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ARTICLE

Association of Kir genes with blood pressure responses to dietary sodium intervention: the GenSalt study

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Abstract

Blood pressure (BP) responses to dietary sodium intervention vary among individuals. The inwardly rectifying potassium channel (Kir) is a potassium-selective ion channel that allows potassium ions to move more easily into rather than out of the cell. We aimed to investigate the associations of Kir genes with BP responses to dietary sodium intervention. A 7-day low-sodium intervention followed by a 7-day high-sodium intervention was conducted among 1906 participants. BP measurements were obtained at baseline and during each dietary intervention. Both single-marker and gene-based analyses were performed to explore the associations between Kir gene variants and BP responses to dietary sodium interventions. The genetic risk score (GRS) was used to assess the cumulative effect of the variants on the BP response to the sodium interventions. During the low-sodium intervention, markers rs858009, rs2835904, and rs860795 in *KCNJ6* were significantly associated with the systolic BP (SBP) response ($P = 8.82 \times 10^{-6}$, 3.32×10^{-6}, and 2.39×10^{-4}, respectively), whereas rs858009 and rs2835904 were significantly correlated with the mean arterial pressure (MAP) response ($P = 1.64 \times 10^{-4}$ and 2.72×10^{-4}, respectively). Marker rs2836023 showed a significant association with the SBP response ($P = 5.72 \times 10^{-5}$) to the high-sodium intervention. The GRS stratified by quartile grouping or as a continuous variable was associated with the BP response to the sodium interventions. Gene-based analyses consistently revealed that *KCNJ6* was significantly associated with the BP response to the sodium interventions. These findings suggest that *KCNJ6* may contribute to variation of BP responses to dietary sodium interventions. Future studies are warranted to confirm these findings and to identify functional variants for salt sensitivity.

Keywords Blood pressure · Salt sensitivity · Genetic association · Kir channel · Polymorphism

Introduction

Over the last decade, the global prevalence of hypertension in adults has increased to 31.1% [1]. Hypertension remains one of the most important worldwide health problems and a major risk factor of cardiovascular disease [2]. Excessive sodium intake has been shown to be associated with increased blood pressure (BP) by various studies, and sodium restriction has resulted in BP reduction [3]. However, the BP response to sodium intake varies considerably among individuals (termed BP salt sensitivity) [4]. Studies have shown that Asians are more likely to be salt-sensitive and have a high sodium intake than Western populations [5]. The heritability of BP salt sensitivity ranges from 20 to 33% in the Chinese population [6]. Multiple genetic loci have been reported to be associated with BP salt sensitivity [7, 8]. However, the genetic mechanisms of potassium and salt sensitivity are unknown.

Potassium supplementation can reduce BP in salt-sensitive but not salt-resistant individuals [9]. The inwardly rectifying potassium channel (Kir) is a specific subset of potassium-selective ion channels that allow potassium ions to move more easily into rather than out of

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the cell. Seven subfamilies have been identified in various mammalian cell types, including the classic Kir channel (Kir2.x), G protein-gated Kir channel (GIRK) (Kir3.x), ATP-sensitive K channel (Kir6.x), and K-transport channels (Kir1.x, Kir4.x, Kir5.x, and Kir7.x). These channels share the same basic topology and have the same basic physiological function [10]. They may have a vital role in vasodilatation and sympathetic nervous system regulation, resulting in BP regulation [11]. In additionally, the sympathetic nervous system is involved in salt-sensitive hypertension [12, 13]. Thus, Kir is a potentially important factor in regulation of the BP response to sodium intake. To examine this relationship, we selected 14 Kir genes and conducted both single-marker and gene-based analyses to systematically examine the associations of Kir gene variants with the BP response to sodium interventions among Chinese participants in the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) Study.

Methods

Study population

Details of the GenSalt study have been published elsewhere [14]. In brief, the study was conducted in six rural areas of northern China, in which populations had a habitual dietary intake of high salt and low potassium. A community-based BP screening program was performed in people aged 18–60 years to identify potential probands together with their families. Individuals with a mean systolic BP (SBP) between 130 and 160 mm Hg and/or a mean diastolic BP (DBP) between 85 and 100 mm Hg and no current use of antihypertensive medications (< 1 month before the screening visit) were recruited as volunteers for the dietary intervention study together with their spouses, siblings, and offspring. Individuals with stage 2 hypertension, current use of antihypertensive medications, secondary hypertension, a history of clinical cardiovascular disease, diabetes, and chronic kidney disease, pregnant women, heavy alcohol users, or those currently on a low-sodium diet were excluded from the GenSalt study. This study was approved by the Institution Review Boards at all participating institutions. All participants signed the informed consent form after receiving a detailed explanation of the baseline observation and the intervention program.

Dietary intervention

All eligible participants received a dietary intervention including a 7-day low-sodium intervention (3 g of salt or 51.3 mmol of sodium per day), followed by a 7-day high-sodium intervention (18 g of salt or 307.8 mmol of sodium per day). All foods were cooked without salt and then prepackaged salt was added to the individual meals served by the study staff. Each participant was required to have their breakfast, lunch, and dinner at the study kitchen under close supervision of the study staff during the entire study period. The participants were also instructed to avoid consuming any foods and beverages (except water) that were not provided by the study. Three timed urinary specimens (one 24-hour and two overnight) were collected at baseline and at the last 3 days in each phase of the intervention. The overnight urinary sodium excretion was converted to 24-hour values based on the formula developed from a random sample of GenSalt participants [15]. The mean (SD) 24-hour urinary excretions of sodium were 242.4 (66.7) mmol, 47.5 (16.0) mmol, and 244.3 (37.7) mmol at baseline, during the low-sodium intervention, and during the high-sodium intervention, respectively. These results showed excellent compliance with the intervention program. Among the 1906 eligible participants, 1871 (98.2%) individuals completed the low-sodium intervention, and 1860 (97.6%) individuals completed the high-sodium intervention.

Phenotypic measurements

Standard questionnaires were used to collect information from the participants on structure, demographic characteristics, personal and family medical histories, and lifestyle risk factors by trained staff during the 3 days of the baseline examination. On the morning of each day at baseline and the last 3 days during the intervention periods (days 5, 6, and 7 on the low-sodium and high-sodium interventions), BP was measured three times according to a standard protocol by trained staff [16]. Random-zero sphygmomanometers (Hawksley & Sons Ltd, Lancing, UK) were used in the GenSalt study. The BP was measured after the participant had remained in a seated position for 5 minutes. All participants were instructed to avoid cigarette smoking, alcohol, coffee/tea, and exercise for at least 30 minutes before their BP measurements. Mean arterial pressure (MAP) was calculated as $DBP + (SBP - DBP)/3$. BP responses were defined continuously as absolute changes in SBP, DBP, and MAP when switching from baseline to the low-sodium intervention and from the low-sodium to the high-sodium intervention.

Genotyping, quality control and genetic risk score (GRS)

Single-nucleotide polymorphisms (SNPs) located in the Kir genes and their ± 5000 base-pair flanking regions were genotyped among all participants. Within the 14 Kir genes shown in Table 1, 393 SNPs were genotyped using the

Table 1 Characteristics of the Kir genes

Gene symbol	Gene location	Physical position[a] ± 5 kb	Tag SNPs	Encoded protein (channel or subunits)
KCNJ10	1q23.2	(160002257, 160045051)	4	Potassium voltage-gated channel subfamily J member 10, Kir4.1
KCNJ9	1q23.2	(160046360, 160064212)	4	Potassium voltage-gated channel subfamily J member 9, Kir3.3
KCNJ3	2q24.1	(155550093, 155719864)	28	Potassium voltage-gated channel subfamily J member 3, Kir3.1
KCNJ13	2q37.1	(233625512, 233646275)	2	Potassium voltage-gated channel subfamily J member 13, Kir7.1
KCNJ11	11p15.1	(17401795, 17415878)	3	Potassium voltage-gated channel subfamily J member 11, Kir6.2
KCNJ1	11q24.3	(128702909, 128742268)	9	Potassium voltage-gated channel subfamily J member 1, Kir1.1
KCNJ5	11q24.3	(128756313, 128796060)	16	Potassium voltage-gated channel subfamily J member 5, Kir3.4
KCNJ12	17p11.2	(21274699, 21328184)	3	Potassium voltage-gated channel subfamily J member 12, Kir2.2
KCNJ16	17q24.3	(68066348, 68136749)	12	Potassium voltage-gated channel subfamily J member 16, Kir5.1
KCNJ2	17q24.3	(68159757, 68181189)	5	Potassium voltage-gated channel subfamily J member 2, Kir2.1
KCNJ14	19q13.33	(48953964, 48974367)	2	Potassium voltage-gated channel subfamily J member 14, Kir2.4
KCNJ6	21q22.13	(38991778, 39293741)	68	Potassium voltage-gated channel subfamily J member 6, Kir3.2
KCNJ15	21q22.13-q22.2	(39596837, 39680043)	15	Potassium voltage-gated channel subfamily J member 15, Kir4.2
KCNJ4	22q13.1	(38817332, 38856205)	9	Potassium voltage-gated channel subfamily J member 4, Kir2.3

Kir inwardly rectifying potassium channel, *SNPs* single-nucleotide polymorphisms

[a]Genomic locations are based on NCBI Build 37 of the genome

Affymetrix 6.0 platform (Affymetrix, Santa Clara, CA, USA). SNPs with a genotype call rate < 95%, minor allele frequency < 1% or deviation from the Hardy–Weinberg equilibrium (HWE) after Bonferroni correction for multiple testing were excluded. Among the remaining 326 qualified SNPs, 180 SNPs were selected as taggers using the Haploview software (version 4.2, http://www.broad.mit.edu/mpg/haploview) with an r^2 threshold of 0.8. Quality control information for the tagged SNPs, including their genomic locations, major/minor alleles, MAFs, HWE *P* values and call rates, is listed in Supplementary Table 1. The GRS was used to assess the cumulative effect of the variants on the BP response to the sodium interventions. Variants comprising the GRS included those robustly associated with the BP response to the sodium interventions in this analysis.

Statistical analysis

The Mendelian consistency of the SNP genotype data was checked using PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/). Families with Mendelian inconsistencies were set as the missing genotypes of the related SNPs. The weighted MAP GRS was calculated for each participant as the sum of the products of the participant's dosage score for each SNP weighted by the effect size (β-coefficient) of the MAP from the present study. Then, the participants were grouped into quartiles of risk for the MAP GRS. The baseline characteristics of all participants were presented as the mean ± SD for continuous variables or as percentages for categorical variables. Additive associations between SNPs and BP responses to the dietary sodium interventions were assessed using a mixed-effects linear regression model with the PROC MIXED procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA). Age, gender, body mass index, and 24-hour urinary sodium were adjusted in the mixed models. A sandwich estimator was used to account for the non-independence of family members. For significant SNPs, the mean effect size and 95% confidence interval were estimated for each genotype using a mixed linear regression model. Gene-based associations of each gene with BP responses to the dietary sodium interventions were evaluated using the truncated product method (TPM) with the R software (version 3.3.1; http://www.r-project.org) [17]. The truncation point was set as $\tau = 0.10$, and the *P* value for TPM was estimated by 1,000,000 simulations. Sensitivity analyses were conducted after excluding significant SNPs within each gene to eliminate their influence on the gene-based association. Additionally, the Versatile Gene-based Association Study (VEGAS) was used to evaluate the robustness of the TPM results [18]. The Bonferroni procedure was used to account for multiple comparisons. The thresholds for the single SNP-based and gene-based analyses were $\alpha = 0.05/180 = 2.78 \times 10^{-4}$ and $\alpha = 0.05/14 = 3.57 \times 10^{-3}$, respectively.

Results

The baseline characteristics of the 1906 participants are shown in Supplementary Table 2. In summary, the mean age of the participants was 38.7 years, and ~ 53% were men. The baseline SBP, DBP, and MAP were 116.9, 73.7, and 88.1 mm Hg, respectively. The BP was significantly

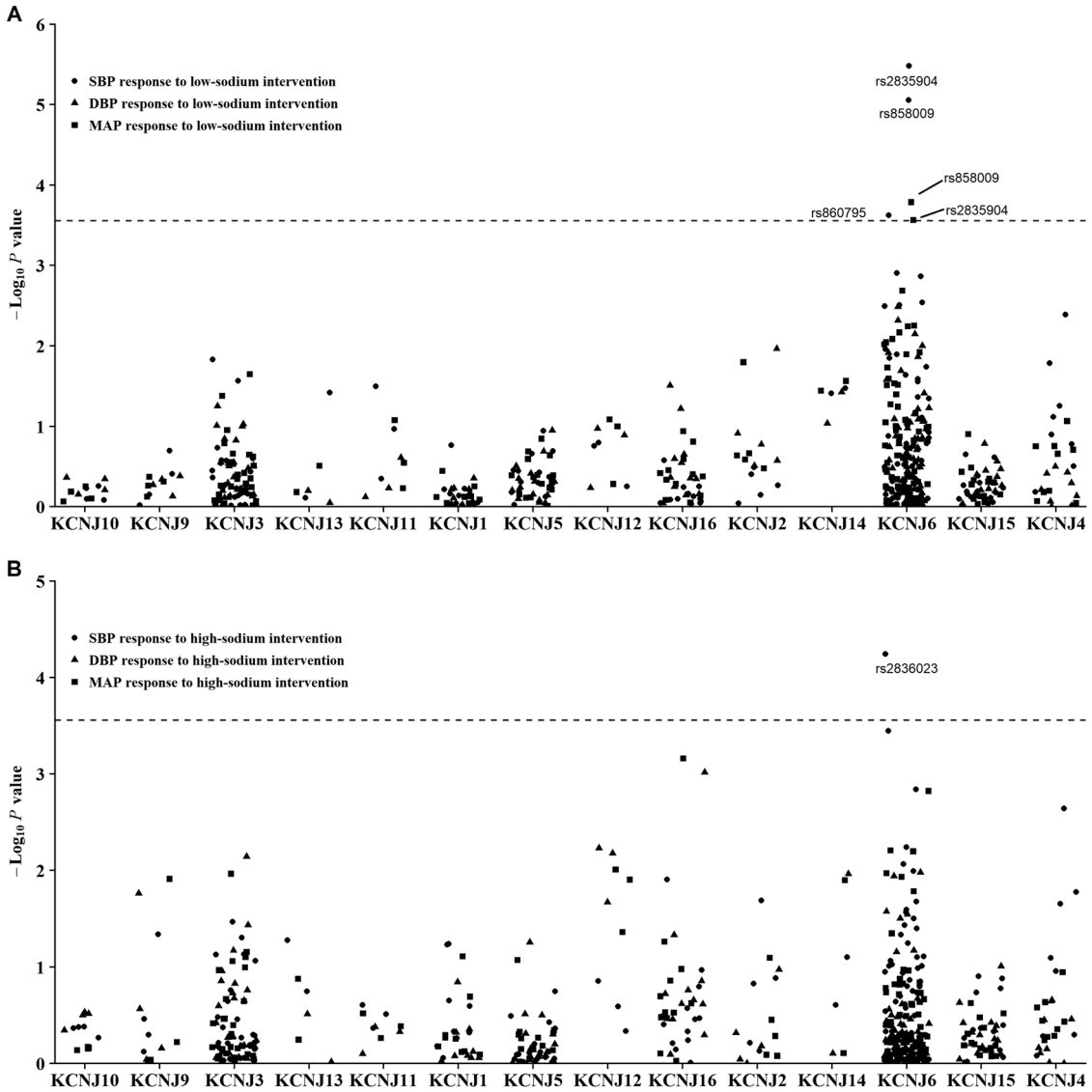

Fig. 1 $-\mathrm{Log}_{10}$ *P* values for the associations of 180 tagged SNPs in Kir genes with BP responses to the low-sodium **a** and high-sodium **b** interventions. Labeled SNPs were significant after corrections for multiple comparisons. The horizontal dashed lines indicate the corrected significance level ($\alpha = 2.78 \times 10^{-4}$). Abbreviations: Kir, inwardly rectifying potassium channel; SNP, single-nucleotide polymorphism

reduced after the low-sodium intervention and elevated after the subsequent high-sodium intervention.

Figure 1 presents the associations of each SNP with the BP responses to the dietary sodium interventions. Four SNPs (rs858009, rs2835904, rs860795, and rs2836023) in *KCNJ6* were significantly associated with the BP responses to the sodium interventions after adjustment for multiple testing. SNP rs858009 was significantly associated with the SBP ($P = 8.82 \times 10^{-6}$) and MAP ($P = 1.64 \times 10^{-4}$) responses to the low-sodium intervention. The number of minor C alleles of marker rs858009 was associated with decreased BP responses to the low-sodium intervention. The mean SBP responses (95% CIs) were −7.52 (−8.46, −6.58), −5.48 (−5.97, −5.00), and −4.93 (−5.41, −4.45) mm Hg and the mean MAP responses (95% CIs) were −4.93 (−5.64,−4.22), −3.74 (−4.13,−3.35), and −3.31 (−3.68,−2.94) mm Hg for the AA, AC, and CC genotypes, respectively (Table 2). The associations of marker rs2835904 with the BP responses to the low-sodium intervention were similar ($P = 3.32 \times 10^{-6}$ for SBP and 2.72×10^{-4} for MAP, respectively). The number of minor G allele of marker rs2835904 was associated with a reduced response of BP to the low-sodium intervention (Table 2). In addition, rs860795 was associated with the SBP response to the low-sodium intervention ($P = 2.39 \times 10^{-4}$) (Table 2). Marker rs2836023 was also significantly associated with the SBP response to the high-sodium intervention ($P = 5.72 \times 10^{-5}$) after the multiple comparison adjustment. The

Table 2 Blood pressure response to dietary sodium intervention according to genotypes (mean (95%CI))

SNP	Genotype	Low-sodium intervention			High-sodium intervention DBP		
		SBP	DBP	MAP	SBP	DBP	MAP
rs858009	AA	−7.52 (−8.46,−6.58)	−3.63 (−4.37,−2.88)	−4.93 (−5.64,−4.22)	5.59 (4.82,6.36)	1.93 (1.26,2.60)	3.16 (2.54,3.77)
	AC	−5.48 (−5.97,−5.00)	−2.86 (−3.27,−2.45)	−3.74 (−4.13,−3.35)	5.02 (4.59,5.44)	2.04 (1.64,2.44)	3.03 (2.67,3.40)
	CC	−4.93 (−5.41,−4.45)	−2.51 (−2.90,−2.11)	−3.31 (−3.68,−2.94)	4.45 (4.01,4.90)	1.91 (1.48,2.33)	2.75 (2.36,3.14)
	P	8.82×10^{-6} [a,b]	9.96×10^{-3} [b]	1.64×10^{-4} [a,b]	5.72×10^{-3} [b]	0.79	0.18
rs2835904	AA	−6.58 (−7.18, −5.97)	−3.16 (−3.63, −2.69)	−4.30 (−4.76, −3.85)	5.42 (4.94,5.90)	1.96 (1.53,2.39)	3.12 (2.72,3.51)
	AG	−5.30 (−5.77, −4.83)	−2.79 (−3.19, −2.39)	−3.62 (−4.00, −3.25)	4.78 (4.34,5.22)	2.03 (1.61,2.44)	2.94 (2.55,3.33)
	GG	−4.51 (−5.17, −3.85)	−2.37 (−2.88, −1.86)	−3.08 (−3.57, −2.59)	4.25 (3.68,4.82)	1.86 (1.31,2.41)	2.65 (2.16,3.14)
	P	3.32×10^{-6} [a,b]	0.03 [b]	2.72×10^{-4} [a,b]	1.45×10^{-3} [b]	0.83	0.15
rs860795	GG	−5.03 (−5.51, −4.55)	−2.55 (−2.97, −2.13)	−3.38 (−3.77, −2.99)	4.44 (4.00,4.88)	1.82 (1.39,2.24)	2.69 (2.30,3.08)
	CG	−5.56 (−6.06, −5.07)	−2.96 (−3.34, −2.58)	−3.83 (−4.20, −3.45)	5.09 (4.65,5.52)	2.15 (1.75,2.54)	3.13 (2.76,3.50)
	CC	−7.06 (−7.94, −6.18)	−3.17 (−3.96, −2.39)	−4.47 (−5.18, −3.75)	5.43 (4.68,6.17)	1.82 (1.12,2.51)	3.02 (2.39,3.64)
	P	2.39×10^{-4} [a,b]	0.09	5.73×10^{-3} [b]	8.55×10^{-3} [b]	0.57	0.15
rs2836023	CC	−5.18 (−5.61,−4.76)	−2.66 (−3.00,−2.32)	−3.50 (−3.82,−3.18)	4.43 (4.06, 4.80)	1.74 (1.39, 2.09)	2.63 (2.31, 2.95)
	CT	−6.04 (−6.65,−5.44)	−3.03 (−3.52,−2.53)	−4.03 (−4.51,−3.56)	5.46 (4.96, 5.96)	2.36 (1.89, 2.83)	3.39 (2.96, 3.82)
	TT	−6.30 (−7.90,−4.70)	−3.74 (−5.10,−2.37)	−4.59 (−5.89,−3.29)	6.69 (5.17, 8.20)	2.54 (1.16, 3.91)	3.95 (2.62, 5.28)
	P	0.02 [b]	0.08	0.03 [b]	5.72×10^{-5} [a,b]	0.03 [b]	1.50×10^{-3} [b]

CI confidence interval, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *P P* values, *SBP* systolic blood pressure, *SNP* single-nucleotide polymorphism

[a] $P < 2.78 \times 10^{-4}$ (significance level after adjustment for multiple testing using Bonferroni, $\alpha = 0.05/180 = 2.78 \times 10^{-4}$)

[b] $P<0.05$ (nominal significance level)

Table 3 BP levels at baseline and responses to dietary intervention by MAP GRS quartile

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
Baseline					
SBP	116.5 (115.1, 118.0)	116.6 (115.3, 118.0)	117.1 (116.0, 118.2)	118.0 (116.8, 119.1)	0.11
DBP	73.54 (72.54, 74.54)	73.38 (72.38, 74.37)	73.79 (73.03, 74.54)	74.15 (73.30, 74.99)	0.30
MAP	87.86 (86.81, 88.92)	87.78 (86.76, 88.80)	88.20 (87.40, 89.01)	88.74 (87.88, 89.61)	0.17
Low-sodium intervention					
SBP	−4.51 (−5.16, −3.85)	−5.06 (−5.71, −4.42)	−5.49 (−6.07, −4.90)	−6.59 (−7.21, −5.97)	2.59×10^{-6}
DBP	−2.37 (−2.93, −1.80)	−2.51 (−3.08, −1.94)	−2.96 (−3.42, −2.50)	−3.22 (−3.73, −2.71)	0.01
MAP	−3.08 (−3.60, −2.55)	−3.36 (−3.88, −2.85)	−3.80 (−4.25, −3.36)	−4.34 (−4.83, −3.86)	1.94×10^{-4}
High-sodium intervention					
SBP	3.83 (3.26, 4.40)	4.92 (4.29, 5.55)	4.74 (4.21, 5.26)	5.74 (5.23, 6.25)	1.86×10^{-6}
DBP	1.62 (1.07, 2.17)	2.17 (1.59, 2.75)	1.76 (1.26, 2.27)	2.35 (1.87, 2.83)	0.03
MAP	2.34 (1.84, 2.84)	3.08 (2.56, 3.61)	2.75 (2.29, 3.21)	3.49 (3.06, 3.92)	9.13×10^{-4}

Data are mean (95% CI). *BP* blood pressure, *CI* confidence interval, *DBP* diastolic blood pressure, *GRS* genetic risk score, *MAP* mean arterial pressure, *P*, *P* values, *SBP* systolic blood pressure. Age, gender, body mass index (BMI), and 24-hour urinary sodium were adjusted. * Linear trend between BP and GRS was calculated in the mixed effect model after adjusted the age, gender, BMI, and 24-h urinary sodium

number of minor T alleles of rs2836023 was related to the increased SBP response to the high-sodium intervention. The SBP responses (95% CIs) were 4.43 (4.06, 4.80), 5.46 (4.96, 5.96), and 6.69 (5.17, 8.20) mm Hg for the CC, CT, and TT genotypes, respectively (Table 2). Markers rs858009, rs2835904, and rs860795 were also associated with the SBP response to the high-sodium intervention, and rs2836023 was associated with the BP response to the low-sodium intervention, although these associations were nominal after multiple test correction. The exact *P* values for all SNP association tests are shown in Supplementary Table 3.

The risk alleles and effect sizes of the SNPs (rs858009, rs2835904, rs860795, and rs2836023) selected for the GRS analysis are shown in Supplementary Table 4. The cumulative effects of the variants on the baseline BP and the BP response to the sodium interventions by MAP GRS quartiles are shown in Table 3. The baseline BP did not significantly differ according to the MAP GRS quartiles. The MAP GRS was inversely associated with the magnitude of the SBP and the MAP response to the low-sodium intervention ($P = 2.59 \times 10^{-6}$ and 1.94×10^{-4}, respectively). Those with higher GRSs had significant increases in the SBP response to the high-sodium intervention ($P = 1.86 \times 10^{-6}$). β coefficients, standard errors, and *P* values corresponding to the GRS increase for baseline BP and the BP responses to the dietary interventions are shown in Table 4.

Table 5 presents the gene-based analysis results. Genes with more than one SNP were examined. In the TPM results, *KCNJ6* was significantly associated with the BP responses to the low-sodium intervention (all $P < 1.00 \times 10^{-6}$ for SBP, DBP, and MAP) and the SBP responses to the high-sodium intervention ($P < 1.00 \times 10^{-6}$). In the sensitivity analyses, after excluding significant markers (rs858009, rs2835904, and rs860795 in the low-sodium intervention analyses and rs2836023 in the high-sodium intervention analyses), the *KCNJ6* gene was still strongly associated with the BP responses to the low-sodium intervention ($P < 1.00 \times 10^{-6}$ for SBP, $P = 3.00 \times 10^{-6}$ for DBP and $P < 1.00 \times 10^{-6}$ for MAP) and the SBP response to the high-sodium intervention ($P < 1.00 \times 10^{-6}$). The VEGAS analysis showed consistent associations of *KCNJ6* with the BP responses to the low-sodium intervention ($P = 4.70 \times 10^{-4}$ for SBP and $P = 1.14 \times 10^{-3}$ for MAP). The TPM analysis also revealed associations of *KCNJ4* with the SBP responses to the low- and high-sodium interventions ($P = 2.40 \times 10^{-5}$ and $P = 3.00 \times 10^{-6}$, respectively). However, these associations of *KCNJ4* were not significant in the VEGAS analysis after multiple testing.

Discussion

This study is a candidate gene study that investigates the associations between Kir genes and BP salt sensitivity in a large Han Chinese population sample. Markers of the *KCNJ6* gene (rs858009, rs2835904, rs860795, and rs2836023) were significantly associated with the BP

Table 4 BP Levels at baseline and responses to dietary Intervention by GRS

Study phase	SBP			DBP			MAP		
	β	SE	*P*	*β*	SE	*P*	β	SE	*P*
Baseline	0.61	0.28	0.03	0.30	0.20	0.14	0.40	0.21	0.06
Low-sodium intervention	−0.75	0.14	4.85×10^{-8}	−0.33	0.11	3.01×10^{-3}	-0.48	0.11	8.22×10^{-6}
High-sodium intervention	0.53	0.11	3.10×10^{-6}	0.12	0.10	0.23	0.26	0.09	4.74×10^{-3}

BP blood pressure, *DBP* diastolic blood pressure, *GRS* genetic risk score, *MAP* mean arterial pressure, *P P* values, *SBP* systolic blood pressure, *SE* standard error. Age, gender, body mass index (BMI) and 24-hour urinary sodium were adjusted

Table 5 Gene-based associations of Kir genes with blood pressure responses in the GenSalt study (TPM)

	Low-sodium intervention			High-sodium intervention		
	SBP	DBP	MAP	SBP	DBP	MAP
KCNJ10	0.18	0.17	0.19	0.13	0.14	0.11
KCNJ9	0.38	0.18	0.18	0.19	0.08	0.06
KCNJ3	0.62	0.46	0.67	0.17	0.35	0.24
KCNJ13	0.08	0.16	0.16	0.09	0.20	0.18
KCNJ11	0.11	0.22	0.20	0.14	0.19	0.15
KCNJ1	0.46	0.64	0.32	0.19	0.47	0.36
KCNJ5	0.63	0.47	0.52	0.57	0.57	0.65
KCNJ12	0.21	0.24	0.20	0.18	8.10×10^{-3}	0.01
KCNJ16	0.36	0.23	0.45	0.41	0.12	0.05
KCNJ2	0.30	0.13	0.14	0.13	0.46	0.34
KCNJ14	0.04	0.05	0.03	0.12	0.02	0.03
KCNJ6	$< 1.00 \times 10^{-6}$ [a]	$< 1.00 \times 10^{-6}$ [a]	$< 1.00 \times 10^{-6}$ [a]	$< 1.00 \times 10^{-6}$ [a]	0.35	0.11
KCNJ15	0.40	0.41	0.46	0.61	0.71	0.35
KCNJ4	2.40×10^{-5} [a]	0.68	0.54	3.00×10^{-6} [a]	0.68	0.26

DBP diastolic blood pressure, *Kir* inwardly rectifying potassium channel, *MAP* mean arterial pressure, *SBP* systolic blood pressure, *SNP* single-nucleotide polymorphism, *TPM* truncated product method

[a]Significant after adjustment for multiple testing using Bonferroni correction ($\alpha = 0.05/14 = 3.57 \times 10^{-3}$)

responses to the sodium interventions. These markers had consistent effects on the BP responses to the dietary low- and high-sodium interventions, although some associations were nominal after multiple test correction. This study also identified a significant relationship between the MAP GRS and BP response to the sodium interventions. Similar results were obtained whether examining the MAP GRS according to quartiles or as a continuous variable. Furthermore, gene-based analyses consistently revealed that *KCNJ6* was significantly associated with the SBP and MAP responses to the low-sodium intervention and the SBP response to the high-sodium intervention. These results may highlight the effect of Kir genes on BP regulation.

Kir channels have been found in a wide variety of cell types, including endothelial cells and smooth muscle cells, which are the major constituents of the vasculature [10]. Classical Kir channels generate a large K^+ conductance and play key roles in maintenance of the resting membrane potential. In addition, these channels may contribute to vasodilatation via the nitric oxide pathway in vascular endothelial cells and reduce the intracellular Ca^{2+} concentration, resulting in vasodilation in vascular smooth muscle cells. Animal studies demonstrated that Kir channels played a vital role in activity of the sympathetic nervous system and then regulated BP [11], as excitation of the sympathetic nervous system is a mechanism underlying BP elevation. In addition, experiments in rats and humans confirmed that the sympathetic nervous system was involved in salt-sensitive hypertension [12, 13]. Salt-sensitive men showed an increased heart rate and reduced heart rate variability determined by the balance between sympathetic and parasympathetic influences [19].

KCNJ6 is a major component of GIRK. GIRK, which is one downstream target of G proteins, can regulate a wide range of electrophysiological activities (i.e., the heart rate) [20]. Activation of GIRK channels requires G proteins together with phosphatidylinositol 4,5 bisphosphate (PIP_2) [21]. Na^+ and hormones that are involved in the regulation of GIRK activity play important roles in the modulation of BP and BP salt sensitivity [22, 23]. *KCNJ6* was initially reported to regulate insulin secretion in pancreatic beta cells [24]. *KCNJ6* opening is not absolutely dependent on Na^+ but increases with the Na^+ concentration [25]. Amino-acid mutations in *KCNJ6* cause changes in ion sensitivity, such as the presence of aspartic acid rather than asparagine, which accounts for the Na^+ sensitivity [26]. Our study provides the first evidence that SNPs (rs858009, rs2835904, rs860795, and rs2836023) in *KCNJ6* are significantly associated with BP responses to sodium intake. All four SNPs are located in the intron region of *KCNJ6*. We used the web tools ENCODE, HaploReg, and RegulomeDB to speculate their functions [27–29]. Variations rs2835904, rs860795, and rs2836023 potentially regulate the chromatin state as an enhancer of histone methylation or acetylation in

different cell types and tissues, such as skeletal muscle and pancreatic islets. Strongly linked with rs2836023, both rs3787857 ($r^2 = 0.92$ and $D' = 0.97$) and rs743293 ($r^2 = 0.94$ and $D' = 0.98$) are likely to affect c-FOS protein binding. FOS proteins are members of the activating protein 1 transcription factor complex. Because it encodes the FOS protein, *c-fos* is more highly expressed in vascular smooth muscle cells from spontaneously hypertensive rats than in normotensive Wistar-Kyoto rats [30]. This information suggested that rs3787857 and rs743293 might be functional sites involved in BP regulation, whereas rs2836023 might simply function as a marker. *KCNJ4* is widely expressed in the heart and arteries. I_{K1} is mainly comprised of Kir2.1 and maintains the resting membrane potential and contributes to phase 3 repolarization in human ventricles [31]. Kir2.3, which is encoded by *KCNJ4*, is the molecular correlate of I_{K1} in the heart and has been implicated in arrhythmias [32]. Few population studies have found evidence that variants of Kir channel-encoding genes are associated with hypertension or salt sensitivity. Therefore, additional study is warranted to confirm these findings.

Strengths and limitations

Our study possessed several important strengths. First, the participants in our study were recruited from rural regions in North China, which minimized the genetic and environmental heterogeneity of the population. Second, good compliance of the participants to the sodium interventions and stringent quality control procedures were conducted for data collection, such as nine BP measurements to limit measurement error. In addition, correction procedures were performed to account for multiple testing.

However, this study has potential limitations. Populations in northern China have a relatively high sodium intake in daily life. These novel associations reported here need to be replicated in other populations with different genetic and environment backgrounds. Furthermore, the present study only included common polymorphisms of the candidate genes, and thus low frequency and structural variants might have been missed. Future studies that repeat the associations between variants in these genes and BP salt sensitivity are still needed.

Conclusion

In our study, we found that variants of the Kir genes were associated with the BP response to the sodium interventions. Further genetic and functional studies are needed to delineate the role of Kir in BP salt sensitivity.

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Compliance with ethical standards

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

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