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SALT-SENSITIVITY OF BLOOD PRESSURE IS ASSOCIATED WITH POLYMORPHISMS IN THE SODIUM-BICARBONATE CO-TRANSPORTER

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Abstract

Past studies have demonstrated that single nucleotide polymorphisms (SNPs) of the sodium-bicarbonate co-transporter gene (SLC4A5) are associated with hypertension. We tested the hypothesis that SNPs in SLC4A5 are associated with salt-sensitivity of blood pressure (BP) in 185 Caucasians consuming an isocaloric constant diet with a randomized order of 7 days low Na⁺ (10 mmol/d) and 7 days high Na⁺ (300 mmol/d) intake. Salt-sensitivity was defined as a 7mm Hg increase in mean arterial pressure (MAP) during a randomized transition between high and low Na⁺ diet.

A total of 35 polymorphisms in 17 candidate genes were assayed, 25 of which were tested for association. Association analyses with salt-sensitivity revealed three variants that associated with salt-sensitivity, two in SLC4A5 (P < 0.001), and one in GRK4 (P = 0.020). Of these, two SNPs in SLC4A5 (rs7571842 and rs10177833) demonstrated highly significant results and large effects sizes, using logistic regression. These two SNPs had P values of 1.0×10^{-4} and 3.1×10^{-4} with odds ratios of 0.221 and 0.221 in unadjusted regression models, respectively, with the G allele at both sites conferring protection. These SNPs remained significant after adjusting for BMI and age, (P = 8.9×10^{-5} and 2.6×10^{-4} and odds ratios 0.210 and 0.286, respectively). Further, the association of these SNPs with salt-sensitivity was replicated in a second hypertensive population. Meta-analysis demonstrated significant associations of both SNPs with salt-sensitivity [rs7571842 (P = 1.2×10^{-5}); rs1017783 (P = 1.1×10^{-4})]. In conclusion, SLC4A5 variants are strongly associated with salt-sensitivity of BP in two separate Caucasian populations.

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Keywords

Genetics-human; Genetics-association studies; Cardiovascular disease; Hypertension (Kidney); Blood Pressure; Sodium-bicarbonate co-transporter; Salt-sensitivity; Hypertension; Genetics

Salt-sensitivity is defined as a quantitative trait in which an increase in sodium (Na^+) load leads to an increase in blood pressure (BP)^{1, 2}. While universal consensus does not exist on the exact quantity of the Na^+ load, magnitude of the pressure increase or the specific protocol details required for this designation, the presence of salt-sensitivity is clearly associated with an increased prevalence of cardiovascular events and mortality, irrespective of unchallenged BP levels³⁻⁵. Indeed, normotensive salt-sensitive (SS) individuals have a cumulative mortality rate similar to that of hypertensive patients⁴. In addition, SS individuals, even those with normal BP levels when initially studied, have a greater age-related increase in BP⁶. Overall, salt-sensitivity has been estimated to be present in 51% of hypertensive and 26% of normotensive subjects, posing a major public health problem in the United States and other Westernized societies⁷.

The underlying pathophysiological mechanisms of salt-sensitivity are currently unknown⁸. However, there is strong evidence that genetic mechanisms may underlie variations in the BP response to dietary salt intake^{2, 9-11}. In particular, there is tight maternal-offspring association of BP change with dietary Na^+ restriction⁹. Salt-sensitivity, hypertension and related cardiovascular diseases are thought to result from the interaction of genes with the environment (e.g., stress and diet), but the specific genes involved in susceptibility to salt-sensitivity have not been completely clarified².

The Na^+ -bicarbonate co-transporter gene (SLC4A5) on chromosome 2 encodes a protein that transports Na^+ and HCO_3^- electrogenically across the basolateral membranes of many cell types, including renal tubule cells, into the interstitial fluid and ultimately into the circulation^{12, 13}. SNPs of SLC4A5 have been associated with baseline and 10-year follow-up BP in previous studies¹⁴⁻¹⁷. In the present study, we tested the hypothesis that SNPs in several candidate genes, but especially SLC4A5, are associated with salt-sensitivity in two Caucasian populations examined by independent investigators.

METHODS

Study Participants

The present analyses were restricted to subjects with genotype and BP data: 55 hypertensive and 130 normotensive Caucasian subjects from the University of Virginia (UVA) discovery cohort and 211 Caucasian hypertensive subjects for the replication cohort (HyperPATH).

Human Subjects Protocol (UVA Cohort)

The study protocol and informed consent documents were approved by the University of Virginia Institutional Review Board for Health Sciences Research. Tests for genetic association for BP traits were performed in 185 subjects of European ancestry ages 18–70 years and body mass index (BMI) of 18–30. The subjects were deemed healthy as determined by screening history, physical examination, standard 12-lead electrocardiogram and laboratory testing (complete blood count, fasting comprehensive metabolic panel, lipid panel and urinalysis with microscopy). The subjects were classified either as normotensive (NT; BP <140 SBP and <90 DBP mm Hg) or hypertensive (HT; history of prior diagnosis of hypertension or diastolic BP 90–114 mm Hg following a 3-week withdrawal from all antihypertensive medications or average BP readings during the pre-study screening period 140/90 mm Hg). Subjects with a history of malignant or accelerated hypertension,

secondary causes of hypertension, contraindication to discontinuing antihypertensive medications, serum creatinine >1.5 mg/dL, urinary protein excretion > 500 mg/day, continuing active urinary sediment, previous myocardial infarction, stroke or transient ischemic episode, congestive heart failure or severe small vessel disease or concurrent pregnancy were excluded. HT subjects with systolic BP >180 mm Hg or diastolic BP >114 mm Hg after withdrawal from antihypertensive medications also were excluded.

The study entailed 2 screening visits (genetic and pre-study) and a 3-week wash out period for HT subjects taking antihypertensive medications with weekly visits for BP monitoring. After completion of screening procedures and/or washout period, the subjects were placed on an isocaloric constant diet containing 1 g protein/kg body weight/day and 60 mmol/day of potassium (K⁺) for 2 consecutive weeks. Individual caloric intake was determined by the subject's height, weight, age and activity level. All food was weighed, measured, prepared and obtained from the Research Diet Kitchen of the General Clinical Research Center of the University of Virginia Health System. On study days 1–7, Na⁺ intake was either 10 mmol/day or 300 mmol/day and on study days 8–14 Na⁺ intake was the opposite; the order of low and high Na⁺ intake was randomized. BP, heart rate, height, and body weight were measured at each of 7 visits over the 2-week study period. BP measurements for the last day of each diet (3 separate measurements of BP taken over 45 minutes in the right arm with the subject in the sitting position) were averaged to identify salt-sensitivity. Salt-sensitivity was defined as a mean arterial pressure (MAP) increase of ≥ 7 mm Hg with the subject on high as compared with low Na⁺ intake. Na⁺ metabolic balance was confirmed with 3 consecutive 24-hr urine collections for Na⁺ on the last 3 days of each diet. Subjects failing to achieve metabolic balance after a maximum of 7 days of the constant low or high Na⁺ diet were excluded. HT subjects were excluded for systolic BP >180 mm Hg or diastolic BP > 114 mm Hg or for persistent cardiovascular symptoms while on the diets. To assess whether or not the order of diets affected the diagnosis of salt-sensitivity, we compared those receiving a high salt diet on the first week with those receiving a low salt diet on the first week. There was no evidence that the order of the diets affected the results ($P = 0.365$, Wilcoxon Rank Sum test).

HyperPATH Cohort and Study Protocol—The HyperPATH Cohort consists of subjects with mild hypertension studied from four international centers [Brigham and Women's Hospital, University of Utah Medical Center, Vanderbilt University, and Hospital Broussais (Paris, France)]. The HyperPATH inclusion/exclusion criteria and the detailed phenotyping protocol have been previously described and are briefly detailed below^{18,19}. Although results from the HyperPATH have been reported previously^{19,20}, the present analyses are original.

All subjects received a screening history, and physical and laboratory examinations. Hypertension was defined as a seated diastolic BP of ≥ 100 mmHg off antihypertensive medications, ≥ 90 mmHg while taking ≥ 1 medication, or treatment with ≥ 2 medications. Subjects requiring ≥ 4 anti-hypertensive medications were excluded. A diagnosis of diabetes mellitus (previously diagnosed, fasting glucose >126mg/dl, or glucose >200mg/dl at 180 minutes after 75gm glucose load), overt renal insufficiency (serum creatinine > 1.5mg/dL), stroke, psychiatric illness, any form of secondary hypertension, obesity (BMI > 30 kg/m²), current tobacco, or illicit drug use were excluded. Subjects with abnormal laboratory values or electrocardiographic evidence of heart block, ischemia, or prior coronary events at screening were excluded. All subjects were between the ages of 18–65 years. Race was self-defined by each subject.

To control for the influence that medications may play on the renin-angiotensin-aldosterone system, all angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers

(ARB), or mineralocorticoid receptor antagonists were discontinued 3 months and β -adrenergic receptor blockers were discontinued 1 month prior to the start of the study. If necessary, subjects were placed on amlodipine or hydrochlorothiazide for BP control. All medications were discontinued 2 weeks prior to the start of the study.

All participants received two alcohol- and caffeine-free isocaloric diets for 5–7 days each on an outpatient basis: high sodium (HS) (200mmol/day) and low sodium (LS) (10mmol/day) with each diet also containing 100mmol/day potassium and 20mmol/day calcium. The order of the diets was random. Subjects were then admitted overnight to the in-patient research center (PRC) after each diet. On the final day of each diet, a 24-hour urine collection was obtained. All studied participants had a urine sodium 150mmol/24 hours on the HS diet and 30mmol/24 hours on the LS diet. BP measurements were obtained using an automated device (DINAMAP; Critikon, Tampa, FL). Three consecutive readings separated by 5 minutes were obtained after each diet and measured while the subject was supine. Salt-sensitivity was defined as a mean arterial pressure (MAP) increase of ≥ 7 mm Hg with the subjects on high as compared with low Na⁺ intake.

Candidate Gene and SNP Selection and Genotyping

Candidate SNPs were selected based on prior association with salt-sensitivity or hypertension. For the rationale for candidate gene and SNP selection, please see the Online Data Supplement at <http://hyper.ahajournals.org>.

In the UVA cohort, each SNP was genotyped using a fluorescent allele-specific PCR (AS-PCR) based assay²¹. This method has also been referred to in the literature as PCR amplification of specific alleles (PASA). Reaction components were assembled on a 384-well array tape platform (<http://www.douglascientific.com>) using nanoliter volumes (500–1000nl). PCR was carried out in a water bath thermocycler using standard 3-stage parameters (denature, primer annealing, primer extension). The specific parameters of each PCR varied depending on the nature of the primers and the SNP being genotyped. The 384 well array tape was scanned after PCR amplification and the ratio of fluorescent signals was used to determine the genotype (homozygous for one allele or heterozygous).

In the HyperPATH cohort two SNPs in the SLC4A5 gene were genotyped for replication purposes: rs7571842 and rs10177833. The SNPs were genotyped using a 7600-SNP Illumina iSelect platform (Illumina, San Diego, CA). These genotyped SNPs had a completion rate of greater than 90% and were in Hardy-Weinberg equilibrium (HWE) in HyperPATH. Repeat genotyping demonstrated concordance with the original genotype call.

Statistical Analysis

Statistical analyses related to the UVA cohort were performed using STATA (Version 11). Genotypic association was assessed for hypertension status at baseline and salt-sensitivity (defined as ≥ 7 mmHg increase in MAP on a high salt diet) using Chi squared or Fisher's Exact test, where appropriate²². For SNPs that showed suggestive evidence of association ($P < 0.1$) logistic regression analyses were performed without adjustments for covariates and then adjusted for body mass index (BMI), age and gender for the hypertension comparison and for BMI and age for salt-sensitivity analyses. All SNPs were tested for HWE in cases and controls separately. Results were not adjusted for multiple comparisons; instead significant results were tested for replication to assess for false positives in the HyperPATH data.

Statistical analyses related to the HyperPATH cohort were performed using SAS 9.1 (SAS Institute). HWE was evaluated using a chi-square test. Population characteristics are displayed as mean values with standard deviations. Non-normally distributed variables are

shown as median values with the inter-quartile range. Logistic regression analyses were performed for salt-sensitivity analyses and adjusted for age, BMI, sibling relatedness, and study site. A dominant genetic model (major allele homozygotes versus heterozygotes plus minor allele homozygotes) was conducted for all analyses.

Meta-analysis

Genetic meta-analysis was conducted using a weighted Z-score method and implemented in the freely available METAL software package (<http://www.sph.umich.edu/csg/abecasis/metal/>). This approach accounts for the direction of association relative to a chosen reference allele and the sample size of each cohort. First, P values from each study are converted to Z scores. A weighted sum of Z scores is calculated where each statistic is weighted by the square root of the sample size for each study. The resulting sum is divided by the square root of the total sample size to obtain an overall Z statistic^{20,23}

RESULTS

Characteristics of the Discovery (UVA) Study Subjects

General characteristics—Tests for genetic association for BP traits were performed in 185 UVA subjects. Of the 185 UVA subjects, 55 were HT at the initiation of the study. The HT group was heavier, older and had a larger proportion of males than the NT subjects (P=0.004 for all comparisons; Table 1A). There were 34 subjects in the SS group and 151 subjects in the salt-resistant (SR) group (Table 1B). The SS subjects were older than the SR subjects. There was no significant difference in BMI between SS and SR subjects. As expected, the distribution of SS subjects differed between the HT and NT subjects (P=0.004; Table 1C). Demographic and clinical descriptions of the HyperPATH data are presented in Table S1 (Please see Online Data Supplement at <http://hyper.ahajournals.org>).

High Na⁺ intake—After 7 days of high Na⁺ intake, urinary Na⁺ excretion was similar in the UVA SS and SR groups at 226.3 ± 37.8 and 218.8 ± 57.3 mmol/24hr, respectively (P=0.52). With subjects on high Na⁺ intake, systolic, diastolic and mean arterial pressures were higher in the SS than the SR group (all P<0.0001) and plasma renin activity was higher in SR than SS (P<0.05). There were no differences in resting heart rate or urine creatinine excretion between the SS and SR groups for either population.

Low Na⁺ intake—Following 7 days of low Na⁺ intake, urinary Na⁺ excretion also was similar in the SS and SR groups at 18.6 ± 6.2 and 17.4 ± 7.4 mmol/24hr, respectively (P=0.31). With subjects on low Na⁺ intake, diastolic BP was higher in the SR group (P=0.02), but there was no significant difference in systolic or mean arterial pressure between the two groups. There were no differences in resting heart rate or total urine creatinine excretion between the SS and SR groups. However, on low Na⁺ intake plasma renin activity was higher in the SR subjects (P=0.03). The mean change in blood pressure from high salt to low salt diet did not differ from that of low salt to high salt diet (P = 0.365).

Effect of high and low Na⁺ intake on BP, plasma renin activity and plasma aldosterone concentrations—For both SS and SR groups, urinary Na⁺ excretion was more than 10-fold higher with subjects on high as opposed to low Na⁺ intake (P<0.0001 for both groups). In the SS group, systolic, diastolic, and mean arterial pressures were higher during high as compared with low Na⁺ intake (all P<0.0001). However, for the SR group BP was not influenced by Na⁺ intake. Plasma renin activity and aldosterone concentrations were both higher with low than high Na⁺ intake (P<0.0001).

Genetic Association Studies

A total of 35 SNPs in 17 candidate genes were assayed in the UVA population, but of these eight were monomorphic in one phenotypic class and/or near monomorphic in the other, and therefore not presented. (Table 2; Tables S2 and S3; see Online Data Supplement at <http://hyper.ahajournals.org> for the rationales for the choice of candidate genes and/or SNPs). Association analyses with salt-sensitivity demonstrated four variants that were marginally associated with salt-sensitivity, the two SNPs in SLC4A5, one SNP in the dopamine D₂ receptor (DRD2, rs6276) and one SNP in G protein-coupled receptor kinase-4 (GRK4, rs2960306) ($P < 0.1$; Table 2). Association with hypertension at baseline revealed two SNPs with a P value below 0.10: rs4961 in *ADD1* ($P = 0.027$) and rs1801058 in GRK4 ($P = 0.018$).

We examined each of these SNPs for association using logistic regression to determine effect sizes, and to assess whether or not adjusting for covariates affected the results. For salt-sensitivity only the two SNPs in SLC4A5 (rs7571842 and rs10177833) demonstrated significant results using logistic regression. These two SNPs had P values of 1.04×10^{-4} and 3.1×10^{-4} and odds ratios of 0.221 and 0.221, respectively (Table 3A). After adjusting for BMI and age, the associations remained ($P = 8.9 \times 10^{-5}$ and 2.55×10^{-4} and odds ratios 0.210 and 0.286, respectively). These two SLC4A5 SNPs were in linkage disequilibrium in both SS and SR UVA subjects, but the strength of the linkage disequilibrium was greater in SR than SS subjects ($r^2 = 0.93$ and 0.61 respectively), further supporting the conclusion of an association of SLC4A5 with SS. The DRD2 SNP was still close to significant after adjusting for covariates ($P = 0.052$). For the hypertension analysis, the associations between GRK4 rs1801058 and ADD1 rs4961 remained significant ($P < 0.05$) after adjusting for covariates (Table 3B) Because ADD1 and GRK4 are within 40 kb of each other, we adjusted each SNP in the logistic regression analyses for the other SNPs in addition to gender, BMI and age to assess independence of these two SNPs. After adjustment for rs4961, rs1801058 remained statistically significant ($p = 0.016$, OR 0.545, 0.330–0.891 95% CI), but the ADD1 SNP rs4961 did not remain significant after adjusting for GRK4 genotypes ($p = 0.524$, OR 0.968, 0.877–1.07 95%, CI).

Replication of the Association of SLC4A5 SNPs and Salt Sensitivity of BP: HyperPATH Protocol

Replication—Associations between the two SLC4A5 SNPs from the UVA cohort and salt-sensitivity were also tested in the HyperPATH cohort (Table 3A) and significant associations were found for rs7571842 ($P = 0.02$). SNP rs1017783 manifested trends for salt-sensitivity that did not reach statistical significance (Table 3A, $P = 0.06$). The effects were in the same direction as for the UVA analyses (ORs 0.32 and 0.36, respectively).

Meta-Analysis—A meta-analysis of the two cohorts was carried out for salt-sensitivity. As expected, both SNPs demonstrated highly significant associations with increased salt-sensitivity (Table 4) [rs7571842 ($P = 1.2 \times 10^{-5}$), rs1017783 ($P = 1.1 \times 10^{-4}$)].

DISCUSSION

Despite numerous genome-wide association studies (GWAS), the specific genetic causes of hypertension and/or salt-sensitivity of BP have remained elusive. Several genes identified from GWAS have been shown collectively only to influence 2% of BP variability²⁴. It is likely that the lack of variance is because GWAS was not designed to account for epistatic or gene-environment interactions. Salt-sensitivity is of particular interest since the prevalence is estimated to be as high as 73% in hypertensive and 36% in normotensive Blacks¹⁰. In Koreans, the incidence of salt-sensitivity is 28%²⁶. Retrospective studies have demonstrated that normotensives with salt-sensitivity have mortality estimates that are

equivalent to those of hypertensive subjects⁴. Thus, the identification of gene variants associated with salt-sensitivity might provide predictive testing to identify subjects for further workup and/or treatment.

Various DNA polymorphisms have been associated with salt-sensitivity of BP. In Koreans, 4 gene variants were strongly associated with salt-sensitivity: cytosolic branched chain aminotransferase 1 (BCAT1, rs7961152, odds ratio = 4.9, P=0.007); ATPase, Ca⁺⁺ transporting, plasma membrane 1 (ATP2B1, rs2681472, odds ratio = 3.8, P=0.040); fibroblast growth factor 5 (FGF5, rs16998073, odds ratio = 2.1, P=0.042); and LOC100132798 (rs2398162; odds ratio = 3.5, P=0.023)²⁶. In elderly Amish, the Ste29-related proline-alanine-rich kinase (SPAK) protein contains two polymorphisms in the STK39 locus (rs3754777 and rs6749447) that are associated with salt-sensitivity²⁷. We previously demonstrated that SNPs in GRK4 are highly associated with salt-sensitive hypertension in Japanese²⁸ and Italians²⁹ which was recapitulated in transgenic mice expressing the human GRK4 variants². Additional polymorphisms in α -adducin (ADD1), angiotensin converting enzyme (ACE), angiotensinogen (AGT), cytochrome P450/11B polypeptide 2 (CYP11B2), G-protein β 3 subunit (GN β 3), and neural precursor cell expressed developmentally down-regulated 4-like protein (NEDD4L) have been linked to salt-sensitive hypertension^{2,30}. Since it is of interest to determine if candidate genes are associated with salt-sensitivity, independently of hypertension, we examined polymorphisms in a Caucasian cohort, containing subjects who were both NT and HT that had been phenotyped for salt-sensitivity using controlled diets. Of importance most of the variants studied were non-synonymous coding variants or those already shown to have biological function. Our results demonstrate that SLC4A5 variants are strongly associated with salt-sensitivity in Caucasians.

SLC4A5 was originally cloned from human heart in 2000³¹. Chromosome 2, the location of SLC4A5, was linked to increased BP in Blacks and Caucasians^{14, 32,33}. Building on this evidence, SLC4A5 was identified as a candidate hypertension susceptibility gene using several combined positional candidate gene methods³⁴. Selected single nucleotide polymorphisms at positions SLC4A5 (rs6731545), SLC4A5 A/C (rs1017783) and SLC4A5 A/G (rs7571842) were associated with elevated heart rate and BP in both Caucasians and African-Americans^{15,16,30}. Our results are consistent in that the allele associated with increased BP in Hunt *et al.*¹⁵ was also our risk allele (A) for salt sensitivity of BP.

The SLC4A5 gene codes for Na⁺-bicarbonate co-transporter-4 (NBC4), also termed NBCe2, an electrogenic Na⁺-bicarbonate co-transporter that helps maintain the homeostasis of intracellular pH by co-transporting three bicarbonate anions for each Na⁺ cation independently of chloride³⁵. Not much is known about the role of NBCe2 in renal physiology. NBCe2 is expressed in various organs including the kidney, heart, brain³⁶. Within the human kidney, NBCe2 mRNA is expressed in the thick ascending limb of Henle and the protein has been localized in the luminal plasma membranes of the cortical collecting duct^{37,38}. A recent study demonstrated that SLC4A5 knockout mice are hypertensive and have compensated metabolic acidosis³⁹. It was reasoned that deletion of SLC4A5 initiates compensatory bicarbonate reabsorption via other Na⁺-bicarbonate transporters at the expense of increased tubule Na⁺ uptake.³⁹ Investigators also have speculated that NBCe2 might be a promising candidate for the strong linkage signal found in response to renin-angiotensin system inhibiting drugs, highlighting its potential importance in SS hypertension⁴⁰.

Increased Na⁺ transport is involved in genetic hypertension⁴¹. Even if SLC4A5 plays a minor role in Na⁺ balance, a decrease of only 0.1% in Na⁺ excretion can in the long run lead to hypertension. For example, a human excretes 1% of filtered Na⁺ (~250 mmol/day). A

decrease in Na⁺ excretion of only 0.1% leads to Na⁺ retention of 25 mmol/day or 250 mmols in 10 days, the cumulative effect of which can be substantial. Our results provide a new line of strong evidence that this gene is associated with salt-sensitivity of BP.

The association of SLC4A5 with salt-sensitivity has heretofore not been identified in GWAS. Effects of the sizes we detected would likely have been seen in GWAS, but this would only have been true if the same phenotype(s) were measured. For BP *per se*, our effect sizes are small and likely would not have been observed in GWAS. Our results for salt-sensitivity could not have been detected in GWAS because our data were based on a totally different approach emphasizing phenotype based on restricted dietary Na⁺ intake. Comparable data are not available in any GWAS.

This study has some limitations. First, results for hypertension *per se* could have been affected by the fact that the hypertensive subjects were not severely hypertensive, possibly reducing our power to detect an effect. Second, the sample sizes of both populations are relatively small; however, both populations have similar meticulous phenotyping protocols that are unavailable in most large-scale BP cohorts. Further, the meta-analysis adds power and validity to the salt-sensitivity finding. The cross-sectional nature of this analysis limits our ability to draw conclusions of causality and additional studies are necessary to determine the functionality of this genetic variant with salt-sensitivity. Strengths of the analysis include (1) use of extensive phenotyping including dietary control of Na⁺, (2) use of a meta-analysis approach to validate prior findings and (3) the fact that similar results were found in two independent studies⁴².

PERSPECTIVES

GWAS have identified genes that influence only 2% of BP variability and have not identified genes that influence the salt-sensitivity of BP. Only a few genes have been found to be associated with SS hypertension using candidate gene association studies. The strong association of two SLC4A5 SNPs with salt-sensitivity of BP may provide an impetus to study gene variants that are associated with salt-sensitivity. These results may have bearing on the recommendation of low Na⁺ diet in the prevention and treatment of cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NOVELTY AND SIGNIFICANCE

What is New

- Na⁺-bicarbonate co-transporter (SLC4A5) gene variants are strongly associated with salt-sensitivity of BP in two independent Caucasian populations.

What is Relevant

- Salt-sensitivity is a quantitative trait in which an increase in Na⁺ load induces an increase in BP.
- Salt-sensitivity is associated with an increased prevalence of cardiovascular events and mortality independently of the BP level.
- Salt-sensitivity is present in approximately one-half of hypertensive and one-quarter of normotensive individuals, thus posing a major public health problem.
- The genetic basis for salt-sensitivity has not been fully elucidated.
- The present study demonstrates that SNPs of SLC4A5 are strongly associated with salt-sensitivity independently of baseline BP.
- Identification of gene variants such as SLC4A5 with the pathogenesis of salt-sensitivity may have important implications for dietary Na⁺ restriction in subjects with these variants.

Summary

This study demonstrates a strong association of SLC4A5 variants with salt-sensitivity of BP in two independent Caucasian populations and suggests a genetic basis for salt-sensitivity in humans. These results may have implications for dietary salt restriction in the prevention of cardiovascular disease and hypertension.

TABLE 1

Characteristics of the Discovery Study Population *

A. Hypertension Status of UVA Study Subjects (Mean ± SD)				
Pre-study screen	Hypertensive Group	Normotensive Group		
Number of subjects	55	130		
Male	29	43		
Female	26	87		
Age (yrs)	52.4±12.3	45.0±13.9		
BMI	25.8±2.9	24.1±2.9		
Systolic BP (mmHg)	136±11.5	118.9±11.6		
Diastolic BP (mmHg)	80.5±9.1	71.5±7.1		
MAP (mmHg)	99.0±9.2	87.3±7.9		
B. Salt-Sensitivity Status of UVA Study Subjects (Mean ± SD):				
Pre-study (Visit 1 or pre-study screen)	Salt-Sensitive Subjects	Salt - Resistant Subjects	P value	
Number of subjects	34	151		
Male	10	62	0.208 [†]	
Female	24	89		
Age (yrs)	52.3 ± 13.9	46.0± 13.6	0.0156	
BMI	25.4 ± 2.7	24.4 ± 3.0	0.0740	
<u>After High Salt Diet</u>				
Systolic (mmHg)	133.5 ± 11.6	120.1 ± 13.2	<0.0001	
Diastolic (mmHg)	79.4 ± 7.6	71.7 ± 8.9	<0.0001	
MAP (mmHg)	97.4 ± 7.5	87.8 ± 9.7	<0.0001	
Heart Rate (Beats/min)	65.8 ± 9.3	68.8 ± 11.1	0.1552	
Plasma Renin Activity (ng/mL/hr)	0.39± 0.20	0.58 ± 0.49	0.0352	
Plasma Aldosterone (ng/dL)	4.02 ± 2.85	3.65 ± 3.29	0.5520	
Urinary Sodium Excretion (mmol/24 hrs)	226.3 ± 37.8	218.8 ± 57.3	0.5186	
Total Urine Creatinine (g/24hrs)	1.31 ± 0.4	1.31 ± 0.4	0.4576	
<u>After Low Salt Diet</u>				
Systolic (mmHg)	118.6 ± 11.5	119.2 ± 15.4	0.8510	
Diastolic (mmHg)	70.3 ± 5.2	74.1 ± 9.0	0.0192	
MAP (mmHg)	86.4 ± 6.4	89.1 ± 9.5	0.1078	
Heart Rate (Beats/min)	72.5 ± 9.0	74.1 ± 10.9	0.4100	
Plasma Renin Activity (ng/mL/hr)	4.5 ± 3.4	6.1 ± 3.7	0.0303	
Plasma Aldosterone (ng/dL)	27.9 ± 12.8	34.1 ± 19.8	0.0830	
Urinary Sodium Excretion (mmol/24 hrs)	18.6 ± 6.2	17.4 ± 7.4	0.3076	
Total Urine Creatinine (g/24 hrs)	1.3 ± 0.3	1.3 ± 0.4	0.6872	

C. Distribution of Salt-Sensitivity Status between UVA Hypertensive and Normotensive Study Subjects

BP Status	SS (Case)	SR (Control)	Total
HTN	17(50%, 30.9%)	38(25.2%, 69.1%)	55
NTN	17(50%, 13.1%)	113(74.8%, 86.9%)	130
Total	34	151	185 (100%)

* If continuous variables were normally distributed, a t-test was used to compare the two groups. If not normally distributed, a Wilcoxon-Rank Sum was used to compare the two groups. The non-normal variables were age, MAP (low salt), systolic (low salt), and diastolic (high and low salt). Also, note that BP measurements were uniformly higher in the hypertensive vs. normotensive group (all $P < 0.0001$).

+ Comparison of distribution of gender in SS vs. SR subjects

TABLE 2

Associations of Candidate Gene SNPs with Salt-Sensitivity and Blood Pressure

Gene Name	Gene Designation	SNP Designation	rs Number	SS Status P value*	HTN Status P Genotype P value*
Aldosterone synthase	<i>CYP11B2</i>	C-344T, 5' near gene	rs1799998	0.8504	0.331
Alpha Adducin 1	<i>ADD1</i>	G460W	rs4961	0.443 ⁺	0.027 ⁺
Alpha Adducin 2	<i>ADD2</i>	Intron 2	rs1541582	0.194 ⁺	0.897 ⁺
Angiotensinogen	<i>AGT</i>	M268T	rs699	0.326	0.885
Angiotensin II Receptor, Type I	<i>AGTR1</i>	A1166C, 3' UTR	rs5186	0.936 ⁺	0.135
Angiotensin Converting Enzyme	<i>ACE</i>	Intron 15	rs1799752	0.610	0.228
Caveolin 1	<i>CAVI</i>	Intron 2	rs3807990	0.576	0.767 ⁺
Cholecystokinin A Receptor	<i>CKKAR</i>	Intron 2	rs3840634	0.813 ⁺	0.271 ⁺
Cholecystokinin B Receptor	<i>CKKBR</i>	H210Y	rs41267457	NA	NA
Cytochrome P450-4A11	<i>CYP4A11</i>	L37F	rs1805000	NA	NA
		V125I	rs1805002	0.247 ⁺	0.908
		F434S	rs1126742	0.623 ⁺	0.334 ⁺
Dopamine Receptor D2	<i>DRD2</i>	-141C, 5' near gene	rs1799732	0.574 ⁺	0.374 ⁺
		E713K	rs1800497	0.721 ⁺	0.603 ⁺
		Intron 1	rs1079597	0.721 ⁺	0.302 ⁺
		3' UTR	rs6276	0.074 ⁺	0.584 ⁺
		R65L	rs2960306	0.020	0.227
		A142V	rs1024323	0.389	0.500
G Protein-coupled Receptor Kinase 4	<i>GRK4</i>	A486V	rs1801058	0.846	0.018
Nitric Oxide Synthase 3 (endothelial cell)	<i>eNOS</i>	E298D	rs1799983	0.170	0.376
		T146T	rs35368770	NA	NA
		T204T	rs35410672	NA	NA
Protein Phosphatase 2, Regulatory Subunit B, Gamma Isoform	<i>PPP2R2C</i>	I429I	rs3796403	0.892	0.281
		A431A	rs11545013	NA	NA
Sodium Bicarbonate Cotransporter, Member 5	<i>SLC4A5</i>	Intron 17	rs7571842	< 0.001 ⁺	0.825

Gene Name	Gene Designation	SNP Designation	rs Number	SS Status vs Genotype P value*	HTN Status vs Genotype P value*
Sorting Nexin 1		Intron 20	<u>rs10177833</u>	<0.001 [†]	0.849
		E33R, frameshift	<u>rs34910981</u>	NA	NA
Sorting Nexin 5	<i>SNX1</i>	P211S	<u>rs1130604</u>	NA	NA
		D466N	<u>rs1802376</u>	NA	NA
		P92L	<u>rs6045116</u>	NA	NA
Sorting Nexin 19	<i>SNX19</i>	V361L	<u>rs3751037</u>	0.198 [†]	0.231 [†]
		S407G	<u>rs3190345</u>	0.319	0.434 [†]
		L618F	<u>rs681982</u>	NA	NA
		N753S	<u>rs4414223</u>	0.230 [†]	0.185 [†]
		L878R	<u>rs2298566</u>	0.361	0.320 [†]

* P values based on 2 × 3 Chi square analyses unless otherwise noted.

[†]Based on pooling rare homozygotes with heterozygotes due to there being too few homozygotes.

NA – Not analyzed because minor allele frequency is too low

TABLE 3

Logistic Regression Analyses for Both Cohorts

A. Logistic Regression Analyses for Salt-Sensitivity										
Gene	SNP	Referent Allele	OR unadjusted	95% CI	P value	OR*	95% CI	P value	OR*	P value
DRD2 UVA	rs6276	A	2.3	0.991–5.340	0.053	2.316	0.994–5.394	0.052		
GRK4 UVA	rs2960306	G	1.098	0.656–1.838	0.721	1.096	0.652–1.842	0.730		
SLC4A5 UVA	rs7571842	A	0.221	0.155–0.576	0.000104	0.210	0.0962–0.458	0.0000894		
SLC4A5 HyperPATH	rs7571842	A				0.32	0.21–0.47	0.02		
SLC4A5 UVA	rs1017783	A	0.221	0.103–0.473	0.000310	0.286	0.146–0.559	0.000255		
SLC4A5 HyperPATH	rs1017783	A	-	-	-	0.36	0.23–0.51	0.06		

B. Logistic Regression Analyses for Hypertension										
Gene	SNP	Referent Allele	OR unadjusted	95% CI	P value	OR ⁺	95% CI	P value	OR ⁺	P value
GRK4	rs1801058	C	0.607	0.385–0.957	0.032	0.544	0.335–0.883	0.014		
ADD1	rs4961	G	0.422	0.208–0.855	0.017	0.406	0.191–0.861	0.019		

* Adjusted for BMI and age.

⁺ Adjusted for gender, BMI and age.

TABLE 4

Meta-Analysis for SNP-Salt-Sensitive Blood Pressure Association *

Cohort	SNP	N	Single Association P-value	Meta P-value
UVA	rs7571842	185	0.000104	1.2×10^{-5}
HyperPATH	rs7571842	211	0.02	
UVA	rs1017783	185	0.000310	1.1×10^{-4}
HyperPATH	rs1017783	211	0.06	

* P-value for the association of both SNPs with salt-sensitive blood pressure accounts for age and BMI.

UVA=University of Virginia, SNP=single nucleotide polymorphism, BMI=body mass index.