GENOME-WIDE GENE-SODIUM INTERACTION ANALYSES ON BLOOD PRESSURE: THE GENSALT STUDY

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Abstract

We performed genome-wide analyses to identify genomic loci that interact with sodium to influence blood pressure (BP) using single marker (one and two degree-of-freedom joint tests) and gene-based tests among 1,876 Chinese participants of the Genetic Epidemiology Network of Salt-Sensitivity (GenSalt) study. Among GenSalt participants, the average of three urine samples was used to estimate sodium excretion. Nine BP measurements were taken using a random-zero-sphygmomanometer. A total of 2.05 million SNPs were imputed using Affymetrix 6.0 genotype data and the Chinese Han of Beijing and Japanese of Tokyo HapMap reference panel. Promising findings ($P<1.00\times10^{-4}$) from GenSalt were evaluated for replication among 775 Chinese participants of the Multi-ethnic Study of Atherosclerosis (MESA). SNP and gene-based results were meta-analyzed across the GenSalt and MESA studies to determine genome-wide significance. The one degree-of-freedom tests identified interactions for *UST* rs13211840 on diastolic BP ($P=3.13\times10^{-9}$). The two degree-of-freedom tests additionally identified associations for *CLGN* rs2567241 ($P=3.90\times10^{-12}$) and *LOC105369882* rs11104632 ($P=4.51\times10^{-8}$) with

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Conflict of Interest

The authors declare no conflict of interest.

systolic BP. The *CLGN* variant rs2567241 was also associated with diastolic BP (P=3.11×10⁻²²) and mean arterial pressure (P= 2.86×10⁻¹⁵). Genome-wide gene-based analysis identified *MKNK1* (P=6.70×10⁻⁷), *C2orf80* (P<1.00×10⁻¹²), *EPHA6* (P=2.88×10⁻⁷), *SCOC-AS1* (P=4.35×10⁻¹⁴), *SCOC* (P=6.46×10⁻¹¹), *CLGN* (P=3.68×10⁻¹³), *MGAT4D* (P=4.73×10⁻¹¹), *ARHGAP42* (P=<1.00×10⁻¹²), *CASP4* (P=1.31×10⁻⁸), and *LINC01478* (P=6.75×10⁻¹⁰) that were associated with at least one BP phenotype. In summary, we identified 8 novel and 1 previously reported BP loci through the examination of SNP and gene-based interactions with sodium.

Keywords

Genome-wide; Gene-sodium interaction; Blood pressure; One degree-of-freedom test; Two degree-of-freedom joint test; Gene-based analysis

Introduction

High blood pressure (BP) is a major public health challenge worldwide due to its high prevalence and associated mortality and morbidity[1, 2]. As a complex trait, BP is influenced by both genetic and environmental factors as well as their interactions. Sodium intake has been demonstrated as an important environmental determinant of hypertension and varies greatly within and across populations[3, 4]. Numerous genome-wide association studies (GWAS) have identified robust associations between genomic loci and BP[5–9]. However, these findings explain only a small proportion of the estimated heritability of this complex phenotype[10]. Because the marginal effects of variants modified by environmental factors may be missed by traditional single-marker analyses, scientists postulate that additional genetic factors may be detected through the analysis of gene-environment interactions. Relatedly, an important interrelationship of sodium and genetic factors on BP has been demonstrated through the identifications of genes responsible for Mendelian forms of hypertension, with the vast majority of recognized genes exerting their effects via renal sodium handling[11]. Since genes may modify the effect of sodium on BP, examination of gene-dietary sodium intake interactions may help to identify novel genomic loci underlying BP regulation[12]. Furthermore, gene-based analyses, which test the joint contributions of SNPs with individually modest effects, may have higher power to identify BP loci[13, 14]. Although the examination of gene-sodium interactions in single marker and gene-based analyses could provide important insights into the molecular mechanisms underlying BP regulation, there has been a paucity of research in this area.

The objective of the current analysis was to identify novel genetic variants and genes influencing BP regulation by conducting genome-wide SNP-based and gene-based gene-sodium interaction analyses on BP among 1,876 Chinese participants of the Genetic Epidemiologic Network of Salt Sensitivity (GenSalt) study.

Methods

Study population

GenSalt is a family-based dietary intervention study designed to identify susceptibility genes that influence BP responses to environmental risk factors for hypertension in human populations. The study includes 1,906 Han Chinese participants aged 16 years and older from 633 families recruited from 6 study sites in rural, north China[15]. In the current study, genotypes, BP, and covariates at baseline were available for a total of 1,876 participants (98.4%).

Genotyping and Genotype Imputation

A total of 868,158 autosomal SNPs across the entire genome were genotyped using the Affymetrix 6.0 platform for each participant. Quality control excluded SNPs with Hardy-Weinberg Equilibrium $P<1\times10^{-6}$, missing rate >25% or MAF<1%. In addition, PLINK[16, 17] and PedCheck[18] were used to identify and correct any non-Mendelian inheritance errors. After quality control, a total of 820,017 genotyped SNPs remained for genotype imputation. Imputation of 2,416,663 SNPs from the HapMap release 22 build 36 CHB+JPT samples was conducted using MACH software[19]. After imputation, SNPs with $r^2<0.30$, MAF<1%, or HWE $P<1\times10^{-6}$ were removed, and a total of 2,216,774 SNPs, with fractional values ranging from 0 to 2, remained for the analysis.

Urinary Sodium and Covariate Measurement

Dietary sodium intake was measured from 24 hour urinary sodium level. During the three-day baseline examination, each GenSalt participant collected three urine samples, including one 24 hour urine sample and 2 overnight urine samples. Among a random sample of 238 participants, 24-hour urine samples were collected in two containers, one for day time and the other for overnight. Using this random sample, an equation was generated to estimate 24 hour urinary sodium level from the overnight urine sample. This equation was applied to the remaining overnight urine samples to estimate 24 hour urinary sodium levels among all study participants. The mean of one measured 24 hour urinary sodium level and two estimated 24 hour urinary sodium levels was used in the current analysis.

Demographic variables, including age, gender, and medication use were collected using standard questionnaires by trained study staff. Body weight and height were measured twice during the baseline examination with the participants in light indoor clothing without shoes. Body mass index (BMI) was calculated as kilograms per meters squared.

BP Measurement and Imputation

BP was measured three times at the same time each morning during the three-day baseline examination by trained and certified observers using a random zero sphygmomanometer according to a standard protocol. For participants taking anti-hypertensive medications, BP was imputed by adding 10 mm Hg to systolic BP (SBP) and 5 mm Hg to diastolic BP (DBP)[20]. After imputation, the mean of the 9 BP measures was used in the current analysis. Mean arterial pressure (MAP) was calculated as SBP/3+2DBP/3, and pulse pressure (PP) was calculated as SBP-DBP.

Replication Study and Genotype Imputation

We attempted to replicate promising GenSalt findings ($P<1\times10^{-4}$) among Chinese participants of the Multi-Ethnic Study of Atherosclerosis (MESA). MESA participants were recruited between July 2000 and August 2002 from 6 field centers around the US. Participants were aged 45–84 and free of cardiovascular diseases at the baseline examination. Phenotype and Affymetrix 6.0 genotype data from MESA participants were made publicly available through the NCBI's database of Genotypes and Phenotypes (dbGaP) and were downloaded for the current analysis. Among 777 Chinese MESA participants, genotype, urinary sodium, BP, and all covariables at baseline were available for 775 (99.7%) participants. Similar to GenSalt, we imputed BP for participants taking antihypertensive medication by adding 10 and 5 mm Hg to SBP and DBP, respectively. Since sodium was measured from spot urine using baseline urine samples, 24-hour urine sodium was estimated using Tanaka's equation[21].

A total of 909,622 autosomal SNPs across the entire genome were genotyped using the Affymetrix 6.0 platform for each participant. We excluded SNPs with Hardy-Weinberg Equilibrium $P<1\times10^{-6}$, missing rate>5% and MAF<1%. Minimac software was used to perform targeted imputation of the 3 Mb region surrounding each identified SNP based on the ALL ancestry panel from the 1000G Phase I Integrated Release Version 3 Haplotypes, which contains haplotypes of 1,092 individuals of all ethnic background. After imputation, SNPs with $r^2<0.30$, MAF<1%, or HWE $P<1\times10^{-6}$ were removed.

Statistical Analysis

Single Marker Analysis—To accommodate familial relationships in both GenSalt (discovery) and MESA (replication) studies, mixed effect models were used to examine SNP-sodium interactions on BP, after adjustment for age, gender, and BMI. Principal components analysis revealed population substructure in MESA (but not GenSalt). Therefore, ancestry was also accounted for in MESA by adjusting for the first three principal components. Both 1 degree of freedom (df) interaction and 2 df joint tests were explored[22]. Lead SNPs (defined as the most significant SNP at a locus) with interaction term $P<1.0\times10^{-4}$ (for the 1 df test) or joint $P<1.0\times10^{-4}$ (for the 2 df test) after genomic control in the discovery stage analyses were further evaluated for replication in Chinese MESA participants. For the 1 df interaction test, inverse-variance-weighted meta-analysis was conducted to combine results from GenSalt and MESA using METAL software[23]. For the 2 df joint test, meta-analysis was performed using methods described by Manning and colleagues[24] which were implemented in METAL software[23] with a patch source code provided by Manning and colleagues[24]. After ensuring the effect direction of interaction terms were consistent, SNPs with replication stage $P<0.05$ and meta-analysis $P<5.0\times10^{-8}$ were considered significant for the 1 df interaction test. SNPs with consistent effect directions in the main effect and interaction term, replication stage $P<0.05$, and meta-analysis $P<5.0\times10^{-8}$ were considered significant for the 2 df joint test.

Gene-based Analysis—SNPs within the 5 kilo bp flanking regions of a gene were first mapped to the gene according to physical position. SNPs within 5 kilo bp flanking regions of two genes were assigned to both genes. P values of both 1 df interaction and 2 df joint

tests in single marker analyses were used to generate gene-based P values using the extended Simes procedure (GATES) method[13]. Genes with $P<1.0\times10^{-4}$ in the discovery stage analysis in GenSalt were further evaluated for replication among Chinese MESA participants. Among MESA participants, SNPs from promising genes were tested for SNP-sodium interactions using methods described in the above single marker analysis, and P values of these SNPs were again used to generate gene-based p values using the GATES method[13]. Fisher's method was applied to combine gene-based p values across GenSalt and MESA[25]. Genes with replication stage $P<0.05$ and combined $P<2.5\times10^{-6}$ (correcting for approximately 20,000 genes across the genome: $0.05/20,000=2.5\times10^{-6}$) were considered significant.

Results

Characteristics of both GenSalt participants and Chinese MESA participants are shown in Table 1. MESA participants were older, had lower urinary sodium level and a higher proportion of hypertension. Participants in both studies had optimal BP levels.

In the discovery stage analyses of genome-wide gene-sodium interactions, a total of 141, 149, 150, and 165 independent loci ($r^2<0.3$) had 1 df test $P<1.0\times10^{-4}$ for SBP, DBP, MAP, and PP, respectively. A total 145, 130, 131, and 207 independent loci were identified by the 2 df joint test for SBP, DBP, MAP and PP, respectively. We identified 454 loci using the 1 df interaction test. The 2 df test identified 701 loci, including 260 which were also identified by the 1 df test. In aggregate, we identified 895 loci in the discovery stage analysis. Genome-wide analysis results from both 1 df test and 2 df joint test are shown in supplementary Figures S1–S4.

Loci that reached genome-wide significance in the meta-analysis of GenSalt and MESA results from the 1 df interaction test are shown in Table 2. Variant rs13211840 at the novel *UST* locus interacted with sodium on DBP (GenSalt $P=7.29\times10^{-8}$, MESA $P=1.19\times10^{-2}$, and Meta-analysis $P=3.13\times10^{-9}$).

Significant findings from the meta-analysis of results from the 2 df joint test are presented in Table 3. Consistent with findings from the 1 df test, the novel *UST* locus variants were again identified for DBP (rs13211840: GenSalt $P=4.41\times10^{-7}$, MESA $P=1.52\times10^{-2}$, and Meta-analysis $P=7.02\times10^{-9}$). In addition, variant at the novel *LOC105369882* loci was associated with SBP (rs11104632: GenSalt $P=1.46\times10^{-6}$, MESA $P=2.16\times10^{-2}$, and Meta-analysis $P=4.51\times10^{-8}$); and novel *CLGN* variant rs2567241 was associated with SBP (GenSalt $P=1.50\times10^{-6}$, MESA $P=4.34\times10^{-5}$, and Meta-analysis $P=3.90\times10^{-12}$), DBP (GenSalt $P=3.85\times10^{-6}$, MESA $P=3.36\times10^{-10}$, and Meta-analysis $P=3.11\times10^{-22}$), and MAP (GenSalt $P=2.01\times10^{-7}$, MESA $P=1.49\times10^{-6}$, and Meta-analysis $P=2.86\times10^{-15}$). Regional association plots for the 3 novel loci (*CLGN, UST*, and *LOC105369882*) with genome-wide significance in the meta-analyses are presented in Figure 1. Although some loci (e.g., rs2567241) achieved genome-wide significance for more than two interactions, only one regional association plot is shown for each loci using the most significant interaction.

Discovery stage genome-wide gene-based analysis results are shown in supplementary Figures S5–S8. A total of 134 promising genes were identified and tested for replication among MESA participants. Genome-wide significant findings from the combined analysis of GenSalt and MESA participants (including both the 1 df interaction test and 2 df joint test) are shown in Table 4. Gene-based analysis for the 1 df interaction test identified one *CASP4* gene for MAP phenotype. Gene-based analysis of the 2 df joint test identified 9 genes at 6 loci, including *MKNK1* at 1p33, *C2orf80* at 2q34, *EPHA6* at 3q11.2, *SCOC-AS1, SCOC, CLGN,* and *MGAT4D* at 4q31.1, *ARHGAP42* at 11q22.1, and *LINC01478* at 18q12.3. Locus 4q31.1 was also identified in the single-marker analysis.

Discussion

In the first genome-wide gene-sodium interaction analysis conducted in the Chinese population, we identified consistent interactions of *UST* rs13211840 at 6q25.1 with sodium intake on the DBP phenotype using both 1 df and 2 df interaction tests. The 2 df test also revealed associations of novel *LOC105369882* rs11104632 variant at 12q21.32 with SBP, while the novel *CLGN* rs2567241 variant was associated with SBP, DBP and MAP in the 2 df test. Furthermore, genome-wide gene-based analysis of both 1 df and 2 df interaction tests identified *MKNK1* at 1p33, *C2orf80* at 2q34, *EPHA6* at 3q11.2, *SCOC-AS1, SCOC, CLGN,* and *MGAT4D* at 4q31.1, *ARHGAP42* at 11q22.1, and *LINC01478* at 18q12.3 that were associated with at least one BP phenotype.

Novel *UST, LOC105369882* variants rs13211840 and rs11104632, respectively, showed significant interactions with sodium on BP in the current analysis. The *UST* signal was consistent across the 1 df and 2 df interaction tests, while the *LOC105369882* locus was identified only in the 2 df test. Although the potential role of *UST* in BP is unclear, other genes at this locus represent interesting BP candidates. For example, the *SASH1* gene has been associated with thyroid function[26] and suggestively associated with responses to rate control therapy among patients with atrial fibrillation[27]. A more recent study indicated that the *SASH1* gene may interact with smoking on SBP[28]. While the role of *LOC105369882* in BP is unclear, another gene at this locus, *CEP290*, is potentially relevant to BP regulation[29], with mutations of this gene shown to associate with many disease phenotypes characterized by renal impairment[30, 31]. Considering the important roles of the thyroid in blood pressure regulation and kidney in sodium filtration, both loci warrant further studies to identify the true causal variant(s).

Association of *CLGN* at 4q28.3-q31.1 with blood pressure phenotypes was identified consistently in the single marker and gene-based analyses. In single marker analysis, three genome-wide significant exonic missense mutations were identified at the *CLGN* locus, including lead *CLGN* SNP rs2567241 along with two other SNPs, rs358304 of *CLGN1* and rs2175563 of nearby *SCOC*. Although the physiologic relevance of the *CLGN1* and *SCOC* genes with BP regulation is unclear, future studies are warranted to determine the potential functional relevance of these promising single-marker findings. In gene-based analysis, *CLGN1* represented one of a cluster of genes including *SCOC-AS1, SCOC and MGAT4D* which interacted with sodium to influence BP. It is unlikely that they are all etiologically

relevant to BP regulation. Future works are warranted to delineate the causal genes involved in BP regulation at this locus.

Genome-wide gene-based analysis also identified novel genes *MKNK1* at 1p33, *C2orf80* at 2q34, *EPHA6* at 3q11.2, *CASP4* at 11q22.3, and *LINC01478* at 18q12.3, as well as previously reported BP gene *ARHGAP42* at 11q22.1[5, 6]. *MNK1* encodes a Ser/Thr protein kinase that interacts with ERK1 and p38 mitogen-activated protein kinase (MAPK)[32], a pathway that is involved in BP regulation through norepinephrine and angiotensin II[33]. Despite its potential biological relevance, our study provides the first evidence that the *MKNK1* gene is involved in BP regulation in human populations. The *CASP4* gene encodes a protein in the cysteine-aspartic acid protease family and plays an import role in inflammation and innate immunity through activation of caspase-1[34]. Pathophysiological studies showed that overexpression of the *CASP4* gene was involved in the loss of proximal tubules and renal injury in nephropathic cystinosis patients[35]. Considering kidney's key roles in sodium filtration and reabsorption, future studies of *CASP4* genes are warranted to identify the causal variant(s).. Previous studies have identified associations of the *EPHA6* gene with obesity-related traits[36]. The *EPHA6* locus also includes the *ARL6* gene, which encodes ADP-ribosylation factor-like 6. Mutations of the *ARL6* gene cause Bardet-Beidl syndrome[37], a heterogeneous disorder which increases the risk of hypertension and diabetes[38]. Gene-based analysis also revealed novel associations of *C2orf80 at 2q34*, and *LINC01478 at 18q12.3*. However, the mechanisms linking these genes to blood pressure regulation are unclear. In aggregate, the identification of these 6 novel loci in gene-based analyses highlights the power and potential utility of gene-based analysis in genomic study.

Our study has several strengths. First, stringent quality control methods were used in measuring genotype, phenotype and covariate data for both the discovery and replication stage samples. This can reduce phenotype measurement error and increase statistical power in identifying both single SNPs and genes underlying BP regulation. Second, since we limited our analysis exclusively to Chinese participants, population stratification should be minimized. Finally, a total of 9 BPs were measured using a random zero sphygmomanometer at the same time during the 3 day baseline examination in the discovery stage. This can greatly reduce measurement error of BP, and increase statistical power to detect genetic variants for BP. Certain limitations should also be acknowledged. The MESA replication study has a smaller sample size than that of GenSalt, potentially limiting our ability to replicate and identify important gene-sodium interactions. In addition, 24 hour urinary sodium was estimated from spot urine among the Chinese MESA participants using Tanaka's equation. Although Tanaka's equation has been widely validated[39–41], there might be some measurement error that could reduce statistical power to replicate genetic loci identified in GenSalt participants, increasing false negative findings. MESA had a higher proportion of participants taking antihypertensive medication. We imputed BP by adding 10 and 5 mm Hg to SBP and DBP, respectively. Although this approach is widely applied to genetic studies of BP[6, 7, 9], any resulting inaccuracy may dilute associations between genetic factors and BP. Furthermore, additional factors such as age and urbanization level differ between the GenSalt and MESA samples. If these factors influence gene-sodium interactions on BP, our power to detect (or replicate) SNP-BP associations could again be reduced. Although the SNPs identified in GenSalt have low MAFs, the findings were

verified through replication in MESA participants. Still, future replication studies with larger sample size, more homogeneous populations and better measurement of urinary sodium levels are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Perspectives

In the first genome-wide gene-sodium interaction analyses of BP in a Chinese population, which included both single marker and gene-based analyses, we identified 9 novel loci that interacted with dietary sodium intake on BP phenotypes. In addition to the 1 novel locus identified through the traditional 1 df single marker interaction analysis (*UST* rs13211840), another two loci were documented using the 2 df test (*CLGN* rs2567241, and *LOC105369882* rs11104632). Gene-based analysis provided consistent support for the *CLGN* gene (plus several other genes clustered at the *CLGN* locus), and identified an additional 5 novel genes, including *MKNK1, C2orf80, EPHA6, CASP4*, and *LINC01478*. In comparison to the previous large scale GWAS meta-analyses of BP conducted in East Asians, the current study was able to robustly identify several novel loci with a relatively small sample size. Such findings highlight the importance of examining gene-sodium interactions and conducting gene-based analysis to identify genomic mechanisms influencing BP. Further, these findings contribute to understanding the mechanisms of BP regulation. Sequencing along with functional studies are needed to help delineate causal variants underlying the strong signals identified here.

Novelty and Significance

What Is New?

- By examining genome-wide gene-sodium interactions on BP through both single makers based and gene-based analyses in a Chinese population, with replication in Chinese participants of the MESA study, we identified 3 novel loci from single marker analysis that interacted with dietary sodium intake on BP phenotypes.

- At the *CLGN* locus, 3 missense variants rs2567241, rs358304 and rs2175563 were identified for SBP, DBP, and MAP phenotypes.

- Genome-wide gene-based analysis additionally identified 5 novel BP loci.

What is Relevant?

- Our findings highlighted the utility of examining gene-environment (sodium) interactions and conducting gene-based analyses to identify genes and variants that influence blood pressure.

Summary

Only a small proportion of the genetic factors influencing blood pressure have been found. Scientists postulate that additional genetic factors may be identified through the analysis of gene-environment interactions. In addition, gene-based analysis methods may also increase power to detect important genetic determinants of BP. Very few studies have explored the two approaches. We examined the interactions between dietary sodium intake and genetic factors on blood pressure using data from 1,906 Chinese participants of the GenSalt study. To avoid false positive results, we evaluated findings from GenSalt among 775 Chinese participants of the MESA study. We robustly identified 3 novel genetic loci that interacted with dietary sodium intake on blood pressure in single-marker analyses. In gene-based analyses, we additionally identified 5 novel genes that were associated with blood pressure phenotypes. These novel loci were successfully replicated among the Chinese participants of MESA. In aggregate, our findings highlighted the utility of examining gene-environment interactions and conducting gene-based analyses to identify genes and variants that influence blood pressure.
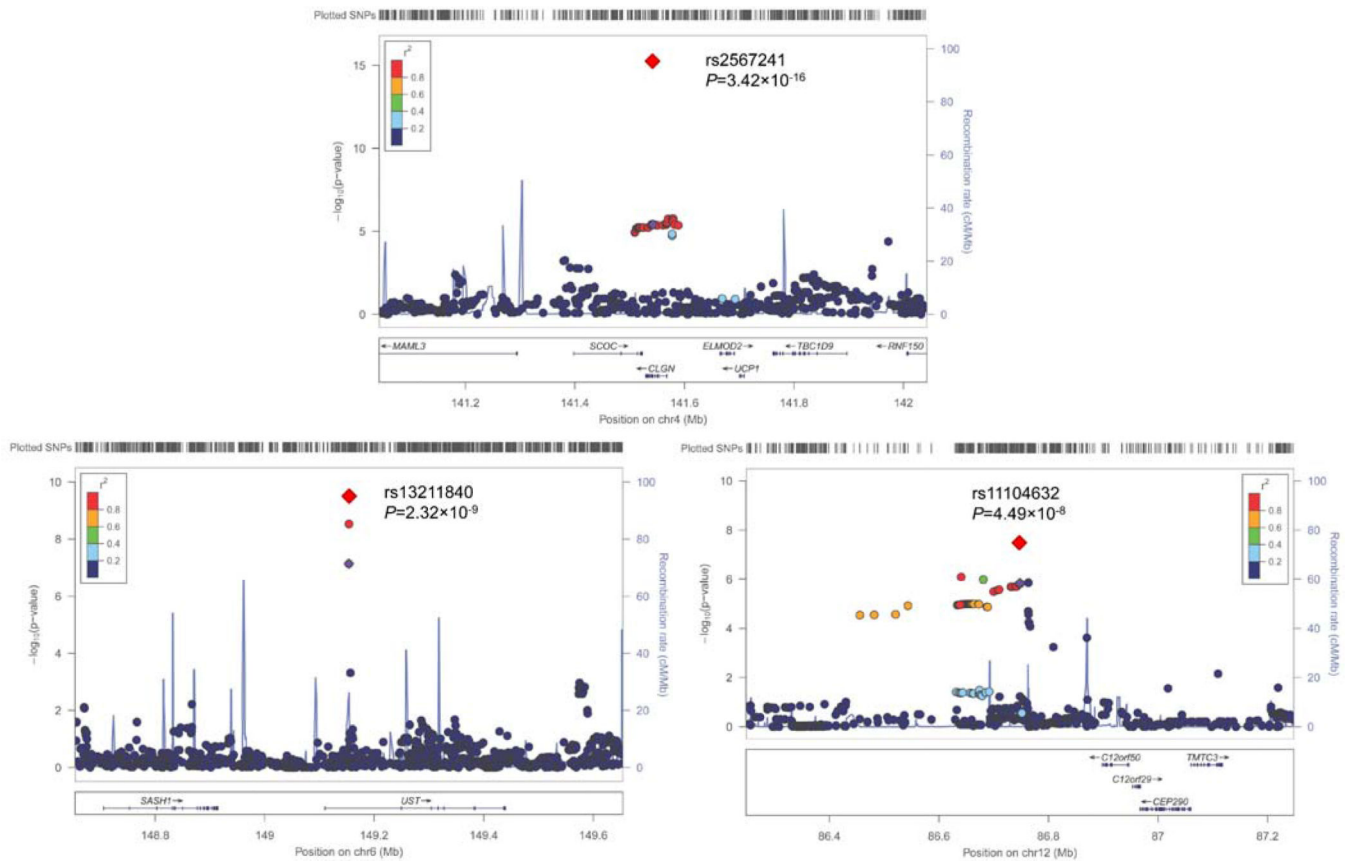
Figure 1.
Regional Association Plots for Loci Achieving Genome-Wide Significance in the Meta-Analysis of both 1 degree of freedom (df) Interaction Test (a,b) and 2 df Joint Test (c) Results. For the SNPs associated with multiple phenotypes, the results for the most significant phenotype are shown. The index SNPs are shown in purple diamond; the correlation (r^2) of each of the surrounding SNPs to the index SNP are shown on a scale from minimal (blue) to maximal (red), The final P values for the index SNPs are shown and also indicated by the red diamonds. The genes in the 1 Mb regions around the index SNPs (500 kb on each side) are indicated at the bottom, and recombination rates are shown in light blue line. The regional plots are drawn using LocusZoom online software.

Table 1

Characteristics of GenSalt and Chinese MESA participants

Variables	GenSalt (n=1876)	MESA (n=775)
Age, y, mean (SD)	38.7 (9.5)	62.4 (10.4)
Women, %	47.2	50.8
Hypertension, %	9.5	40.8
Hypertension medication, %	4.4	29.0
BMI, kg/m2, mean (SD)	23.3 (3.2)	24 (3.3)
24-h urinary Na, gram, mean (SD)	5.6 (1.5)	3.5 (0.8)
Baseline SBP, mmHg, mean (SD)	116.9 (14.2)	127.4 (23.8)
Baseline DBP, mmHg, mean (SD)	71.0 (9.7)	74.8 (12.0)

SD=standard deviation; SBP=systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index;

Table 2

Results Achieving Genome-Wide Significance in the Meta-Analysis of Gene-Sodium Interactions Using the 1 Degree of Freedom Test.

rsID	Chr	Position (Build 36)	CA	CAF	Region	Genes	Studies	Beta	SE	P
Diastolic blood pressure										
rs13211840	6	149153883	T	0.985	intronic	*UST*	GenSalt	−0.09	0.02	1.02E-07
							MESA	−0.06	0.02	1.19E-02
							Meta	−0.08	0.01	3.13E-09

Chr=chromosome; CA=coded allele; CAF=coded allele frequency; SE=standard error

Table 3

Results Achieving Genome-Wide Significance in the Meta-Analysis of Gene-Sodium Interactions Using the 2 Degree of Freedom Joint Test.

rsID	Chr	Position (Build 36)	CA	CAF	region	Nearest Gene	Studies	Main Effect			Interaction Effect			Joint P
								Beta	SE	P	Beta	SE	P	
Systolic blood pressure														
rs2567241	4	141542612	C	0.990	exonic	*CLGN*	GenSalt	3.02	2.10	1.49E-01	−0.10	0.02	2.04E-05	2.24E-06
							MESA	11.33	4.62	1.82E-02	−0.05	0.13	7.06E-01	4.34E-05
							Meta				−0.09	0.02		3.90E-12
rs11104632	12	86747816	G	0.982	intergenic	*LOC105 369882*	GenSalt	4.07	1.46	5.40E-03	0.08	0.02	2.48E-04	2.19E-06
							MESA	6.79	2.98	2.74E-02	0.08	0.10	4.36E-01	2.16E-02
							Meta				0.09	0.02		4.51E-08
Diastolic blood pressure														
rs2567241	4	141542612	C	0.990	exonic	*CLGN*	GenSalt	2.37	1.50	1.13E-01	−0.07	0.01	8.16E-07	5.47E-06
							MESA	7.34	2.98	1.77E-02	−0.04	0.08	6.46E-01	3.36E-10
							Meta				−0.06	0.01		3.11E-22
rs13211840	6	149153883	T	0.985	intronic	*UST*	GenSalt	1.42	1.42	3.20E-01	−0.09	0.02	7.29E-08	6.52E-07
							MESA	6.45	3.11	4.39E-02	−0.06	0.02	1.19E-02	1.52E-02
							Meta				−0.08	0.01		7.02E-09
Mean arterial pressure														
rs2567241	4	141542612	C	0.990	exonic	*CLGN*	GenSalt	2.59	1.62	1.10E-01	−0.08	0.01	1.45E-07	3.01E-07
							MESA	8.51	3.40	1.58E-02	−0.04	0.09	7.05E-01	1.49E-06
							Meta				−0.07	0.01		2.86E-15

Chr=chromosome; CA=coded allele; CAF=coded allele frequency; SE=standard error

Table 4

Gene-based Analysis Results for Gene-Sodium Interaction Analysis

Gene	Chr	Start (Build 36)	Gene Length (bp)	GenSalt P	MESA P	Meta P
Mean Arterial Pressure			1 DF Test			
CASP4	11	104318803	25733	4.23E-08	4.80E-02	4.27E-08
			2 DF Joint Test			
Systolic Blood Pressure						
MKNK1	1	46795665	46889	4.20E-06	4.08E-02	2.80E-06
SCOC	4	141484064	39097	9.40E-05	4.70E-05	9.00E-08
CLGN	4	141529056	39210	3.40E-06	1.30E-04	1.00E-08
MGAT4D	4	141583978	55004	2.30E-06	2.57E-03	1.20E-07
LINC01478	18	40157397	208264	1.50E-08	6.51E-03	2.30E-09
Diastolic Blood Pressure						
SCOC	4	141484064	39097	7.47E-05	2.50E-11	6.40E-14
SCOC-AS1	4	141424329	89668	9.95E-05	1.95E-11	6.77E-14
CLGN	4	141529056	39210	1.38E-05	1.09E-09	4.94E-13
MGAT4D	4	141583978	55004	6.74E-05	3.79E-08	7.07E-11
Mean Arterial Pressure						
SCOC-AS1	4	141424329	89668	1.57E-05	1.51E-07	6.58E-11
SCOC	4	141484064	39097	1.16E-05	1.83E-07	5.92E-11
CLGN	4	141529056	39210	1.08E-06	4.69E-06	1.37E-10
MGAT4D	4	141583978	55004	1.36E-06	1.10E-04	3.53E-09
Pulse Pressure						
C2orf80	2	208738315	24704	6.38E-07	1.03E-12	<4.72E-24
EPHA6	3	98641126	429464	1.11E-05	9.85E-03	1.86E-06
ARHGAP42	11	100063616	303251	2.64E-07	4.72E-24	<4.72E-24

DF=degree of freedom;