Common Variants in Epithelial Sodium Channel Genes Contribute to Salt Sensitivity of Blood Pressure

The GenSalt Study

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Background—Rare mutations of the epithelial sodium channel (ENaC) lead to mendelian forms of salt-sensitive hypertension or salt-wasting hypotension. We aimed to examine the association between common variants in the ENaC genes and salt sensitivity of blood pressure (BP).

Methods and Results—A total of 1906 Han Chinese participated in the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study, which includes a 7-day low-sodium intake (51.3 mmol sodium/d) followed by a 7-day high-sodium intake (307.8 mmol sodium/d). Nine BP measurements were obtained at baseline and each intervention period using a random-zero sphygmomanometer. Single-nucleotide polymorphisms, both tagging and functional, from the 3 ENaC subunits, α, β, and γ (*SCNN1A, SCNN1B,* and *SCNN1G*), were genotyped. Multiple common single-nucleotide polymorphisms in *SCNN1G* were significantly associated with BP response to low-sodium intervention (rs4073930, $P=1.7\times10^{-5}$; rs4073291, $P=1.1\times10^{-5}$; rs7404408, $P=1.9\times10^{-5}$; rs5735, $P=3.0\times10^{-4}$; rs4299163, $P=0.004$; and rs4499238, $P=0.002$) even after correcting for multiple testing. For example, under an additive model, the minor allele G of SNP rs4073291 was associated with 1.33 mm Hg lower systolic BP reduction during low-sodium intervention.

Conclusions—This large dietary sodium intervention study indicates that common variants of ENaC subunits may contribute to the variation of BP response to dietary sodium intake. Future studies are warranted to confirm these findings in an independent population and to identify functional variants for salt sensitivity.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00721721.

(*Circ Cardiovasc Genet*. 2011;4:375-380.)

Key Words: blood pressure ■ epithelial sodium channel ■ genetic variant ■ salt sensitivity

Interactions among numerous genetic and environmental factors play an important role in blood pressure (BP) regulation. High-dietary sodium intake is one of the most important environmental risk factors for elevated BP.[1–3] However, BP response to dietary sodium intake varies considerably among individuals, a phenomenon known as salt sensitivity.[4,5] Salt sensitivity of BP has been associated with an increased risk of hypertension, cardiovascular disease, and premature death.[6,7] Previous studies have suggested that an individual's genetic profile may contribute to their BP responses to dietary sodium intake.[8–13] Thus, the investigation of genetic determinants of salt sensitivity will help to understand the potential interaction between genetic factors and dietary sodium intake on the regulation of BP.

Received October 6, 2010; accepted May 2, 2011.

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Guest Editor for this article was Barbara V. Howard, PhD.

The online-only Data Supplement is available at http://circgenetics.ahajournals.org/cgi/content/full/CIRCGENETICS.110.958629/DC1.

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Circ Cardiovasc Genet **is available at http://circgenetics.ahajournals.org** **DOI: 10.1161/CIRCGENETICS.110.958629**

Clinical Perspective on p 380

The renal epithelial sodium channel (ENaC) mediates net renal reabsorption of sodium in the distal tubule and is critical for the control of sodium balance, blood volume, and thereby of BP.[14,15] ENaC is composed of 3 partly homologous subunits, α, β, and γ.[16] It has been documented that gain-of-function mutations of ENaC genes lead to mendelian forms of hypertension or hypotension. For example, mutations in the cytoplasmic C terminus of either the β- or γ-subunit of ENaC cause Liddle syndrome, which is an autosomal dominant form of salt-sensitive hypertension and associated with increased ENaC activity and sodium reabsorption of the kidney.[17–20] On the other hand, mutations in ENaC subunits may also cause pseudohypoaldosteronism type I, which is an inherited form of severe hypotension and salt wasting.[21]

Recently, researchers have tried to identify common variants of ENaC genes that may influence the risk of essential hypertension and reported inconsistent results.[22–25] Iwai et al[22] reported that single-nucleotide polymorphism (SNP) rs5718, also known as G(−173)A, in the promoter region of the ENaC γ-subunit gene was associated with low systolic BP (SBP) in a Japanese population. However, this finding was not replicated in 2 independent studies of white Australians.[23,26] In addition, 2 studies conducted in Chinese Kazakhs reported inconsistent findings about the association of rs879605 in the α-subunit gene with hypertension.[24,25] These discrepancies might reflect differences in genetic architecture and linkage disequilibrium (LD) patterns among different ethnicities and heterogeneity of hypertension etiology among individuals even within the same ethnicity. Furthermore, none of these studies took into account the gene-sodium interaction on BP. Animal experiments suggested that variants of the ENaC genes and sodium intake might interactively determine the development of hypertension.[27] We examined the association between common variants in ENaC genes and BP responses to dietary sodium intervention in the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study.

Methods

Study Participants

The GenSalt study was a family-based dietary feeding study conducted in rural areas of northern China from 2003 to 2005. A community-based BP screening was conducted among persons ages 18 to 60 years in the study villages to identify potential probands and their families. The probands who had a mean SBP between 130 to 160 mm Hg and/or a diastolic BP (DBP) between 85 to 100 mm Hg and no use of antihypertensive medications and their spouses, siblings, and offspring were recruited for the dietary feeding study. All participants were of Han Chinese ethnicity. Individuals who had stage-2 hypertension, secondary hypertension, a history of clinical cardiovascular disease or diabetes, used antihypertensive medications, or were pregnant, heavy alcohol drinkers, or currently on a low-sodium diet were excluded from the study. More information about study design and participants has been published elsewhere.[28] Among 1906 eligible participants for the dietary intervention, 1871 (98.2%) and 1860 (97.6%) completed the low-sodium and high-sodium interventions, respectively, and were included in the current analysis. Institutional review boards or ethics committees at all participating institutes approved the study protocol. Written informed consent for the baseline observation and for the intervention was obtained from each participant before data collection or intervention, respectively.

Dietary Intervention

After a 3-day baseline observation, the study participants received a 7-day low-sodium diet (3 g of sodium chloride or 51.3 mmol of sodium or 1,179.9 mg of sodium per day) followed by a 7-day high-sodium diet (18 g of sodium chloride or 307.8 mmol of sodium or 7079.4 mg of sodium per day). All foods were cooked without salt, and prepackaged salt was added to the individual study participant's meal when it was served by the study staff. To ensure study participants' compliance to the intervention program, they were required to avoid consuming any foods and beverages that were not provided by the study. Dietary compliance by the participants was confirmed by measurements of 24-hour urinary excretion of sodium and potassium. One 24-hour and 2 overnight urine specimens were collected at the baseline and the last 3 days of each intervention phase. The overnight urinary sodium excretion was converted to 24-hour values through the use of the formulas developed from a random subsample of 238 subjects who collected overnight and 24-hour urine samples on the same days. The mean (standard deviation) of 24-hour urinary excretions of sodium and potassium were 242.4 (66.7) mmol and 36.9 (9.6) mmol at baseline, 47.5 (16.0) mmol, and 31.4 (7.7) mmol during the low-sodium intervention and 244.3 (37.7) mmol and 35.7 (7.5) mmol during the high-sodium intervention, respectively.

BP Measurements

Three sitting BP measurements were obtained each morning of the 3-day baseline observation and on days 5, 6, and 7 of each intervention period by the trained and certified observers, using a random-zero sphygmomanometer according to a standard protocol.[29] BP levels at baseline and during intervention were calculated as the mean of 9 measurements from each period.

Previous GenSalt analysis showed that BP responses to low-sodium and to high-sodium interventions shared some but not all genetic determinants (genetic correlation coefficients $\rho=-0.43$, $P=0.03$ for SBP and $\rho=-0.58$, $P=0.0003$ for DBP).[30] BP response to high-sodium intervention from low-sodium intervention may provide a more valid phenotype measure for salt sensitivity because participants' sodium intake was controlled during both phases. On the other hand, identifying genetic determinants of BP response to low-sodium from usual diet should have more direct clinical and public health implications. Therefore, we used BP responses to low-sodium intervention from baseline and responses to high-sodium intervention from low-sodium intervention. In the text and tables, the terms "BP response to low-sodium intervention from baseline" and "BP response to high-sodium intervention from low-sodium intervention" are simplified as "BP response to low-sodium" and "BP response to high-sodium," respectively.

SNP Selection and Genotyping

Both *SCNN1B* and *SCNN1G* genes are located on chromosome 16, whereas *SCNN1A* is located on chromosome 12. Tag SNPs from these genes were selected on the basis of empirical patterns of LD structure in the Chinese Han of Beijing HapMap sample, using Tagger software. The r^2 threshold used for selecting each tagSNP was 0.8. We also included SNPs that were previously reported to be associated with BP or hypertension. SNPs were genotyped with the use of SNPlex assays (Applied Biosystems, Foster City, CA), based on oligonucleotide ligation assay for capillary electrophoresis on ABI 3700 DNA Analyzers (Applied Biosystems). To provide better coverage of these 3 genes, we included additional SNPs genotyped on the Affymetrix 6.0 platform (Affymetrix, Santa Clara, CA), which had minor allele frequencies >0.01. In total, 46 SNPs passed quality control and were included in this analysis. The detailed information for these SNPs concerning their genome and gene locations, allele frequencies, and probability values for the Hardy-Weinberg equilibrium test is presented in online-only Data Supplement Table SI (please see http://hyper.ahajournal.org).

Table 1. Characteristics of 1906 Participants

Variable	Mean±SD	Median (Range)*
Age, y	38.7±9.6	39.0 (16.0–62.0)
Male, n (%)	1010 (53.0)	
BMI, kg/m^2	23.3±3.2	22.9 (14.5–37.8)
Baseline BP, mm Hg		
Systolic	116.9±14.2	115.8 (80.9–176.9)
Diastolic	73.7±10.3	73.3 (38.9–109.3)
BP during low-sodium intervention, mm Hg		
Systolic	111.4±12.2	110.0 (82.4–159.3)
Diastolic	71.0±9.7	70.7 (41.1–112.2)
BP during high-sodium intervention, mm Hg		
Systolic	116.3±13.6	114.4 (82.0–181.1)
Diastolic	72.9±10.3	72.4 (41.3–116.0)
BP response to low-sodium, mm Hg†		
Systolic	−5.5±7.0§	−4.4 (−45.8 to 24.2)
Diastolic	−2.8±5.5§	−2.6 (−23.1 to 18.2)
BP response to high-sodium, mm Hg‡		
Systolic	4.9±6.0§	4.7 (−11.3 to 36.2)
Diastolic	1.9±5.4§	1.8 (−20.0 to 27.1)

BMI indicates body mass index.

*Median and range is for continuous variables.

†BP change from baseline to the low-sodium intervention.

‡BP change from the low-sodium to high-sodium intervention.

§*P* value was <0.0001 when compared with no BP change during sodium interventions.

Statistical Analysis

The mendelian consistency of the SNP genotype data was assessed by PLINK (version 1.05; http://pngu.mgh.harvard.edu/~purcell/plink/).[31] We used Haploview software (version 4.2; http://www.broadinstitute.org/haploview) to test the Hardy-Weinberg equilibrium for each SNP and estimate the extent of pairwise LD between SNPs.[32] The solid spine LD method, as implemented in Haploview, was used to define LD blocks.

Baseline characteristics and BP response variables of intervention participants were summarized as means (standard deviations) and medians (ranges) for continuous variables and as percentages for categorical variables. Analyses of the association between SNPs and BP response phenotypes were conducted using a linear mixed-effects model implemented in the Proc Mixed procedure of SAS (version 9.1; SAS Institute, Cary, NC). A "sandwich" option was used to compute the estimated variance-covariance matrix of the fixed-effects (genetic variant effects) parameters by using the asymptotically consistent estimator. Because most of the studied families (random effects) only included sib pairs, we selected compound symmetry as the covariance structure, which assumes the same degree of dependency among family members. Age, sex, BP measurement room temperature, and study site were adjusted in multivariable analyses. Additive genetic models were assumed. The false discovery rate method was used to adjust for multiple testing.[33] A false discovery rate *q* value is calculated in this method to estimate the proportion of rejected null hypotheses which are erroneously rejected. We used the Proc Mult test procedure, along with the false discovery rate option, in SAS to calculate *q* value for each SNP. A *q* value of 0.05 was used as the threshold for statistical significance in our study.

Results

Table 1 shows study participants' baseline characteristics, BP levels during low-sodium and high-sodium interventions, and BP responses to dietary sodium interventions. Overall, BP levels decreased from baseline to low-sodium intervention and increased from low-sodium to high-sodium intervention. BP levels were similar at baseline and during the high-sodium intervention. BP responses to dietary sodium intervention were significantly different from zero.

The Figure shows the association of each SNP with SBP and DBP responses to low-sodium and high-sodium interventions. After adjusting for age, sex, BP measurement, room temperature, and study site, multiple SNPs in the *SCNN1A, SCNN1B,* and *SCNN1G* genes were associated with BP responses to

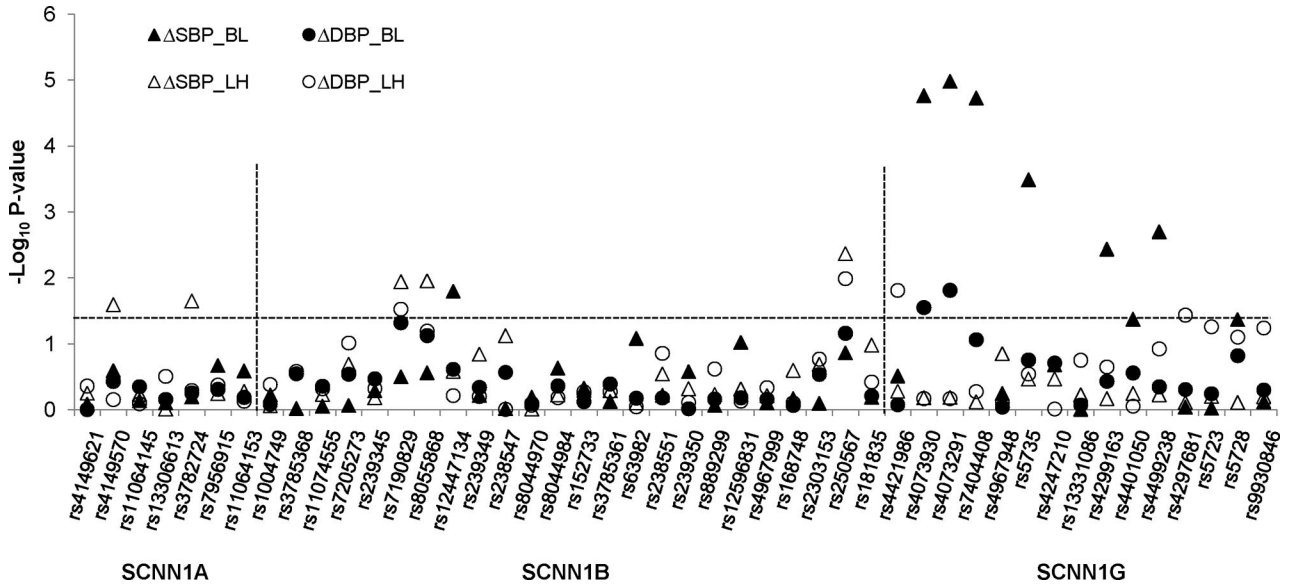

Figure. Log probability values for the association between 46 SNPs of *SCNN1A*, *SCNN1B*, and *SCNN1G* genes and BP responses to low- and high-sodium interventions. Horizontal dashed line indicates probability value of 0.05. ΔSBP_BL indicates systolic BP changes from baseline to low-sodium intervention; ΔDBP_BL, diastolic BP changes from baseline to low-sodium intervention; ΔSBP_LH, systolic BP changes from low- to high-sodium intervention; and ΔDBP_LH, diastolic BP changes from low- to high-sodium intervention.

Table 2. Regression Coefficients (Standard Errors) of BP Responses to Low-Sodium and High-Sodium Interventions Associated With Minor Alleles From Linear Mixed-Effects Models

			BP Response to Low Sodium, mm Hg†				BP Response to High Sodium, mm Hg†			
			Systolic		Diastolic		Systolic		Diastolic	
SNP	Alleles*	MAF	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
SCNN1A										
rs4149570	C:A	0.50	0.23 (0.21)	0.26	0.16 (0.17)	0.37	0.43 (0.19)	0.03	0.07 (0.18)	0.71
rs3782724	A:G	0.24	0.12 (0.26)	0.64	−0.13 (0.22)	0.56	−0.48 (0.21)	0.02	−0.13 (0.20)	0.51
SCNN1B										
rs7190829	A:G	0.18	−0.27 (0.27)	0.32	−0.47 (0.24)	0.05	0.59 (0.23)	0.01	0.50 (0.23)	0.03
rs8055868	G:A	0.20	−0.29 (0.27)	0.28	−0.41 (0.23)	0.08	0.61 (0.24)	0.01	0.41 (0.22)	0.06
rs12447134	A:C	0.08	0.96 (0.40)	0.02	0.39 (0.34)	0.24	−0.38 (0.34)	0.26	0.16 (0.31)	0.61
rs250567	G:A	0.10	0.58 (0.39)	0.14	0.58 (0.32)	0.07	−0.95 (0.33)	0.004	−0.82 (0.32)	0.01
SCNN1G										
rs4421986	C:T	0.06	−0.51 (0.50)	0.31	−0.08 (0.39)	0.84	0.29 (0.45)	0.53	−0.88 (0.36)	0.02
rs4073930	T:C	0.16	1.28 (0.30)	1.7E-5/3.0E-4‡	0.56 (0.26)	0.03	0.11 (0.24)	0.66	0.11 (0.26)	0.68
rs4073291	T:G	0.16	1.33 (0.30)	1.1E-5/3.0E-4‡	0.63 (0.26)	0.02	0.11 (0.24)	0.65	0.11 (0.27)	0.68
rs7404408	C:T	0.16	1.29 (0.30)	1.9E-5/3.0E-4‡	0.44 (0.26)	0.09	0.07 (0.24)	0.76	0.16 (0.26)	0.53
rs5735	T:C	0.17	1.18 (0.33)	3.0E-4/0.004‡	0.36 (0.27)	0.18	0.24 (0.26)	0.34	0.29 (0.27)	0.29
rs4299163	G:C	0.10	1.09 (0.38)	0.004/0.02‡	0.29 (0.32)	0.37	0.14 (0.33)	0.68	0.38 (0.31)	0.23
rs4401050	C:T	0.09	0.86 (0.42)	0.04	0.36 (0.33)	0.28	0.20 (0.33)	0.57	0.05 (0.35)	0.89
rs4499238	G:A	0.10	1.11 (0.36)	0.002/0.03‡	0.23 (0.31)	0.45	0.17 (0.31)	0.59	0.47 (0.30)	0.12
rs4297681	C:A	0.06	−0.05 (0.47)	0.91	0.25 (0.36)	0.50	0.11 (0.41)	0.78	−0.72 (0.34)	0.04
rs5728	A:G	0.19	0.60 (0.30)	0.04	0.36 (0.25)	0.15	0.07 (0.26)	0.78	−0.43 (0.25)	0.08

MAF indicates minor allele frequency; SE, standard error.

*Major allele:minor allele.

†BP response to low-sodium equals mean BP of last 3 days during low-sodium intervention minus mean BP during baseline; BP response to high sodium equals mean BP of last 3 days during high-sodium intervention minus mean BP of last 3 days during low-sodium intervention.

‡FDR *q* values <0.05. β is the effect size on BP response (in mm Hg) per minor allele, based on the additive genetic model. Age, sex, BP measurement room temperature, and study site were adjusted in the mixed-effects models.

dietary sodium intervention, with probability values <0.05. The regression coefficient estimates and standard errors are shown in Table 2. Several SNPs in the *SCNN1A* and *SCNN1B* genes were associated with BP responses to high-sodium intervention, although the statistical significance did not remain after correcting for multiple comparisons. For example, the minor A allele of SNP rs250567 of *SCNN1B* was associated with 0.95 mm Hg (P=0.004, q=0.18) and 0.82 mm Hg (P=0.01, q=0.36) decreased SBP and DBP responses to high-sodium intervention, respectively, compared with its major G allele. After adjusting for multiple testing, 6 SNPs from *SCNN1G* were significantly associated with SBP response to low-sodium intervention. The 4 SNPs with the smallest probability values (rs4073930, rs4073291, rs7404408, and rs5735) were in the same LD block and highly correlated with each other (online-only Data Supplement Figure SI, please see http://circgenetics.ahajournals.org). The minor alleles of these 4 SNPs were associated with a smaller SBP decrease in response to low-sodium intervention. For example, the minor allele G of SNP rs4073291 was associated with 1.33 mm Hg lower SBP reduction during low-sodium intervention ($P=1.1\times10^{-5}$, $q=3.0\times10^{-4}$).

Discussion

Our study identified several common variants in the ENaC genes that may contribute to the BP response to dietary sodium intake. To our knowledge, this is the first report of a comprehensive analysis of association between common variants in the ENaC genes and BP response to dietary sodium intervention.

A previous study in an animal model indicated that ENaC genes might play a critical role in salt-sensitive hypertension.[20] Pradervand et al[27] generated a mouse model of Liddle syndrome with deletion of the C terminus of ENaC β-subunit by Cre/loxP-mediated recombination. Under normal sodium diet, mice heterozygous (L/+) and homozygous (L/L) for Liddle mutation (L) develop normally during the first 3 months of life and have BP levels similar to wild-type. Under high sodium intake, the mice with Liddle syndrome mutation have development of high BP, metabolic alkalosis, and hypokalemia accompanied by cardiac and renal hypertrophy.

Multiple mutations in the ENaC β- and γ-subunits have been related to Liddle syndrome in humans. Most of them cause the deletion of a conserved proline-rich PY motif (codon 611 to 623) in the cytoplasmic C-terminus region of the β- or γ-subunits, resulting in increased ENaC activity.[34] A mutation Asn530Ser of the γ-subunit, outside of the PY motif, was also reported to be associated with Liddle syndrome.[35] In the single SNP analysis of our study, 6 SNPs of the *SCNN1G* gene, 1 in an exon and 5 in introns, showed

significant association with SBP response to low-sodium intervention. The significant exonic SNP rs5735 is synonymous and does not change the amino acid encoded. A study of a Japanese population and 2 studies of Caucasian populations did not find an association between this synonymous mutation and BP.[22,26,36] To speculate on the functional implication of the 5 significant intronic SNPs, we used the Web tool FastSNP, which could analyze potential SNP function using a variety of biological databases and analytic tools.[37] Two of the 5 SNPs, rs4073930 and rs4299163, are possible intronic enhancers for the regulation of gene expression. On the other hand, it is a possibility that none of these SNPs are causal and they are simply in high LD with the true causal variant, which was not tested in the current study. To fully interpret these associations, further dissection of the regions surrounding these SNPs and functional studies are warranted in the future.

Investigators have expended much effort to sequence the exons of the *SCNN1B* gene to find mutations associated with BP in the general population. T594 mol/L is the most studied mutation and has been implicated as a gain-of-function mutation causing impaired renal sodium excretion and salt-sensitive hypertension.[38,39] However, this mutation appears to be unique for people of African descent and was absent in selected European, American, or Japanese populations.[38,40] T594M was not genotyped in our study. In addition, some other mutations of the *SCNN1B* gene were detected in a Japanese population, but none of them was associated with BP levels.[41]

Recently, several common variants of *SCNN1B* were reported to be associated with BP or hypertension in white Europeans (rs239345), Chileans (a GT short tandem repeat in intron 8), and Koreans (rs7205273 and rs8044970).[42–44] In our study, these SNPs were not significantly associated with salt sensitivity of BP. However, SNP rs250567 of the *SCNN1B* gene showed a trend toward association with BP response to high-sodium intervention. This SNP is located at the 3′ flanking region and has no inferred functional implication, based on the analysis by FastSNP. Further resequencing to discover new genetic variants around this SNP may help to detect stronger signals and identify functional variants.

Mutations in the *SCNN1A* gene could lead to pseudohypoaldosteronism type I.[21] However, we did not observe any associations between common variants of *SCNN1A* and salt sensitivity of BP after adjusting for multiple testing. Inconsistent results on the association of BP to a common promoter SNP rs879605, also known as G2139A, have been reported across studies. The G2139A allele was associated with higher BP level in a Japanese population.[45] A haplotype containing this allele was found to be associated with essential hypertension in a sample of Kazakhs, a Chinese minority group.[25] However, neither single-marker nor haplotype analysis of this SNP was associated with hypertension in another sample of Chinese Kazakhs.[24] In our study, the association between one of its adjacent SNPs, rs11064153, and salt sensitivity of BP was not significant.

To the best of our knowledge, we report for the first time significant associations between common variants of the ENaC genes and BP response to dietary sodium intervention. These findings suggest that the variants in ENaC genes play an important role in the development of salt-sensitive hypertension. Furthermore, low-sodium intervention might be particularly effective in lowering BP among individuals with specific variants of ENaC genes. Replication of these results in other populations and further functional studies are critically important for identifying true causal variants.

Sources of Funding

The Genetic Epidemiology Network of Salt Sensitivity (GenSalt) is supported by research grants (U01HL072507, R01HL087263, and R01HL090682) from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.

Disclosures

None.

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CLINICAL PERSPECTIVE

Rare mutations of the epithelial sodium channel (ENaC) genes lead to mendelian forms of salt-sensitive hypertension or salt-wasting hypotension. However, the role of common variants in the ENaC genes on salt-sensitive hypertension has not been well examined. This large dietary intervention study aimed to examine the association between common variants in the ENaC genes and salt-sensitivity of blood pressure in a homogenous Chinese population. This study identified several common variants in the ENaC subunit-γ (*SCNN1G*), which were significantly associated with blood pressure responses to dietary sodium intervention. These findings suggest that the variants in ENaC genes play an important role in the development of salt-sensitive hypertension. Furthermore, low-sodium intervention might be particularly effective in lowering blood pressure among individuals with specific variants of ENaC genes. Replication of these results in other populations and further functional studies are critically important for identifying true causal variants for salt-sensitive hypertension.

Common Variants in Epithelial Sodium Channel Genes Contribute to Salt Sensitivity of Blood Pressure: The GenSalt Study

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Circ Cardiovasc Genet. 2011;4:375-380; originally published online May 11, 2011;
doi: 10.1161/CIRCGENETICS.110.958629
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268

SUPPLEMENTAL MATERIAL

Table S1. Information on Genotyped SNPs of SCNN1A, SCNN1B, and SCNN1G

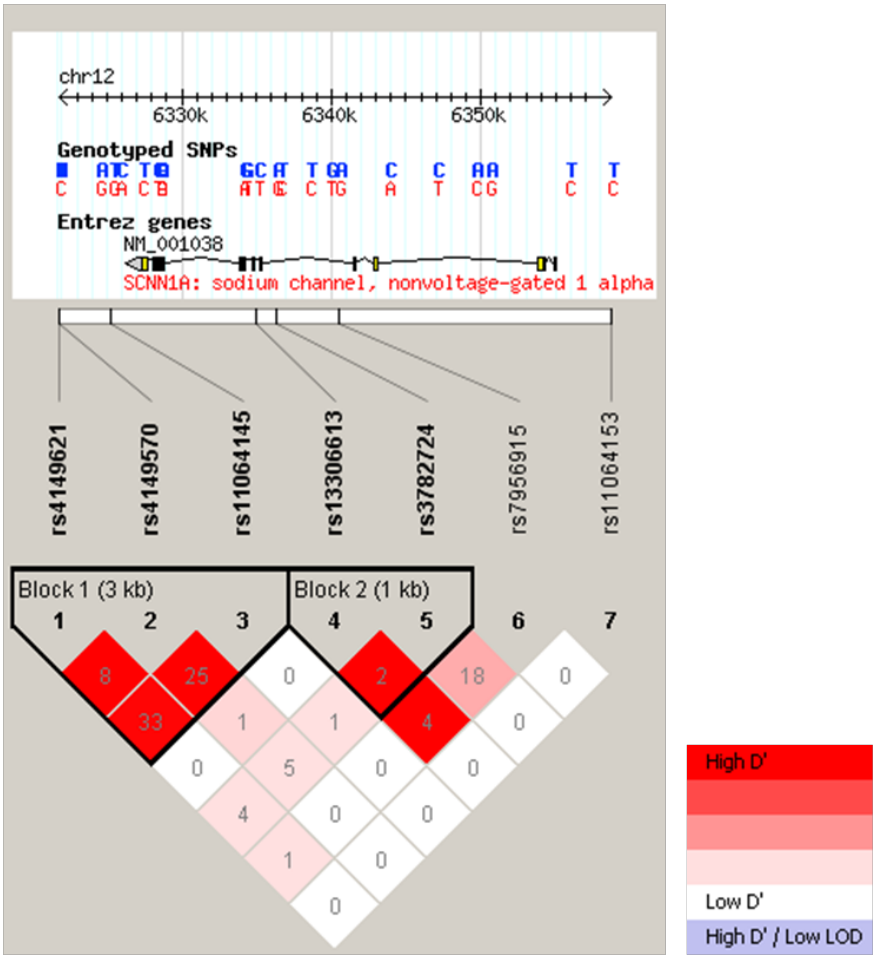
Gene	Chr	SNP	Position	Region in Gene	MAF	Alleles*	HW p-values
SCNN1A	12	rs4149621	6321822	3' Flanking	0.08	T:C	0.48
		rs4149570	6321851	3' Flanking	0.50	C:A	0.63
		rs11064145	6325359	3' Flanking	0.21	T:G	0.33
		rs13306613	6335070	Intron	0.06	C:T	0.26
		rs3782724	6336342	Intron	0.24	A:G	0.90
		rs7956915	6340521	Intron	0.40	C:T	0.40
		rs11064153	6358711	5' Flanking	0.35	G:A	0.05
SCNN1B	16	rs1004749	23222352	Intron	0.34	A:C	0.22
		rs3785368	23228281	Intron	0.35	G:A	0.32
		rs11074555	23231527	Intron	0.30	A:G	0.18
		rs7205273	23232350	Intron	0.26	C:T	0.20
		rs239345	23253439	Intron	0.28	T:A	0.10
		rs7190829	23254805	Intron	0.18	A:G	0.70
		rs8055868	23255752	Intron	0.20	G:A	0.88
		rs12447134	23258170	Intron	0.08	A:C	0.39
		rs239349	23260349	Intron	0.48	G:A	0.04
		rs238547	23267700	Exon (syn)	0.03	C:T	1
		rs8044970	23269331	Intron	0.38	T:G	0.33
		rs8044984	23269354	Intron	0.31	A:C	0.11
		rs152733	23270179	Intron	0.45	A:G	0.46
		rs3785361	23270900	Intron	0.29	T:A	0.49
		rs63982	23271582	Intron	0.38	G:T	1

		rs238551	23283422	Intron	0.50	A:G	0.32
		rs239350	23286980	Intron	0.13	G:A	0.02
		rs889299	23289415	Intron	0.34	G:A	0.63
		rs12596831	23292758	Intron	0.13	C:T	0.04
		rs4967999	23295036	Intron	0.45	C:T	0.92
		rs168748	23295756	Intron	0.45	G:A	0.29
		rs2303153	23297702	Intron	0.11	G:C	0.76
		rs250567	23301900	3' Flanking	0.10	G:A	0.29
		rs181835	23304614	3' Flanking	0.33	A:G	0.76
SCNN1G	16	rs4421986	23098751	5' Flanking	0.06	C:T	0.04
		rs4073930	23103147	Intron	0.16	T:C	0.03
		rs4073291	23103494	Intron	0.16	T:G	0.02
		rs7404408	23104239	Intron	0.16	C:T	0.01
		rs4967948	23106745	Intron	0.01	G:A	1
		rs5735	23108349	Exon (syn)	0.17	T:C	0.09
		rs4247210	23113158	Intron	0.16	G:C	0.57
		rs13331086	23115618	Intron	0.06	A:C	0.59
		rs4299163	23119520	Intron	0.10	G:C	0.35
		rs4401050	23124903	Intron	0.09	C:T	0.85
		rs4499238	23128880	Intron	0.10	G:A	0.25
		rs4297681	23132542	Intron	0.06	C:A	1
		rs5723	23134288	Exon (syn)	0.06	C:G	0.62
		rs5728	23134863	3' UTR	0.19	A:G	1
		rs9930846	23136036	3' Flanking	0.06	T:C	0.53

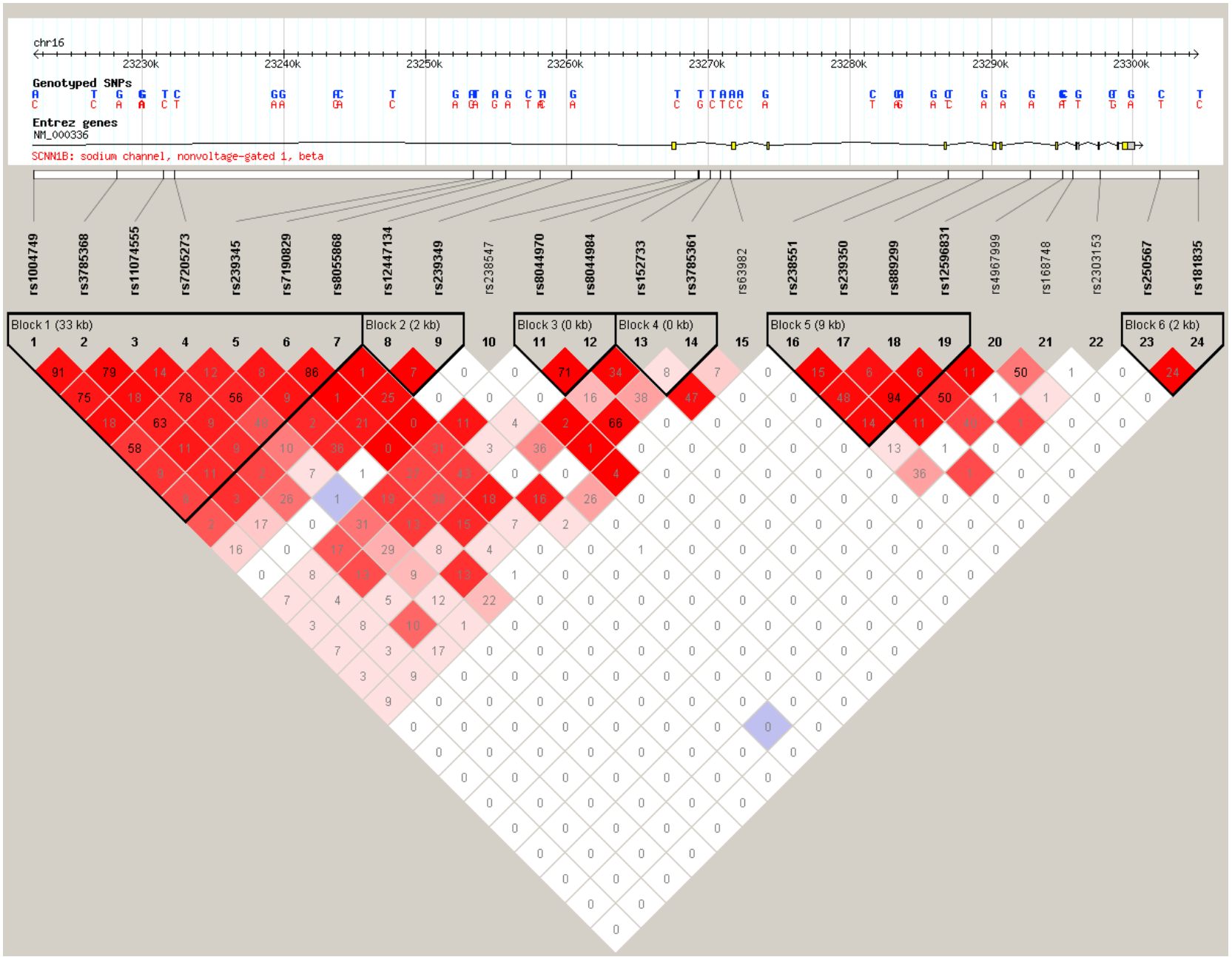
*Major allele: minor allele.

Figure S1. Linkage disequilibrium structure of the SCNN1A (a), SCNN1B (b), and SCNN1G (c) genes. The D' color scheme of Haploview was applied and $r^2 \times 100$ are shown in each cell (r^2 values of 1.0 are not shown).

a.



b.


chr16
23230k
23240k
23250k
23260k
23270k
23280k
23290k
23300k
Genotyped SNPs
Entrez genes
NM_000336
SCNN1B: sodium channel, nonvoltage-gated 1, beta
rs1004749
rs3785368
rs11074555
rs7205273
rs239345
rs7190829
rs8055868
rs12447134
rs239349
rs238547
rs8044970
rs8044984
rs152733
rs3785361
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rs250567
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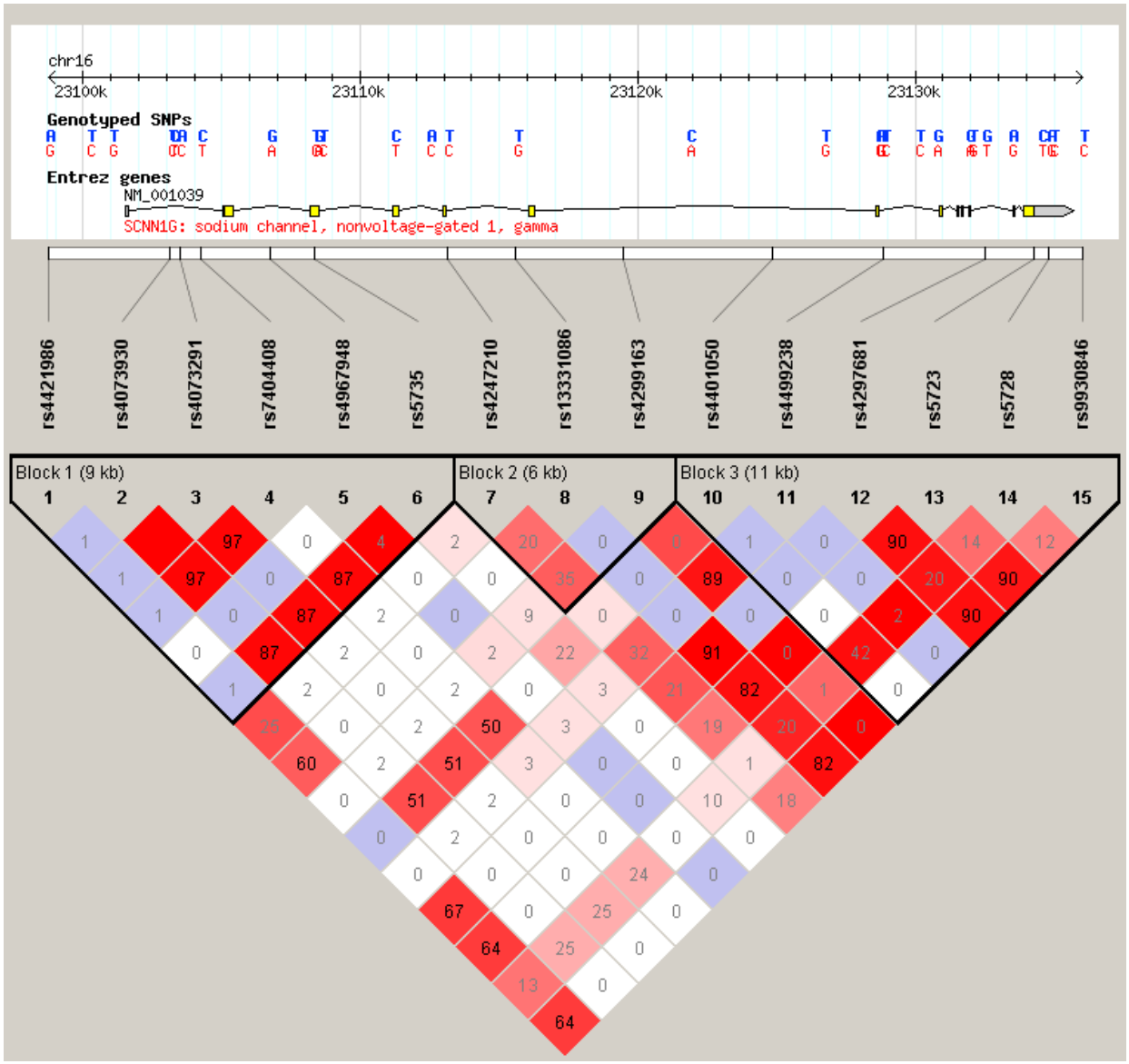
Block 4 (0 kb)
Block 5 (9 kb)
Block 6 (2 kb)

C.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23100k
23110k
23120k
23130k
Genotyped SNPs
Entrez genes
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SCNN1G: sodium channel, nonvoltage-gated 1, gamma
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rs4073930
rs4073291
rs7404408
rs4967948
rs5735
rs4247210
rs13331086
rs4299163
rs4401050
rs4499238
rs4297681
rs5723
rs5728
rs9930846
Block 1 (9 kb)
Block 2 (6 kb)
Block 3 (11 kb)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

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