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Genetic variants in the apelin system and blood pressure responses to dietary sodium interventions: a family-based association study

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Abstract

Objective—We examined the association between genetic variants in the apelin system and blood pressure (BP) responses to low- and high-sodium interventions in the GenSalt Study.

Methods—A 7-day low-sodium intervention (51.3 mmol sodium/day) followed by a 7-day high-sodium intervention (307.8 mmol sodium/day) was conducted among 1,906 participants from 637 Han Chinese families. BP measurements were obtained at baseline and following each intervention using a random-zero sphygmomanometer. Twenty-three single nucleotide polymorphisms (SNPs), including both tag and functional SNPs, were selected from three candidate genes (APLN, APLNR, and ACE2). Single marker and haplotype analyses were conducted using the FBAT program. The false discovery rate method was used to correct for multiple testing.

Results—SNPs rs2282623 and rs746886 of the APLNR gene were significantly associated with diastolic (DBP) (both P=0.002) and mean arterial pressure (MAP) (P=0.001 and 0.005, respectively) responses to low-sodium intervention. Six SNPs of the ACE2 gene were significantly associated with systolic (SBP), DBP or MAP responses to low-sodium intervention. Three of them, rs1514283, rs1514282, and rs4646176, were also significantly associated with MAP response to high-sodium (all $P \leq 0.006$). Haplotype analysis indicated the A-T-T haplotype of APLNR SNPs rs721608-rs2282623-rs746886 was associated with decreased DBP and MAP responses to low-sodium

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intervention (P=0.001 and 0.003, respectively), while G-C-C was associated with increased SBP and MAP responses to high-sodium intervention (P=0.004 and 0.01, respectively).

Conclusions—This large family-based study indicates that genetic variants in the APLNR and ACE2 genes are significantly associated with BP responses to dietary sodium intervention.

Keywords

ACE2; apelin; apelin receptor; blood pressure; polymorphism; dietary sodium; salt sensitivity

Introduction

Blood pressure (BP) response to changes in dietary sodium intake varies considerably between individuals, a phenomenon known as salt-sensitivity [1,2]. Salt-sensitivity of BP has been associated with increased risk of hypertension, cardiovascular disease (CVD) and premature death [3,4]. Previous studies suggested that genetic factors might play an important role in the salt-sensitivity of BP [5–8]. Family studies also documented a moderately high heritability of salt-sensitivity [5,6]. Examining the genetic determinants of salt-sensitivity will enhance our understanding of its mechanism and thus facilitate the development of targeted prevention and treatment strategies for reducing hypertension and related CVD burden.

The apelin system, including apelin and its receptor APJ, is a peptidic signaling pathway which is implicated in the regulation of cardiovascular function and fluid homeostasis [9]. APJ belongs to a family of seven-transmembrane domain receptors and is coupled to inhibitory G-proteins. Apelin is synthesized as a 77 amino acid pre-propeptide that can be cleaved into fragments of different sizes that activate APJ [10,11]. Although physiological experiments in animal models have yielded conflicting results about the peripheral role of apelin in regulating vascular tone, acting as vasodilator or vasoconstrictor, a recent study showed that exogenous apelin caused nitric oxide-dependent arterial vasodilation *in vivo* in man [12–16]. In addition, there is accumulated evidence suggesting the central effect of apelin system in neural control of cardiovascular function. Most recent studies indicate that overexpression of the apelin gene in the rostral ventrolateral medulla results in chronic BP elevation and cardiac hypertrophy in normotensive rats [17] and APJ knockout mice do not show decreased urine volume and urine osmolality during water deprivation, suggesting an anti-diuretic effect of apelin *in vivo* [18]. Apelin peptides are specific substrates of angiotensin-converting enzyme 2 (ACE2), an enzyme which acts as an important regulator of the renin-angiotensin-aldosterone system (RAAS) [19,20]. Single nucleotide polymorphisms (SNPs) of the apelin and APJ genes have recently been reported to be associated with hypertension [21]. The aim of this study was to examine the association between genetic variants in the apelin system (apelin and APJ) and ACE2 and salt-sensitivity measured by BP responses to changes in dietary sodium intake among 637 Chinese families.

Methods

Study Participants

The Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study was conducted in rural areas of northern China from October 2003 to July 2005. All study participants were of Han Chinese ethnicity. The GenSalt sampling design targeted families at high risk for developing hypertension, who might be more sensitive to sodium intervention [22]. Probands with a mean systolic BP (SBP) between 130–160 mmHg and/or diastolic BP (DBP) between 85–100 mmHg were identified by community-based BP screening among all 18–60 years in the study villages. Both 2-generation (probands, their parents and siblings) and 3-generation (additionally including the probands' spouse and offspring) families were recruited for the study. Probands, along with their siblings, spouses, and offspring participated in the dietary intervention. Individuals who had stage-2 hypertension, secondary hypertension, a history of clinical CVD, diabetes, or chronic kidney disease (including microproteinuria), use of antihypertensive medications, or were pregnant, heavy alcohol drinkers or currently on a low-sodium diet were excluded from the dietary intervention. Among the 1,906 participants eligible for dietary intervention, 1,871 (98.2%) and 1,860 (97.6%) completed the low and high dietary sodium interventions, respectively, and were included in the current analysis. Institutional Review Boards at all participating institutes approved the study. Written informed consents for the baseline observation and for the intervention program were obtained from each participant.

Dietary Sodium Intervention

After a 3-day baseline observation, the study participants received a 7-day low-salt diet (3 grams of sodium chloride or 51.3 mmol of sodium per day) followed by a 7-day high-salt diet (18 grams of sodium chloride or 307.8 mmol of sodium per day). During the period of sodium intervention, dietary potassium intake remained unchanged. Total energy intake was varied according to each participant's baseline energy intake. All foods were cooked without salt, and pre-packaged 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 have their breakfast, lunch, and dinner at the study kitchen under supervision of the study staff during the entire study period. The study participants were instructed to avoid consuming any foods that were not provided by the study. In addition, three timed urinary specimens (one 24–hour and two overnight) were collected at baseline and in each phase of intervention (days 5, 6, and 7) to monitor participants' compliance to dietary sodium intervention. The timed overnight urinary excretions of sodium and potassium were converted to 24-hour values based on a formula developed from data in this study. The results from the 24-hour urinary excretions of sodium showed excellent compliance with the study diet: the mean (standard deviation) 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) and 31.4 (7.7) during the low-sodium intervention, and 244.3 (37.7) and 35.7 (7.5) during the high-sodium intervention, respectively.

Phenotype Measurement

A standard questionnaire was administered by trained staff at the baseline examination just preceding dietary salt intervention. Information on family structure, demographic characteristics, personal and family medical history, and lifestyle risk factors were collected. Body weight, height, and waist circumference were measured twice in light indoor clothing without shoes during the baseline examination. Body mass index (BMI) was calculated as kilograms per meters squared (kg/m^2). Three 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 [23]. BP was measured with the participant in the sitting position after 5 minutes of rest. In addition, participants were advised to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 minutes prior to their measurement. All BP observers were blinded to the dietary intervention.

SNP Selection and Genotyping

Both the apelin coding gene (APLN) and ACE2 are located on the X chromosome and the APJ coding gene (APLNR) on chromosome 11. TagSNPs from these three genes were selected based on empirical patterns of linkage disequilibrium (LD) structure in the Chinese Han of Beijing (CHB) HapMap sample using Tagger software. We also included SNPs which were previously reported to be associated with BP or hypertension. SNPs were genotyped using SNPlex assays (Applied Biosystems, Foster City, CA, USA) based on oligonucleotide ligation assay for capillary electrophoresis on ABI 3700 DNA Analyzers (Applied Biosystems, Foster City, CA, USA). To provide better coverage of the candidate genes, we included additional SNPs genotyped on the Affymetrix 6.0 platform (Affymetrix, Santa Clara, CA, USA), whose minor allele frequencies were no less than 0.01. Finally, a total of 23 SNPs (14 SNPlex SNPs and 9 Affymetrix SNPs) were analyzed in this study (Table 1).

Statistical Analysis

BP levels at baseline and during intervention were calculated as the mean of 9 measurements from 3 clinical visits during the 3-day baseline observation or on days 5, 6, and 7 of each intervention period. Mean arterial pressure (MAP) was calculated as (SBP-DBP)/3 + DBP. The percent changes in BP from baseline to low-sodium and low to high-sodium interventions were used to measure BP responses to changes in dietary sodium intake. Percent changes in BP were adjusted for the effects of age and examination room temperature separately within sex-field center groups. In summary, each measure was regressed on the covariates in a stepwise manner, and only significant terms (0.05 level) were retained. The residual variance was also examined by regressing the squared residual from the first regression on the same covariates (stepwise) and retaining significant terms. The final adjusted phenotype was computed as the residual from the first regression, divided by the square root of the predicted score from the second regression. A final standardization step was taken to ensure a mean of 0 and an SD of 1. These adjusted and standardized scores were used as the phenotype variables in the association analyses.

The Mendelian consistency of the SNP genotype data was assessed by PLINK and PedCheck [24,25]. We used Haploview software (version 4.0, http://www.broad.mit.edu/mpg/haploview) to test Hardy-Weinberg Equilibrium (HWE) for each SNP and estimate the extent of pairwise LD between SNPs [26]. The solid spine LD method, as implemented in Haploview software, was used to define LD blocks.

We used the Family Based Association Test (FBAT) program (version 2.0.2, http://www.biostat.harvard.edu/~fbat/default.html) to test the association of single marker and haplotypes with adjusted phenotypes. FBAT performs family-based tests of association that are efficient and robust to population admixture, phenotype distribution and ascertainment based on phenotype. Three genetic models (additive, dominant, and recessive) were tested. To assess the effect of genetic variants on the trait value, a univariate FBAT test was performed for each allele and haplotype. This test provides a Z-statistic with its corresponding p-value. In our study, a positive Z-statistic for an allele or a haplotype indicates a decreased response to low-sodium intervention and an increased response to high-sodium intervention. The false discovery rate (FDR) method was used to correct for multiple testing [27].

Results

Table 2 shows the baseline characteristics and BP responses to the low- and high-sodium intervention among family members. The probands had higher mean baseline levels of BMI, SBP, and DBP than their siblings, spouses, and offspring whereas the parents had the highest baseline BP levels among all of the groups. Overall, BP levels decreased from baseline to low-sodium intervention but increased from low-sodium to high-sodium intervention. For both low- and high-sodium interventions, the probands had greater percentage changes in MAP than their siblings, spouses and offspring.

Table 3 shows all SNPs which were significantly associated with BP response to low-sodium or high-sodium intervention after adjustment for multiple testing (FDR <0.05). SNPs rs2282623 and rs746886 of APLNR were significantly associated with DBP (both P =0.002) and MAP (P =0.001 and 0.005, respectively) responses to low-sodium intervention. Compared to their major alleles, the minor alleles were associated with decreased BP response during the low-sodium intervention. Six SNPs of the ACE2 gene were also significantly associated with BP responses to low-sodium intervention. Among them, SNPs rs2074192, rs714205 and rs2285666 were associated with SBP response to low-sodium intervention (P =0.0007, 0.003 and 0.003, respectively). The other three SNPs were significantly associated with MAP response to both low-sodium and high-sodium interventions. Their minor alleles predicted greater MAP response to changes in dietary sodium intake. We did not find any association between APLN SNPs and BP responses.

We also performed haplotype analysis based on LD blocks of the three genes defined by Haploview (Figure 1). For the APLNR gene, there were three major haplotypes (≥0.05) in block1 which were formed by rs721628, rs2282623, and rs746886. Of the three haplotypes, haplotype ATT was significantly associated with DBP and MAP responses to low-sodium intervention (P =0.001 and 0.003, respectively) (Table 4). Another haplotype of this block (GCC) was significantly associated with SBP and MAP responses to high-sodium intervention (P =0.004 and 0.01, respectively). For the ACE2 gene, only one block was defined, within which two haplotypes (TTTCCCCGGC and TTCGCCCGGT) were significantly associated with SBP response to low-sodium intervention. Haplotype TTTCCCCGGC was associated with reduced SBP response to low-sodium, whereas haplotype TTCGCCCGGT was associated with increased SBP response. These haplotypes only differed at three loci, rs2074192, rs714205, and rs2285666, which also showed significant associations with SBP response to low-sodium in the single marker analysis.

Discussion

This large family-based association study identified multiple SNPs in the apelin receptor and ACE2 genes associated with BP responses to changes in dietary sodium intake. These results indicated that the apelin system and ACE2 genes might be mechanistically involved in BP salt-sensitivity and their genetic variants might contribute to the variation of this complex phenotype.

It is well-known that BP is a complex phenotype and influenced by multiple genetic and environmental factors and their interactions. Although recent large-scale genome-wide association studies have identified some genetic variants highly significantly associated with BP, they only explained a very small proportion of the total variation of BP [28,29]. Failure to measure gene and environment interactions may result in the inability to fully detect genetic contribution to BP variability in those studies. The dietary sodium intervention in our study provides a good opportunity to identify genes related to salt-sensitive hypertension.

Based on its physiological function in the cardiovascular system, we selected candidate genes in the apelin system and examined their association with BP responses to changes in dietary sodium intake. This novel pathway has broad effects in the cardiovascular system and is emerging as an important mediator of cardiovascular homeostasis [30]. Recently, the apelin system has been implicated in BP regulation and reported to be associated with hypertension [14,21].

In this study, we observed that two SNPs in the 3’ UTR region of APLNR, rs2282623 and rs746886, were significantly associated with BP response to low-sodium intervention. The haplotype analysis of the block including these two SNPs further confirmed their association with BP response to salt interventions. To speculate on the possible function of these significant SNPs, we analyzed them using the web tool SNPseek, a database of putatively functional human SNPs (http://snp.wustl.edu/cgi-bin/SNPseek/index.cgi). SNPseek shows that both of them are in regulatory potential regions where a marked resemblance of typical regulatory elements exists. This annotation of SNPseek is integrated from a regulatory potential score, which is computed from alignments of human DNA with that of six other species and provided by UCSC Genome browser. It is based on the principle that regions conserved across long evolutionary timescales are likely to be functionally significant [31]. Thus, it is possible that these two SNPs of APLNR influence BP response to salt intake through their impact on gene expression of APLNR. On the other hand, we still cannot exclude the possibility that these two SNPs are in strong LD with the true functional SNP which was not analyzed in our study. These results highlight a potential region for targeted resequencing in the future.

We found multiple intronic SNPs of the ACE2 gene associated with BP responses to changes in salt intake. Of them, SNP rs2285666, also known as 8790G/A, has been reported to be associated with hypertension among those with metabolic syndrome [32]. SNPseek analysis shows SNP rs2285666 is located in a splice site of the ACE2 gene and may alter splicing patterns. We could not find functional support for the association of the other SNPs, which are also not in strong LD with any other SNPs based on the HapMap data. However, there is accumulating evidence that intronic polymorphisms may cause complex disorders [33–35]. In order to fully explain these associations, further dissection of the regions surrounding these intronic SNPs and functional studies are warranted.

Large sample size, homogenous population, and excellent compliance to the dietary intervention highlight the strengths of the study. A possible concern of this study is the definition of BP response to low-sodium intervention. We took the BP difference between low-sodium intervention and baseline observation as the BP response to low-sodium intervention. Since the baseline dietary salt intake was not controlled, it might influence the study power and validity. However, all study participants were living in a homogenous environment with high dietary salt intake. Through measuring urine sodium excretion, we found that the baseline dietary sodium intake was much higher than that during low-sodium intervention and even as high as that during the high-sodium intervention. This might reduce the variation of BP response to low-sodium caused by the inter-individual variation in baseline sodium intake. Furthermore, some associations observed for BP response to low-sodium intervention were replicated by BP response to high-sodium intervention in our study.

In summary, we reported here for the first time significant associations between genetic variants of the APLNR and ACE2 genes and BP responses to changes in dietary sodium intake. These findings may contribute to a better understanding of the genetic mechanisms underlying BP regulation and may have potential clinical and public health implications. Replication of these results in other populations and further functional studies to identify causal variants are critically important.

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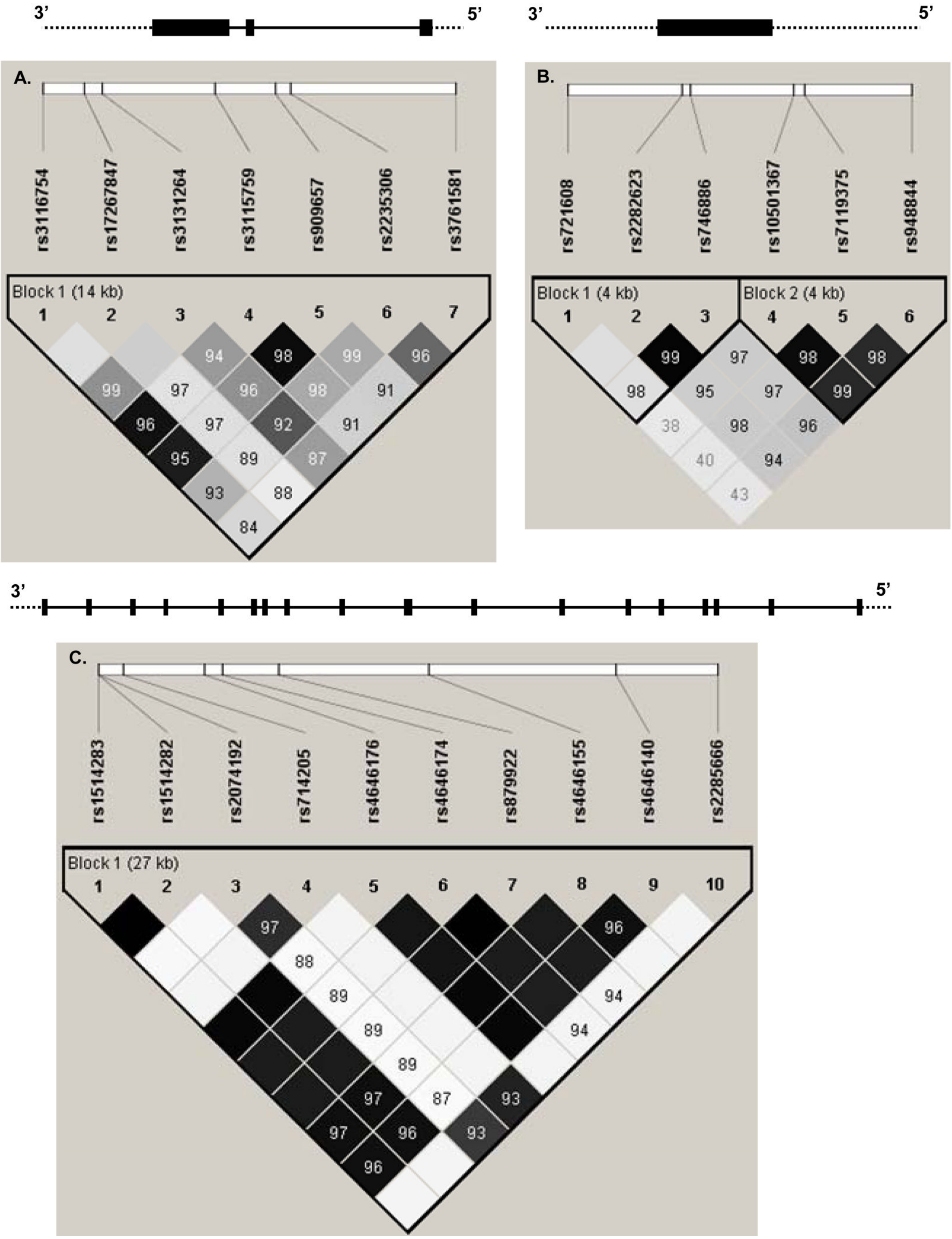

Figure 1.

The gene structures of APLN (A), APLNR (B), and ACE2 (C) and the position of single nucleotide polymorphisms. The black boxes in each gene are exons. Solid lines show introns and dashed lines show flanking regions. Pairwise linkage disequilibrium (LD) coefficients D'×100 are shown in each cell (D' values of 1.0 are not shown). The r^2 color scheme of Haploview was applied ($r^2 = 0$ shown in white, $0 < r^2 < 1$ shown in shades of grey, and $r^2 = 1$ shown in black).

Table 1

Characteristics of all SNPs analyzed in this study

Gene	Chr	SNP	Physical Location	Region in Gene	Alleles[a]	MAF	P-Values[b]
APLN	X	rs3116754	128603256	3'flanking	A:T	0.337	0.1649
		rs17267847	128604715	3'flanking	C:T	0.209	0.6554
		rs3131264	128605307	3'flanking	G:T	0.454	0.8702
		rs3115759	128609197	3'UTR	A:G	0.333	0.0768
		rs909657	128611248	intron1	G:A	0.332	0.4509
		rs2235306	128611779	intron1	T:C	0.408	0.0652
		rs3761581	128617402	5'flanking	T:G	0.314	0.0186
APLNR	11	rs721608	56754499	3'flanking	A:G	0.149	0.9602
		rs2282623	56758402	3'UTR	C:T	0.411	0.4338
		rs746886	56758687	3'UTR	C:T	0.411	0.3791
		rs10501367	56762163	5'flanking	C:T	0.226	0.8741
		rs7119375	56762540	5'flanking	G:A	0.223	0.8671
		rs948844	56766174	5'flanking	T:G	0.259	0.5169
ACE2	X	rs1514283	15492668	intron16	T:C	0.034	0.757
		rs1514282	15492684	intron16	T:C	0.034	0.757
		rs2074192	15492711	intron16	C:T	0.417	0.308
		rs714205	15493825	intron16	G:C	0.451	0.2775
		rs4646176	15497425	intron14	C:G	0.035	0.0891
		rs4646174	15498192	intron14	C:G	0.037	0.0961
		rs879922	15500728	intron11	C:G	0.036	0.0687
		rs4646155	15507430	intron8	G:A	0.033	0.0406
		rs4646140	15515773	intron6	G:A	0.029	0.0879
		rs2285666	15520269	intron3	T:C	0.446	0.8699

Chr, chromosome; SNP, single nucleotide polymorphism; MAF, minor allele frequency.

[a] Alleles are presented as major : minor allele.

[b] P values of Hardy-Weinberg Equilibrium test.

Table 2

Characteristics at baseline and BP responses to sodium intervention for all study participants

	Probands	Siblings	Spouses	Offspring	Parents
No. of subjects	676	956	69	205	1236
Age, yrs	41.0 ± 8.3	39.6 ± 7.7	49.1 ± 6.7	23.5 ± 6.5	67.6 ± 8.4
Male, %	60.4	51.1	33.3	44.4	48.6
Body mass index, kg/m^2	24.2 ± 3.3	23.1 ± 2.8	23.4 ± 3.7	21.5 ± 3.3	22.8 ± 3.4
BP at baseline, mm Hg					
Systolic	128.0 ± 11.4	111.6 ± 11.5	112.6 ± 14.9	106.6 ± 10.3	136.6 ± 23.9
Diastolic	80.3 ± 9.0	71.0 ± 8.9	72.6 ± 10.0	65.3 ± 9.0	75.0 ± 11.7
Mean arterial pressure	96.7 ± 8.5	84.5 ± 9.2	85.9 ± 11.2	79.1 ± 8.7	95.6 ± 14.3
BP response to low salt, %					
Systolic	−6.1 ± 6.0	−3.7 ± 5.0	−4.4 ± 5.8	−2.0 ± 4.5	–
Diastolic	−5.1 ± 7.2	−2.4 ± 7.4	−3.4 ± 6.8	−2.4 ± 8.1	–
Mean arterial pressure	−5.6 ± 5.8	−3.0 ± 5.5	−3.8 ± 5.8	−2.3 ± 5.5	–
BP response to high salt, %					
Systolic	4.9 ± 5.6	4.4 ± 5.5	5.4 ± 5.5	2.5 ± 4.2	–
Diastolic	3.6 ± 8.1	2.8 ± 7.7	2.9 ± 6.5	1.7 ± 8.5	–
Mean arterial pressure	4.1 ± 6.2	3.4 ± 6.0	3.9 ± 5.4	2.0 ± 5.3	–

Continuous variables are indicated as mean ± standard deviation. BP, blood pressure.

Table 3

Summary of individual SNPs significantly associated with BP response to low- and high-sodium interventions

Gene	SNP	Allele	SBP Response		DBP Response		MAP Response	
			Z	P-value	Z	P-value	Z	P-value
Low-sodium Intervention								
APLNR	rs2282623	T	1.851	0.06	3.100	0.002^{a*}	3.275	0.001^{a*}
	rs746886	T	0.993	0.32	3.165	0.002^{a*}	2.786	0.005^{a*}
ACE2	rs1514283	C	0.031	0.98	−2.787	0.005^{b*}	−2.863	0.004^{b*}
	rs1514282	C	0.031	0.98	−2.787	0.005^{b*}	−2.863	0.004^{b*}
	rs2074192	T	3.376/3.066	0.0007^{c*}/0.002^{a*}	2.682	0.007^{b*}	2.723/2.903	0.006^{a*}/0.004^{b*}
	rs714205	C	2.990	0.003^{c*}	1.272	0.20	1.999	0.045
	rs4646176	G	−0.004	1	−2.787	0.005^{b*}	−2.863	0.004^{b*}
	rs2285666	C	2.923	0.003^{c*}	1.886	0.06	2.409	0.02
High-sodium Intervention								
ACE2	rs1514283	C	−0.851	0.40	0.734	0.46	2.738	0.006^{b*}
	rs1514282	C	−0.851	0.40	0.734	0.46	2.738	0.006^{b*}
	rs4646176	G	−0.890	0.37	0.614	0.54	2.738	0.006^{b*}

For associations that were not significant under any model, Z and P values for an additive model are listed. All genetic models are based on the minor allele of each SNP. SNP, single nucleotide polymorphism; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure. Z indicates test statistic for FBAT.

[a] dominant model;

[b] recessive model;

[c] additive model.

[*] P-values are significant after correction for multiple testing (FDR <0.05).

Table 4

Summary of haplotypes significantly associated with BP response to low- and high-sodium interventions

Gene	Haplotype	Frequency	SBP Response		DBP Response		MAP Response	
			Z	P-value	Z	P-value	Z	P-value
Low-sodium Intervention								
APLNR	ATT[a]	0.38	1.183	0.24	2.607/3.241	0.009[b]*/0.001[c]*	2.924	0.003[c]*
ACE2	TTCGCCCGGT[d]	0.52	−2.153	0.03[b]*	−0.929	0.35	−1.391	0.17
	TTTCCCCGGC[d]	0.40	2.395/2.365	0.02[b]*/0.02[c]*	0.558	0.58	1.206	0.23
High-sodium Intervention								
APLNR	GCC[a]	0.14	2.483/2.868	0.01[b]*/0.004[c]*	1.887	0.06	2.519/2.437	0.01[b]*/0.01[c]*

Z indicates test statistic for FBAT. For association not significant under any model, Z and P values for additive model are listed. BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[a] rs721608-rs2282623-rs746886.

[b] additive model.

[c] dominant model.

[d] rs1514283-rs1514282-rs2074192-rs714205-rs4646176-rs4646174-rs879922-rs4646155-rs4646140-rs2285666.

[*] P-values are significant after correction for multiple testing (FDR <0.05).