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ORIGINAL ARTICLE

Genetic susceptibility to salt-sensitive hypertension in a Han Chinese population: a validation study of candidate genes

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Salt-sensitive hypertension is a complex disease associated with genetic factors. This study aimed to identify the association between 29 candidate single-nucleotide polymorphisms and salt-sensitive hypertension in a Han Chinese population. Sixty-three participants with salt-sensitive hypertension and 279 controls with salt-resistant hypertension were recruited. A modified Sullivan's acute oral saline load and diuresis shrinkage test was used to detect blood pressure salt sensitivity. Lifestyle risk factors were obtained via a questionnaire. We used the Sequenom Mass ARRAY Platform to genotype the 29 candidate single-nucleotide polymorphisms, and the cumulative genetic risk score was used to evaluate the joint genetic effect. The frequencies of eight genotypes and five alleles in *CYP11B2*, *PRKG1*, *ADRB2*, *FGF5*, *SLC8A1* and *BCAT1* genes differed significantly between the salt-sensitive and salt-resistant hypertension groups. Multiple logistic regression adjusted for age and sex showed that subjects carrying rs7897633-A (*PRKG1*), rs434082-A (*SLC8A1*) and rs1042714-G (*ADRB2*) risk alleles had 1.83-, 2.84- and 2.40-fold increased risk for salt-sensitive hypertension, respectively. Combined risk allele analysis using the cumulative genetic risk score showed that subjects carrying one risk had 2.30-fold increased risk, and those carrying 2–4 risks had 3.32-fold increased risk for salt-sensitive hypertension. Among 29 candidate single-nucleotide polymorphisms, rs7897633-A in *PRKG1*, rs434082-A in *SLC8A1* and rs1042714-G in *ADRB2* were significantly associated with salt-sensitive hypertension. A joint effect of single-nucleotide polymorphisms from different pathways contributed to a high risk of salt-sensitive hypertension.
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INTRODUCTION

Essential hypertension is one of the most common cardiovascular diseases that poses a considerable threat to human health. It is influenced by both genetic and environmental factors.[1] High salt intake is the most important environmental risk factor for hypertension. Epidemiological, animal and clinical experimental studies consistently identify the positive correlation between high dietary sodium and elevated blood pressure (BP). The heterogeneity of BP response to sodium is defined as BP salt sensitivity. Salt-sensitive hypertension (SSH) can be regarded as an intermediate inheritance phenotype of essential hypertension with significant individual differences and ethnic specificity. Svetkey *et al.*[2] examined 20 African-American families, and reported heritability of ~26–84% for mean arterial blood pressure (MAP) responses to salt sensitivity. The Genetic Epidemiology Network of Salt Sensitivity (GenSalt) dietary feeding study indicated that ~39% of Chinese adults were salt-sensitive (SS).[3] Salt sensitivity is more common in women, older individuals and those with higher readings of basic blood pressure.[4] A 27-year cohort study reported that SSH is an independent risk factor for cardiovascular disease that increases morbidity and mortality.[5]

There has been substantial evidence to elucidate the genetic determinants underlying BP salt sensitivity,[6,7] but the associated pathologic mechanisms are not completely clear. Polygenic diseases such as hypertension are postulated to arise from epistatic interactions of many single-nucleotide polymorphisms (SNPs).[8,9] Most reports have focused on the renin–angiotensin–aldosterone system genes and their association with salt sensitivity, including the well-known angiotensin-converting-enzyme (*ACE*) insertion–deletion polymorphism,[10] as well as the *AGT* M235T and G6A polymorphisms.[11] *ETBR* 1065AA+GA (rs5351) has been reported to occur more frequently in salt-resistant (SR) hypertensive individuals, whereas *ETBR* 1065GG occurs more frequently in SS hypertensive individuals.[12,13]

Genome-wide association studies (GWAS) and candidate gene studies have made great strides in delineating genomic mechanisms associated with BP regulation that have been well established in the following pathways: renin–angiotensin–aldosterone system,[14] ion and

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water channels, transporters and exchangers,[15] the endothelial system,[16] intracellular messengers,[17] the sympathetic nervous system,[18] the apelin–APJ system[19] and the kallikrein–kinin system,[20] among many others[21,22] that are related to BP salt sensitivity. Until recently, three GWAS have been conducted on salt sensitivity. In a large family-based, dietary-based, genome-wide linkage scan study, the *FAM84A* gene SNP rs11674786 was significantly associated with diastolic blood pressure (DBP) and MAP responses, and rs16983422 of the *VSNL1* gene was marginally associated with DBP and MAP responses. The present study provides new evidence of genetic factors that might be partially responsible for salt sensitivity of BP.[23] One meta-analysis identified eight novel loci for BP phenotypes that were physically mapped in or near the following genes: *PRMT6*, *CDCA7*, *PIBF1*, *ARL4C*, *IRAK1BP1*, *SALL1*, *TRPM8* and *FBXL13*. The polymorphism rs7577262 in the *TRPM8* gene showed genome-wide significance for its association with systolic blood pressure (SBP), and the intronic *FBXL13* marker rs17135875 achieved genome-wide significant associations with MAP responses to the cold pressor test.[24] Another GWAS study on Caucasians with mild hypertension identified that SNPs located in the first intron of the *cGMP-dependent protein kinase 1* (*PRKG1*) gene are associated with variations in DBP, whereas *SLC24A3* and *SLC8A1* are associated with variations in SBP following acute salt loading.[25] Although GWAS are valuable for uncovering novel mechanisms underlying BP salt sensitivity, most of the findings require evidence of replication, and some biological pathways warrant further investigation.

In the present study, we used a modified Sullivan's acute salt loading and diuresis shrinkage test[26–28] to identify the responses in BP salt sensitivity among a community of patients with essential hypertension in Beijing. We summarized the pathologic pathway of 29 candidate SNPs in SSH. Literature retrieval of association studies on candidate SNPs and previous GWAS results of salt sensitivity were also used to validate the effects of candidate SNPs with environmental risk factors of SSH.

METHODS

Subjects

Sixty-three individuals with SSH and 279 individuals with SR hypertension (SRH) were recruited from a community of individuals with essential hypertension in Beijing, in a case–control study. The essential hypertension group was defined as those with SBP $\geqslant$ 140 mm Hg and/or DBP $\geqslant$ 90 mm Hg, and included those who used antihypertensive medications, according to the 2010 Chinese guidelines for the management of essential hypertension.[29] Participants who were pregnant or who abused alcohol, as well as those with cardiovascular disease, heart failure, cerebrovascular disease, secondary hypertension, resistant hypertension or Liddle syndrome were excluded. The study was approved by the Ethical Committee of Capital Medical University, Beijing, China. All participants gave informed consent before participation.

Measurement of anthropometric parameters

Information on the history of hypertension, physical examination, personal behavior and use of antihypertensive medications was obtained, using a standard questionnaire. Body weight, height, waist circumference, hip circumference, SBP and DBP were measured by well-trained community doctors. Blood pressure was measured using a mercury sphygmomanometer on the right arm of each participant, who was seated in a comfortable position after at least 5 min rest.

After overnight fasting, peripheral venous blood samples were collected the following morning, to evaluate biochemical parameters, such as fasting plasma glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. Daily sodium intake was evaluated using a food frequency questionnaire and 24-h urinary Na excretion.

Acute oral saline load test

A modified Sullivan's acute salt loading and diuresis shrinkage test was used to identify SSH and SRH.[26–28] The modified Sullivan's acute salt loading and diuresis shrinkage test entailed the following process: for the first day, an acute salt load of 1 L of oral saline (155 mmol NaCl) was administered within 30 min in the morning. After 2 h, the diuresis shrinkage test was performed and each patient was administered oral furosemide (40 mg). Blood pressure was measured using a standard procedure three times at 5-min intervals, before loading, 2 h after the salt load test and 2 h after the diuresis shrinkage test. The mean blood pressure values of the three readings were used for further analysis. MAP was calculated according to the equation: $MAP = (SBP + 2 \times DBP)/3$.[30] Individuals with an increased MAP of at least 5 mm Hg after 2 h of the salt load, or those with a reduction by more than 10 mm Hg after 2 h of the diuresis shrinkage test, were categorized as SS, whereas all other individuals were categorized as SR.

Tag-SNP selection

The selection of 29 candidate SNPs was performed in a comprehensive manner that included the evaluation of pathologic mechanisms of SSH, and retrieval of published epidemiologic studies that used evidence-based methods and GWAS results. We downloaded data on the Han Chinese population SNPs from the database of the international HapMap Project (HapMap Data Rel 24/phase II Nov08, on NVBI B36 assembly, dbSNP b126). To achieve a power $\geqslant$ 80% in the present study, the SNPs that were significantly associated with SSH, and minor allele frequencies $>$ 0.05 in the Chinese population of the HapMap database, were selected by the Haploview 4.0 software (version 4.0; Mark Daly's Laboratory, Broad Institute; http://sourceforge.net/projects/haploview/).

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood leukocytes, using the QIAamp DNA Blood Mini Kit (Tiangen Inc., Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of the isolated DNA were measured using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. If the value of OD260/OD280 was between 1.7 and 2.0, and the DNA concentration was $>10\ ng\ \mu l^{-1}$, the result was considered more favorable. All candidate SNPs were genotyped on the Sequenom Mass ARRAY Platform (Sequenom, San Diego, CA, USA). Based on the manufacturer's instructions, the entire process included multiplex PCR amplification, shrimp alkaline phosphatase treatment, iPLEX primer extension, cleaning of the resin, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and data analysis.[31,32]

Statistical analysis

Statistical analyses were carried out using SPSS version 19.0 for Windows (SPSS, Chicago, IL, USA). The independent two-sample *t*-test was used for continuous variables with normal distribution, and the rank-sum test was used to analyze variables with non-normal distribution. The χ^2 test was used to analyze Hardy–Weinberg equilibrium, and to compare the distributions of allelic and genotypic frequencies. The association between a polymorphism and SSH at a single locus was analyzed by multiple logistic regression adjusted for age and sex. A multivariable model was developed based on 1000 bootstrap samples on the original data, using multiple logistic regression analysis. A cumulative genetic risk score (cGRS) was applied, to analyze the combined effect of multiple SNPs on SSH. This score was calculated for each individual, by adding the number of risk alleles at each locus. A value of 2 was assigned to subjects with double risk alleles, and a value of 0 was assigned to all other subjects. The cGRS ranged from 0 to 8 among the subjects. Multiple logistic regression was used to evaluate the association between cGRS and SSH as a binary dependent variable. Power analysis was performed, using the Quanto software version 1.2.4 (University of Southern California, Los Angeles, CA, USA). Assuming a minor allele frequency of 0.15 and disease prevalence of 20.0%, statistical power $>$80% was used to detect genetic effects at an odds ratio of 2.03–3.53 in an additive model. The significance level in all the tests was $P < 0.05$ for two sides. The analysis was carried out using additive, dominant, recessive and allele models.

Table 1 Characteristics of 342 participants based on salt-sensitive and salt-resistant hypertension

Variable	*Total*	*SSH*	*SRH*	P-*value*
Number (%)	342	63 (18.4)	279 (81.6)	—
Sex (male,%)	101 (29.8)	15 (24.2)	86 (31.0)	0.286$^{\#}$
Age (years)	57.61 ± 8.50	59.62 ± 8.95	57.16 ± 8.35	0.038$^{\Delta *}$
BMI (kg m^{-2})	27.51 ± 3.62	27.47 ± 3.61	27.52 ± 3.62	0.916$^{\Delta}$
MAP_1 (mm Hg)	104.26 ± 10.70	104.56 ± 11.19	104.20 ± 10.60	0.806$^{\Delta}$
UNaE (mmol per 24 h)	193.64 ± 90.94	179.47 ± 87.59	196.84 ± 91.53	0.182$^{\&}$
UKE (mmol per 24 h)	48.11 ± 21.29	47.98 ± 19.58	48.13 ± 21.70	0.878$^{\&}$
Salt intake (g)	10.80 ± 5.72	10.98 ± 5.77	10.00 ± 5.48	0.220$^{\Delta}$

Abbreviations: BMI, body mass index; MAP_1, baseline mean arterial blood pressure; SSH, salt-sensitive hypertension; SRH, salt-resistant hypertension; UNaE, 24- h urine sodium content; UKE, 24- h urinary potassium content.
Values are means plus/minus s.d or numbers and percentages. *P*-values are calculated by χ^2 ($^{\#}$) or *T*-test ($^{\Delta}$) or rank-sum test ($^{\&}$).
$^{*}P<0.05$.

RESULTS

Baseline characteristics of the subjects

The baseline characteristics of the study subjects are presented in Table 1. The average age of the SSH group was higher than that of the SRH group ($P=0.038$). No significant differences in sex, body mass index level, baseline mean arterial blood pressure, salt intake, 24-h urinary sodium content and 24-h urinary potassium content were noted between the two groups.

Association between candidate SNPs and SSH

We genotyped 29 SNPs in 342 participants (SSH/SRH = 63/279), and the distribution of genotypes and alleles for the 29 SNPs are listed in Table 2. No deviation from Hardy–Weinberg equilibrium was observed among these SNPs in the control group ($P>0.05$). Univariate analysis indicated that the frequencies of eight SNPs in the six genes *CYP11B2*, *PRKG1*, *ADRB2*, *FGF5*, *SLC8A1* and *BCAT1*, and five alleles in the *CYP11B2*, *PRKG1*, *ADRB2*, *FGF5* and *SLC8A1* genes differed significantly between the SSH and SRH groups. After adjustments for age and sex, only the SNP rs7961152 in the *BCAT1* gene failed to show any significant differences (Table 3).

Multivariable analysis of candidate SNPs in SSH

Multiple logistic regression analysis adjusted for age and sex revealed significant differences between the SSH and SRH groups in the frequencies of risk allelic distributions of rs7897633 (*PRKG1*) ($P=0.027$), rs434082 (*SLC8A1*) ($P<0.001$) and rs1042714 (*ADRB2*) ($P=0.004$). Carriers of rs7897633-A, rs434082-A and rs1042714-G risk alleles had a 1.83-fold (OR (95% CI): 1.07–3.14), 2.84-fold (OR (95% CI): 1.65–4.87) and 2.40-fold (OR (95% CI): 1.32–4.35) increased risk for SSH, respectively. Older females showed greater risk for SSH. The final logit model was $(P=\mathrm{SSH}) = 0.03 \times \mathrm{age} + 0.58 \times \mathrm{sex} + 0.61 \times$ *PRKG1* rs7897633-A allele $+ 1.04 \times$ *SLC8A1* rs434082-A allele $+ 0.88 \times$ *ADRB2* rs1042714-G allele $- 4.85$. After 1000 bootstrap samples were used to confirm the results of multiple logistic regression based on increasing the sample size, similar results were observed (Table 4).

A cGRS was applied to analyze the combined effect of multiple SNPs on SSH. The risk score for each individual was calculated by adding the number of risk alleles (1-risk indicated subjects with one homozygous risk genotype of more than five significant alleles; 2–4 risks indicated subjects with more than two homozygous risk genotypes; 0-risk indicated subjects without homozygous risk genotypes). In multiple logistic regression analysis adjusted for age and sex, subjects carrying 1-risk (with rs1799998/CC, rs7897633/AA, rs1904694/GG, rs434082/AA or rs1042714/GG) had a 2.30-fold (OR (95% CI): 1.18–4.48, $P=0.014$) increased risk for SSH, whereas the risk was increased 3.32-fold (OR (95% CI): 95% CI 1.51–7.30, $P=0.003$) among subjects carrying 2–4 risks (Table 3).

DISCUSSION

Epidemiologic studies have shown that genetic factors can considerably affect blood pressure. Kawasaki *et al.*[33] and Weinberger[34] were among the first to recognize the heterogeneity of BP response to sodium, and proposed the concept of salt sensitivity in humans. The GenSalt study, which is the largest dietary sodium-feeding study to date, was designed to examine gene–sodium interactions associated with BP.[35] Candidate gene studies have made considerable progress in revealing the genetic mechanisms of BP response to salt intake. Extensive efforts have been made to identify the genes in different pathways, such as renin–angiotensin–aldosterone system, ion and water channels, transporters and exchangers, the endothelial system, apelin–APJ system, sympathetic nervous system, intracellular messengers and the kallikrein–kinin system.[36] Several studies have tried to the identify genetic factors associated with salt sensitivity; however, there have been inconsistent results.[12] Genetic studies in SSH generally focus on exploration of the functions of renal sodium excretion and its related regulation genes. We first systematically reviewed the literature to select candidate genes involved in SSH, and then explored the association between candidate genes and SSH. Seven SNPs were verified as having an association with the development of salt sensitivity: rs1799998 in *CYP11B2*; rs7897633 and rs1904694 in *PRKG1*; rs434082 and rs11893826 in *SLC8A1*; rs1042714 in *ADRB2*; and rs16998073 in *FGF5*.

The aldosterone synthase gene, *CYP11B2*, encodes a cytochrome *P*450 enzyme that is involved in the terminal steps of aldosterone synthesis in cells of the zona glomerulosa in the adrenal glands of humans, and its expression is regulated by angiotensin II and potassium.[37] One polymorphism in this gene, rs1799998, is located 344 bp upstream. Studies have shown that the C allele binds to the steroidogenic factor-1 site, five times stronger than it does to the T allele,[38] a phenomenon that might modify the effects of aldosterone, and affect the cardiovascular system. A dietary intervention study that entailed a 7-day low-sodium regimen followed by a 7-day high-sodium regimen reported no significant associations between this SNP and salt sensitivity of blood pressure.[39] A population-based, cross-sectional study suggested that the frequency of the C allele was significantly lower in people of African origin than in those of white and South Asian origins.[40] Furthermore, the TT genotype was associated with higher plasma aldosterone levels, and higher SBP and DBP than was the CC genotype. These results might reflect an association with various races. Iwai *et al.*[41] reported that the *CYP11B2* rs1799998 polymorphism in a Japanese population is associated with salt sensitivity. In the present study, the participants who carried the CC genotype and C allele were at greater risk of SSH, as compared with those with the TT genotype and T allele.

Many researchers suggest that SSH is related to a disordered mechanism of sodium and calcium ion transport and impaired endothelial function. After salt loading, inhibition of the *PRKG1* isoenzyme reduces the activity of nitric oxide, a process that affects the regulation of vascular smooth muscle cells. The *PRKG1* gene might influence BP either by increasing the concentration of free intracellular calcium ions or increasing the sensitivity of contractile cells to calcium ions. Calcium ions have an important role in the control of vascular tone, and make a significant contribution to the regulation of systemic blood pressure.[42,43] PRKG1 proteins have central roles in the regulation of cardiovascular and neuronal functions, relaxation of smooth muscle tone,[44,45] prevention of platelet aggregation and

Table 2 Distributions of genotypic and single factor analysis of 29 tag-SNPs in SSH candidate genes

					χ^2 test			χ^2 test			
dbSNP	*Gene symbol*	*Genomic position (bp)*	*Models*	*Genotype*	P-*value*	*OR (95% CI)*	*Minor allele*	P-*value*	*OR (95% CI)*	*MAF*	P_{HWE}
Rs4961	*ADD1*	2 906 707	Additive	TT *vs.* TG *vs.* GG	0.707	—	G	0.443	1.17 (0.79–1.74)	0.45	0.884
			Dominant	(TT+TG) *vs.* GG	0.421	1.31 (0.68-2.52)					
			Recessive	TT *vs.* (TG+GG)	0.641	1.16 (0.62–2.18)					
Rs699	*AGT*	230 845 794	Additive	TT *vs.* TC *vs.* CC	0.371	—	T	0.196	1.35 (0.86–2.14)	0.20	0.521
			Dominant	(TT+TC) *vs.* CC	0.285	0.74 (0.42–1.29)					
			Recessive	TT *vs.* (TC+CC)	0.425	0.49 (0.15–1.66)					
Rs2681472	*ATP2B1*	90 008 959	Additive	CC *vs.* CT *vs.* TT	0.967	—	C	0.866	1.04 (0.69–1.56)	0.34	0.859
			Dominant	(CC+CT) *vs.* TT	0.953	0.98 (0.56–1.72)					
			Recessive	CC *vs.* (CT+TT)	0.796	0.90 (0.39–2.05)					
Rs7961152	*BCAT1*	24 981 611	Additive	AA *vs.* AC *vs.* CC	0.044*	—	A	0.591	1.18 (0.64–2.16)	0.10	0.054
			Dominant	(AA+AC) *vs.* CC	0.988	0.10 (0.51–1.96)					
			Recessive	AA *vs.* (AC +CC)	0.034*	5.55 (4.41–6.98)					
Rs848307	*CLCNKA*	16 319 232	Additive	TT *vs.* TC *vs.* CC	0.472	—	T	0.242	0.77 (0.49–1.20)	0.31	0.541
			Dominant	(TT+TC) *vs.* CC	0.221	1.42 (0.81–2.48)					
			Recessive	TT *vs.* (TC+CC)	0.626	1.28 (0.47–3.46)					
Rs1739843	*CLCNKA*	16 343 254	Additive	TT *vs.* TC *vs.* CC	0.201	—	T	0.069	0.65 (0.41–1.04)	0.31	0.544
			Dominant	(TT+TC) *vs.* CC	0.113	1.59 (0.89–2.81)					
			Recessive	TT *vs.* (TC+CC)	0.191	2.22 (0.65–7.58)					
Rs1010069	*CLCNKA*	16 352 937	Additive	CC *vs.* CT *vs.* TT	0.856	—	C	0.617	0.90 (0.58–1.38)	0.32	0.592
			Dominant	(CC+CT) *vs.* TT	0.738	1.10 (0.63–1.92)					
			Recessive	CC *vs.* (CT+TT)	0.596	1.31 (0.48–3.53)					
Rs1799998	*CYP11B2*	143 999 600	Additive	CC *vs.* CT *vs.* TT	0.107	—	C	0.042*	1.54 (1.02–2.32)	0.26	0.348
			Dominant	(CC+CT) *vs.* TT	0.148	0.67 (0.38–1.16)					
			Recessive	CC *vs.* (CT+TT)	0.051	0.46 (0.20–1.02)					
Rs1126742	*CYP4A11*	47 398 496	Additive	CC *vs.* CT *vs.* TT	0.312	—	C	0.815	0.94 (0.57–1.55)	0.20	0.459
			Dominant	(CC+CT) *vs.* TT	0.835	0.94 (0.53–1.67)					
			Recessive	CC *vs.* (CT+TT)	0.321	1.23 (1.17–1.30)					
Rs1799983	*eNOS*	150 696 111	Additive	TT *vs.* TG *vs.* GG	0.189	—	T	0.239	1.42 (0.79–2.54)	0.10	0.566
			Dominant	(TT+TG) *vs.* GG	0.402	0.76 (0.39–1.46)					
			Recessive	TT *vs.* (TG+GG)	0.154	0.22 (0.03–1.58)					
Rs5351	*EDNBR*	78 475 313	Additive	GG *vs.* GA *vs.* AA	0.560	—	G	0.462	1.16 (0.78–1.73)	0.37	0.866
			Dominant	(GG+GA) *vs.* AA	0.804	0.93 (0.53–1.65)					
			Recessive	GG *vs.* (GA+AA)	0.285	0.68 (0.33–1.39)					
Rs16998073	*FGF5*	81 184 341	Additive	TT *vs.* TA *vs.* AA	0.054	—	T	0.177	0.76 (0.51–1.13)	0.47	0.301
			Dominant	(TT+TA) *vs.* AA	0.026*	1.91 (1.08–3.40)					
			Recessive	TT *vs.* (TA+AA)	0.879	0.95 (0.48–1.87)					
Rs1129649	*GNB3*	6 948 468	Additive	CC *vs.* CT *vs.* TT	0.874	—	C	0.604	1.12 (0.73–1.72)	0.29	0.813
			Dominant	(CC+CT) *vs.* TT	0.661	0.88 (0.51–1.54)					
			Recessive	CC *vs.* (CT+TT)	0.689	0.83 (0.32–2.12)					
Rs1024323	*GRK4*	3 006 043	Additive	AA *vs.* AG *vs.* GG	0.750	—	A	0.647	1.12 (0.69–1.83)	0.18	0.630
			Dominant	(AA+AG) *vs.* GG	0.803	0.93 (0.52–1.66)					
			Recessive	AA *vs.* (AG+GG)	0.718	0.60 (0.15–2.31)					
Rs1801058	*GRK4*	3 039 150	Additive	CC *vs.* CT *vs.* TT	0.654	—	C	0.933	0.98 (0.66–1.46)	0.43	0.259
			Dominant	(CC+CT) *vs.* TT	0.578	1.18 (0.66–2.13)					
			Recessive	CC *vs.* (CT+TT)	0.599	0.83 (0.41–1.68)					

Table 2 (Continued)

dbSNP	Gene symbol	Genomic position (bp)	Models	Genotype	χ^2 test P-value	χ^2 test OR (95% CI)	Minor allele	χ^2 test P-value	χ^2 test OR (95% CI)	MAF	P_{HWE}
Rs2398162	*LOC100132798*	96 830 550	Additive	AA *vs.* AG *vs.* GG	0.333	—	A	0.179	0.76 (0.50–1.14)	0.40	0.945
			Dominant	(AA+AG) *vs.* GG	0.138	1.52 (0.87–2.65)					
			Recessive	AA *vs.* (AG+GG)	0.573	1.26 (0.56–2.84)					
Rs2288774	*NEDD4L*	55 983 330	Additive	CC *vs.* CT *vs.* TT	0.680	—	C	0.430	1.18 (0.79–1.77)	0.34	0.449
			Dominant	(CC+CT) *vs.* TT	0.581	0.85 (0.48–1.51)					
			Recessive	CC *vs.* (CT+TT)	0.414	0.72 (0.32–1.60)					
Rs4149601	*NEDD4L*	55 816 791	Additive	AA *vs.* AG *vs.* GG	0.625	—	A	0.339	0.75 (0.42–1.35)	0.17	0.835
			Dominant	(AA+AG) *vs.* GG	0.339	1.38 (0.71–2.66)					
			Recessive	AA *vs.* (AG+GG)	1.000	1.52 (0.18–12.60)					
Rs7897633	*PRKG1*	52 957 721	Additive	AA *vs.* AC *vs.* CC	0.041*	—	A	0.012*	1.66 (1.12–2.47)	0.48	0.973
			Dominant	(AA+AC) *vs.* CC	0.072	0.52 (0.25–1.07)					
			Recessive	AA *vs.* (AC+CC)	0.023*	0.51 (0.28–0.92)					
Rs1904694	*PRKG1*	52 905 494	Additive	GG *vs.* GA *vs.* AA	0.007*	—	G	0.003*	1.81 (1.22–2.69)	0.36	0.478
			Dominant	(GG+GA) *vs.* AA	0.038*	0.52 (0.28–0.97)					
			Recessive	GG *vs.* (GA+AA)	0.003*	0.37 (0.19–0.73)					
Rs5735	*SCNN1G*	23 200 848	Additive	TT *vs.* TC *vs.* CC	0.338	—	T	0.414	0.80 (0.46–1.38)	0.18	0.676
			Dominant	(TT+TC) *vs.* CC	0.250	1.45 (0.77–2.74)					
			Recessive	TT *vs.* (TC+CC)	0.908	0.72 (0.19–2.71)					
Rs3790261	*SLC24A3*	19 560 664	Additive	GG *vs.* GA *vs.* AA	0.595	—	G	0.379	0.83 (0.55–1.26)	0.36	0.554
			Dominant	(GG+GA) *vs.* AA	0.563	1.18 (0.68–2.06)					
			Recessive	GG *vs.* (GA+AA)	0.331	1.62 (0.61–4.33)					
Rs434082	*SLC8A1*	40 485 074	Additive	AA *vs.* AG *vs.* GG	0.097	—	A	0.036*	1.68 (1.03–2.74)	0.15	0.727
			Dominant	(AA+AG) *vs.* GG	0.032*	0.54 (0.30–0.95)					
			Recessive	AA *vs.* (AG+GG)	0.827	0.55 (0.10–2.91)					
Rs11893826	*SLC8A1*	40 564 647	Additive	AA *vs.* AG *vs.* GG	0.045*	—	A	0.309	1.26(0.81–1.96)	0.31	0.093
			Dominant	(AA+AG) *vs.* GG	0.059	1.72 (0.98–3.03)					
			Recessive	AA *vs.* (AG+GG)	0.311	0.63 (0.25–1.55)					
Rs1937506	—	68 035 371	Additive	AA *vs.* AG *vs.* GG	0.218	—	A	0.359	1.29 (0.75–2.25)	0.12	0.235
			Dominant	(AA+AG) *vs.* GG	0.551	0.83 (0.44–1.54)					
			Recessive	AA *vs.* (AG+GG)	0.157	0.22 (0.03–1.60)					
Rs3754777	*STK39*	169 015 914	Additive	AA *vs.* AG *vs.* GG	0.459	—	A	0.399	1.21 (0.78–1.88)	0.24	0.874
			Dominant	(AA+AG) *vs.* GG	0.259	0.73 (0.42–1.27)					
			Recessive	AA *vs.* (AG+GG)	1.000	1.14 (0.32–4.08)					
Rs6749447	*STK39*	169 041 386	Additive	TT *vs.* TG *vs.* GG	0.523	—	T	0.523	1.12 (0.74–1.68)	0.35	0.464
			Dominant	(TT+TG) *vs.* GG	0.995	1.00 (0.57–1.76)					
			Recessive	TT *vs.* (TG+GG)	0.278	0.65 (0.30–1.42)					
Rs1042714	*ADRB2*	148 206 473	Additive	GG *vs.* GC *vs.* CC	0.004*	—	G	0.001*	2.38 (1.38–4.08)	0.09	0.367
			Dominant	(GG+GC) *vs.* CC	0.003*	0.40 (0.22–0.74)					
			Recessive	GG *vs.* (GC+CC)	0.086	0.11 (0.01–1.21)					
Rs1042713	*ADRB2*	148 206 440	Additive	GG *vs.* GA *vs.* AA	0.204	—	G	0.081	1.45 (0.95–2.20)	0.40	0.884
			Dominant	(GG+GA) *vs.* AA	0.097	0.57 (0.29–1.12)					
			Recessive	GG *vs.* (GA+AA)	0.249	0.65 (0.32–1.35)					

Abbreviations: 95% CI, 95% confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odd ratio; SNP, single-nucleotide polymorphism.
P-values are calculated by χ^2.
*$P<0.05$.

Table 3 Multiple logistic regression analysis of associations between eight tag-SNPs and SSH

Genes/dbSNP	*Polymorphism*	*SSH*[a]	*SRH*[a]	P-*value*[b]	*OR*	*95% CI*
CYP11B2	Genotype			0.096		
Rs1799998	CC	10 (16.1)	22 (8.1)	0.031*	2.59	1.09–6.14
	CT	24 (38.7)	100 (36.6)	0.409	1.29	0.70–2.38
	TT	28 (45.2)	151 (55.3)	—	1	—
	(CC+CT) *vs.* TT	34 (54.8)/28 (45.2)	122 (44.7)/151 (55.3)	0.146	0.66	0.38–1.16
	CC *vs.* (CT+TT)	10 (16.1)/52 (83.9)	22 (8.1)/251 (91.9)	0.044*	0.43	0.19–0.98
	Allele					
	C (risk)	44 (35.5)	144 (26.4)	0.038*	1.56	1.03–2.37
	T	80 (64.5)	402 (73.6)	—	1	—
PRKG1	Genotype			0.039*		
Rs7897633	AA	23 (37.1)	63 (23.1)	0.018*	2.72	1.19–6.22
	AC	29 (46.8)	136 (49.8)	0.337	1.47	0.67–3.22
	CC	10 (16.1)	74 (27.1)	—	1	—
	(AA+AC) *vs.* CC	52 (83.9)/10 (16.1)	199 (72.9)/74 (27.1)	0.101	0.54	0.26–1.13
	AA *vs.* (AC+CC)	23 (37.1)/39 (62.9)	63 (23.1)/210 (76.9)	0.016*	0.48	0.27–0.88
	Allele					
	A(risk)	75 (60.5)	262 (48.0)	0.002*	2.07	1.30–3.31
	C	49 (39.5)	284 (52.0)	—	1	—
PRKG1	Genotype			0.008*		
Rs1904694	GG	16 (26.2)	32 (11.7)	0.002*	3.53	1.57–7.93
	GA	29 (47.6)	131 (47.8)	0.274	1.45	0.74–2.84
	AA	16 (26.2)	111 (40.5)	—	1	—
	(GG+GA) *vs.* AA	45 (73.8)/16 (26.2)	163 (59.5)/111 (40.5)	0.055	0.54	0.29–1.01
	GG *vs.* (GA+AA)	16 (26.2)/45 (73.8)	32 (11.7)/242 (88.3)	0.003*	0.35	0.18–0.71
	Allele					
	G(risk)	61 (50)	195 (35.6)	0.004*	1.81	1.21–2.70
	A	61 (50)	353 (64.4)	—	1	—
ADRB2	Genotype			0.010*		
Rs1042714	GG	2 (3.3)	1 (0.4)	0.050	11.79	1.00–139.65
	GC	19 (31.7)	47 (17.4)	0.014*	2.25	1.18–4.31
	CC	39 (65.0)	222 (82.2)	—	1	—
	(GG+GC) *vs.* CC	21 (35.0)/39 (65.0)	48 (17.8)/222 (82.2)	0.005*	0.41	0.22–0.77
	GG *vs.* (GC+CC)	2 (3.3)/58 (96.7)	1 (0.4)/269 (99.6)	0.072	0.11	0.01–1.22
	Allele					
	G(risk)	23 (19.2)	49 (9.1)	0.003*	2.35	1.35–4.10
	C	97 (80.8)	491 (90.9)	—	1	—
FGF5	Genotype			0.062		
Rs16998073	TT	13 (21.3)	56 (20.4)	0.249	0.63	0.29–1.38
	TA	23 (37.7)	145 (52.9)	0.019*	0.47	0.25–0.88
	AA	25 (41.0)	73 (26.6)	—	1	—
	(TT+TA) *vs.* AA	36 (59.0)/25 (41.0)	201 (73.4)/73 (26.6)	0.025*	0.51	0.29–0.92
	TT *vs.* (TA+AA)	13 (21.3)/48 (78.7)	56 (20.4)/218 (79.6)	0.963	1.02	0.50–2.05
	Allele					
	T	49 (40.2)	257 (46.9)	0.136	0.73	0.49–1.10
	A	73 (59.8)	291 (53.1)	—	1	—
SLC8A1	Genotype			0.051		
Rs434082	AA	2 (3.3)	5 (1.8)	0.280	2.58	0.46–14.40
	AG	23 (37.7)	69 (25.3)	0.021*	2.03	1.11–3.71
	GG	36 (59.0)	199 (72.9)	—	1	—
	(AA+AG) *vs.* GG	25 (41.0)/36 (59.0)	74 (27.1)/199 (72.9)	0.015*	0.49	0.27–0.87
	AA *vs.* (AG+GG)	2 (3.3)/59 (96.7)	5 (1.8)/268 (98.2)	0.410	0.49	0.09–2.68
	Allele					
	A(risk)	27 (22.1)	79 (14.5)	0.017*	1.83	1.11–3.01
	G	95 (77.9)	467 (85.5)	—	1	—

Table 3 (Continued)

Genes/dbSNP	*Polymorphism*	*SSH*[a]	*SRH*[a]	P-*value*[b]	*OR*	*95% CI*
SLC8A1	Genotype			0.066		
Rs11893826	AA	7 (11.7)	21 (7.7)	0.699	1.21	0.47–3.10
	AG	18 (30.0)	130 (47.4)	0.033*	0.51	0.27–0.95
	GG	35 (58.3)	123 (44.9)	—	1	—
	(AA+AG) *vs.* GG	25 (41.7)/35 (58.3)	151 (55.1)/123 (44.9)	0.085	1.66	0.93–2.93
	AA *vs.* (AG+GG)	7 (11.7)/53 (88.3)	21 (7.7)/253 (92.3)	0.306	0.62	0.25–1.55
	Allele					
	G(risk)	88 (73.3)	376 (68.6)	0.374	1.23	0.78–1.92
	A	32 (26.7)	172 (31.4)	—	1	—
BCAT1	Genotype			0.951		
Rs7961152	AA	2 (3.2)	0 (0.0)	0.999	<0.01	—
	AC	11 (17.7)	57 (20.9)	0.751	0.89	0.43–1.83
	CC	49 (79.0)	216 (79.1)	—	1	—
	(AA+AC) *vs.* CC	13 (21.0)/49 (79.0)	57 (20.9)/216 (79.1)	0.892	0.95	0.48–1.89
	AA *vs.* (AC+CC)	2 (3.2)/60 (96.8)	0 (0.0)/273 (100.0)	0.999	<0.01	—
	Allele					
	A(risk)	15 (12.1)	57 (10.4)	0.525	1.22	0.66–2.25
	C	109 (87.9)	489 (89.6)	—	1	—
Combined genotypes				0.003*	—	—
(cGRS)	0-risk	27 (45.8)	181 (67.8)	—	1	—
	1-risk	19 (32.2)	57 (21.3)	0.014*	2.30	1.18-4.48
	2–4 risks	13 (22.0)	29 (10.9)	0.003*	3.32	1.51–7.30

Abbreviations: 95% CI, 95% confidence interval; cGRS, cumulative genetic risk score; OR, odds ratio; SNP, single-nucleotide polymorphism; SRH, salt-resistant hypertension; SSH, salt-sensitive hypertension.
*$P<0.05$.
[a]Numbers are frequencies and percentage.
[b]*P*-value was calculated by multiple logistic regression (adjusted by age and gender).

Table 4 Multivariate logistic regression model in allele for salt-sensitive hypertension

Variable	B	*S.e.*	*Wals*	*P*-value[a]	*OR*	95% *CI*	*P*-value[b]
Age	0.03	0.01	4.84	0.028*	1.03	1.00–1.06	0.032*
Sex	0.58	0.25	5.33	0.021*	1.79	1.09–2.94	0.021*
Rs7897633-A	0.61	0.28	4.86	0.027*	1.83	1.07–3.14	0.028*
Rs434082-A	1.04	0.28	14.24	0.001*	2.84	1.65–4.87	0.001*
Rs1042714-G	0.88	0.30	8.27	0.004*	2.40	1.32–4.35	0.004*
Rs1799998-C	0.30	0.24	1.54	0.215	1.35	0.84–2.17	0.227
Rs1904694-G	0.35	0.23	2.24	0.134	1.42	0.90–2.25	0.148
Constant	–4.85	0.92	27.76	0.001*	0.01	—	0.001*

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.
*$P<0.05$.
[a]*P*-value was calculated by multiple logistic regression model.
[b]*P*-value was based on 1000 bootstrap resamples by multiple logistic regression model.

modulation of cell growth. The *PRKG1* gene is most strongly expressed in all types of smooth muscle, platelets, cerebellar Purkinje cells, hippocampal neurons and the lateral amygdalae.[46] The pathologic effects of the *PRKG1* gene on SSH have not been clarified. In 2011, Citterio *et al.*[25] conducted a genome-wide association study in Italians, and reported a strong association between a cluster of tag-SNPs mapped in the first introns of the *PRKG1* gene (rs7897633) and DBP after acute salt loading. On the other hand, a subsequent study by Citterio *et al.*[47] demonstrated that the *PRKG1* risk haplotype GAT (rs1904694, rs7897633 and rs7905063) is associated with a rightward shift of the pressure–natriuresis curve compared with the ACC haplotype, indicating that *PRKG1* risk alleles are associated with salt sensitivity related to a loss of inhibitory control of renal Na^+ reabsorption, suggestive of a blunt pressure–natriuresis response.

SLC8A1, a gene that codes for the Na^+/Ca^{2+} exchanger type 1, is involved in the control of peripheral vascular resistance. *SLC8A1* affects essential hypertension and salt sensitivity by regulating intracellular Ca^{2+} and the tubular response to salt loading.[48] Citterio *et al.*[25] also focused on this gene, and reported that rs434082 was associated with variations in SBP. The rs11893826 polymorphism was significantly associated with urinary Ca^{2+} excretion 2 h after acute salt loading, suggesting that reduced Ca^{2+} excretion could affect BP response. Indeed, we verified that the polymorphic locus rs434082 was significantly associated with SSH. Subjects who carried the rs434082-A allele and the AA/GA genotype were at high risk for salt sensitivity that might have been influenced by the regulation of Ca^{2+} transport.

ADRB2 encodes the β-2-adrenergic receptor, which is a member of the G-protein-coupled receptor superfamily. This receptor is directly associated with one of its ultimate effectors, the class C L-type calcium channel. The *ADRB2* gene is strongly implicated in the regulation of blood pressure. In an African-American sib-pairs study, preliminary evidence of a link between the *ADRB2* gene and salt sensitivity was reported.[49] In Dietary Approaches to Stop Hypertension-sodium trials,[20] the association between two SNPs of *ADRB2* (rs1042713 and rs1042714) and BP response to sodium intake, strongly suggests that this locus modulates dietary sodium sensitivity. Consistent with the present results, Pojoga *et al.*[18] reported that salt sensitivity is associated with the A allele of rs1042713 and the C allele of rs1042714.

FGF5 is a member of the fibroblast growth factor (FGF) family that mediates a variety of biological processes, including embryonic

development, cell growth, morphogenesis, tissue repair and tumor growth and invasion. A Han Chinese population study suggested that variation in upstream regions of the *FGF5* gene was associated with altered susceptibility to essential hypertension, and reported that individuals with rs16998073 had a 72% increased risk for hypertension under a codominant model.[50] Effects of *FGF5*-rs16998073 on SBP and essential hypertension were significantly more pronounced in Han Chinese than in white Europeans.[51] However, few studies have focused on the association between rs16998073 in *FGF5* and SSH. Rhee *et al.*[52] reported that rs16998073 in *FGF5* was associated with the development of salt sensitivity in a Korean population. Our study also demonstrated that rs16998073 might have a role in salt sensitivity.

A GRS is widely used for the prediction of diabetes,[53] breast cancer[54] and cardiovascular disease.[55,56] It is especially useful in earlier life, when knowledge of other risk factors is limited.[53] In the present study, it was used to combine the effects of five SNPs on SSH and could provide a statistically significant improvement over the existing model. We used a modified Sullivan's acute salt loading and diuresis shrinkage test to determine the BP response to salt sensitivity. Previously, there has been no gold standard to identify salt sensitivity. A variety of protocols have been used to test for salt sensitivity, including acute salt loading,[57] and chronic low- and high-sodium dietary intervention.[3] However, the established methods of salt sensitivity determination are too complicated for screening at the level of the population. A greater number of studies that focus on an easier, more acceptable method of salt sensitivity testing is crucial. Some limitations affected the present study. First, our study sample was relatively small. Thus, a multivariable model was developed based on 1000 bootstrap samples. This method was used to perform the internal validation of predictive accuracy. Second, all associations suggested in this study were derived from a population-genetics-based approach supported by statistical analyses, and the underlying biological mechanisms of SSH require further research.

In conclusion, the present study aimed to identify the association between 29 candidate SNPs and SSH in a Han Chinese population. Eight genotypes and five alleles in the *CYP11B2*, *PRKG1*, *ADRB2*, *FGF5*, *SLC8A1* and *BCAT1* genes showed significant differences between the SSH and SRH groups. A joint effect of SNPs from different pathways contributed to a higher risk of SSH. The polymorphisms rs7897633-A in the *PRKG1* gene, rs434082-A in the *SLC8A1* gene and rs1042714-G in the *ADRB2* gene, in addition to increasing age and the female sex, were all risk factors for SSH. Subjects carrying 2–4 risks had 3.32-fold increased risk compared with those without risk alleles for SSH.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

LZ designed the study, and wrote and revised the manuscript. ZL performed the analysis and interpretation of the data, and drafted the manuscript. HQ, BL and JW contributed to acquisition of the data (population studies) and reviewed the manuscript. KL and JZ contributed to acquisition of data (genetics) and reviewed the manuscript. HC, YY and YH provided technical support for the analysis of the data and critical revision of the manuscript. All authors read and approved the final manuscript.

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