $r^2 \geq 0.5$ and minor allele frequency (MAF) ≥ 0.05 values, and 15 tagSNPs were selected according to the block of LD in our study, including rs4784733, rs2304478, rs13306673, rs2289119, rs8043560, rs2304483, rs5803, rs7187932, rs6499858, rs11644728, rs8049280, rs7204044, rs2010501, rs2399594, and rs711746.

A 1-mL peripheral blood sample was collected from each subject. Genomic DNA was extracted using an AxyPrep-96 DNA Extraction Kit (Axygen, Union City, CA, USA). The method of multiplex-polymerase chain reaction (PCR)-ligase detection reaction (LDR) was used for genotyping. The sequences of the PCR primers (Takara, Dalian, China) used in the current study are listed in Table 1. The target DNA sequences were amplified using a multiplex PCR method. PCR was carried out in a final volume of 10 μL, containing 1X PCR buffer, 3.0 mM $MgCl_2$, 2.0 mM deoxynucleotide triphosphate, 0.4 μL primers, 0.2 μL Qiagen HotStarTaq Polymerase (QIAGEN, Shenzhen, China), 4 μL 1X Q-solution, and 10-20 ng genomic DNA. Thermal cycling was performed for all SNP loci in Gene Amp PCR system 9600 (PerkinElmer, Waltham, MA, USA). The SNPs selected were divided into two groups for multiple PCR based on annealing temperature. For Group A SNPs, PCR was run at 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 45°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 10 min. For Group B SNPs, the PCR was run at 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. The ligation reactions were carried out in a final volume of 10 μL, containing 1X NEB Taq DNA ligase buffer, 12.5 pmol of each probe mix, 0.05 μL Taq DNA ligase (NEB Biotechnology, Beijing, China), and 1 μL multi-PCR product. A total of 35 cycles for ligase detection reaction were performed with 95°C for 2 min, 94°C for 30 s, and 60°C for 2 min. The fluorescent products of ligase detection reaction were differentiated by Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, CA, USA). To verify the accuracy of the genotyping results for tagSNPs, we randomly sequenced 20 samples.

Table 1. PCR primers used in the current study.

SNPs	Forward: (5'-3')	Reverse: (5'-3')
Group A		
rs7204044	CATTTCCACTGTCTTGCGCT	CCAACACCTAGCAGAGAATG
rs11644728	CAAGGTGCCCCACATTTTCA	TGCTTGTTCAGAGCCTGAAG
rs8043560	ACTGACAGTGTGCACTTTAC	GGAAAAGACTGGAAAACCCC
rs5803	GGAAGATCAAGGCCTTCTAC	ACCTGCATGAGGATCTGGAC
rs13306673	TGCAGGTCAGTGGGCTGGAT	AGAGACCACAAGTCAGGCAG
rs8049280	TTTCCCCCTACTTCTGTTGC	AGAGGGTATTGCAGACACAG
rs6499858	GCTGGTTCTTCATCCTTGAC	GAGAGAAGGGAAAAGGGTCA
Group B		
rs2304478	CTGCAGCTCCACTATTTCAC	TCTGTCATCTGTGCCATGGG
rs2304483	CTAGAAAGAGGCTCGACTGC	ATTGGAATGAAGGTCTCCAC
rs2289119	ATAATGAAGGGACAGCTGCC	TTACTCTGTCCTCTTCCAGC
rs2399594	AGACCCTCCTGGAGCCCAGA	GACTTTTCTACTATCTGGAG
rs4784733	CTCCACAATCAAATGGTGTT	TTGGCCTAAACCGCCCGTG
rs2010501	CACAAAGCTTCACCTGGGAC	GATTACAGGTGTGACCATGC
rs711746	CTGACTCAAAGGAAGTGACC	CACTTGACGTTCTCCCCA
rs7187932	CTTGCCCCAGGATTATGTAG	AGGTTAGGTAACCCTTCCTC

Statistical analysis

The baseline data of hypertensive and normotensive subjects were assessed by ANOVA using the SPSS13.0 software (SPSS Inc., Chicago, IL, USA). LD was analyzed with the Haploview 4.0 software (Barrett et al., 2005). The association of alleles and genotypes of single selected SNPs and haplotypes with hypertension was examined using the UNPHASED3.0.13 software and the Haploview 4.0 software (Barrett et al., 2005). The association of allele distributions of the selected SNPs with SBP and DBP was evaluated using the UNPHASED3.0.13 software. The association of the genotype distribution of the SNPs with SBP and DBP was examined using additive, dominant, and recessive models with the SPSS13.0 software. Hardy-Weinberg equilibrium for genotypic distributions was examined using the χ^2 goodness-of-fit test. In this study, SBP and DBP were treated as a continuous variable. Statistical significance was set at $P < 0.05$ (2-sided).

RESULTS

Demographic and baseline characteristics of the study subjects

A total of 754 subjects (508 hypertensive patients and 246 normotensive subjects) were included in this study. Forty patients with hypertension (7.9%) had elevated SBP only, 65 (12.8%) had elevated DBP only, and 403 (79.3%) had both elevated SBP and DBP. The demographic and baseline data of the study subjects are presented in Table 2. The normotensive and hypertensive subjects showed significant differences in gender, age, BMI, waistline, hipline, SBP, DBP, and TGs. Overall, hypertensive subjects were markedly older and had significantly higher BMIs than normotensive subjects ($P < 0.01$). The mean SBPs were 162.02 ± 25.67 and 118.31 ± 12.98 mmHg and the DBPs were 101.52 ± 15.12 and 75.49 ± 8.51 mmHg for the hypertensive and normotensive subjects, respectively ($P < 0.001$).

Table 2. Demographic and baseline characteristics of the study participants.

Variables	Normotensives	Hypertensives	P value
No.	225	496	
Male gender, N (%)	91 (40.4)	245 (49.4)	0.030*
Age (years)	40 ± 16.04	52.54 ± 12.56	0.000**
BMI (kg/m^2)	23.53 ± 4.53	26.84 ± 5.21	0.000**
Waistline (cm)	82.65 ± 13.25	91.94 ± 14.14	0.000**
Hipline (cm)	95.15 ± 13.46	102.61 ± 10.52	0.000**
SBP (mmHg)	118.31 ± 12.98	162.02 ± 25.67	0.000**
DBP (mmHg)	75.49 ± 8.51	101.52 ± 15.12	0.000**
Fasting blood glucose	5.67 ± 1.65	5.78 ± 1.77	0.583
Heart rate	78.18 ± 12.52	71.66 ± 9.66	0.239
HDL-C (mM)	1.46 ± 0.54	1.46 ± 0.55	0.744
LDL-C (mM)	3.01 ± 1.32	3.20 ± 1.38	0.124
TG (mM)	1.51 ± 1.31	1.84 ± 1.76	0.001*
TC (mM)	4.74 ± 1.80	4.99 ± 1.91	0.205
Smoking, N (%)	55 (24.9)	131 (30.3)	0.169
Alcohol consumption, N (%)	42 (20.1)	148 (34.4)	0.000**

BMI = body mass index; DBP = diastolic blood pressure; HDL = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride. *P < 0.05, **P < 0.001.

Genotype and allele distributions of the *SLC12A3* SNPs

The genotype distributions of all selected polymorphisms were consistent with Hardy-Weinberg equilibrium. The allele distributions of *SLC12A3* rs7187932 and rs2399594 polymorphisms were significantly different between the hypertensive and normotensive subjects (Table 3). The presence of the G allele in rs7187932 was associated with an increased risk of hypertension (OR: 1.30; 95%CI = 1.00-1.38; P = 0.048) while the presence of the G allele in rs2399594 was associated with a reduced risk for hypertension (OR: 0.76; 95%CI = 0.60-0.97; P = 0.030). No significant difference was observed in other allele distributions.

Table 3. Association of single SNP of the *SLC12A3* gene with hypertension.

SNPs	Sites	Case	Control	OR (95%CI)	χ^2	df	P
rs4784733	TT	300 (0.61)	155 (0.64)	1	2.94	2	0.230
	CT	179 (0.36)	75 (0.31)	1.23 (0.88-1.72)			
	CC	14 (0.03)	11 (0.05)	0.66 (0.29-1.48)			
	T	779 (0.79)	385 (0.80)	1	0.15	1	0.699
	C	207 (0.21)	97 (0.20)	1.06 (0.80-1.38)			
rs2304478	AA	8 (0.02)	6 (0.02)	1	1.05	2	0.590
	AG	103 (0.21)	55 (0.23)	1.41 (0.46-4.25)			
	GG	382 (0.77)	180 (0.75)	1.59 (0.54-4.66)			
	A	119 (0.12)	67 (0.14)	1	0.97	1	0.325
	G	867 (0.88)	415 (0.86)	1.18 (0.85-1.62)			
rs13306673	CC	3 (0.01)	5 (0.02)	1	3.26	2	0.196
	CT	89 (0.18)	39 (0.16)	3.80 (0.87-16.71)			
	TT	411 (0.82)	200 (0.82)	3.43 (0.81-14.48)			
	C	95 (0.09)	49 (0.10)		0.13	1	0.714
	T	911 (0.91)	439 (0.90)	1.07 (0.74-1.54)			
rs2289119	AA	46 (0.09)	27 (0.11)	1	0.93	2	0.628
	AG	194 (0.39)	88 (0.37)	1.29 (0.76-2.22)			
	GG	253 (0.51)	126 (0.52)	1.18 (0.7-1.98)			
	A	286 (0.29)	142 (0.29)	1	0.03	1	0.857
	G	700 (0.71)	340 (0.71)	1.02 (0.80-1.30)			
rs8043560	CC	251 (0.50)	120 (0.49)	1	0.34	2	0.843
	CT	212 (0.42)	107 (0.44)	0.95 (0.69-1.30)			
	TT	40 (0.08)	17 (0.07)	1.13 (0.61-2.07)			
	C	714 (0.71)	347 (0.71)		0.00	1	0.958
	T	292 (0.29)	141 (0.29)	1.01 (0.79-1.28)			
rs2304483	AA	82 (0.17)	30 (0.12)	1	2.42	2	0.298
	AG	233 (0.47)	116 (0.48)	0.73 (0.46-1.18)			
	GG	178 (0.36)	95 (0.39)	0.69 (0.42-1.12)			
	A	397 (0.40)	176 (0.37)	1	1.92	1	0.165
	G	589 (0.60)	306 (0.63)	0.85 (0.68-1.07)			
rs5803	CC	39 (0.08)	20 (0.08)	1	0.56	2	0.755
	CT	204 (0.41)	92 (0.38)	1.14 (0.63-2.06)			
	TT	260 (0.52)	132 (0.54)	1.01 (0.57-1.80)			
	C	282 (0.28)	132 (0.27)	1	0.95	1	0.690
	T	724 (0.72)	356 (0.73)	0.95 (0.75-1.21)			
rs7187932	AA	24 (0.05)	14 (0.06)	1	4.65	2	0.098
	AG	158 (0.32)	95 (0.39)	0.97 (0.48-1.97)			
	GG	311 (0.63)	132 (0.55)	1.37 (0.69-2.74)			
	A	206 (0.21)	123 (0.26)	1	3.93	1	0.048*
	G	780 (0.79)	359 (0.74)	1.30 (1.00-1.38)			
rs6499858	CC	46 (0.09)	33 (0.14)	1	3.27	2	0.195
	CT	221 (0.44)	104 (0.43)	1.52 (0.92-2.52)			
	TT	236 (0.47)	107 (0.44)	1.58 (0.96-2.61)			
	C	313 (0.31)	170 (0.35)	1	2.07	1	0.150
	T	693 (0.69)	318 (0.65)	1.18 (0.94-1.49)			

Continued on next page

Table 3. Continued.

SNPs	Sites	Case	Control	OR (95%CI)	χ^2	df	P
rs11644728	TT	4 (0.01)	5 (0.02)	1	2.78	2	0.249
	TG	99 (0.20)	41 (0.17)	3.02 (0.77-11.81)			
	GG	400 (0.80)	198 (0.81)	2.53 (0.67-9.51)			
	T	107 (0.11)	51 (0.10)	1	0.01	1	0.913
	G	899 (0.89)	437 (0.90)	0.98 (0.69-1.40)			
rs8049280	TT	364 (0.72)	182 (0.75)	1	4.88	2	0.087
	TC	133 (0.26)	54 (0.22)	1.23 (0.86-1.77)			
	CC	6 (0.01)	8 (0.03)	0.38 (0.13-1.10)			
	T	861 (0.86)	418 (0.86)	1	0.00	1	0.972
	C	145 (0.14)	70 (0.14)	1.01 (0.74-1.37)			
rs7204044	AA	7 (0.01)	1 (0.004)	1	1.84	2	0.399
	AG	122 (0.24)	62 (0.25)	0.28 (0.04-2.37)			
	GG	374 (0.74)	181 (0.74)	0.29 (0.04-2.42)			
	A	136 (0.14)	64 (0.13)	1	0.05	1	0.829
	G	870 (0.86)	424 (0.87)	0.97 (0.70-1.33)			
rs2010501	TT	288 (0.58)	139 (0.58)	1	0.58	2	0.749
	TC	181 (0.37)	87 (0.36)	1.00 (0.72-1.39)			
	CC	24 (0.05)	15 (0.06)	0.77 (0.39-1.52)			
	T	757 (0.77)	365 (0.76)	1	0.20	1	0.657
	C	229 (0.23)	117 (0.24)	0.94 (0.73-1.22)			
rs2399594	AA	283 (0.57)	123 (0.51)	1	5.16	2	0.076
	AG	180 (0.37)	93 (0.39)	0.84 (0.61-1.17)			
	GG	30 (0.06)	25 (0.10)	0.52 (0.29-0.92)			
	A	746 (0.76)	339 (0.70)	1	4.70	1	0.030*
	G	240 (0.24)	143 (0.30)	0.76 (0.60-0.97)			
rs711746	AA	194 (0.39)	86 (0.36)	1	5.81	2	0.055
	AG	240 (0.49)	110 (0.46)	0.97 (0.69-1.36)			
	GG	59 (0.12)	45 (0.18)	0.58 (0.37-0.92)			
	A	628 (0.64)	282 (0.59)	1	3.68	1	0.055
	G	358 (0.36)	200 (0.41)	0.80 (0.64-1.00)			

Haplotype analysis

LD analysis indicated that 11 of the 15 examined SNPs fell into three high LD blocks: block 1 (rs4784733, rs2304478, and rs13306673), block 2 (rs8043560, rs2304483, rs5803, rs7187932, rs6499858, and rs11644728), and block 3 (rs2399594 and rs711746). A haplotype analysis based on the three blocks with high LD values showed that haplotype GG consisting of rs2399594 and rs711746 was associated with a markedly reduced risk for hypertension (OR: 0.76; 95%CI = 0.60-0.97; P = 0.029) (Table 4). No significant association was observed between other haplotypes and hypertension. Furthermore, we found that only rs2304478 (P = 0.028) and rs2399594 (P = 0.029) (Table 5) were significantly associated with the SBP of patients with hypertension. No alleles of the selected SNPs were associated with DBP. Using the recessive model, we found that rs2304478 (P = 0.035) and rs2399594 (P = 0.037) were significantly associated with SBP whereas we failed to observe the association of any SNPs with BP using the additive and dominant models. Furthermore, DBP was not associated with any genotype distributions of the selected SNPs in any of the three genetic models (Table 6).

Table 4. Estimated haplotype frequency and its correlation with hypertension.

	Haplotype	Frequency	OR (95%CI)	χ^2	P
Block 1[a]	TGC	0.67	1.04 (0.83-1.31)	0.12	0.733
	TAC	0.13	0.85 (0.62-1.18)	0.95	0.329
	CGC	0.11	1.18 (0.83-1.68)	0.81	0.369
	CGT	0.10	0.96 (0.67-1.38)	0.16	0.688
Block 2[b]	CACGCG	0.29	1.04 (0.81-1.32)	0.08	0.777
	TGTGCG	0.27	1.06 (0.82-1.35)	0.19	0.666
	TGCATG	0.22	0.78 (0.60-1.01)	3.61	0.058
	TGCGTT	0.10	1.09 (0.76-1.58)	0.22	0.636
	TACGCG	0.10	1.33 (0.91-1.95)	2.16	0.142
	TGCGCG	0.01	0.70 (0.26-1.83)	0.58	0.448
Block 3[c]	AA	0.62	1.24 (1.00-1.56)	3.69	0.055
	GG	0.26	0.76 (0.60-0.97)	4.77	0.029*
	AG	0.12	1.01 (0.72-1.42)	0.01	0.937

[a]Block 1: rs4784733-rs2304478-rs13306673. [b]Block 2: rs8043560-rs2304483-rs5803-rs7187932-rs6499858-rs11644728. [c]Block 3: rs2399594-rs711746. *P < 0.05.

Table 5. Effects of allele distributions on blood pressure.

SNPs	Allele	Frequency	SBP (N = 754)		DBP (N = 754)	
			χ^2	P (df = 1)	χ^2	P (df = 1)
rs4784733	T	0.80				
	C	0.20	0.03	0.866	0.12	0.732
rs2304478	A	0.13				
	G	0.87	4.85	0.028	3.30	0.069
rs13306673	C	0.10				
	T	0.90	0.15	0.696	0.11	0.735
rs2289119	A	0.29				
	G	0.71	0.03	0.860	0.00	0.990
rs8043560	C	0.71				
	T	0.29	0.00	0.976	0.06	0.800
rs2304483	A	0.39				
	G	0.61	0.38	0.537	0.06	0.811
rs5803	C	0.28				
	T	0.72	0.00	0.979	0.91	0.341
rs7187932	A	0.22				
	G	0.78	0.42	0.515	1.05	0.305
rs6499858	C	0.32				
	T	0.68	0.29	0.592	0.41	0.522
rs11644728	T	0.11				
	G	0.89	0.08	0.773	0.04	0.832
rs8049280	T	0.85				
	C	0.15	0.40	0.529	0.06	0.810
rs7204044	A	0.13				
	G	0.87	0.01	0.929	0.33	0.563
rs2010501	T	0.77				
	C	0.23	0.36	0.551	0.37	0.543
rs2399594	A	0.74				
	G	0.26	4.77	0.029	3.83	0.050
rs711746	A	0.62				
	G	0.38	1.11	0.293	0.87	0.352

DBP and SBP = diastolic and systolic blood pressure.

Table 6. Effects of genotype distributions on diastolic (DBP) and systolic blood pressure (SBP) by different models.

		SBP	P	DBP	P	Dominant model	P (SBP)	P (DBP)	Recessive model	P (SBP)	P (DBP)
rs4784733	CC	140.00 ± 6.24	0.265	88.83 ± 4.15	0.368	CC+CT *vs* TT	0.725	0.918	CC *vs* CT+TT	0.145	0.180
	CT	150.26 ± 1.86		94.29 ± 1.09							
	TT	148.48 ± 1.48		93.64 ± 0.89							
rs2304478	AA	141.31 ± 7.79	0.100	91.92 ± 4.40	0.170	GG+AG *vs* AA	0.366	0.721	GG *vs* AG+AA	0.035*	0.060
	AG	144.74 ± 2.36		91.33 ± 1.48							
	GG	150.13 ± 1.32		94.41 ± 0.78							
rs13306673	CC	148.26 ± 1.24	0.345	93.49 ± 0.74	0.425	CC+CT *vs* TT	0.282	0.330	CC *vs* CT+TT	0.473	0.527
	CT	151.20 ± 2.84		95.06 ± 1.75							
	TT	137.25 ± 12.50		87.50 ± 8.27							
rs2289119	AA	145.60 ± 3.46	0.504	92.17 ± 2.06	0.579	GG+AG *vs* AA	0.349	0.457	GG *vs* AG+AA	0.736	0.666
	AG	150.14 ± 1.83		94.48 ± 1.10							
	GG	148.42 ± 1.61		93.41 ± 0.97							
rs8043560	CC	151.22 ± 3.94	0.675	95.69 ± 2.26	0.401	CC+CT *vs* TT	0.700	0.440	CC *vs* CT+TT	0.513	0.400
	CT	147.67 ± 1.70		92.72 ± 1.05							
	TT	149.09 ± 1.65		94.22 ± 0.97							
rs2304483	AA	151.56 ± 2.90	0.575	94.58 ± 1.65	0.829	AA+AG *vs* GG	0.908	0.944	AA *vs* AG+GG	0.304	0.581
	AG	148.04 ± 1.63		93.36 ± 1.04							
	GG	148.62 ± 1.92		93.76 ± 1.09							
rs5803	CC	148.65 ± 1.59	0.997	93.05 ± 0.92	0.604	TT+CT *vs* CC	0.996	0.316	TT *vs* CT+CC	0.942	0.705
	CT	148.58 ± 1.79		94.38 ± 1.13							
	TT	148.93 ± 3.63		94.57 ± 2.14							
rs7187932	AA	154.56 ± 6.83	0.110	95.81 ± 3.22	0.140	GG+AG *vs* AA	0.238	0.471	GG *vs* AG+AA	0.187	0.119
	AG	145.75 ± 1.92		91.84 ± 1.14							
	GG	150.00 ± 1.42		94.55 ± 0.89							
rs6499858	CC	149.63 ± 1.66	0.673	94.06 ± 1.04	0.801	CC+CT *vs* TT	0.892	0.562	CC *vs* CT+TT	0.428	0.623
	CT	147.52 ± 1.64		93.59 ± 0.98							
	TT	149.09 ± 4.19		92.54 ± 2.18							
rs11644728	GG	148.70 ± 1.29	0.737	93.61 ± 0.78	0.955	TT+GT *vs* GG	0.923	0.796	TT *vs* GT+GG	0.437	0.922
	GT	148.93 ± 2.31		94.10 ± 1.31							
	TT	140.89 ± 14.91		93.11 ± 10.44							
rs8049280	CC	139.64 ± 9.58	0.526	90.36 ± 6.69	0.779	CC+CT *vs* TT	0.727	0.959	CC *vs* CT+TT	0.259	0.485
	CT	148.65 ± 2.11		93.89 ± 1.18							
	TT	148.89 ± 1.35		93.72 ± 0.82							
rs7204044	AA	150.25 ± 5.93	0.975	97.00 ± 5.07	0.598	AA+AG *vs* GG	0.892	0.447	AA *vs* AG+GG	0.880	0.603
	AG	148.29 ± 2.15		92.61 ± 1.26							
	GG	148.74 ± 1.34		93.99 ± 0.81							
rs2010501	CC	150.22 ± 6.40	0.832	94.11 ± 3.33	0.796	TT+CT *vs* CC	0.770	0.887	TT *vs* CT+CC	0.553	0.500
	CT	149.51 ± 1.95		94.26 ± 1.10							
	TT	148.22 ± 1.43		93.30 ± 0.91							
rs2399594	AA	150.97 ± 1.54	0.096	94.65 ± 0.94	0.133	AA+AG *vs* GG	0.239	0.088	AA *vs* AG+GG	0.037*	0.124
	AG	146.59 ± 1.75		93.11 ± 1.07							
	GG	144.02 ± 5.27		89.55 ± 2.76							
rs711746	AA	149.10 ± 1.77	0.189	93.71 ± 1.12	0.158	AA+AG *vs* GG	0.074	0.067	AA *vs* AG+GG	0.831	0.987
	AG	150.01 ± 1.66		94.58 ± 0.98							
	GG	143.66 ± 3.30		90.55 ± 1.82							

DISCUSSION

The causes of hypertension are heterogeneous and genetic factors are thought to explain 30-50% of BP variation in the general population (Coffman, 2011). The *SLC12A3* gene encodes a thiazide-sensitive Na^+-Cl^- cotransporter that is involved in renal sodium reabsorption in the DCT. Mounting evidence has implicated the *SLC12A3* gene as one of the underlying causes of hypertension (Matsuo et al., 2004). However, there have been few studies examining the association of polymorphisms in the *SLC12A3* gene with hypertension. An earlier study revealed nominal evidence of association of *SLC12A3* polymorphisms at genome-wide significance levels with one or more BP traits in the CHARGE genome-wide association (GWA) meta-analysis (Levy et al., 2009). The current study showed that three SNPs of the *SLC12A3* gene are significantly associated with hypertension or SBP in the Mongolian population: rs2304478 and r7187932 were found to be associated with hypertension whereas rs7187932 and rs2399594 were associated specifically with SBP. In particular, the presence of the G allele in rs7187932 was associated with an increased risk of hypertension while the presence of the G allele in rs2399594 was associated with a reduced risk of hypertension. Wang et al. (2014) studied the associations of the rs2304483, rs5804, rs8063291, and rs6499857 polymorphisms of the *SLC12A3* gene with hypertension in 1009 hypertensive patients and 756 normotensive controls of Han Chinese descent in northeastern China. They found that the *SLC12A3* gene rs5804 polymorphism plays a predominant role in determining hypertension risk among northeastern Han Chinese. The same study failed to find any association between *SLC12A3* rs2304483 and hypertension, a finding also seen in our current study.

Mongolians are one of the ethnic groups in China with a high prevalence of hypertension. Family aggregation of hypertension in Mongolians suggests that genetic factors might play an important role in the etiology of hypertension in Mongolians. Our previous study confirmed that the chloride channel Kb gene, *CLCNKB*, and the *PPARγ* gene are involved in the genetic susceptibility to hypertension in Mongolians (Gao et al., 2010; Su et al., 2012). Furthermore, in a small study of 385 unrelated Mongolians (158 patients with hypertension *vs* 227 normotensive subjects), we previously examined the association of the *SLC12A3* gene with hypertension in Mongolians using nine tagSNPs of the *SLC12A3* gene (rs6499858, rs2289119, rs13306673, rs2010501, rs7188995, rs7204044, rs4784733, rs8063406, and rs2304478) (Chang et al., 2011b). We found that only the single rs7204044 SNP was significantly associated with hypertension in Mongolians; this result, however, was not confirmed by our current study. The two studies recruited study subjects from different areas of Inner Mongolia; however, more importantly, they differed in sample size, with the number of hypertensive subjects in the current study being 3-fold as many as that of our previous study. On the other hand, both studies failed to demonstrate an association of rs4784733, rs2304478, rs13306673, rs2289119, rs6499858, and rs2010501 with hypertension in Mongolians.

In conclusion, the results of this study support that the *SLC12A3* gene is a susceptibility gene for hypertension in the Mongolian population.

Conflicts of interest

The authors declare no conflict of interest.

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