Genetic Variation in the β2 Subunit of the Voltage-Gated Calcium Channel and Pharmacogenetic Association With Adverse Cardiovascular Outcomes in the INternational VErapamil SR-Trandolapril STudy GENETic Substudy (INVEST-GENES)

Yuxin Niu, PhD*; Yan Gong, PhD*; Taimour Y. Langae, PhD; Heather M. Davis, PharmD; Hazem Elewa, PhD; Amber L. Beitselshees, PharmD, MPH; James I. Moss, PhD; Rhonda M. Cooper-DeHoff, PharmD; Carl J. Pepine, MD; Julie A. Johnson, PharmD

Background—Single-nucleotide polymorphisms (SNPs) within the regulatory β subunit of the voltage-gated calcium channel (CACNB2) may contribute to variable treatment response to antihypertensive drugs and adverse cardiovascular outcomes.

Methods and Results—SNPs in CACNB2 from 60 ethnically diverse individuals were identified and characterized. Three common SNPs (rs2357928, rs7069292, and rs61839258) and a genome-wide association study-identified intronic SNP (rs11014166) were genotyped for a clinical association study in 5598 hypertensive patients with coronary artery disease randomized to a β-blocker (BB) or a calcium channel blocker (CCB) treatment strategy in the INternational VErapamil SR-Trandolapril STudy GENETic Substudy (INVEST-GENES). Reporter gene assays were conducted on the promoter SNP, showing association with clinical outcomes. Twenty-one novel SNPs were identified. A promoter A→G SNP (rs2357928) was found to have significant interaction with treatment strategy for adverse cardiovascular outcomes (P for interaction, 0.002). In whites, rs2357928 GG patients randomized to CCB were more likely to experience an adverse outcome than those randomized to BB treatment strategy, with adjusted hazard ratio (HR) (CCB versus BB) of 2.35 (95% CI, 1.19 to 4.66; P=0.014). There was no evidence for such treatment difference in AG (HR, 1.16; 95% CI, 0.75 to 1.79; P=0.69) and AA (HR, 0.63; 95% CI, 0.36 to 1.11; P=0.11) patients. This finding was consistent in Hispanics and blacks. CACNB2 rs11014166 showed similar pharmacogenetic effect in Hispanics, but not in whites or blacks. Reporter assay analysis of rs2357928 showed a significant increase in promoter activity for the G allele compared to the A allele.

Conclusions—These data suggest that genetic variation within CACNB2 may influence treatment-related outcomes in high-risk patients with hypertension.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00133692.

Key Words: genetic variations ▪ CACNB2 ▪ hypertension ▪ cardiovascular diseases ▪ pharmacogenetics

High blood pressure is 1 of the major risk factors for cardiovascular disease, which is the leading cause of morbidity and mortality in the United States.1 β-blockers (BBs) and calcium channel blockers (CCBs) are among the most widely used antihypertensive drugs that are either used alone or in combination with other antihypertensive drugs to lower blood pressure and the incidence of coronary events (myocardial infarction [MI], death from coronary disease), strokes, and congestive heart failure.2,3 However, limited pharmacogenetic data exist on CCBs in the literature.4,5

Clinical Perspective on p 555

Calcium channels mediate Ca2+ entry into cells in response to action potentials and subthreshold depolarizing signals and are composed of 5 subunits: the pore-forming α1 subunit and the auxiliary α2, δ, β, and γ subunits.6 All CCBs work by...

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From the Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics (Y.N., Y.G., T.Y.L., H.M.D., H.E., J.I.M., R.M.C.-D., J.A.J.), College of Pharmacy, University of Florida, Gainesville, Fla; Division of Endocrinology, Diabetes and Nutrition (A.L.B.), University of Maryland School of Medicine, Baltimore, Md; and Division of Cardiovascular Medicine (R.M.C.-D., C.J.P., J.A.J.), University of Florida College of Medicine, Gainesville, Fla.

*Drs Niu and Gong contributed equally to this work.

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Correspondence to Julie A. Johnson, PharmD, Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida, PO Box 100486, Gainesville, FL 32610. E-mail Johnson@cop.ufl.edu
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binding to the $\alpha_1$ subunit of the voltage-gated L-type calcium channel (LTCC) to block transmembrane flow of Ca$^{2+}$ through the channel. The $\beta_2$ subunit of the LTCC, encoded by CACNB2 and expressed in cardiovascular tissue, is responsible for targeting the cell surface expression of the $\alpha_1$ pore-forming subunit. Based on this physiological role, we hypothesized that CACNB2 may contain variability important to response to antihypertensive drugs, particularly those with mechanisms derived from intracellular Ca$^{2+}$ (especially CCBs and BBs). Since the beginning of this study, a genome-wide association study (GWAS) of blood pressure and hypertension reported CACNB2 rs11014166 as 1 of the loci associated with both systolic and diastolic blood pressure and hypertension.\(^7\) This finding strengthened our hypothesis about the potential influence of this gene on antihypertensive treatment response. The purpose of this study was to investigate the pattern of genetic variation within CACNB2 and to explore whether this genetic variation plays a role in interpatient variability in risk of adverse cardiovascular outcomes either independently or relative to antihypertensive drug treatment.

**Methods**

**Single-Nucleotide Polymorphism Discovery**

**DNA Samples**

Genomic DNA isolated from lymphoblastic cell lines from 60 individuals of 3 racial/ethnic groups was obtained from the Human Genetic Cell Repository sponsored by the National Institutes of Health housed at the Coriell Institute (Camden, NJ).\(^6\) The 60 individuals were 20 European Americans, 20 African Americans, and 20 Native Americans. We also resequenced CACNB2 from a chimpanzee (DNA obtained from Coriell) to assist in assessment of phylogenetic patterns associated with the discovered single-nucleotide polymorphisms (SNPs).

**Resequencing and Detection of Variants**

Because the size of CACNB2 is >400 kb, we focused our resequencing effort on exons, intron-exon boundaries, and 3' and 5' untranslated regions (UTRs). These regions of CACNB2 were amplified in fragments of ~217 to 658 bp. Four alternative promoter regions were amplified in fragments of ~1973 to 2879 bp. The purified polymerase chain reaction (PCR) products were evaluated by direct sequencing with Amersham Biosciences ET-terminator chemistry method. DNA sequence data were compiled, and polymorphic sites were identified using PolyPhred.\(^8\) Sequence variations were called if both forward and reverse sequence reads were consistent.

The Polymorphism Database Mining, Annotation Programs (PolyMAPr),\(^9\) was used to predict the functional effects of nonsynonymous coding-region SNPs (PolyPhen) and any variants that might alter exon splicing enhancer sites (ESEfinder), putative transcription factor binding sites (JASPAR), or intron-exon splice sites (Alternative Splicing Database, splice junction sequences).\(^10\) SNPs were selected for analysis in the genetic association study on the basis of a PolyMAPr function score of >80% of the maximum possible and a minor allele frequency (MAF) of >0.05 in all 3 populations and of at least 0.20 in 1 population.

**Computational Methods**

Comparative analysis of CACNB2 sequences from multiple species (rat and mouse versus human) was performed with the VISTA genome browser tools (http://pipeline.lbl.gov/cgi-bin/gateway2).\(^11-13\) From the University of California, Santa Cruz (UCSC) Genome Browser, the human (Human May 2004, UCSC hg17), mouse (May 2004, UCSC mm5), and rat (June 2003, UCSC rn3) genome sequences were obtained.

**Clinical Association Study**

**International VErapamil SR-Trandolapil STudy**

The International VErapamil SR-Trandolapil STudy (INVEST) evaluated adverse cardiovascular outcomes occurring after randomized treatment with either an atenolol-based BB strategy or a verapamil SR-based CCB strategy in 22,576 patients with documents for coronary artery disease (CAD) and hypertension.\(^14,15\) The design, protocol, and primary outcome have been published in detail elsewhere.\(^14,15\) In brief, patients were eligible if they were aged ≥50 years and had documented CAD, with essential hypertension as defined by the Sixth Joint National Committee.\(^16\) requiring drug therapy. Patients were randomly assigned to a verapamil SR- or an atenolol-based treatment strategy, Hydrochlorothiazide, trandolapril, or both were added as needed to achieve Joint National Committee VI blood pressure targets.\(^16\)

The primary outcome of INVEST was the first occurrence of all-cause mortality, nonfatal MI, or nonfatal stroke. Secondary outcomes were the individual components of the primary outcome (ie, all-cause mortality, nonfatal MI, and nonfatal stroke). Three members of the events committee, masked to treatment assignment, confirmed all outcome events by reviewing documentation and other pertinent patient records.

**INVEST Genetic Substudy Cohort**

Genetic samples were collected from 5979 INVEST patients from 213 sites in the United States and Puerto Rico who provided genomic DNA samples and additional written informed consent for genetic studies. Genomic DNA was collected using buccal cells from mouthwash samples as previously described.\(^7\) The 5598 samples with sufficient quality and quantity DNA were tested here. All patients provided written informed consent for participation in the main INVEST and in the genetic substudy (INVEST-GENES), and both studies were approved by the University of Florida Institutional Review Board. We conducted a nested case-control study that included the 258 INVEST-GENES patients who experienced a primary outcome event during study follow-up (cases) and 774 individuals who did not have an event during study follow-up (controls). Controls were frequency matched to cases for age (by decades), sex, and race/ethnicity in a ratio of approximately 3:1. We have previously documented the nested case-control approach to yield essentially identical results to analyses of the entire genetics cohort.\(^4\)

**Genotyping**

Four SNPs selected for clinical association study (based on potential functional significance and MAFs of >0.10) — rs7069292, rs2357928, rs61839258, and rs7909119 — were genotyped for INVEST-GENES case-control samples by pyrosequencing.\(^18\) One SNP (rs7909119) deviated from Hardy-Weinberg equilibrium, which appeared to be due to assay problems that could not be solved and was dropped from further consideration. PCR reactions were carried out using HotStar Taq mix (Qiagen; Valencia, Calif), and 5-μL reactions were performed for pyrosequencing assays according to the manufacturer’s recommendations.

During the final stages of our study, a GWAS reported an intronic CACNB2 SNP, rs11014166, as being associated with blood pressure and hypertension.\(^7\) We elected to genotype rs11014166 and rs2357928 (which showed a pharmacogenetic association in the case-control sample analysis) in the entire INVEST-GENES cohort. Genotyping of the additional samples was performed with a TaqMan SNP genotyping assay. The following TaqMan probes were used for CACNB2 rs11014166 and rs2357928, respectively: C_2740542_10 and TaqMan Custom Assay Part #4331349. Genotype accuracy was verified by genotyping at least 5% randomly selected duplicate samples for each SNP on both TaqMan and pyrosequencing genotyping platforms, and results showed high concordance between the 2 platforms.
Transcript variants and urinary exosome exomes were used for transcriptome sequencing. Details are shown in the online-only Data Supplement materials.

Fold Luciferase Activity
Fold luciferase activity was used for the relative promoter activity. The relative luciferase activity of the CACNB2 reporter construct was represented as the ratio of the firefly luciferase activity to that of Renilla. The fold changes from negative control (pGL4/Basic) were calculated as the ratio of relative luciferase (pGL4/+58G and pGL4/+58A) to the mean relative luciferase (pGL4/Basic).

Statistical Analysis
Baseline characteristics were compared using $\chi^2$ test or ANOVA, as appropriate. Hardy-Weinberg equilibrium was evaluated separately by race/ethnicity using $\chi^2$ test with 1 degree of freedom. Linkage disequilibrium (LD) analysis was performed through use of the Haploview version 3.2 program.

To minimize the potential population stratification in our racially and ethnically diverse population in INVEST, all analyses were conducted by race/ethnicity separately. To control for potential of population admixture in each race/ethnicity group, we used a total panel of 87 ancestral informative markers selected to show large allele frequency differences across 3 parental populations (West Africans, indigenous Americans, and Europeans) selected from a panel of $>10,000$ SNPs. Maximum likelihood was used to estimate each patient’s individual genomic ancestry proportions on these 3 axes, and 2 of these 3 terms were included in the models to control for ancestry. These 87 ancestral informative markers were genotyped at Prevention Genetics (Marshfield, Wis) using either allele-specific PCR with universal energy-transfer-labeled primers or competitive allele-specific PCR. To ensure accurate ancestry proportion estimates, at least 48 (50%) ancestry informative markers had to be genotyped successfully in each individual to be included in the analyses. For the case-control samples, unadjusted and adjusted odds ratios (ORs) and 95% CIs for occurrence of the primary outcome were calculated using logistic regression. The adjusted model controlled for the prespecified covariates (age, sex, history of heart failure, and history of MI) and for other potential confounders selected by a stepwise selection procedure ($P<0.2$ for entry; $P<0.05$ for stay) as follows: treatment strategy, ancestry, body mass index (BMI), mean on-treatment systolic blood pressure, diabetes, history of stroke or transient ischemic attack, renal insufficiency, and smoking history. Interaction between genotype and treatment strategy also was evaluated. If an SNP showed a main effect of $P<0.10$ for the composite outcome, secondary outcomes also were tested.

For the cohort analysis, Cox proportional hazard modeling was performed to test the association of the SNPs and the risk for primary outcome and secondary outcomes, adjusting for variables selected using the same model-building process as mentioned previously. In addition, time-varying exposure was used for verapamil, atenolol, hydrochlorothiazide, and trandolapril. The assumption of the proportion hazards was tested in each Cox regression model.

To account for multiple comparisons, we performed the false discovery rate (FDR) adjustment according to the method of Benjamini and Hochberg. Nonparametric Wilcoxon rank sum test was used to assess promoter activity indicated by luciferase activity ratios in the reporter assays. A 2-sided $P<0.05$ was considered significant for the reporter assay analyses. All statistical analyses were conducted using SAS version 9.2 (SAS Institute; Cary, NC).

Results

SNP Discovery
The genomic structure of the CACNB2 gene is depicted in online-only Data Supplement Figure 1. Exon 1A, 1B, 2C, and 2D are the alternative first exons in 11 transcript isoforms in the CACNB2 gene. Exon 7A, 7B, and 7C are mutually exclusive alternative exons in 8 transcript isoforms. The VISTA plot of the multiple species comparative analysis of CACNB2 sequence from mouse and rat versus human is shown in online-only Data Supplement Figure 1D. Comparison of the human CACNB2 locus with those of mouse and rat genomic sequences showed strongly conserved (>75%) regions of the protein coding (in blue) and 5' UTR (in light green).

A total of 74 SNPs were identified in regions including exons and intron-exon boundaries and 3' and 5' UTRs of CACNB2 by direct sequencing (online-only Data Supplement Table 1) as follows: 65 in African Americans and 45 in European Americans and in Native Americans. MAFs of the CACNB2 SNPs ranged from 0.025 to 0.5. Forty-two SNPs were found in all 3 populations.

Fifty-three (72%) of the SNPs have been reported previously in the National Center for Biotechnology Information SNP database, whereas 21 (28%) were novel. Only 10 of the previously reported SNPs had been validated by multiple independent submissions with allele frequency information in the public database. Detailed genotype data for each of the 60 individuals is available online at the Pharmacogenetics and Pharmacogenomics Knowledge Base Web site (http://www.pharmgkb.org/).

All SNPs in the 3 populations were in Hardy-Weinberg equilibrium. Pairwise LD of the 74 SNPs in CACNB2 for the 3 race/ethnicity groups obtained from Haploview software are shown in online-only Data Supplement Figure 2. A relative lack of strong LD was observed for all 3 groups, minimizing the value of a tagSNP approach in our association studies.

In silico tests of potential functional significance of the 74 SNPs of CACNB2 were analyzed using PolyMAPr software. Fourteen SNPs suggested by PolyMAPr to have potential functional significance (eg, potential alternative promoter) are shown in online-only Data Supplement Table 2. One of the potential functional SNPs was located in the 3' UTR, and the other 13 were located in 4 candidate alternative promoter regions. Based on the PolyMAPr function score of >80% of maximum possible and MAF >0.05 in all 3 populations and >0.20 in at least 1 population, 4 SNPs (rs7069292, rs2357928, rs61839258, and rs7909119) were selected for clinical association study, but rs7909119 was abandoned because of assay difficulties. SNP rs2357928 was found to have significant pharmacogenetic association in the case-control samples and, therefore, was genotyped in the rest of the INVEST-GENES samples. Also genotyped in the INVEST-GENES cohort samples was the newly reported hypertension and blood pressure GWAS SNP rs11014166, which was not found in our discovery effort because of its intronic location. LD between these 2 SNPs was minimal, with D' and $r^2$,
respectively, of 0.12 and 0 in whites, 0.20 and 0.02 in blacks, and 0.07 and 0.002 in Hispanics.

Clinical Association Study

Study Population and Baseline Characteristics

Baseline characteristics and medical history for the INVEST-GENES participants are shown in Table 1. Consistent with the overall INVEST participants, these hypertensive patients with CAD were elderly (age, 66.1 ± 9.6 years), mostly overweight (BMI, 29.4 ± 5.5 kg/m²), and racially diverse (41% white, 12% black, 46% Hispanic), with slightly fewer men (45%) than women (55%). Patients in atenolol-based BB and verapamil-based CCB treatment strategies were similar in baseline characteristics, medical history, and nonstudy medication use (Table 1).

Genetic and Pharmacogenetic Association of CACNB2 Polymorphism With Primary Outcome

Genotype data were complete for 1006 patients (97.5% of the case-control) for rs7069292, 1022 (99.0% of the case-control) for rs61839258, 5537 (98.9% of the cohort) for rs2357928, and 5460 (97.6% of the cohort) for rs11014166. All genotype frequencies were in Hardy-Weinberg equilibrium in the 3 race/ethnicity groups. For all 4 SNPs, the genotype frequencies differed significantly by race/ethnicity (Table 2).

Table 1. Baseline Characteristics for INVEST-GENES Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case-Control (n=1032)</th>
<th>Cohort (n=5598)</th>
<th>CCB (n=2777)</th>
<th>BB (n=2821)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>70.5 ± 9.5</td>
<td>66.1 (9.6)</td>
<td>66.1 (9.6)</td>
<td>66.2 (9.6)</td>
<td>0.66</td>
</tr>
<tr>
<td>Age &gt;70 y</td>
<td>566 (54.8)</td>
<td>1902 (34.0)</td>
<td>932 (33.6)</td>
<td>970 (34.4)</td>
<td>0.52</td>
</tr>
<tr>
<td>Women</td>
<td>524 (50.8)</td>
<td>3090 (55.2)</td>
<td>1537 (55.4)</td>
<td>1553 (50.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td>White</td>
<td>616 (59.7)</td>
<td>2315 (41.4)</td>
<td>1174 (42.3)</td>
<td>1141 (40.5)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>131 (12.7)</td>
<td>657 (11.7)</td>
<td>323 (11.6)</td>
<td>334 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>261 (25.3)</td>
<td>2589 (46.3)</td>
<td>1261 (45.4)</td>
<td>1328 (47.1)</td>
<td></td>
</tr>
<tr>
<td>Other/multiracial</td>
<td>24 (2.3)</td>
<td>37 (0.7)</td>
<td>19 (0.7)</td>
<td>18 (0.6)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.6 ± 5.4</td>
<td>29.4 ± 5.5</td>
<td>29.3 ± 5.5</td>
<td>29.5 ± 5.6</td>
<td>0.14</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>148.2 ± 19.0</td>
<td>148.0 ± 18.4</td>
<td>147.6 ± 18.6</td>
<td>148.4 ± 18.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Diastolic</td>
<td>83.4 ± 11.1</td>
<td>85.4 ± 10.7</td>
<td>85.1 ± 10.8</td>
<td>85.7 ± 10.7</td>
<td>0.71</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
<td>74.8 ± 9.3</td>
<td>74.8 ± 9.6</td>
<td>74.9 ± 9.6</td>
<td>74.7 ± 9.6</td>
<td>0.33</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>148.2 ± 19.0</td>
<td>148.0 ± 18.4</td>
<td>147.6 ± 18.6</td>
<td>148.4 ± 18.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Heart failure (NYHA class I-III)</td>
<td>326 (31.6)</td>
<td>1317 (23.5)</td>
<td>668 (24.1)</td>
<td>649 (23.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Stroke or TIA</td>
<td>107 (10.4)</td>
<td>395 (7.1)</td>
<td>222 (8.0)</td>
<td>173 (6.1)</td>
<td>0.0065</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>99 (9.6)</td>
<td>187 (7.0)</td>
<td>204 (7.4)</td>
<td>187 (6.6)</td>
<td>0.29</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>182 (17.6)</td>
<td>842 (15.0)</td>
<td>401 (14.4)</td>
<td>441 (15.6)</td>
<td>0.21</td>
</tr>
<tr>
<td>PVD</td>
<td>131 (12.7)</td>
<td>620 (11.1)</td>
<td>310 (11.2)</td>
<td>310 (11.0)</td>
<td>0.84</td>
</tr>
<tr>
<td>Diabetes</td>
<td>107 (10.4)</td>
<td>395 (7.1)</td>
<td>222 (8.0)</td>
<td>173 (6.1)</td>
<td>0.0065</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>646 (62.6)</td>
<td>3068 (54.8)</td>
<td>1536 (55.3)</td>
<td>1532 (54.3)</td>
<td>0.45</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>32 (3.1)</td>
<td>88 (1.6)</td>
<td>52 (1.9)</td>
<td>36 (1.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Cancer</td>
<td>66 (6.4)</td>
<td>230 (4.1)</td>
<td>125 (4.5)</td>
<td>105 (3.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin/other antiplatelet agent</td>
<td>613 (59.4)</td>
<td>2590 (46.3)</td>
<td>1291 (46.5)</td>
<td>1299 (46.1)</td>
<td>0.74</td>
</tr>
<tr>
<td>Antidiabetic medication</td>
<td>274 (26.5)</td>
<td>1392 (24.9)</td>
<td>666 (24.0)</td>
<td>726 (25.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Lipid-lowering drugs</td>
<td>437 (42.3)</td>
<td>2034 (36.3)</td>
<td>1033 (37.2)</td>
<td>1001 (35.5)</td>
<td>0.18</td>
</tr>
<tr>
<td>Nitrates</td>
<td>324 (31.4)</td>
<td>1568 (28.0)</td>
<td>770 (27.7)</td>
<td>796 (28.3)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Data are presented as no. (%) or mean ± SD. LV indicates left ventricular; NYHA, New York Heart Association; PVD, peripheral vascular disease; TIA, transient ischemic attack.

*By t test for continuous variables and χ² test for categorical variables.
Table 3. Primary Outcome by Race* and P Value for SNP-Treatment Interaction†

<table>
<thead>
<tr>
<th>SNP</th>
<th>White</th>
<th>Hispanic</th>
<th>Black</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted HR/OR</td>
<td>SNPs-Treatment</td>
<td>Adjusted HR/OR</td>
<td>SNPs-</td>
<td>Adjusted HR/OR</td>
<td>SNPs-</td>
</tr>
<tr>
<td></td>
<td>(95% CI)†</td>
<td>P</td>
<td>(95% CI)</td>
<td>Treatment P</td>
<td>(95% CI)</td>
<td>Treatment P</td>
</tr>
<tr>
<td>rs2357928 (G)</td>
<td>0.72 (0.52–1.01)</td>
<td>0.06</td>
<td>0.002§</td>
<td>0.45 (0.20–1.0)</td>
<td>0.049</td>
<td>0.32</td>
</tr>
<tr>
<td>rs11014166 (T)</td>
<td>0.91 (0.57–1.45)</td>
<td>0.68</td>
<td>0.4</td>
<td>1.03 (0.59–1.79)</td>
<td>0.91</td>
<td>0.47</td>
</tr>
<tr>
<td>rs61839258 (T)</td>
<td>1.13 (0.77–1.66)</td>
<td>0.54</td>
<td>0.54</td>
<td>0.16 (0.03–1.04)</td>
<td>0.055</td>
<td>0.98</td>
</tr>
<tr>
<td>rs7069292 (C)</td>
<td>1.01 (0.74–1.38)</td>
<td>0.97</td>
<td>0.24</td>
<td>0.75 (0.33–1.69)</td>
<td>0.48</td>
<td>0.37</td>
</tr>
</tbody>
</table>

| Minor alleles shown in parentheses.  
*Adjusted for age, sex, history of MI, heart failure, ancestry, CCB treatment or BB treatment strategy.  
†HR reported for rs2357928 and rs11014166 per each minor allele; OR reported for rs61839258 and rs7069292 for each minor allele.  
‡The FDR-adjusted P=0.024.

Pharmacogenetic Effect of rs2357928  
CACNB2 rs2357928 was associated with a significantly increased risk of the primary outcome (HR, 1.67; 95% CI, 1.02 to 2.73; P=0.043) when compared to the CC genotype (HR, 1.00; 95% CI, 0.63 to 1.57; P=0.96). The interaction between SNP and treatment was not significant in any of the 3 race/ethnicity groups (Table 3).

Discussion  
CACNB2 is a gene of increasing interest because it is one of the few that has recently been replicated as a hypertension
Here, we report the common DNA sequence variations in the CACNB2 coding region and flanking 5′ and 3′ UTR obtained from 20 European American, 20 African American, and 20 Native American samples. A total of 74 SNPs were identified in these CACNB2 gene regions, of which 28% were novel SNPs not previously reported in National Center for Biotechnology Information SNP database. In this study, only 3 nonsynonymous mutations were found in the last coding exon of the CACNB2 gene, and we did not find any novel nonsynonymous SNP in any of the 3 populations, indicating that CACNB2 is highly conserved at the protein level. The comparison of the human CACNB2 gene sequence with those of mouse and rat also showed strong conservation in the protein-coding region and flanking 5′/UTR. Based on in silico functional analysis and MAFs, we genotyped 3 SNPs for study in our genetic association analysis and later included the hypertension GWAS SNP (rs11014166) in our analysis.

In our clinical association study, we identified 1 promoter SNP (rs2357928) in CACNB2 that was associated with a significant SNP-treatment interaction in whites, that for minor allele (GG) homozygous patients, a verapamil SR-based BB treatment was associated with significantly increased risk for the primary outcome compared with an atenolol-based BB treatment, whereas for AA and AG individuals, the CCB and BB treatment strategies were equivalent. This effect was validated for Hispanic patients, but not AA homozygotes. In the blood pressure and hypertension GWAS analysis of the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium,7 the T allele of rs11014166 was associated with lower systolic and diastolic blood pressure and lower risk for hypertension. INVEST, a cardiovascular outcome study of hypertensive patients with CAD, is not an appropriate replication cohort for the hypertension and blood pressure association. Nevertheless, our data suggest that this SNP may be associated with adverse cardiovascular outcomes in hypertensive patients with CAD in a treatment-specific manner.

Verapamil SR, like all marketed CCBs, binds directly to the α subunit of the LTCC, which is modulated by the auxiliary β subunit studied herein. Thus, the validated association with rs2357928, including preliminary functional data, suggests that the CACNB2 gene (and SNP) deserve further study.

**Limitations**

There are limitations worth noting in this study. First, the genetic association findings were not replicated in an independent sample of whites. We did validate the finding in INVEST-GENES Hispanics, which is an independent sample from the white sample. However, the number of events in Hispanic patients was small. This validation in a different ethnic group, along with the in vitro data that support a functional basis, suggest that rs2357928 is a functional SNP. Additionally, pharmacogenetic analyses conducted within a randomized controlled clinical trial such as INVEST present clear advantages over cohort studies in that the randomization substantially reduces biases and confounding. The recent finding in the GWAS of the association of CACNB2 with hypertension and blood pressure adds to the likelihood that this gene is very important in hypertension and hypertension-related treatment outcomes.

Although we did not observe this association in the black samples, only 1 black individual harboring the genotype with the most prominent effect (GG) experienced an adverse cardiovascular outcome. However, the pattern in heterozygotes and major allele homozygotes was consistent with whites and Hispanics. Thus, it is not clear whether the lack of...
association in this population is due to inadequate power, or an absence of genetic effect for this SNP in this population, or that the studied SNP is a tagSNP not in LD with the functional SNP in blacks.

Conclusions

Our association study suggests significant pharmacogenetic effects for the promoter SNP rs2357928 in CACNB2 such that for minor allele homozygotes, a verapamil SR-based CCB treatment strategy is associated with substantially higher risk for adverse cardiovascular outcome compared with an atenolol-based BB treatment strategy. These findings were validated in a second ethnic group and further supported by in vitro studies suggesting differential transcriptional activity with this promoter SNP. Additional studies in other cohorts are required, but these data suggest that this CACNB2 SNP may have future potential for guiding selection of antihypertensive drug therapy among patients with CAD.

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Disclosures

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13. Brudno M, Do CB, Cooper GM, Kim MF, Davydov E, Green ED, Sidow A, Batzoglou S. LAGAN and multi-LAGAN: efficient tools for...
Selection of a hypertension treatment strategy in patients with stable coronary artery disease is largely empirical given that randomized trials have demonstrated equivalent outcomes with β-blocker (BB)- or calcium channel blocker (CCB)-based treatment. In the context of 1 of these clinical trials, the INternational VErapamil SR-Trandolapril STudy, we have shown the regulatory β2 subunit of the voltage-gated calcium channel (CACNB2) to contain a polymorphism that associates with adverse cardiovascular outcomes in a treatment-specific manner. More specifically, white patients harboring the rs2357928 GG genotype randomized to a CCB-based treatment strategy were more likely to experience an adverse cardiovascular outcome than those randomized to a BB-based treatment strategy. There was no evidence for such treatment difference in AG and AA individuals. This finding was consistent in Hispanics and blacks. Reporter assay analysis of this polymorphism showed a significant increase in promoter activity for the G allele compared to the A allele. These data suggest that instead of empirical treatment, patients with the CACNB2 rs2357928 GG genotype might benefit most from treatment with BBs and that either BBs or CCBs could be used in those with the AG and AA genotype. Importantly, CACNB2 was among a small number of genes recently identified in a hypertension genome-wide association study. Taken together, these data suggest that CACNB2 is important in hypertension and treatment-associated outcomes in patients with hypertension. Replication of these findings in an independent clinical trial is warranted.
Genetic Variation in the β2 Subunit of the Voltage-Gated Calcium Channel and Pharmacogenetic Association With Adverse Cardiovascular Outcomes in the INInternational VÉrapamil SR-Trandolapril STudy GENEtic Substudy (INVEST-GENES)
Yuxin Niu, Yan Gong, Taimour Y. Langaaee, Heather M. Davis, Hazem Elewa, Amber L. Beitelshees, James I. Moss, Rhonda M. Cooper-DeHoff, Carl J. Pepine and Julie A. Johnson

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