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Hypertension

Genetic Implication of a Novel Thiamine Transporter in Human Hypertension

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Objectives This study coupled 2 strategies—trait extremes and genome-wide pooling—to discover a novel blood pressure (BP) locus that encodes a previously uncharacterized thiamine transporter.

Background Hypertension is a heritable trait that remains the most potent and widespread cardiovascular risk factor, although details of its genetic determination are poorly understood.

Methods Representative genomic deoxyribonucleic acid (DNA) pools were created from male and female subjects in the highest- and lowest-fifth percentiles of BP in a primary care population of >50,000 patients. The peak associated single-nucleotide polymorphisms were typed in individual DNA samples, as well as in twins/siblings phenotyped for cardiovascular and autonomic traits. Biochemical properties of the associated transporter were evaluated in cellular assays.

Results After chip hybridization and calculation of relative allele scores, the peak associations were typed in individual samples, revealing an association between hypertension, systolic BP, and diastolic BP and the previously uncharacterized solute carrier *SLC35F3*. The BP genetic association at *SLC35F3* was validated by meta-analysis in an independent sample from the original source population, as well as the International Consortium for Blood Pressure Genome-Wide Association Studies (across North America and western Europe). Sequence homology to a putative yeast thiamine (vitamin B_1) transporter prompted us to express human SLC35F3 in *Escherichia coli*, which catalyzed [^{3}H]-thiamine uptake. *SLC35F3* risk-allele homozygotes (T/T) displayed decreased erythrocyte thiamine content on microbiological assay. In twin pairs, the *SLC35F3* risk allele predicted heritable cardiovascular traits previously associated with thiamine deficiency, including elevated cardiac stroke volume with decreased vascular resistance, and elevated pressor responses to environmental (cold) stress. Allelic expression imbalance confirmed that *cis* variation at the human *SLC35F3* locus influenced expression of that gene, and the allelic expression imbalance peak coincided with the hypertension peak.

Conclusions Novel strategies were coupled to position a new hypertension-susceptibility locus, uncovering a previously unsuspected thiamine transporter whose genetic variants predicted several disturbances in cardiac and autonomic function. The results have implications for the pathogenesis and treatment of systemic hypertension. (J Am Coll Cardiol 2014;63:1542–55) © 2014 by the American College of Cardiology Foundation

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Hypertension is the most common and fatal of the cardiovascular risk factors (1), but despite pharmacological advances, it remains inadequately controlled by medications (2). Blood pressure (BP) displays substantial heritability (typically in the range of ∼30% to 50%) (3), but its genetic determinants remain largely elusive, a problem referred to as the "missing heritability" (4). Although rare Mendelian syndromes of familial hypertension have been documented, usually in association with renal or electrolyte disturbances (5), the bulk of common heritable BP variation in the population remains unexplained (6). Genetic-linkage (meiotic cosegregation) approaches generally possess insufficient resolution for very complex traits such as hypertension (7), and even recent successes in genome-wide association studies have detected loci with high significance but small effect sizes (8).

We took a different approach to genetic determinants of BP variation in the population: harnessing the power of trait extremes (9), coupled with pooling for genome-wide association (10). The use of phenotypic extremes is a powerful approach both theoretically and practically (11) in genetic association studies, and genomic deoxyribonucleic acid (DNA) pooling has been an efficient strategy in genome-wide searches for susceptibility alleles. Here we coupled these 2 strategies, discovering and verifying the role of a novel solute transporter in association with systemic hypertension.

Methods

Human subjects. Subjects were volunteers, and each subject gave informed, written consent; the protocols were approved by the institutional review board. Recruitment procedures, definitions, and confirmation of subject diagnoses were according to previous reports. Genomic DNA of each individual was prepared from blood leukocytes. Characteristics of the 3 human population samples are given in Table 1.

BLOOD PRESSURE: POPULATION TRAIT–EXTREME SAMPLES IN SAN DIEGO. The power of an association study on trait-extreme samples was computed (9) under varying disease allele frequencies for Type I error rate (alpha). We thus determined that this sample had >90% power to detect genotype association with a trait if a genotype contributed as little as 3% to the total variation in men; the power was even higher in the female cohort. We began by studying subjects of white (European, by self-identification) ancestry, recruited from a large adult primary care (Kaiser Permanente) population in San Diego, California, as previously described (9). In this primary care population, ∼81% attended the clinic, and ∼46% consented to participation in the study, with collection of blood for preparation of genomic DNA. Subjects who did or did not participate were similar in self-reported age- and sex-specific history of myocardial infarction (men: 4.6% vs. 4.0%; women: 1.4% vs. 1.3%) or in other conditions, including hypertension, diabetes, obesity, or high cholesterol level. BP was measured by trained health assistants in the same health appraisal office within a single facility (Kaiser Permanente, Clairemont Mesa Medical Offices, San Diego, California), in seated subjects with a regularly calibrated aneroid system, using a manual BP cuff and auscultation of the brachial artery at the antecubital fossa. The protocol included cuff inflation to 200 mm Hg, with pressure then gradually released. Systolic blood pressure (SBP) was recorded as the pressure at which the first sound is heard (i.e., Korotkoff-1), whereas diastolic blood pressure (DBP) was recorded as the pressure at which the sounds disappear (i.e., Korotkoff-5). If BP was >150 mm Hg (SBP), >90 mm Hg (DBP), or both, a second reading was taken. From consented participants, the subjects in this study were selected, based on measurement of DBP, to represent the highest- and lowest-fifth DBP percentiles in that population (Fig. 1). Subjects were ascertained by using DBP as the trait because twin and family studies have provided evidence that DBP is substantially heritable (12–14), and SBP correlates highly with DBP. In the trait-extreme subjects, there was substantial and nonoverlapping separation between both the DBP and SBP values. A second sampling of different patients from within BP extremes of the same population allowed for replication. The statistical power of association between bi-allelic DNA markers and human quantitative trait loci can be substantially augmented by the sampling patients from opposite (highest and lowest) ends of the trait distribution (11), and analyses of the quantitative trait in trait-extreme subjects (as opposed to dichotomization of the trait) further enhances power (9,15). This population sample afforded us >90% power to detect genotype association with a trait when the genotype contributed as little as ∼2.5% to the total variation in men (even at $p < 10^{-8}$); the power was even higher in women. To accomplish this, lower-BP subjects were selected from the lowest-fifth percentiles of DBP, whereas the higher-BP group was selected from the highest-fifth percentiles of DBP. Both SBP and DBP differed significantly between the BP-extreme groups ($p < 0.0001$). Forty-one percent of

Abbreviations and Acronyms

AEI = allelic expression imbalance

ANOVA = analysis of variance

bp = base pairs

BP = blood pressure

cDNA = complementary deoxyribonucleic acid

CEU = Caucasian of European descent

CI = cardiac index

CO = cardiac output

DBP = diastolic blood pressure

DNA = deoxyribonucleic acid

HR = heart rate

ICBP = International Consortium for Blood Pressure Genome-Wide Association Studies

LD = linkage disequilibrium

MAF = minor allele frequency

mRNA = messenger ribonucleic acid

NCBI = National Center for Biotechnology Information

QTL = quantitative trait locus

RNA = ribonucleic acid

SBP = systolic blood pressure

SHR = spontaneously hypertensive rat

SNP = single-nucleotide polymorphism

SVR = systemic vascular resistance

SVRI = systemic vascular resistance index

TCDB = Transporter Classification Database

Table 1 Human Subject Groups Evaluated in this Study of *SLC35F3* Genetic Variation and BP, by Group Genotyped for *SLC35F3* (N = 32,063)

	Purpose of Study	Age, yrs	Female	Hypertension*
UCSD Twins/Siblings (n = 616)	"Intermediate" (early) traits	40.0 ± 0.7	71.8	11.2
San Diego (Kaiser) population BP extremes (n = 1,994)	Discovery of BP association (pooled → individual)	58.2 ± 0.3	60.8	48.0
ICBP (n = 29,453)	Replication of BP association	49.9 ± 1.1	54.7	32.5

Values are mean ± SEM or %. *Defined as SBP ≥140 mm Hg or DBP ≥90 mm Hg, or on BP treatment.
BP = blood pressure; ICBP = International Consortium for Blood Pressure Genome-Wide Association Studies; SBP = systolic blood pressure; UCSD = University of California at San Diego.

patients in the higher-BP group were taking 1 or more antihypertensive medications (including diuretics and angiotensin-converting enzyme inhibitors), whereas none in the lower-BP group were on such treatment. Further BP/single-nucleotide polymorphism (SNP) association studies were replicated in an independent sample of different patients from the same primary health care provider, using the same recruitment criteria as with the first cohort, described earlier.

EXTENSION OF HYPERTENSION ASSOCIATION INTO AN ADDITIONAL POPULATION SAMPLE: REPLICATION IN INTERNATIONAL CONSORTIUM FOR BP GENOME-WIDE ASSOCIATION STUDIES. Extension of associations in our BP-extreme cohorts was sought in the International Consortium for Blood Pressure Genome-Wide Association Studies (ICBP), across North America and Western Europe. Complete details of ICBP methodology have previously been presented (6). In short, ICBP data were analyzed separately for SBP and DBP, with local *SLC35F3* region data plotted using LocusZoom (Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan). For the peak BP-associated SNP in the second intron (rs16842784; C > A), the numbers of available ICBP subjects for SBP/DBP were 27,865 and 29,452, respectively.

TWIN PAIRS AND SIBLINGS FOR AUTONOMIC AND CARDIAC PHENOTYPING (UNIVERSITY OF CALIFORNIA AT SAN DIEGO). From 235 nuclear families, 399 subjects including twin and sibling pairs were recruited from southern California to participate in the study. Zygosity was confirmed by extensive microsatellite and SNP genotyping, as described (16). Twins ranged in age from 15 to 84 years; 10% were hypertensive. Thus, the twin/sibling analyses were adjusted for sex and age. All of the twins in these allelic/haplotype association studies were self-identified as being of European (Caucasian) ancestry.

CARDIAC AND VASCULAR FUNCTION. Baseline/resting cardiovascular traits (SBP, DBP, heart rate [HR], cardiac index [CI], and systemic vascular resistance index [SVRI]) were estimated in twins/siblings noninvasively using the DynaPulse oscillometric device (PulseMetric, Vista, California), as previously described (17). Flow parameters (cardiac

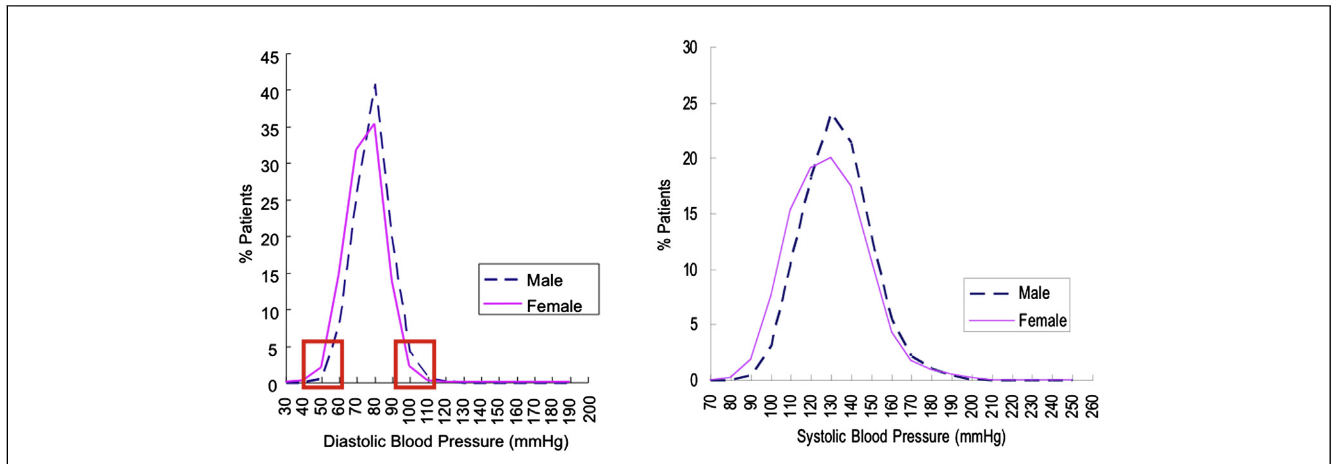


Figure 1 Population BP Extremes: A Powerful Approach to Genotypes Underlying Hypertension

Blood pressure (BP) distribution in a primary care database of over 53,000 adults (27,478 women; 25,528 men). We ascertained age-, sex-, and ethnicity-matched subjects with extremely high and low diastolic BP (DBP) (from the upper and lower fifth percentiles of DBP distribution); this approach has >90% power to detect loci contributing >2.5% of BP variance. **Red rectangles** = DBP range for upper- and lower-percentile (extreme) sample selection. Trait extremes method from Schork et al. (11).

output [CO], SVR) were normalized to body surface area, to yield CI and SVRI. The correspondence between DynaPulse and more conventional or invasive determinations of CO and SVR were documented recently (18).

ENVIRONMENTAL (COLD) STRESS TEST. To probe the functional significance of common variation at *SLC35F3,* we examined the potential influence of polymorphisms on BP response during an environmental (cold) stress test (19) in 399 twin (monozygotic or dizygotic) and sibling subjects. During the stressor, the subject immersed the nondominant hand into ice (0°C) water for 1 min, with averaged measurements of SBP, DBP, and HR stable over 3 beats pre- and post-procedure.

AUTONOMIC NEURAL FUNCTION. Sensitivity of the baroreceptor reflex arc (in ms/mm Hg) was tested by the ramp (time domain) method, using spontaneous excursions of SBP upward (with reflex bradycardia) and downward (with reflex tachycardia), as described (20,21). BP (in mm Hg) and pulse interval (R-R interval or heart period, in ms/beat) were recorded continuously and noninvasively for 5 min in seated subjects with a radial artery applanation device and dedicated sensor hardware (Colin Pilot, Colin Medical Instruments Corporation, San Antonio, Texas) and software (ATLAS, WR Medical Electronics Company, Stillwater, Minnesota; Autonomic Nervous System, Tonometric Data Analysis, Colin Medical Instruments Corporation) calibrated every 5 min against ipsilateral brachial arterial pressure with a cuff sphygmomanometer. HR was recorded continuously with thoracic electrocardiographic electrodes to the Colin Pilot. BP and HR were recorded continuously with the same devices during spontaneous excursions of BP with reciprocal HR changes: upward excursions of BP with reflex bradycardia and downward excursions of BP with reflex tachycardia. In each case, baroreceptor slope in the "time domain" was calculated with the Autonomic Nervous System, Tonometric Data Analysis software, with beat-by-beat regression of change in pulse interval (R-R interval; ms/beat) as a function of change in SBP (mm Hg) on the preceding beat (phase lag: 1 beat). Time windows of >4 beats were used, with change in SBP of >1 mm Hg and change in R-R of >6 ms. Baroreceptor slope (ms/mm Hg) values were recorded for regressions with correlation coefficients (r) > 0.9. The slopes for 3 such regressions, if each was within ±10% of the mean value, were averaged to yield the final value for baroreceptor slope.

SECRETONEURIN ASSAY. Human plasma treated with ethylenediaminetetraacetic acid as an anticoagulant was frozen and stored at −70°C prior to assay. The biologically active secretoneurin fragment of human SCG2 (TNEIVEEQYTPQSLATLESVFQELGKLTGPNNQ) was measured by radioimmunoassay, as previously described (22), with a rabbit polyclonal antibody directed against synthetic human secretoneurin ($SCG2_{154-165}$) and iodination on the endogenous tyrosine. The assay sensitivity in plasma was <40 pmol/l, with a reference range of <220 pM (<0.22 nM).

Genome-wide association. The rationale, methodology, and quality metrics for the pooling approach to genome-wide association have been presented in detail (10). Briefly, we formed representative DNA pools from 4 groups of subjects with the most extreme (highest- and lowest-fifth percentiles) of BP in a large primary care population: highest male (n = 277), highest female (n = 283), lowest male (n = 241), and lowest female (n = 330). Genomic DNA was prepared from blood leukocytes in each individual, then quantified by PicoGreen fluorescence (Molecular Probes, Inc., Eugene, Oregon), and then equivalent amounts of DNA from each individual were incorporated into each of the 4 pools. The quality of the pooled DNA was verified by appearance on 1% agarose gel followed by ethidium bromide staining. The 4 pooled DNA samples were then hybridized (after digestion with either Nsp I or Sty I) to replicate Affymetrix 5.0 (~500 K) SNP arrays (Affymetrix, Inc., Santa Clara, California) (4 arrays per pooled sample). In addition, the same samples were hybridized to replicate HumanHap300 (~300 K) SNP arrays (Illumina, Inc., La Jolla, California).

Relative allele scores were calculated for bi-allelic SNPs using the formula A/(A + B), where *A* = major allele and *B* = minor allele, to highlight the most significantly associated SNPs. Relative allele scores were calculated in the higher- versus lower-BP groups, which were then ranked for each SNP. SNP loci with relative allele scores most different between extreme cases and extreme controls, for both the Affymetrix 5.0 and Illumina HumanHap300, were pursued further. First, we ranked all SNPs by their allele-frequency differences between the 2 BP groups. Second, we took the top 250 SNPs from each array (Affymetrix and Illumina) and computed the absolute (in base pairs [bp]) distances between each neighboring SNP in these top lists. For those SNPs that were on different arrays but were ranked in the top 250 SNPs and that had a nearby neighbor SNP from the opposite array, we ranked (Online Table 1) and selected candidates for follow-up replication genotyping, resulting in 5 selected SNP pairs. Because the initial decision for locus/SNP follow-up was based on genotyped pools, standard statistical tests were not applicable at this stage.

Individual SNP genotyping. Diploid rs17514104 (*SLC35F3* tagging SNP in intron 2: TGGAA[C/T]TTGAC; alleles C > T) or rs16842784 (C > A, also in intron 2) genotypes were determined by either of 2 extension-based techniques—the matrix-assisted laser desorption ionization mass spectrometry method of Sequenom, Inc. (La Jolla, California), or the luminescent base incorporation method of Pyrosequencing (Biotage, Qiagen, Germantown, Maryland), in which genotypes were verified by visual inspection, with exclusion of artefactual data from further analysis—or by the TaqMan primer/probe system (Premier Biosoft, Palo Alto, California) on an ABI-7900HT device (AB Sciex, Foster City, California [formerly Applied Biosystems]). Reproducibility of diploid genotypes was verified with blinded replicate samples, indicating 98.8% concordance. In the twin/sibling sample, genotypes were acquired as part of a genome-wide

scan (Illumina-610-Quad; Illumina, Inc.) and verified in each subject by TaqMan. rs17514104 Diploid genotypes were in Hardy-Weinberg equilibrium (chi^2: 0.001; p = 0.966; minor allele frequency [MAF]: ~27%–28%). Human *SLC35F3* genetic variants considered in this study are tabulated in Online Table 2.

Statistics and bioinformatics. STATISTICS. Estimates are stated as mean ± 1 SD. Traits that were not normally distributed were log_{10}-transformed prior to parametric data analyses. Two-way analysis of variance (ANOVA) or multivariate general linear modeling, as well as post-hoc corrections, were performed in SPSS version 11.5 (IBM SPSS Statistics, IBM Corporation, Armonk, New York) to evaluate the significance of single variants during in vivo association studies as well as in vitro transporter activity. During marker-on-trait associations in vivo, results of descriptive statistics (mean ± SEM for each diploid genotype) were plotted in order to select the optimal model (additive vs. dominant/recessive) for inferential statistical testing (F, chi-square, p). BP analyses were adjusted for age and sex, as well as the effects of antihypertensive treatment, by adding a fixed value (10/5 mm Hg) to each treated BP, as described (23); although such adjustments are necessarily imperfect, their value is reinforced by restoration of familial (sibling/sibling) BP correlations (23). Parametric, general linear model (typically, 2-way ANOVA) analyses tested associations of *SLC35F3* diploid genotypes and continuous BP traits (mm Hg). Dichotomous variables (e.g., hypertension) were analyzed by proportions and chi-square test. For twin pair analyses, descriptive (genotype-specific mean ± SEM) and inferential (chi-square, p value) statistics were computed across all of the twins using generalized estimating equations (PROC GENMOD) in SAS (SAS Institute Inc., Cary, North Carolina) or SPSS, to account for correlated trait values within each twinship, using an exchangeable correlation matrix (24). In twin pairs, heritability (or the fraction of trait variance accounted for by genetic variance [$h^2 = V_G/V_P$]) was estimated by Sequential Oligogenic Linkage Analysis Routines (SOLAR) software (Texas Biomedical Research Institute, San Antonio, Texas) (25). Haplotypes were imputed from unphased diploid genotypes on the SLC35F3 region using an expectation-maximization algorithm in PLINK version 1.07 (Harvard University, Cambridge, Massachusetts). Meta-analyses were carried out with the command META, testing fixed-effects (i.e., genotype as independent variable) models in STATA-12 (StataCorp LP, College Station, Texas), after individual study regression analysis in SPSS or R, incorporating individual study data to derive significance as well as pooled genotype effect size (beta, or slope per allele) and its SE.

FALSE DISCOVERY RATE. In consideration of testing the effects of multiple correlated alleles (or diploid genotypes) at a locus on a trait, we employed estimation of the false discovery rates in order to minimize false negative results while maximizing true positive results, using the Excel calculator of false discovery rates from p values (Microsoft Corporation, Redmond, Washington).

ALTERNATIVE MRNA SPLICING. Based on transcript and expressed sequence tag (short messenger ribonucleic acid [mRNA] → complementary DNA [cDNA] fragment) evidence (with sequence data processing according to Florea et al. [26]), computationally derived alternative splicing patterns were viewed.

SLC35F3 FUNCTIONAL AND STRUCTURAL PREDICTIONS. Transporter homology predictions were conducted within the Transporter Classification DataBase (TCDB) (Saier Laboratory Bioinformatics Group, University of California, San Diego, California), evaluating sequence similarity as well as functional classification and likely orthologs, paralogs, and membrane-spanning secondary structures. Amino acid sequence alignments were conducted in Clustal-W (now Clustal Omega, European Molecular Biology Laboratory, Wellcome Trust Genome Campus, Hinxton, United Kingdom). Cellular localization (organelle) targeting sequences were evaluated using TargetP (Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark).

SLC35F3 AND THIAMINE METABOLISM. *SLC35F3* EXPRESSION. The human *SLC35F3* full-length cDNA (UniGene Hs.158748, RefSeq mRNA XM_001132419.1, Image clone 5273868, National Center for Biotechnology Information [NCBI] accession BC037878.1, IRAK75-e24) was obtained from Geneservice Ltd. (Cambridge, United Kingdom) in pBluescript-R, and subcloned into the prokaryotic expression plasmid pZE12luc (27), under the control of the $P_{LlacO-1}$ promoter. *Escherichia coli* strain MW25113 (with endogenous thiamine transport [ThiBPQ = sfuABC] and biosynthetic [ThiH] genes inactivated) was transformed by this plasmid, under selection by ampicillin, with expression of SLC35F3 triggered by 1-mM isopropyl β-D-1-thiogalactopyranoside (IPTG).

THIAMINE UPTAKE CATALYZED BY SLC35F3. Thiamine [^{3}H(G)] hydrochloride, at 10 to 20 Ci/mmol (0.37–0.74 TBq/mmol) was obtained from American Radiolabeled Chemicals (ART-0710; St. Louis, Missouri). The uptake protocol followed our previous studies (28), with modifications as noted here. *E. coli* grown in minimal medium (M9 with 0.2% glucose, 100-ng/μl ampicillin, and 1-mM isopropyl β-D-1-thiogalactopyranoside [IPTG]) was harvested in the exponential growth phase, pelleted and washed once in ice-cold M9 medium, and resuspended in M9 (plus 0.2% glucose), with cell density adjusted to optical density at 500 nm (OD_{500}) = 1 (light scattering). [^{3}H]-thiamine was added at 20 nM, and uptake studies were conducted at 18°C; in an initial kinetic experiment (time course), cellular net uptake of [^{3}H]-thiamine, catalyzed by SLC35F3 expression, was maximal by 5 min. Aliquots (100 μl) were periodically removed from the 1-ml cell suspensions, diluted 15-fold with M9 buffer, filtered (0.45-μm filters [Millipore

Corporation, Billerica, Massachusetts]) and washed twice with the same buffer. After the filters were dried under a heat lamp for 15 min, radioactivity was measured by scintillation counting using 10 ml of Biosafe NA scintillation fluid (Research Products International, Mt. Prospect, Illinois). Uptake activity attributed to SLC35F3 alone was obtained by subtracting the activity remaining in the absence of SLC35F3 function from the cDNA-expressed activity, assuming that the absence of *SLC35F3* does not activate some other transporter.

HUMAN ERYTHROCYTE THIAMINE (VITAMIN B_1) DETERMINATION. Blood was obtained from subjects of known diploid genotype at *SLC35F3* rs17514104, specifically major allele (C/C) and minor allele (T/T) homozygotes, into ethylenediaminetetraacetic acid anticoagulant tubes, whereupon whole blood was frozen at −70°C prior to thawing and thiamine assay by a microbiological method, as described (29,30), wherein a thiamine–growth-dependent strain of *Lactobacillus fermentum* is used on a coated 96-well microtiter plate in a commercial spectrophotometric kit (Immundiagnostik AG, Bensheim, Germany). After incubation at 37°C for 48 h, the growth of *Lactobacillus fermentum* is measured turbidimetrically at 630 nm in an enzyme-linked immunosorbent assay spectrophotometric reader, and a standard curve is generated from the dilution series. The amount of vitamin B_1 is directly proportional to the turbidity. Assay standards ranged from 0 to 15 μg/l, and blood samples were typically assayed in duplicate or triplicate at 1:10 dilution. The results correlate well with high-performance liquid chromatography methods (r: 0.886), and the coefficients of variation were 2.75% (intra-assay; n = 28) and 3.81% (interassay; n = 5). Results are expressed in micrograms per liter of blood.

ALLELIC EXPRESSION IMBALANCE AT THE *SLC35F3* LOCUS. The rationale for the allelic expression imbalance (AEI) approach was detailed by Pastinen (31); briefly, AEI was employed to provide evidence that a *cis*-quantitative trait locus (QTL) (in particular, a *cis*-eQTL for mRNA expression) exists at the *SLC35F3* locus; that is, the abundance of *SLC35F3* transcripts is tested for dependence on the diploid genotype (both alleles) at the same locus encoding the transcript.

HUMAN AEI. From 53 CEU (Caucasian of European descent) lymphoblastoid cell lines, genomic DNA and double-stranded cDNA were used for the parallel genotyping and AEI analysis on Infinium Human1M or Human1M-Duo SNP bead microarrays (Illumina, Inc., La Jolla, California), as described (32). Briefly, the results of SNP genotyping and allelic transcript ratios, as defined by averaging across all measured SNPs in each sample for the annotated transcripts, were coupled by linear regression to discover *cis*-acting regulatory SNPs. Prior to averaging allele ratios across each transcript, we applied a polynomial normalization to account for intensity-dependent variation in allelic expression ratios observed in cDNA samples, as previously described (33). Multiple testing was addressed by permutation tests at the transcripts; based on permutation tests, significance of $p < 1E\text{-}7$ was considered genome-wide significant, as defined by a false positive rate of ∼50 to 100 per 10,000 measured transcripts (32).

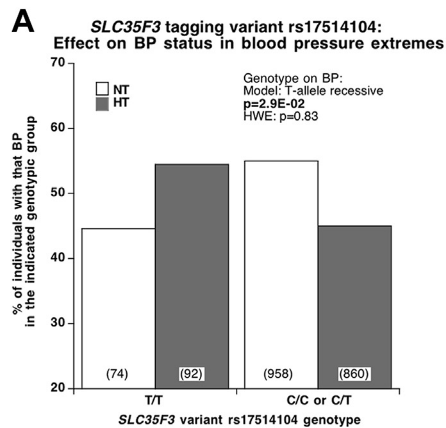
RODENT (MOUSE) AEI. AEI in mouse strains was measured using F1 animals generated from 4 inbred strains with known genetic sequence. Six F1 strains (all possible pairings) were generated from parental strains A/J (A), C3H/HeJ (C), C57BL/6NJ (B), and DBA/2J (D). Liver RNA was obtained from 6 male F1 animals from each F1 strain. The RNA from these 6 animals was pooled into 2 groups of 3, which were hybridized to the mouse diversity array (34), a genotype array containing 600,000 SNPs polymorphic in mouse strains. The genomic DNA from each of the F1s was also hybridized to the mouse diversity array. By comparing the different levels of relative intensity of the alleles in the genomic DNA to the intensity of the alleles in the RNA, we can measure the amount of AEI in each F1 strain.

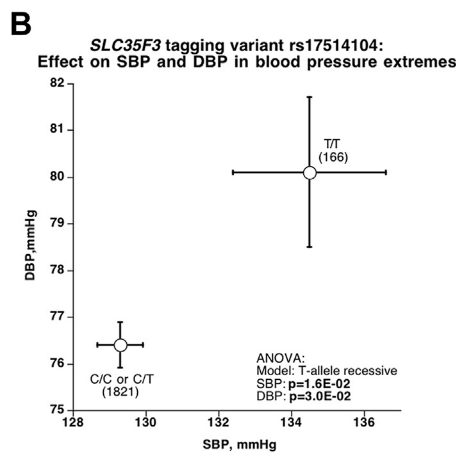
Results

Genome-wide association: population BP-extreme samples in San Diego. Based on relative allele scores in the higher-BP group versus the lower-BP group, on both the Affymetrix 5.0 (∼500 K) and Illumina HumanHap300 (∼300 K) SNP array hybridizations, the variants within each sex analysis were then ranked by allele-frequency differences and prioritized for validation genotyping. At this point, results of pooling were not amenable to standard statistical testing. Tagging SNPs were then selected for further study by individual genotyping in the original (unpooled) population BP-extreme samples (from which the pools had been formed). Because individual genotyping results on *SLC35F3* (a ∼420-kbp locus on chromosome 1q42) achieved statistical significance with BP, that locus was selected for additional evaluation. The BP-associated region of *SLC35F3*, as defined by tag SNPs, was within intron 2 of this ∼420-kbp locus.

SLC35F3 tag SNP rs17514104 (MAF: ∼27% to 28%) was typed in not only the original population BP-extreme individual samples, but also in subjects from a second BP-extreme sample from the same source population. In these samples, examination of the distribution of diploid genotypes by BP status (highest, lowest) (Fig. 2A) revealed an over-representation of T/T (minor allele) homozygosity among hypertensive subjects, with a corresponding depletion of C alleles (among C/C minor allele homozygotes or C/T heterozygotes) in the normotensive subjects (p = 2.9E-02). When BP was evaluated as a continuous trait (Fig. 2B), T/T homozygotes displayed parallel *elevations* in both SBP and DBP (p = 1.6E-02 and p = 3.0E-02, respectively). Meta-analysis indicated that the genotype effects were directionally consistent in each San Diego population sample, for both SBP and DBP (both, p = 1.9E-02) (Table 2).

EXTENSION OF BP ASSOCIATION TO *SLC35F3* IN ICBP. The ICBP program allowed replication into an additional





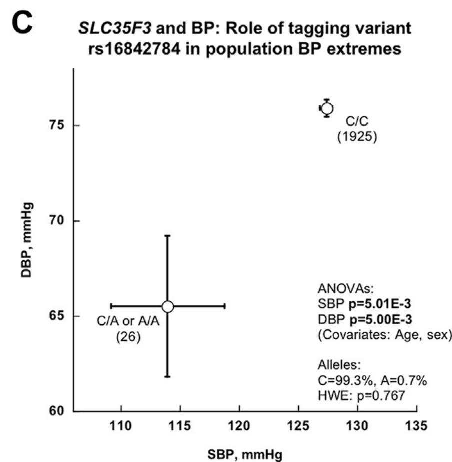


Figure 2 ***SLC35F3* Tagging Variants: Effects on BP in Population-BP-Extreme Subjects, and Replication**

Each of the 2 tagging variants is located within the same linkage disequilibrium block in *SLC35F3* intron 2. Effects of rs17514104 on **(A)** blood pressure (BP) status (hypertensive [HT] or normotensive [NT]) as a dichotomous trait (chi-square) and **(B)** systolic and diastolic BP (SBP and DBP, respectively) as quantitative traits (mm Hg) (analysis of variance [ANOVA], adjusted for age and sex). **(C)** Effect of rs16842784 on SBP and DBP as quantitative traits (mm Hg) (ANOVA, adjusted for sex and age). HWE = Hardy-Weinberg equilibrium.

population sample of >27,000 adults (in North America or western Europe); inspection of the trait-associated region of *SLC35F3* (by LocusZoom plot) (Online Fig. 1) revealed peak BP association in *SLC35F3* intron 2 at rs16842784, which is 17,474 bp downstream (3′) of previous tag SNP rs17514194 (D′: 1.0 in HapMap CEU) (Online Table 2) and within the same linkage disequilibrium (LD) block, as judged by the cM/Mb tracing. Thus we typed rs16842784 (C > A; MAF: ~0.7%–0.8%) in the original San Diego subjects and found that the BP associations were significant for SBP and DBP in both ICBP ($p = 4.18E-04$ and $p = 1.64E-04$, respectively) and the San Diego BP extremes ($p = 1.10E-02$ and $p = 8.00E-03$); subsequently, meta-analysis documented the replication for both SBP and DBP, at $p < 1.00E-04$ and $p < 1.00E-04$ (Table 3).

Figure 2C plots the effects of rs16852784 on SBP and DBP in the San Diego BP-extreme samples. Effects on SBP and DBP were significant, at $p = 5.01E-03$ and $p = 5.00E-03$, respectively, and the effect of the minor/A allele was to *decrease* SBP and DBP by ~13 and ~10 mm Hg. The proportions of trait variance (R^2) explained by rs16852784 in the San Diego population sample on SBP and DBP were 0.063% and 0.096%, highlighting the substantial difference between the effect of a relatively rare (here, MAF: ~0.8%) genetic variant on the trait in subjects segregating the allele (Fig. 2C) versus the effect in the overall population (R^2).

***SLC35F3* HAPLOTYPE AND BP: POPULATION BP-EXTREME SAMPLES IN SAN DIEGO.** Because *SLC35F3* genotype data from both rs16842784 and rs17514104 were available and in LD (D′: 1.0) (Online Table 2), we also examined the effects of rs16842784 (C > A; MAF: ~0.8%) → rs17514104 (C > T; MAF: ~28%) haplotypes on BP. Haplotypes C → C (~71.9%), A → C (~27.6%), and C → T (~0.5%) were observed, whereas doubly minor allele haplotype A → T was not observed (0%). By regression on the BP traits, the rarest haplotype, C → T, had strong effects on both SBP ($p = 0.00103$) and DBP ($p = 0.00031$), whereas haplotype A → C had more modest effects on the traits (SBP: $p = 0.025$; DBP: $p = 0.039$), and the most common haplotype, C → C, did not influence BP.

Bioinformatic analyses of SLC35F3: homology to a thiamine transporter. Within the TCDB international consensus system, human SLC35F3 (UniProt Q8IY50) is a member of the drug/metabolite transporter (category 2.A.7) superfamily of membrane transporters (SLC35F3 classification in TCDB: 2.A.7.24.8).

Sequence similarity analyses within that TCDB group indicate that the closest homologue with functional data is likely to be transporter 2.A.7.24.1, initially represented by Thi74 of yeast (*Saccharomyces cerevisiae*), known as a thiamine-repressible mitochondrial membrane protein (UniProt Q04083; yeast locus YDR438W), although Q04083 did not possess a classic mitochondrial targeting sequence, when tested using TargetP, indeed, Q04083 possessed a hydrophobic amino terminus typical of a signal peptide for the secretory pathway or cell

Table 2 Effects of *SLC35F3* Intronic Variant rs17514104 on the Quantitative BP Traits (SBP and DBP, in mm Hg) in 2 Independent Population Samples: Meta-Analysis

Group	Alleles, Minor/Major	MAF, %	n	Trait	Model	Beta (Slope per Allele)	SE (of Beta)	p Value
KWE1	T/C	27.6	922	SBP	T-recessive	3.492	1.691	–
KWE2	T/C	27.3	1.072	SBP	T-recessive	1.823	1.328	–
Meta-result	T/C	27.5	1.994	SBP	T-recessive	2.460	1.044	1.90E-02
KWE1	T/C	27.6	922	DBP	T-recessive	1.612	1.392	–
KWE2	T/C	27.3	1.072	DBP	T-recessive	1.979	0.964	–
Meta-result	T/C	27.5	1.994	DBP	T-recessive	1.860	0.792	1.90E-02

Covariates in these analyses were age and sex, and BP values in the hypertensive subjects were adjusted for medications, as described in the Methods section.

BP = blood pressure; DBP = diastolic blood pressure; KWE1 = first population BP-extreme sample; KWE2 = second population BP-extreme sample; MAF = minor allele frequency; SBP = systolic blood pressure.

envelope (Online Table 3): MNRVGIDVDHMIGVLLLA VVVVFWV. Sequence homologies between the 2 proteins are tallied in Online Table 3, and secondary structure predictions are given in Online Figures 2A and 2B. Each protein is likely to give rise to 10 membrane-spanning (hydrophobic) domains, in a "2 + 8" configuration, with the most amino-terminal 2 hydrophobic domains somewhat displaced from the remaining 8 hydrophobic domains by a relatively long hydrophilic intervening sequence (between the first 2 and the final 8 hydrophobic domains).

Thiamine metabolism studies. [^{3}H]-THIAMINE UPTAKE CATALYZED BY HUMAN SLC35F3 PROTEIN EXPRESSION IN *E. COLI*. To test whether human SLC35F3 can catalyze thiamine transport, we expressed its cDNA in an *E. coli* strain (previously disabled for thiamine uptake [ThiBPQ] or synthesis [ThiH]; see Methods section), and evaluated cellular uptake of [^{3}H]-thiamine. During SLC35F3 expression, [^{3}H]-thiamine uptake rose substantially, by ~3.3-fold (p = 3.4E-04) (Fig. 3A).

THIAMINE CONTENT OF HUMAN ERYTHROCYTES. STRATIFICATION BY *SLC35F3* GENOTYPE. We measured thiamine in subjects stratified by previously determined rs17514104 genotype, either major allele (C/C) or minor allele (T/T) homozygotes. Subjects with the T/T (i.e., hypertension-associated) genotype displayed a significant reduction in blood thiamine content, from 114.5 ± 0.63 μg/l to 101.2 ± 0.69 μg/l (p = 3.0E-02) (Fig. 3B). Both values were in the range previously reported for healthy subjects.

Heritable cardiac and autonomic effects of human *SLC35F3* genetic variation in twin pairs. Subjects with profound thiamine deficiency experience both cardiovascular (wet beriberi) and neurologic (dry beriberi) manifestations of the deficiency (35). We, therefore, examined the influence of *SLC35F3* on heritable cardiac and autonomic traits in a series of twin pairs. Trait heritability ($h^2 = V_G/V_P$, where V_G is genetic variance and V_P is total phenotypic variance), estimated from twin pair variance components in SOLAR (25), is given in Table 4; each trait displayed substantial heritable determination.

Among cardiac traits (Table 4, Fig. 4A), the rs17514104 T allele conferred an increase in SVI (p = 0.017), with a borderline change in CI (p = 0.065) and a corresponding fall in SVRI (p = 0.027). Among pressor traits (Table 4, Fig. 4B), the T allele conferred an increase in the DBP response to cold stress (p = 0.0036), as well as the post-stress/final DBP (p = 0.027). Among neuroendocrine biochemical traits (Table 4, Fig. 4C), the T allele conferred a decline in secretoneurin (neuroendocrine secretogranin II fragment) secretion (p = 0.0083). Finally, among autonomic neural traits (Table 4), baroreceptor slope (an integrated afferent/efferent reflex arc) was unchanged. False discovery rate analysis indicated that significance was preserved after accounting for the multiple phenotype tests. The second (lower MAF) *SLC35F3* SNP, rs16842784, also affected HR (p = 0.009) and CI (p = 0.007).

Thus, genotype-associated trait changes were primarily cardiac rather than neurological. The relatively young age of this phenotyped twin/sibling cohort (Table 1) should be considered in the interpretation of their cardiovascular data.

Table 3 SBP/DBP Determination by *SLC35F3* Tagging Variant rs16842784: Replication by Meta-Analysis Across 2 Independent Study Groups

Group	Alleles, Minor/Major	MAF. %	n	Trait	Model	Beta (Slope per Allele)	SE (of Beta)	p Value
ICBP	A/C	0.8	27,865	SBP	Additive	−4.986	1.413	4.18E-04
KWE1+2	A/C	0.7	1,951	SBP	Additive	−7.092	3.140	1.10E-02
Overall	A/C	0.8	29,816	SBP	Additive	−5.341	1.289	<1.00E-04
ICBP	A/C	0.8	29,452	DBP	Additive	−3.340	0.886	1.64E-04
KWE1+2	A/C	0.7	1,944	DBP	Additive	−5.394	2.359	8.00E-03
Overall	A/C	0.8	31,396	DBP	Additive	−3.594	0.829	<1.00E-04

Covariates in these analyses were age, sex, and BMI, and BP values in the hypertensive subjects were adjusted for medications, as described in the Methods section.

BMI = body mass index; BP = blood pressure; DBP = diastolic blood pressure; ICBP = International Collaboration for Blood Pressure Genome-Wide Association Studies; KWE1+2 = first and second population BP-extreme samples; MAF = minor allele frequency; SBP = systolic blood pressure.

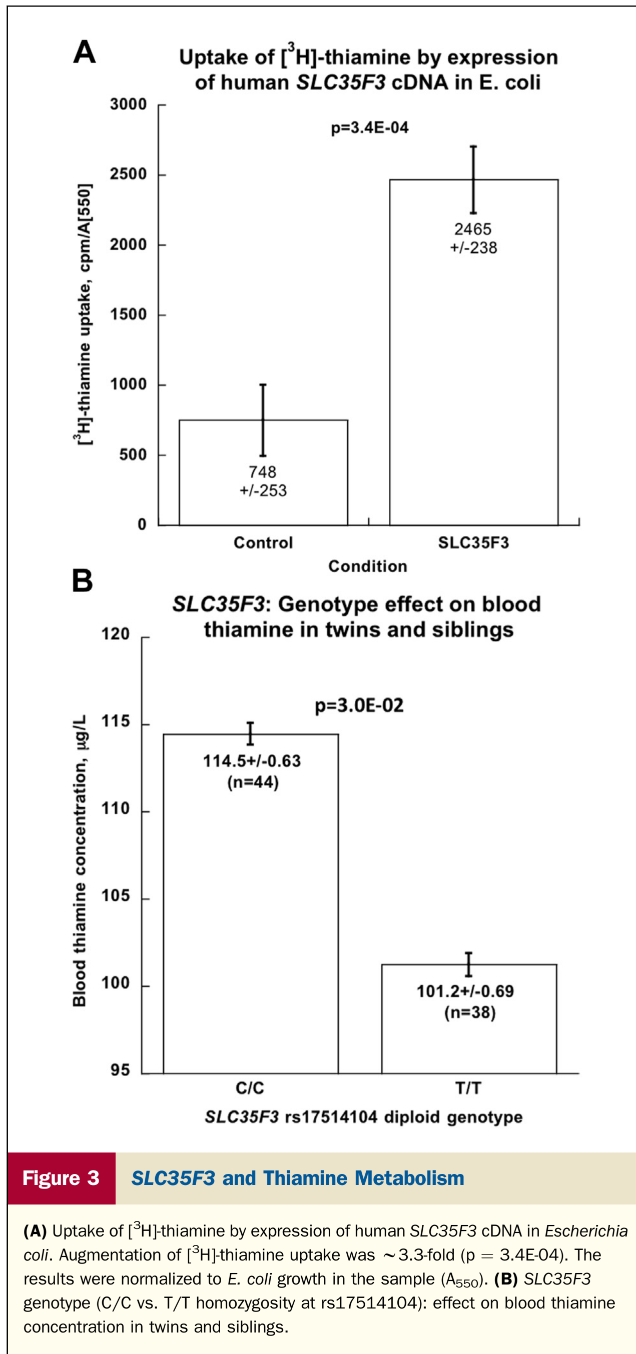


Figure 3 ***SLC35F3* and Thiamine Metabolism**

(A) Uptake of [^{3}H]-thiamine by expression of human *SLC35F3* cDNA in *Escherichia coli*. Augmentation of [^{3}H]-thiamine uptake was ~3.3-fold (p = 3.4E-04). The results were normalized to *E. coli* growth in the sample (A_{550}). **(B)** *SLC35F3* genotype (C/C vs. T/T homozygosity at rs17514104): effect on blood thiamine concentration in twins and siblings.

Physical characteristics of subjects with rs17514104 T/T homozygosity, or altered cardiac and vascular function. We profiled each group we studied (twin pairs; population BP extremes, samples 1 and 2) to test whether there were readily identifiable demographic or clinical signatures of the underlying genotype. The twins were profiled for cardiac and vascular function; stratification of the twins/siblings by rs17514104 diploid genotype did not reveal differences (all, $p > 0.15$ by ANOVA) in age, sex, body mass index, height, or weight. Within the BP-extreme samples, stratification of either cohort 1 (n = 934) or cohort 2 (n = 1,072) by rs17514104 diploid genotype did not reveal any differences (all, $p > 0.05$ by ANOVA) in age, sex, body mass index, height, or weight.

Hematologic effects of human *SLC35F3* genetic variation. Because patients with other thiamine transporter (*SLC19A2, SLC19A3*) mutations may exhibit megaloblastic anemia, our subjects from the population BP-extreme samples (Fig. 1) also had determination of the following hematologic traits: hemoglobin, hematocrit, mean corpuscular volume, ferritin, and transferrin saturation. None of these traits was influenced by *SLC35F3* rs17514104 genotype (all nonsignificant) in our subjects, which is perhaps not surprising in that depletions of erythrocyte thiamine in our subjects (p = 3.0E-02) (Fig. 3B) still remained within the typical range of normal.

AEI (allele-specific expression) at *SLC35F3*. AEI can provide independent evidence that a *cis*-QTL does exist at a locus, such as *SLC35F3* (see Methods section).

HUMAN. Two nearby SNP variants (separated by only 501 bp; LD as D′: 1.0) (Online Table 2) within the *SLC35F3* locus (both in intron 2) achieved substantial significance in their effects on *SLC35F3* transcript abundance in CEU lymphoblastoid cells (Online Table 4): rs10910399 (p = 4.80E-07; R^2: 0.4005; C-allele overexpressed) and rs12029247 (p = 5.92E-07; R^2: 0.3956; A-allele overexpressed). Thus these 2 SNPs accounted for ~40% of the variance in transcript abundance. The relative positions of these 2 AEI SNPs are given in Online Table 2. Notably, these 2 AEI SNPs were within 78,974 bp of the BP-tagging SNPs (all 4 in intron 2 within the same LD block), with LD estimated at D′ 0.406. However, neither of these 2 AEI SNPs predicted either SBP or DBP in the ICBP cohort ($p > 0.2$ in each case).

RODENT (MOUSE). AEI was consistently observed at *SLC35F3* in F1 strains constructed from inbred mouse strains. Six F1 strains (all possible pairings) were generated from parental strains A/J (A), C3H/HeJ (C), C57BL/6NJ (B), and DBA/2J (D). We observed strong signals of AEI in 4 of the 6 groups of F1 animals: AxB, AxD, HxB, and HxD. The AEI signal was observed from 2 SNPs (Chr8:128874317 and Chr8:128904545). For F1s AxH and BxD, the SNPs are homozygous, preventing our ability to observe AEI. However, for the 4 of F1s (AxB, AxD, HxB, and HxD) where the SNPs were heterozygous, the ratio of intensities of the 2 alleles from the RNA hybridization was significantly different from the ratio of intensities of the 2 alleles from the DNA hybridization ($p < 0.0001$), providing evidence of AEI at this locus.

Discussion

Overview. Hypertension is perhaps the most common and ultimately fatal of the cardiovascular risk factors (1). Complex traits (36) such as hypertension remain largely enigmatic in cause, with evidence for both hereditary and environmental determinants. Twin and family studies suggest substantial heritability (h^2) of BP traits, yet the genes

Table 4 *SLC35F3* **Variant rs17514104: Effect on Heritable Autonomic Traits in Twins and Siblings***

Category/Trait	Trait Heritability (h^2)† in Twin Pairs (n = 338) Estimate ± SEM	p Value	rs17514104 (C/T) Diploid Genotype, mean ± SEM (n = 399‡) C/C (n = 225)	C/T (n = 147)	T/T (n = 36)	p Value	FDR§
Biochemical							
Plasma secretoneurin (SCG2 fragment), nM	0.73 ± 0.04	3.08E-26‖	0.137 ± 0.0029	0.126 ± 0.0034	0.124 ± 0.0065	0.0083‖	0.017‖
Physiological/hemodynamic (resting)							
Heart rate, beats/min	0.31 ± 0.07	3.94E-05‖	71.2 ± 1.44	74.6 ± 1.62	72.2 ± 2.42	0.413	0.184
CI, l/min/m^2	0.71 ± 0.04	2.51E-19‖	2.73 ± 0.033	2.78 ± 0.041	2.86 ± 0.081	0.065	0.037‖
SVI,¶ ml/min/m^2	0.66 ± 0.05	1.81E-17‖	39.3 ± 0.31	39.6 ± 0.35	41.1 ± 0.77	0.017‖	0.023‖
SVRI,¶ dynes/s/cm^5/m^2	0.55 ± 0.06	7.70E-12‖	1517 ± 20.7	1500 ± 27.3	1411 ± 49.1	0.027‖	0.027‖
Autonomic: baroreceptor slope, ms/mm Hg							
Upward deflections	0.25 ± 0.08	1.91E-03‖	15.1 ± 0.67	14.2 ± 0.69	14.1 ± 1.06	0.730	0.265
Downward deflections	0.37 ± 0.08	6.30E-06‖	11.8 ± 0.45	11.5 ± 0.50	11.7 ± 0.94	0.718	0.287
Environmental stress (cold pressor test), mm Hg							
Delta-SBP	0.65 ± 0.05	2.60E-16‖	11.0 ± 1.74	14.3 ± 2.03	18.5 ± 5.57	0.053	0.035d
Delta-DBP	0.54 ± 0.06	7.52E-11‖	8.6 ± 1.06	11.5 ± 1.24	16.6 ± 2.82	0.0036‖	0.014‖
Post-SBP	0.29 ± 0.07	1.48E-04‖	128.2 ± 2.4	131.2 ± 3.13	132.6 ± 6.50	0.142	0.071
Post-DBP	0.29 ± 0.08	1.67E-04‖	72.1 ± 1.42	75.5 ± 1.66	77.8 ± 3.68	0.027‖	0.027‖

*Descriptive statistics were computed in twins and siblings using GEE in SAS (SAS Institute Inc., Cary, North Carolina). †Heritability (h^2) was estimated from variance components in twins using Sequential Oligogenic Linkage Analysis Routines (SOLAR) software (Texas Biomedical Research Institute, San Antonio, Texas). ‡Some subjects had >1 genotype. §FDR = false discovery rate: In consideration of testing the effects of multiple correlated alleles (or diploid genotypes) at a locus on a trait, we employed estimation of the FDR to minimize false negative results while maximizing true positive results, using the Excel calculator of FDR from p values, at http://www.rowett.ac.uk/~gwh/fdr.html. Twin and sibling analyses were adjusted for sex and age. ‖$p < 0.05$. ¶Hemodynamic value (cardiac output or systemic vascular resistance) normalized to body surface area (in m^2).

CI = cardiac index; DBP = diastolic blood pressure; SBP = systolic blood pressure; SVI = stroke volume index; SVRI = systemic vascular resistance index.

involved in the bulk of BP variance in the population remain elusive (37). Although particular (and relatively uncommon) families display Mendelian cosegregation of hypertension with alterations in renal electrolyte transport (5), heritable BP variation in the population remains largely unaccounted for, despite recent successes in genome-wide association of large population samples (6).

Here we harnessed the power of trait extremes (38) coupled with a pooled genome-wide association strategy (9) to approach genetic determinants of hypertension in humans. Exploiting the statistical power that is possible with a very large sample size, we selected subjects through a sampling design with the power to detect small contributions to BP variation, ascertaining subjects within the highest- and lowest-fifth percentiles of BP in a primary care setting in southern California using a large community database (>53,000 subjects) of men and women whose BP was measured at routine health maintenance visits. Genomic DNA pooling is an effective initial strategy that has been successful in detecting genetic associations in amyotrophic lateral sclerosis (39), memory performance (40), progressive supranuclear palsy (41), multiple sclerosis (42), and melanoma (43).

By these strategies, we identified a novel BP association on chromosome 1q42, within the *SLC35F3* locus encoding a previously uncharacterized solute transporter with homology to an unspecified thiamine transporter in yeast; we then documented that SLC35F3 can catalyze thiamine (vitamin B_1) transport, and that risk-allele homozygotes displayed depletion in blood thiamine content. AEI confirmed that *cis* variation at the *SLC35F3* locus influenced gene expression, and both the clinical marker-on-hypertension effect and the AEI effect resulted from allelic variation in *SLC35F3* intron 2.

Although the involvement of *SLC35F3* in hypertension is an emerging proposal, the picture now derives from multiple lines of evidence:

- Initial pooled association was found with both Affymetrix and Illumina chips.
- Association was verified by individual genotyping in the same population sample with BP extremes.
- Association was extended by meta-analysis into an independent BP-extreme sample from the same population, as well as a new population.
- "Intermediate" (or pathogenic precursor) phenotypes for hypertension, such as cold stress–induced change in DBP and post-stress DBP observed in the twin and sibling pairs, provide a physiological basis for the *SLC35F3* association.
- *SLC35F3* may encode a novel thiamine transporter, as evidenced by depletion of in vivo blood thiamine stores, and catalysis of in vitro bacterial uptake of $[^3H]$-thiamine.

Role of thiamine transport and *SLC35F3* in hypertension risk. We were able to document that *SLC35F3* can mediate thiamine transport and that risk-allele homozygotes (T/T)

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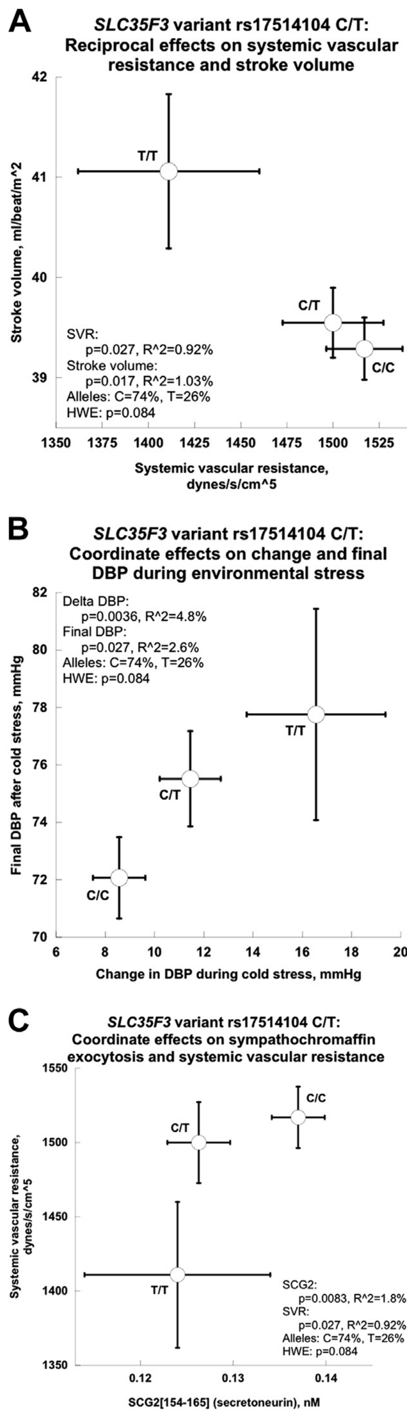

Figure 4 ***SLC35F3* and Hypertension: Effects on Early Pathogenic (or "Intermediate") Traits in Twins and Siblings**

Effects of *SLC35F3* variant rs17514104 on **(A)** systemic vascular resistance (SVR) and stroke volume, **(B)** change in diastolic blood pressure (DBP) and final DBP during cold stress, and **(C)** sympathochromaffin exocytosis (secretion of SCG2 fragment secretoneurin) and SVR. Twin and sibling analyses were adjusted for sex and age. HWE = Hardy-Weinberg equilibrium.

have diminished blood thiamine content. Two other high-affinity thiamine transporters are known: SLC19A2 and SLC19A3. Rare Mendelian deficiency of SLC19A2 leads to thiamine-responsive megaloblastic anemia (with diabetes and deafness) (44); however, our *SLC35F3* risk allele was not associated with alterations in hemoglobin or mean corpuscular volume. Rare variants at *SLC19A3* cause autosomal recessive basal ganglia disease (45). We have not yet characterized the relative affinity or abundance of SLC35F3 in comparison to the better characterized thiamine transporters SLC19A2 and SLC19A3, particularly in relevant cells in brain, heart, and blood vessels.

Because thiamine deficiency can cause a spectrum of phenotypes, from cardiovascular (wet beriberi) to neurologic (dry beriberi), we evaluated such traits in a series of twins and siblings and found that such traits were heritable, and the *SLC35F3* risk (T) allele was associated with alterations of cardiac stroke volume and SVR, as well as pressor responses, although not neural reflex control of the circulation. Thus, the modest thiamine decrement we observed here apparently does have hemodynamic (although not autonomic) consequences, consistent with those seen in wet beriberi.

Thiamine (vitamin B_1) replacement or supplementation reportedly corrects abnormalities of both BP and glucose metabolism in the CD36 (fatty acid translocase)-defective spontaneously hypertensive rat (SHR) (38). In this model, thiamine repletion not only corrected glucose oxidation, hyperinsulinemia, and elevated BP, but also decreased expression of mRNAs encoding angiotensin-converting enzyme, angiotensin, and the angiotensin II type 1 receptor (38). Also of note for our human cardiovascular and autonomic findings on *SLC35F3*, dietary depletion of thiamine in the SHR (38) resulted primarily in cardiovascular signs of wet beriberi, consistent with our cardiac findings in twins and siblings. The *SLC35F3* locus also lies within the confidence interval (rat chromosome 19; human chromosomes 1q42.13–1q42.3) for linkage to SBP at rat BP QTL 32 (Bp32) in recombinant inbred strains from an HXBxBXH cross (46), wherein the hypertensive strain is the SHR.

In humans, thiamine administration reportedly improves endothelium-dependent vasodilation (47); an older report suggested a beneficial effect of thiamine on BP in human hypertension (48), and more recently thiamine lowered SBP in elderly patients with biochemically documented subclinical thiamine deficiency (49), for which diuretic use may be a risk factor (50).

Why might thiamine exert such beneficial effects in hypertension? In addition to potential repair of the wet beriberi lesions noted earlier, analyses of the transcriptome in rodent genetic models of hypertension reveal widespread changes in mRNAs encoding components of carbohydrate intermediary metabolism (51–53), including the thiamine-dependent transketolase step in the pentose phosphate pathway.

Functional genetic variation at *SLC35F3*? What genetic variant(s) at *SLC35F3* confers functional change on the gene,

either qualitative or quantitative? At the human *SLC35F3* locus on chromosome 1q42, common nonsynonymous (amino acid replacement) variation has not been detected at *SLC35F3* (NCBI dbSNP), despite extensive exome sequencing of 2n = 4,504 chromosomes as part of the National Heart, Lung and Blood Institute Exome Sequencing Project, as well as sequencing across the entire region in 2n = 358 chromosomes within the NCBI's 1000 Genomes Project. Thus, the precise genetic variant(s) underlying the thiamine and BP traits in this report remains elusive, but is therefore likely to be noncoding (and perhaps regulatory). Notably, both the BP-associated SNPs (rs16842784, rs17514104) and the SNPs displaying AEI for transcript abundance (rs10910399, rs12029247) (Online Table 2) are contained within a 78,974-bp stretch within intron 2 (Online Table 2), and each is in LD with the others (minimum D′: 0.406). Thus, a relatively circumscribed region of *SLC35F3* seems to harbor alleles' contribution to variance in both BP and transcript abundance.

The peak genetic association within *SLC35F3* intron 2 suggests splice variation as a possible genetic culprit; indeed, based on transcript and expressed sequence tag, splice variation in this region of *SLC35F3* does occur, resulting in alternative retention/exclusion of RefSeq (NM_173508) exon 1, 2, or 3.

A micro-RNA gene encoding hsa-miR-4671 is embedded within intron 3 of *SLC35F3*; however, the downstream targets of hsa-miR-4671 are not yet defined, and thus we did not pursue this micro-RNA in light of our positive findings on thiamine transport and storage.

The relative abundance of *SLC35F3* expression across human tissues has been reported (54): *SLC35F3* is widely expressed across tissues pertinent to BP control, such as brain (maximal in cerebellum), adipocytes, heart, kidney, and smooth muscle. In the mouse transcriptome, *SLC35F3* is most highly expressed in the central nervous system: ciliary bodies > retinal pigment epithelium > iris > dorsal striatum > nucleus accumbens > spinal cord.

Advantages and limitations of this study. STRENGTHS. This study began with a genome-wide approach in subjects with the most extreme BP values in the population, an approach that has enhanced power to detect genetic marker-on-trait association, even when the genotype contributes only modestly to total trait variation (38). Evidence for involvement of the *SLC35F3* gene in hypertension then emerged from multiple lines of evidence: clinical, genetic, and biochemical.

CAVEATS. Our studies on *SLC35F3* in hypertension and autonomic physiology were conducted in subjects of limited diversity in biogeographic ancestry; studies of additional ethnic or population groups would be required to evaluate whether these *SLC35F3* results are of more general importance across the overall population. Caution should be exercised in interpreting the results of pooled genetic association studies; however, we undertook individual genotyping, based on preliminary pooling results, in order to apply appropriate statistical tests to our results. "Intermediate" cardiovascular traits, such as CO, were studied in a relatively

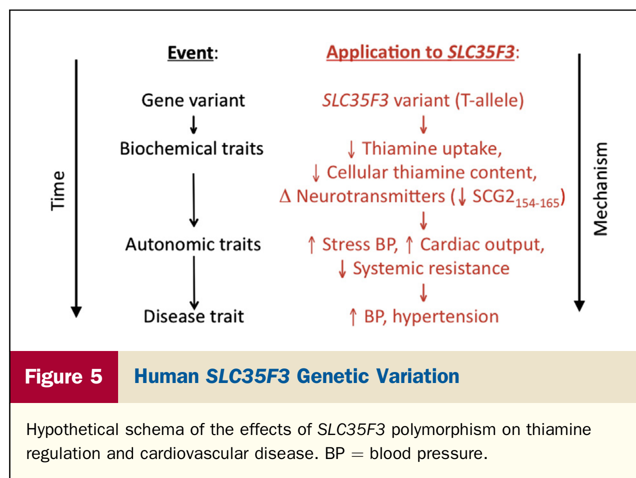

Figure 5 **Human *SLC35F3* Genetic Variation**

Hypothetical schema of the effects of *SLC35F3* polymorphism on thiamine regulation and cardiovascular disease. BP = blood pressure.

young cohort of twins and siblings (Table 1), on average ~18 years younger than the population BP-extreme sample and ~10 years younger than the ICBP; because the relative contribution of elevated CO to hypertension declines with advancing age, additional cardiovascular (output and resistance) data in older subjects would be useful. Despite initial findings that SLC35F3 is involved in thiamine transport, we do not yet understand the details of such action, including the affinity, capacity, and selectivity of SLC35F3 for its thiamine substrate, nor have we examined the effect of thiamine replacement upon BP or its precursor traits, although previous work in animals (38) and humans (48) suggests a beneficial effect.

Conclusions and Perspectives

We coupled 2 novel strategies, quantitative trait-extreme sampling and pooled genome-wide association, to discover a new hypertension-susceptibility locus, uncovering a previously unsuspected thiamine transporter whose genetic variants predicted several disturbances in cardiac and autonomic function. Figure 5 integrates our findings into an overall hypothetical schema. The results have implications for the heritability of the BP trait and the pathogenesis systemic hypertension and open up new approaches to the rational prevention or treatment of the trait.

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