Associations of Variants in the CACNA1A and CACNA1C Genes With Longitudinal Blood Pressure Changes and Hypertension Incidence: The GenSalt Study

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BACKGROUND
We aimed to examine the associations of voltage-dependent calcium-channel genes CACNA1A and CACNA1C with blood pressure (BP) changes and hypertension incidence in a longitudinal family study.

METHODS
A total of 1,768 Han Chinese participants from the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) follow-up study were eligible for the current study. Nine BP measurements were obtained at baseline and each follow-up visit using a random-zero sphygmomanometer. Mixed-effect models were used to assess additive associations of 176 tag single-nucleotide polymorphisms (SNPs) in CACNA1A and CACNA1C with longitudinal BP changes and hypertension incidence. The truncated product method was used for gene-based analysis. The Bonferroni correction was used for adjustment of multiple testing.

RESULTS
During an average of 7.2 years of follow-up, 512 (32.1%) participants developed hypertension. CACNA1A SNP rs8182538 was significantly associated with longitudinal diastolic BP (DBP) change after Bonferroni correction (Pinteraction = 9.90 × 10−5), with mean DBP increases of 0.85, 1.03, and 1.19 mm Hg per year for participants with genotypes C/C, C/T, and T/T, respectively. A similar trend was observed for the association of rs8182538 with systolic BP (SBP) change. In the gene-based analysis, CACNA1A and CACNA1C were significantly associated with DBP change (P = 2.0 × 10−5) and SBP change (P = 1.4 × 10−4) after Bonferroni correction, respectively. The gene-based associations remained significant after removing rs8182538 within CACNA1A and rs758116 within CACNA1C in sensitivity analysis.

CONCLUSIONS
Our findings indicated that CACNA1A and CACNA1C might contribute to BP changes over time in Han Chinese population. Further replication of these findings is warranted.

Keywords: blood pressure; CACNA1A; CACNA1C; genetics; hypertension; longitudinal changes.

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High blood pressure (BP) is the leading risk factor for global burden of disease and mortality, contributing to 7.0% of disability-adjusted life-years and 9.4 million deaths, respectively.1,2 The estimated number of adults with hypertension will be increased to 1.56 billion by 2025.3 As a complex trait, hypertension susceptibility was determined by environmental and genetic factors, as well as their interactions. Approximately 31%–68% of BP variation in populations may be genetically determined.4 Although there has been substantial progress elucidating the genetic determinants underlying BP regulation, the exact genomic mechanisms remain largely unknown.

Voltage-dependent Ca2+ channels (VDCCs) mediating the entry of Ca2+ into excitable cells are involved in a variety of physiological processes, including vascular smooth muscle contraction, which is crucial in BP regulation.5,6 It has been reported that the upregulation of VDCCs expression may contribute to Ca2+ imbalances and result in hypertension.7,8 CACNA1A and CACNA1C genes encode alpha-1a and alpha-1c subunits of VDCCs, respectively, which are targets of calcium-channel blockers (CCBs).6 A large-scale study conducted in 86,588 individuals suggested that polymorphisms in the CACNA1A and CACNA1C genes were potentially associated with cross-sectional BP and hypertension.9

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Further pharmacogenetic analyses had identified several variants in CACNA1C were associated with the efficacy of antihypertensive effects of CCBs in hypertensive patients among different populations. Therefore, we presumed that the CACNA1A and CACNA1C genes had crucial effects on the regulation of BP. However, single marker and aggregate associations of CACNA1A and CACNA1C genes with BP-related phenotypes were not investigated in a cohort study. Furthermore, very few of these studies have been conducted in Han Chinese population. Thus, we aimed to examine the relationships of CACNA1A and CACNA1C with BP changes over time and incident hypertension by using both single-marker and gene-based association analyses. The current study was conducted in a large, homogeneous sample of Han Chinese participants from the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) follow-up study.

METHODS

Study population

The GenSalt study was conducted among Han Chinese population in 6 rural villages in Northern China from 2003 to 2005. Details of the study design and methods have been published elsewhere. Briefly, a community-based BP screening was conducted among persons aged 18–60 years in the study villages to identify potential probands and their families. Those with mean systolic BP (SBP) of 130–160 mm Hg and/or diastolic BP (DBP) of 85–100 mm Hg and no use of antihypertensive medications, as well as their parents, spouses, siblings, and offspring were recruited for the study. Individuals were excluded if they had stage 2 hypertension, secondary hypertension, a history of cardiovascular disease or diabetes, pregnancy, heavy alcohol consumption, or low-sodium diet lifestyle.

Institutional review boards at all of the participating institutions approved the GenSalt study. Written informed consents were obtained from all participants.

GenSalt baseline data collection

A standard questionnaire was administered by trained investigators at the baseline examination to collect information on demographic characteristics, personal and family medical history, and lifestyle risk factors. Three morning BP measurements were obtained according to standard protocol on each of the 3 days of baseline observation by trained and certified observers using a random-zero sphygmomanometer. BP was measured from the right arm of participants in the sitting position after 5 minutes of rest. In addition, participants were advised to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 minutes prior to their BP measurements. The average of the 9 BP readings was used for analysis. Body weight and height were measured twice in light indoor clothing without shoes. Body mass index (BMI) was calculated as kilograms per square meter (kg/m$^2$).

GenSalt follow-up

The GenSalt study participants were re-examined from 2008 to 2009 and 2011 to 2012 in the GenSalt follow-up study. Three BP measurements were obtained in the morning during each of 3 days of follow-up visits according to the same protocol used in the GenSalt study. Hypertension was defined as SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg or the use of antihypertensive medications.

Among 1,906 eligible participants from 633 families who completed the baseline examination, 117 individuals were missing BP information at both of the follow-up visits, and another 21 individuals were missing genotype data. The remaining 1,768 participants (92.8%) were eligible for our analysis.

Genotype data and quality control

The genes CACNA1A and CACNA1C were selected based on their potential effect on BP regulation. Within the 2 candidate genes (±5,000-bp flanking regions), 369 single-nucleotide polymorphisms (SNPs) were genotyped on the Affymetrix 6.0 platform (Affymetrix, Santa Clara, CA). Quality control, including checks of Mendelian consistency, genotyping call rate, minor allele frequency, and Hardy–Weinberg equilibrium, was performed using Plink software (version 1.05; Dr Sean Purcell, http://pngu.mgh.harvard.edu/~purcell/plink/). Sixty-two SNPs were excluded because of minor allele frequency < 1%, genotyping call rate < 95%, or deviation from the Hardy–Weinberg equilibrium after correction for multiple testing. After quality control, we selected tag-SNPs with pairwise $r^2$ thresholds of less than 0.9 using Haploview software (version 4.2, http://www.broad.mit.edu/mpg/haploview/). A total of 176 tag-SNPs in CACNA1A and CACNA1C were included in the current analysis. Characteristics of these SNPs were presented in Supplementary Table 1.

Statistical analysis

The characteristics of study participants were presented as means for continuous variables and as frequencies (percentages) for categorical variables. The additive associations between genotyped SNPs and longitudinal SBP and DBP changes were analyzed using mixed-effect linear regression models to accommodate the longitudinal, family-based design of the GenSalt study.16,17 Autoregressive and compound symmetry covariance matrices were used to account for the correlations of repeated measurements within individuals and of individuals within families, respectively. To evaluate the significance of the association of each SNP with longitudinal BP change, a genotype by follow-up time interaction term and the main effects of these variables were included in the models. Models were additionally adjusted for the fixed effects of age, gender, and BMI using the PROC MIXED procedure in SAS (version 9.3; SAS Institute, Cary, NC). The mixed-effect model we used was as follows:

$$ \gamma_{ijk} = \beta_0 + \beta_1 \times \text{age}_{ij} + \beta_2 \times \text{gender}_{ij} + \beta_3 \times \text{BMI}_{ij} + \beta_4 \times \text{SNP}_{ij} + \beta_5 \times \text{time}_{ijk} + \beta_6 \times \text{SNP}_{ij} \times \text{time}_{ijk} + a_{ijk} + b_{ijk} + e_{ijk} $$

In the formula, $\beta_0$ was the mean after accounting for covariates, genetic effects, and the interaction term. The terms $\text{age}_{ij}$, $\text{gender}_{ij}$, and $\text{BMI}_{ij}$ represented baseline age, gender, and BMI.
RESULTS

The characteristics of 1,768 participants during the initial and 2 follow-up interviews were summarized in Table 1. On average, participants were 39.0 years of age, had a BMI of 23.4 kg/m², and had mean SBP and DBP of 116.9 and 73.8 mm Hg at baseline, respectively. Among the baseline 1,768 participants, 52.3% were male. During a mean follow-up of 7.2 years, 512 (32.1%) participants developed hypertension, and mean SBP and DBP of participants increased by 12.2 and 8.4 mm Hg, respectively.

Figure 1 displays the associations of 176 tag-SNPs in CACNA1A and CACNA1C with BP changes and hypertension incidence. Marker rs8182538 in CACNA1A was significantly associated with longitudinal change in DBP after Bonferroni correction \((P_{\text{interaction}} = 9.9 \times 10^{-5})\). Although no SNP showed significant association with SBP change over-time or hypertension incidence after adjustment for multiple testing, SNP rs8182538 achieved nominal significance for longitudinal SBP change. Sensitivity analyses excluding those participants taking antihypertensive treatment from the analyses of BP changes revealed similar results (data not shown).

The average change in BP per year and odds ratios for hypertension incidence according to genotypes of significant SNP is shown in Table 2. For CACNA1A marker rs8182538, each copy of the minor T allele was associated with larger increases in DBP, with mean DBP increases of 0.85, 1.03, and 1.19 mm Hg for genotypes C/C, C/T, and T/T, respectively. Similar trends were found for SBP changes and odds ratios for hypertension incidence.

Findings of gene-based analysis are presented in Table 3. Genetic variation in CACNA1A and CACNA1C was found to be associated with DBP and SBP changes over time after Bonferroni correction, respectively \((P = 2.0 \times 10^{-5} \text{ for CACNA1A and } P = 1.4 \times 10^{-6} \text{ for CACNA1C})\). No genetic variation in CACNA1A and CACNA1C was significantly associated with incident hypertension. Sensitivity analyses removing the most significant SNP within each gene \((rs8182538 \text{ in CACNA1A and } rs758116 \text{ in CACNA1C})\) also identified significant associations of CACNA1A and CACNA1C with DBP and SBP changes,
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respectively (\(P = 3.5 \times 10^{-4}\) for CACNA1A and \(P = 7.1 \times 10^{-4}\) for CACNA1C).

**DISCUSSION**

To our best knowledge, the current study is the first candidate gene study to investigate the association between CACNA1A and CACNA1C genes with longitudinal BP changes and hypertension incidence in the Chinese population. One marker of the CACNA1A, rs8182538, was found to be significantly associated with DBP change over time after Bonferroni correction for multiple testing (\(P = 9.9 \times 10^{-5}\)).

VDCCs mediate the influx of Ca\(^{2+}\) into excitable cells and are also involved in a variety of calcium-dependent processes, including vascular smooth muscle contraction, which was crucial in BP regulation. For example, upregulation of VDCCs expression may contribute to Ca\(^{2+}\) imbalances and result in hypertension.

Tajada et al.\textsuperscript{20} found that the increased Ca\(^{2+}\) influx finally led to abnormal arterial tone due to the increased VDCCs function in hypertensive mice.

**Table 2.** Association of rs8182538 with BP changes and hypertension incidence among 1,768 GenSalt participants

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Chromosome</th>
<th>Position</th>
<th>Genotype (N)</th>
<th>SBP (\beta) (SE)</th>
<th>DBP (\beta) (SE)</th>
<th>HTN incidence OR</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACNA1A</td>
<td>rs8182538</td>
<td>19</td>
<td>13416170</td>
<td>C/C (N = 528)</td>
<td>1.35 (0.09)</td>
<td>0.85 (0.06)</td>
<td>9.9 \times 10^{-5}</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C/T (N = 900)</td>
<td>1.55 (0.07)</td>
<td>1.03 (0.05)</td>
<td>1.23 (0.87, 1.75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T/T (N = 340)</td>
<td>1.61 (0.12)</td>
<td>1.19 (0.08)</td>
<td>1.56 (1.20, 2.03)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Gene-based associations of CACNA1A and CACNA1C with BP changes and hypertension incidence

<table>
<thead>
<tr>
<th>Gene</th>
<th>SBP change</th>
<th>DBP change</th>
<th>HTN incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACNA1A</td>
<td>0.673</td>
<td>2.0 \times 10^{-4}</td>
<td>0.129</td>
</tr>
<tr>
<td>CACNA1C</td>
<td>1.4 \times 10^{-4}</td>
<td>0.296</td>
<td>0.104</td>
</tr>
</tbody>
</table>

**Figure 1.** \(\log_{10}\) \(P\) values for the 176 single-nucleotide polymorphisms (SNPs) in CACNA1A and CACNA1C with longitudinal changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension (HTN) incidence. The squares and circles indicate \(P\) values for the testing of genotype by follow-up time interactions for SBP and DBP, respectively. The triangles indicate \(P\) values for the testing of SNP effect on HTN incidence. SNP rs8182538 was significantly associated with longitudinal changes in DBP after Bonferroni correction for multiple testing (\(P = 9.9 \times 10^{-5}\)).
Moreover, potential interaction of VDCCs expression and RhoA and ERK/p38 mitogen-activated protein kinase pathways could affect the phenotype of vascular smooth muscle cell, which essentially participate in hypertension mechanism. VDCCs are multisubunit complexes, and the channel activity is directed by the pore-forming alpha-1 subunit. CACNA1A and CACNA1C genes encode alpha-1a and alpha-1c subunits, respectively, which serve as targets of CCBs.

The present study provides the first evidence of association of CACNA1A marker rs8182538 with BP changes over time. Participants with genotypes C/C, C/T, and T/T had mean DBP increases of 0.85, 1.03, and 1.19 mm Hg, respectively. Similar trends of SBP increases were also shown as 1.35, 1.55, and 1.61 mm Hg for C/C, C/T, and T/T, respectively. This marker is located in an intronic region of CACNA1A without known functional relevance. A large study involving 86,588 participants found nominal evidence of association of CACNA1A marker rs1985579 polymorphisms with BP traits. Unfortunately, neither rs1985579 nor its proxy was genotyped by our study. By using gene-based analysis, we found that CACNA1A was significantly associated with longitudinal DBP change. This finding remained even after removal of rs8182538, revealing that gene-based analysis was not only driven by the observed single-marker association. It was worth pointing out that there might be more than 1 variant associated with BP changes in the CACNA1A, which could not be directly identified by less powerful single-marker analysis. It highlighted the importance of considering both singular and joint effects of variants to elucidate the genetic architecture of complex phenotypes such as longitudinal BP changes. Resequencing followed by functional studies will be warranted to determine the causal variant(s) in CACNA1A that may influence long-term BP regulation and hypertension.

In addition, gene-based analysis found that CACNA1C was significantly associated with SBP change over time, although no single marker in the CACNA1C region showed statistical significant association with BP changes or incident hypertension after correction for multiple testing. Several variants in CACNA1C have been reported to affect the efficacy of CCB in the treatment of hypertension. For instance, a retrospective pharmacogenetic analysis involving 120 Caucasian hypertensive participants found that 3 SNPs in CACNA1C, rs2239128, rs2239050, and rs2238032, had significant associations with antihypertensive outcome, combining to yield a positive treatment outcome of about 15%–80%. In addition, a sequence-proven variant, CACNA1C 527974 G>A, was associated with the antihypertensive effects of L-type dihydropyridine CCBs in Japanese patients with hypertension. However, potential functions of these genetic polymorphisms are still unknown. Furthermore, the human CACNA1C gene, spanning > 500 kb, undergoes extensive mRNA splicing that will bring numerous isoforms with various functions of physiological regulation.

REFERENCES
