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REGIONAL ASSOCIATION-BASED FINE MAPPING FOR SODIUM-LITHIUM COUNTERTRANSPORT ON CHROMOSOME 10

Alanna C. Morrison, PhD[a], **Eric Boerwinkle, PhD**[a], **Stephen T. Turner, MD**[b], and **Robert E. Ferrell, PhD**[c]

[a]Human Genetics Center, University of Texas Health Science Center at Houston, 1200 Herman Pressler; Suite 453E, Houston, TX 77030

[b]Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN 55905

[c]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, 130 DeSoto Street, Room 324 Parran Hall, Pittsburgh, PA 15261

Abstract

Background: Increased erythrocyte sodium-lithium countertransport (SLC) has been observed in patients with essential hypertension. Consistent evidence of genetic linkage was shown for SLC on chromosome 10, and a region of interest was localized between 26 and 56 Mb.

Methods: This study surveyed single nucleotide polymorphisms (SNPs) in 54 genes that reside in the region of interest and investigated their association with SLC and blood pressure. These SNPs were genotyped in 1133 non-Hispanic White individuals from 255 pedigrees comprising the second phase of the Rochester Family Heart Study. The variance components-based genetics software package SOLAR was used to evaluate whether a SNP contributes to a significant fraction of the trait heritability.

Results: Of the 77 SNPs surveyed in this study across the region of interest, four SNPs were associated with SLC ($p<0.04$), five SNPs were associated with blood pressure ($p<0.04$), and two SNPs in mannose-binding lectin 2 (*MBL2*) were associated with both phenotypes. In general, the pairwise linkage disequilibrium among the genotyped SNPs was low.

Conclusion: This fine-mapping survey of genetic variation in a linkage region of interest provides overall support for association mapping for SLC on chromosome 10. Genes significantly associated with systolic blood pressure and/or SLC in these families will be prioritized for future studies.

Keywords

sodium-lithium countertransport; blood pressure; association; polymorphism; chromosome

Introduction

Efforts to identify genetic variation influencing common chronic diseases such as hypertension are aided by the evaluation of intermediate traits and the application of well-

Correspondence: Alanna C. Morrison, Ph.D. Human Genetics Center, University of Texas Health Science Center at Houston 1200 Herman Pressler; Suite 453E Houston, TX 77030 Phone: 713-500-9800 Fax: 713-500-0900 E-mail: Alanna.C.Morrison@uth.tmc.edu.

Conflict of Interest
The authors of this manuscript have no conflict of interest to disclose.

designed analytical methodology. Experimental and epidemiological studies have suggested that sodium-lithium countertransport (SLC) may be considered an intermediate phenotype for hypertension.1 Increased erythrocyte SLC has been consistently documented in patients with essential hypertension2, and the reported heritability of SLC ranges from 55% to 88%.[3, 4] Several studies provide evidence of a genetic basis for SLC [3-7], including four genome-wide analyses of SLC in humans.[8-11] In the Rochester Family Heart Study, we observed consistent evidence of linkage (LOD > 2) for SLC on chromosome 10 in two independent samples of non-Hispanic White families.11 Overlap of the 1-LOD confidence intervals for the observed linkage between the two samples defined a genomic region of interest on chromosome 10 between 26 and 56 Mb on the physical map (NCBI Build 34). This study was designed to survey genetic variation in 54 genes that reside in the region of interest and test its association with SLC and blood pressure in the Rochester Family Heart Study cohort. Prioritization of genes on chromosome 10 associated with SLC and/or blood pressure will guide future association mapping efforts with the ultimate goal of localizing the functional genetic variation responsible for inter-individual phenotypic variation.

Methods

Study Population

Study participants were from the Rochester Family Heart Study, the overall objective of which is to identify and characterize genetic variation influencing risk of cardiovascular disease in the general population of Rochester, MN. Individuals were ascertained without regard to health or disease between 1984 and 1991 through index school children in the Rochester, MN, school system and these individuals took part in a detailed physical examination at the Mayo Clinic. The sampling details, clinic examination protocol, and baseline characteristics have been described by Moll et al12 and Turner et al.13 Sampling was done in two phases, differing only with respect to falling under different National Institutes of Health grant cycles, resulting in two independent samples of pedigrees referred to as Phase 1 and Phase 2. Genotyping for this project was completed in 1133 non-Hispanic White parents and children from 255 Phase 2 pedigrees that had available DNA. Restricting the analysis to the lower two generations of the pedigrees limits the potential confounding effects of age and body mass index on the SLC distribution. Appropriate institutional review boards approved the Rochester Family Heart Study, and all participants provided informed consent.

Phenotypic Measures

The standard assay procedure to measure SLC in erythrocytes has been described elsewhere.[1, 11, 14, 15] Blood pressure was measured with a random-zero sphygmomanometer. Three blood pressure readings, at least two minutes apart, were measured in the right arm after the subject had been sitting quietly for at least five minutes. The pressure at Korotkoff phase I sound was taken as systolic blood pressure. Diastolic blood pressure was determined at the occurrence of Korotkoff phase V sound. Blood pressure measures for this study were the averages of the three readings taken for each subject.

Single nucleotide polymorphism (SNP) selection and genotype determination

The region of interest on chromosome 10, identified from linkage analyses in the Rochester Family Heart Study, corresponds to 26 to 56 Mb on the physical map (NCBI Build 34) and contains 54 genes. As an initial survey of genetic variation in these genes, at least one SNP was chosen from public16 or private17 databases for genotyping. Table 1 shows the genes evaluated in this study, the number of SNPs genotyped in each gene, and allele frequencies estimated in a sample of unrelated individuals (N=255) generated by a randomly sampling one individual from each pedigree. Thirty-eight genes contain only one SNP, eleven genes

contain two SNPs, three genes contain three SNPs, and two genes contain four SNPs. Pairwise linkage disequilibrium among the 77 SNPs was evaluated using Haploview.18 Average pairwise linkage disequilibrium measured by r^2 was 0.005. Only two pairs of SNPs had a pairwise r^2 greater than 0.80: rs10899795 in *FYXD4* and rs4597022 in *HNRPF* (r^2=0.84) and rs1657224 in *PARD3* and rs1362999 in *CFP1* (r^2=1.0)

SNPs were genotyped by the fluorescence polarization method described by Chen et al.19 using the L.J.L. Biosystems' Analyst HT Assay Detection System. Data were analyzed using the Allele Caller software package. Genotype clusters generated by Allele Caller are checked visually by the operator and questionable calls are repeated or assigned by direct sequencing of the sample.

Statistical Analyses

Agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested using a χ^2 goodness-of-fit test in a random sample (N=255) comprised of one individual from each pedigree. Association between each SNP and blood pressure or SLC was evaluated using the variance components-based genetics software package SOLAR.20 SOLAR evaluates whether a SNP contributes to a significant fraction of the trait heritability by comparing models including or excluding the SNP genotype, coded as the additive effect of the rare allele, as a covariate. Age, gender and triglyceride levels were included in the variance-components models as potential confounders. No adjustment for multiple comparisons was made within this study given the consistent published evidence11 of the existence of a gene influencing SLC and/or blood pressure in this region of chromosome 10 as well as evidence that variation in multiple genes may contribute to SLC.10 This region of chromosome 10 was identified by evidence of linkage to SLC in the Rochester Family Heart Study.11 Therefore, for each individual SNP significantly associated with SLC in the variance-components model, we evaluated the decrease in the LOD score from the peak LOD of 2.27 at 55 cM originally observed in the Phase 2 pedigrees.

Results

Genotype frequencies of all SNPs agreed with Hardy-Weinberg expectations. Descriptive characteristics of the Rochester Family Heart Study for the phenotypes of interest are shown in Table 2. The proportion of males in the total sample of pedigrees was 51.8 percent.

The results from the variance-components analyses incorporating each SNP are shown in Table 3 for those SNPs demonstrating a significant association with either SLC or blood pressure ($p<0.05$) after taking into account the potential confounding effects of age, gender and triglyceride levels. The proportion of phenotypic variance attributable to each SNP is also reported in Table 3 and for each SNP demonstrating a significant association with SLC, we observed a reduction in the LOD score assessed at the peak evidence for linkage reported in Morrison et el.11 Inclusion of rs1838065 in *MBL2* in the variance components model resulted in the greatest decrease in the LOD to 0.80 at 55 cM. When both rs930507 and rs1838065 in *MBL2* were included in the linkage analysis model, a LOD of 0.77 at 55 cM was observed.

A total of seven SNPs associated with blood pressure and/or SLC were identified in the linkage region of interest on chromosome 10. Two of these SNPs, rs930507 and rs1838065, reside in mannose-binding lectin 2 (*MBL2*) and are the only polymorphisms to show a significant effect on both SLC and blood pressure. Pairwise linkage disequilibrium between the two *MBL2* SNPs is low (r^2=0.15).

Discussion

This study is a survey of genetic variation in 54 genes that reside in a region on chromosome 10 with consistent evidence of linkage for SLC and blood pressure.11 Association-based fine-mapping of this region demonstrates that variation in three genes is associated with SLC, variation in four genes is associated with blood pressure, and variation in one gene is associated with both phenotypes. Of the seven SNPs associated with blood pressure and/or SLC, only two of these SNPs reside in the same gene, mannose-binding lectin 2 (*MBL2*).

It is interesting to note that two SNPs in this study, rs930507 and rs1838065 in *MBL2*, demonstrated a significant association with SLC and blood pressure. These SNPs also appeared to contribute most to the linkage evidence for SLC in this region of chromosome 10, resulting in a reduction in the LOD score from 2.27 to 0.77 at 55 cM when they were both included in the linkage analysis model. *MBL2* encodes the soluble mannose-binding protein found in serum. This protein, secreted by the liver, is a part of the acute-phase response and is involved in innate immune defense. It recognizes mannose and N-acetylglucosamine on bacterial pathogens, and is capable of activating the complement system.21 Three nonsynonymous polymorphisms in exon 1 of *MBL2* have been associated with low serum levels of mannose-binding lectin and increased risk of infections22 as well as worsened prognosis for chronic diseases such as cystic fibrosis23, rheumatoid arthritis24, and systemic lupus erythematosus.25 These SNPs in exon 1 of *MBL2* have also been associated with an increased risk of coronary artery disease26, arterial thrombosis among patients with systemic lupus erythematosus21 and increased systemic arterial stiffness in patients after Kawasaki disease.27 Using available information from the International HapMap Project Caucasian data, we determined that the SNP at codon 54 (rs1800450) is not in linkage disequilibrium with either of the two *MBL2* SNPs evaluated in this study (r^2=0.06 with rs930507 and r^2=0.13 with rs1838065). Although the mechanism by which genetic variation in *MBL2* modulates systemic arterial stiffness is unknown, it is clear that *MBL2* plays a role in the inflammatory pathophysiology of the vasculature. Evidence from animal models and human population-based studies suggest vascular inflammation may be involved in the initiation as well as development of hypertension.28 These observations, coupled with the results from this study, lend support to the prioritization of *MBL2* as a putative candidate gene for SLC and susceptibility to hypertension.

Additional evidence that this region of chromosome 10 harbors a gene associated with blood pressure-related phenotypes comes from a genome-wide SNP scan of the Framingham Heart Study Offspring Cohort. On chromosome 10, a putative association between rs1916565 and diastolic blood pressure was identified (http://gmed.bu.edu).29 Rs1916565 resides between the *CHAT* and *PARG* genes evaluated as a part of our study. Also on chromosome 10, an association was identified between a SNP (rs10508995) proximal to *MBL2* and systolic blood pressure (http://gmed.bu.edu). Utilizing Caucasian data from the International HapMap Project we determined that rs10508995 is not in strong linkage disequilibrium (r^2<0.02) with the two *MBL2* SNPs genotyped in our study, and rs10508995 was not significantly associated with SLC or blood pressure in the Rochester Family Heart Study (data not shown).

A strength of this study is that we have genotyped SNPs in all 54 genes in the region of interest on chromosome 10. However, 70% of the genes surveyed contained only one genotyped SNP. Given this generalized survey of the region, lack of an association with SLC or blood pressure is not a basis for excluding a gene from further study. However, detection of an association with one of the 54 genes in this region may be used to prioritize that gene for future study. A potential limitation of this study is that the pedigrees were not ascertained with regard to health or disease and the children's generation in particular, with a

mean age of 15.5 years, represents low blood pressures (systolic blood pressure 104.6±10.1 mm Hg and diastolic blood pressure 63.0±9.8 mm Hg). Although it is important to note the sample of pedigrees in this study are the same families that contributed to the evidence of linkage for SLC in this region of chromosome 10.11

Evidence of association for rs930507 and rs1838065 in *MBL2* and SLC and systolic blood pressure has been found in a region of linkage for SLC and blood pressure on chromosome 10. *MBL2* may be considered a candidate gene for SLC and susceptibility to hypertension. Prioritization of genes on chromosome 10 associated with SLC and/or blood pressure will guide future association-based fine-mapping efforts, with the ultimate goal of localizing the functional genetic variation responsible for inter-individual variation in these traits.

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Table 1

Description of genetic variation surveyed in a 30 Mb region on chromosome 10

Gene	Gene Name	SNP ID	Location (Mb)	Allele Frequency
MYO3A	Myosin IIIA	rs7911700	26.326372	0.60 (G)/0.40 (A)
GAD2	Glutamate decarboxylase 2	rs8190612	26.552381	0.87 (G)/0.13 (A)
APBB1IP	Amyloid beta (A4) precursor protein-binding	rs1932253	26.769118	0.67 (A)/0.33 (G)
TRPT	Trans-prenyltransferase	rs1748354	27.033395	0.58 (T)/0.42 (A)
SSH3BP1	Abl-interactor 1	rs6482575 rs2018904 rs2505963 rs2505956	27.110610 27.121908 27.200938 27.225292	0.56 (C)/0.44 (G) 0.82 (T)/0.18 (C) 0.62 (G)/0.38 (A) 0.74 (C)/0.26 (T)
YME1L1	YME1-like 1	rs9833 rs11015538	27.440867 27.441062	0.84 (G)/0.16 (A) 0.81 (A)/0.19 (G)
MASTL	Microtubule associated serine/threonine kinase-like	rs2274636	27.483018	0.88 (T)/0.12 (C)
RAB18	Member RAS oncogene family	rs2477343	27.842426	0.63 (A)/0.37 (G)
WAC	WW domain containing adaptor with coiled-coil	rs332136	28.898584	0.54 (T)/0.46 (C)
BAMBI	BMP and activin membrane-bound inhibitor homolog	rs1888085 rs675558	29.006500 29.008312	0.88 (T)/0.12 (G) 0.61 (G)/0.39 (A)
SVIL	Supervillin isoform 2	rs1886999	29.790956	0.52 (A)/0.48 (G)
MAP3K8	Mitogen-activated protein kinase kinase kinase 8	rs306588 rs1042058 rs3034	30.763599 30.768107 30.789901	0.74 (A)/0.26 (G) 0.60 (C)/0.40 (T) 0.87 (T)/0.13 (C)
TCF8	Transcription factor expression 8	rs3758455	31.655255	0.92 (G)/0.08 (A)
ARHGAP12	Rho GTPase activating protein 12	rs2255555 rs2808074	32.154956 32.219190	0.79 (A)/0.21 (G) 0.51 (C)/0.49 (A)
KIF5B	Kinesin family member 5B	rs2286746	32.348400	0.91 (T)/0.09 (G)
EPC1	Enhancer of polycomb homolog 1	rs11592754	32.659578	0.88 (T)/0.12 (G)
ITGB1	Integrin, beta 1	rs1187072	33.283813	0.53 (T)/0.47 (A)
NRP1	Neuropilin 1	rs1888690 rs2804495	33.575802 33.652506	0.86 (G)/0.14 (C) 0.70 (T)/0.30 (G)
PARD3	PAR-3 partitioning defective 3 homolog	rs3781128 rs1657224	34.660226 34.877634	0.52 (C)/0.48 (T) 0.57 (T)/0.43 (A)
CUL2	Cullin2	rs12240347	35.399481	0.67 (A)/0.33 (G)
CREM	cAMP responsive element modulator	rs1148247	35.536952	0.56 (C)/0.44 (T)
CFP1	Cyclin fold protein 1	rs11010188 rs1362999	35.718856 35.792555	0.71 (A)/0.29 (G) 0.58 (T)/0.42 (A)
NYBR1	Breast cancer antigen	cv26944508	NA	0.53 (C)/0.47 (A)
ZNF25	Zinc finger protein 25	rs13503	38.279849	0.53 (A)/0.47 (C)
ZNF33a	Zinc finger protein 33a	rs633400	38.369427	0.88 (G)/0.12 (C)
ZNF11B	Zinc finger protein 11B	rs209390 rs2473116	42.448886 42.456895	0.84 (G)/0.16 (A) 0.59 (G)/0.41 (A)
RET	Ret proto-oncogene	rs1800858	42.915974	0.73 (G)/0.27 (A)
GALNACT2	Chondroitin sulfate	rs7092548	42.990811	0.82 (C)/0.18 (T)
FXYD4	FXYD domain containing ion transport regulator 4	rs10899795	43.189103	0.80 (C)/0.20 (A)

Gene	Gene Name	SNP ID	Location (Mb)	Allele Frequency
HNRPF	Heterogeneous nuclear ribonucleoprotein F	rs7905676 rs4597022	43.201081 43.205226	0.66 (T)/0.34 (C) 0.81 (C)/0.19 (G)
ZNF239	Zinc finger protein 239	rs2230660 rs3763789	43.373019 43.381366	0.92 (C)/0.08 (G) 0.89 (T)/0.11 (C)
ZNF32	Zinc finger protein 32	rs3814561	43.461915	0.75 (A)/0.25 (G)
CXCL12	Chemokine (C-X-C motif) ligand 12	rs2839696	44.186634	0.97 (G)/0.03 (A)
RASSF4	Ras association (RalGDS/AF-6) domain family 4	rs3829908	44.784106	0.77 (G)/0.23 (A)
DEPP	Decidual protein induced by progesterone	rs3740094	44.793323	0.85 (G)/0.15 (A)
ZNF22	Zinc finger protein 22	rs11494	44.820227	0.96 (T)/0.04 (C)
ALOX5	Arachidonate 5-lipoxygenase	rs2291427	45.256230	0.66 (G)/0.34 (A)
CTGLF1	Centaurin, gamma-like family, member 1	rs35963845	45.494334	0.79 (G)/0.21 (A)
GDF2	Growth/differentiation factor 2	rs3781226	48.036264	0.99 (G)/0.01 (A)
GDF10	Growth/differentiation factor 10	rs1902725	48.056248	0.80 (C)/0.20 (T)
MAPK8	Mitogen-activated protein kinase 8	rs1919709	49.190009	0.75 (G)/0.25 (A)
ARHGAP22	Rho GTPase activating protein 22	rs3789320 rs7898936 rs1445151 rs1345107	49.325369 49.376645 49.419580 49.464487	0.53 (T)/0.47 (G) 0.92 (C)/0.08 (T) 0.54 (T)/0.46 (C) 0.82 (G)/0.18 (A)
ERCC6	Excision repair cross-complementing rodent repair deficiency, complementation group 6	rs1917801	50.414312	0.91 (G)/0.09 (A)
SLC18A3	Solute carrier family 18, member 3	rs3729496	50.491197	0.78 (A)/0.22 (C)
CHAT	Choline acetyltransferase	rs1880676	50.494123	0.77 (G)/0.23 (A)
PARG	Poly (ADP-ribose) glycohydrolase	rs7067802	50.708303	0.67 (A)/0.33 (G)
MSMB	Microseminoprotein, beta	rs4630240	51.202534	0.59 (G)/0.41 (A)
NCOA4	Nuclear receptor coactivator 4	rs10761618	51.244612	0.72 (A)/0.28 (G)
ACF	Apobec-1 complementation factor	rs12570156	52.279014	0.68 (A)/0.32 (G)
CSTF2T	Cleavage stimulation factor, 3′ pre-RNA, subunit 2, 64kDa, tau variant	rs11601	53.127130	0.81 (G)/0.19 (A)
PRKG1	cGMP-dependent protein kinase 1, alpha isoenzyme	rs1937652 rs7917364 rs12356995	53.235324 53.283507 53.396390	0.69 (T)/0.31 (C) 0.81 (A)/0.19 (G) 0.75 (G)/0.25 (A)
DKK1	Dickkopf homolog 1	rs2241529 rs1569198	53.744763 53.746277	0.56 (G)/0.44 (A) 0.53 (A)/0.47 (G)
MBL2	Mannose-binding lectin 2, soluble	rs930507 rs1838065	54.198272 54.199263	0.84 (C)/0.16 (G) 0.60 (A)/0.40 (G)
PCDH15	Protocadherin 15	rs4481935 rs9787578 rs978841	55.254249 55.561682 55.734720	0.54 (A)/0.46 (G) 0.75 (C)/0.25 (A) 0.63 (C)/0.37 (T)

Table 2

Descriptive characteristics of the Rochester Family Heart Study sample

Characteristic	N	Mean (SD)	Minimum	Maximum
Age (years)	1133	28.0 (14.6)	6.3	69.1
Triglyceride levels (mg/dL)	1133	93.0 (57.05)	27	799
Sodium-lithium countertransport (μmol/L RBC/hr)	1097	289.5 (121.7)	34.5	1679.6
Systolic blood pressure (mm Hg)	1132	107.6 (11.9)	77.3	183.7
Diastolic blood pressure (mm Hg)	1129	67.4 (10.6)	24.7	107.0

Table 3

SNPs demonstrating a significant association with blood pressure and sodium-lithium countertransport

Gene	SNP ID	SBP	DBP	SLC	
		p-value (proportion of phenotypic variance attributable to the SNP)			LOD score for SLC at 55 cM*
TRPT	rs1748354	NS	0.03 (0.004)	NS	NA
YME1L1	rs9833	NS	NS	0.04 (0.004)	1.93
ZNF239	rs3763789	0.03 (0.005)	NS	NS	NA
ERCC6	rs1917801	NS	NS	0.02 (0.007)	1.94
DKK1	rs2241529	0.04 (0.006)	<0.01 (0.007)	NS	NA
MBL2	rs930507	<0.01 (0.007)	NS	<0.01 (0.006)	1.50
MBL2	rs1838065	0.03 (0.009)	NS	0.04 (0.007)	0.80

Variance-components models for evaluating association were adjusted for age, gender and triglyceride levels.

NS=not significant (p>0.05); NA=not applicable; SBP=systolic blood pressure; DBP=diastolic blood pressure; SLC=sodium-lithium countertransport

*LOD score is reported at 55 cM for a linkage analysis model for SLC that includes each individual SNP.