Pharmacogenomic Association of Nonsynonymous SNPs in SIGLEC12, A1BG, and the Selectin Region and Cardiovascular Outcomes

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Abstract—We sought to identify novel pharmacogenetic markers associated with cardiovascular outcomes in patients with hypertension on antihypertensive therapy. We genotyped a 1:4 case:control cohort (n=1345) on the Illumina HumanCVD Beadchip from the INternational VErapamil SR–Trandolapril STudy (INVEST), where participants were randomized to a β-blocker strategy or a calcium channel blocker strategy. Genome-spanning single nucleotide polymorphism (SNP)×treatment interaction analyses of nonsynonymous SNPs were conducted in white and Hispanic race/ethnic groups. Top hits from whites were tested in Hispanics for consistency. A genetic risk score was constructed from the top 3 signals and tested in the Nordic Diltiazem study. SIGLEC12 rs16982743 and A1BG rs893184 had a significant interaction with treatment strategy for adverse cardiovascular outcomes (INVEST whites and Hispanics combined interaction P=0.0038 and 0.0036, respectively). A genetic risk score, including rs16982743, rs893184, and rs4525 in F5, was significantly associated with treatment-related adverse cardiovascular outcomes in whites and Hispanics from the INVEST study and in the Nordic Diltiazem study (meta-analysis interaction P=2.39×10^{−5}). In patients with a genetic risk score of 0 or 1, calcium channel blocker treatment was associated with lower risk (odds ratio [95% confidence interval]=0.60 [0.42–0.86]), and in those with a genetic risk score of 2 to 3, calcium channel blocker treatment was associated with higher risk (odds ratio [95% confidence interval]=1.31 [1.08–1.59]). These results suggest that cardiovascular outcomes may differ based on SIGLEC12, A1BG, F5 genotypes, and antihypertensive treatment strategy. These specific genetic associations and our risk score provide insight into a potential approach to personalized antihypertensive treatment selection.

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Key Words: antihypertensive agents ▪ β-blockers ▪ calcium channel blockers ▪ genetic variation ▪ hypertension ▪ pharmacogenetics

Hypertension is the most common chronic disease in the United States, affecting approximately one third of the adult population, and is a major risk factor for acute myocardial infarction (MI), stroke, heart failure, and renal failure. Numerous antihypertensive drugs are considered appropriate first-line therapy to lower blood pressure (BP), including diuretics, β-blockers (βB), calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers. These drugs are ultimately prescribed to prevent the long-term cardiovascular complications of hypertension. However, there is great interpatient variability in antihypertensive drug response, with only 50% of patients achieving an adequate BP response to any 1 drug, and limited data are available to guide treatment selection. Why patients respond differently to the same drug and why some patients experience adverse cardiovascular outcomes, despite BP control, while others do not, remains poorly understood. Pharmacogenomics aims to identify genetic markers that are associated with drug response, outcomes, and adverse events.

In the past decade, there have been many advances made in cardiovascular pharmacogenomics. Yet, to date, there have been few functional variants identified that associate with pharmacogenomic or treatment-related cardiovascular outcomes in patients with hypertension. To discover variants that may be functional, we assessed nonsynonymous SNPs (nsSNPs) from the Illumina HumanCVD chip (Illumina, San

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Diego, CA) for association with treatment-related outcomes in the INternational V/Erapamil SR–Trandolapril Study (INVEST), a hypertension outcomes trial. In addition, we replicated our findings in the NORDic DILtaizem (NORDIL) study, another hypertension outcomes trial with similar treatment regimens.6

Methods

Study Participants

The INVEST-GENEtic Substudy (INVEST GENES) collected DNA from 5979 patients with hypertension and coronary artery disease enrolled in INVEST, residing in the United States and Puerto Rico. The genetic substudy was approved by the Institutional Review Board at the University of Florida; all patients provided voluntary, written informed consent, and all study procedures were in accordance with institutional guidelines and adhered to the principles of the Declaration of Helsinki and the US Code of Federal Regulations for Protection of Human Subjects. The methods and results of INVEST have been previously published. Briefly, patients were randomized to a verapamil-SR (CCB) or an atenolol (βB)-based treatment strategy, with hydrochlorothiazide and trandolapril available as add-on treatment for BP control. The primary outcome (PO) was the first occurrence of all-cause death, nonfatal MI, or nonfatal stroke. Secondary outcomes included the individual components of the PO. Overall, BP control and cardiovascular outcomes were similar between the 2 strategies.6

A nested case–control cohort was constructed from INVEST-GENES. Cases (n=269) were defined as those patients experiencing the PO. Each case was frequency matched to 4 controls (n=1076) based on age by decade, sex, and race/ethnicity. Patients in the case–control cohort were primarily classified as white (n=795), Hispanic (n=380), and black (n=170). The results presented here were limited to the white and Hispanic race/ethnic groups because there is limited power in the black race/ethnic group.

DNA was collected on 4200 Swedish subjects from NORDIL, a hypertension outcomes trial comparing a CCB strategy (diltiazem) to conventional antihypertensive treatment using diuretics, βBs, or both. The primary end point was fatal or nonfatal stroke, fatal or nonfatal MI, or other cardiovascular death, and information is available on all death events. The methods and results of NORDIL have been previously published.6

Additional details on the study designs and participants for INVEST and NORDIL are available in the online-only Data Supplement.

Genotyping and Quality Control

All 1345 INVEST patients in the nested case–control cohort were successfully genotyped on the Illumina HumanCVD Beadchip, a gene-centric array containing ≈50000 SNPs in ≈2100 genes involved in cardiovascular, inflammatory, and metabolic processes. Four thousand one hundred ninety-six NORDIL subjects were successfully genotyped on the Illumina 610Quad genome-wide array (Illumina). Additional genotyping and quality control details are available in the online-only Data Supplement.

Statistical Analysis

This analysis was limited to nsSNPs on the HumanCVD Beadchip, with a minor allele frequency >0.03, to identify markers with the greatest likelihood for functional effect and the greatest potential to replicate across race/ethnic groups in INVEST. Genome-spanning nsSNP×treatment interaction analyses were conducted by race/ethnic group under an additive genetic model using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). Analyses were adjusted for age, sex, principal components for ancestry, and history of MI, heart failure, and diabetes mellitus. The primary inference was made from the SNP×treatment interaction analysis in whites. The screening threshold was set at interaction P<0.05 in whites based on the a priori hypothesis that nsSNPs are likely to be functional. SNPs meeting the screening P value were examined in Hispanics for evidence of association. The significance threshold to move forward to our external replication cohort was P<0.05, with consistent direction of association. Thus, significant hits had a P=0.00125 (0.05×0.05×0.5). Gene regions were also examined because patterns of linkage disequilibrium may vary between race/ethnic groups. For SNPs that showed consistent evidence of a pharmacogenomic (treatment interaction) association in both race/ethnic groups, whites and Hispanics were pooled together and a combined treatment interaction analysis was conducted. Adjusted odds ratios (OR) and 95% confidence intervals (CIs) for the occurrence of the PO were assessed by genotype using a logistic regression model, under both additive and dominant genetic models of inheritance, using SAS version 9.2 (Cary, NC). Pairwise measure of r2 and D’ of top hits were assessed using Haploview version 4.2.8

Based on the initial findings in the 3 groups (INVEST whites, INVEST Hispanics, and NORDIL), the final genetic risk score was calculated from rs16982743, rs893184, and rs4525. One point was given for each genotype that conferred higher risk in the CCB arm/group versus the βB arm/group. The potential genetic risk scores ranged from 0 to 3 (1 point each for SIGLEC12 rs16982743 GG [Gln/Gln], A1BG rs893184 GG [Arg/Arg], and F5 rs4525 AA [His/His]). The risk scores were dichotomized as low risk (0–1 points) and high risk (2–3 points) because only a small number of subjects had a score of 0 and 3, and they responded similarly to those with a score of 1 and 2, respectively. Adjusted ORs and 95% CIs for the occurrence of the PO were assessed by genetic risk score using a logistic regression model in SAS version 9.2 (Cary, NC). Secondary outcomes were also tested in INVEST with the final risk score. The significance level for the final risk score×treatment interaction in INVEST whites and Hispanics was P<0.05, and the significance level in NORDIL was a 1-sided P<0.05 because we had a 1-sided hypothesis for replication. Meta-analysis for the risk score was conducted using METAL and PLINK. A weighted model was also tested but discarded because it was more complex but not more informative. Additional details of the statistical methods are available in the online-only Data Supplement.

Results

Study Population and Baseline Characteristics

Baseline demographics and characteristics of the INVEST-GENES case–control cohort and NORDIL subjects are shown in Table 1. On average, INVEST subjects were older than NORDIL subjects. In addition, INVEST subjects had lower systolic BP and diastolic BP, but this is likely explained by the fact that there was no washout of antihypertensive drugs in INVEST and 87.6% of INVEST subjects were treated at entry, whereas there was a washout in NORDIL (Table 1). Furthermore, the baseline demographics and characteristics of the INVEST-GENES case–control cohort and NORDIL subjects stratified by treatment group are shown in Table S1 in the online-only Data Supplement.

Genome-Spanning Interaction Analysis and Between-Race Consistency in INVEST

After SNP quality control procedures and applying a minor allele frequency cutoff of ≥0.03, there were 1313 nsSNPs analyzed in the white race/ethnic group in INVEST. Sixty-five nsSNPs in the white race/ethnic group had a treatment interaction P<0.05. Two nsSNPs from whites showed evidence of consistent association in Hispanics: additive P<0.05 and consistent direction of association (Table 2; Figure S1). Allele counts and Hardy-Weinberg Equilibrium data are shown in Table S2.
The first SNP, rs16982743 in SIGLEC12 (sialic acid–binding Ig-like lectin 12), results in a stop codon at amino acid position 29 (white interaction P=0.0329, Hispanic interaction P=0.0215, and combined interaction P=0.0038; Table 2; Figure S1A). In whites at rs16982743, stop codon carriers (A carriers) showed similar risk for the PO when treated with the CCB versus βB strategy (OR [95% CI]=0.78 [0.43–1.40]; P=0.4015), whereas in those subjects with the Gln/Gln (G/G) genotype, the risk for the PO was higher in the CCB strategy compared with the βB strategy (OR [95% CI]=1.94 [1.21–3.14]; P=0.0065; interaction P=0.0159). There was a consistent association in Hispanics, so the white and Hispanic groups were pooled for a combined analysis. OR (95% CI) for stop codon carriers and Gln/Gln (G/G) subjects were 0.82 (0.51–1.33; P=0.4241) and 1.91 (1.29–2.83; P=0.0013), respectively (interaction P=0.0038; Figure S1A).

The second nsSNP that showed consistent pharmacogenomic association in whites and Hispanics was rs893184, located in A1BG (α-1-B glycoprotein; white interaction P=0.0248, Hispanic interaction P=0.0310, and combined interaction P=0.0036; Table 2; Figure S1B). rs893184 causes a histidine (His) to arginine (Arg) substitution at amino acid position 52 in A1BG. His carriers had a lower risk for the PO in the CCB strategy in whites and Hispanics, whereas Arg/Arg patients had a higher risk for the PO in the CCB strategy. After pooling the race/ethnic groups, the observed association was stronger (combined interaction P=0.0036; Figure S1B).

Linkage disequilibrium was assessed between the 2 nsSNPs because they are located 6.85 Mb apart on chromosome 19. Overall, there was very low linkage disequilibrium between them (r² values of 0 and 0.02 in INVEST whites and Hispanics, respectively, and D’ values of 0.03 and 0.24 in INVEST whites and Hispanics, respectively; Table S3).

In addition, gene regions identified from the interaction analysis in whites were investigated in Hispanics. The F5-SELP-SELL-SELE region on chromosome 1 showed strong evidence of association in both whites and Hispanics: multiple nsSNPs associated in each race group. In this region, there were 3 independent nsSNPs with evidence of association in the white race/ethnic group (rs9332701, rs6131, and rs8361; Figure S2) and 2 independent nsSNPs with evidence of association in the Hispanic race/ethnic group (rs4525 and rs6125; Figure S3). Through linkage disequilibrium assessment and haplotype interaction analysis, we discovered that a 2-nSNP haplotype spanning SELP and SELE, rs6125 and rs8361, was associated with treatment interaction in whites and replicated in Hispanics (white interaction P=0.0097, Hispanic interaction P=0.0028, and combined interaction P=0.0003; Table S4).

### Table 1. Baseline Demographics and Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>INVEST Whites (n=795)</th>
<th>INVEST Hispanics (n=380)</th>
<th>NORDIL (n=4196)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>70.8±9.5</td>
<td>72.3±8.8</td>
<td>60.1±6.6</td>
</tr>
<tr>
<td>Male</td>
<td>415 (52.2)</td>
<td>185 (48.7)</td>
<td>2093 (49.9)</td>
</tr>
<tr>
<td>Primary outcome case</td>
<td>159 (20.0)</td>
<td>76 (20.0)</td>
<td>376 (9.0)</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>28.6±5.4</td>
<td>27.8±4.6</td>
<td>28.2±4.4</td>
</tr>
<tr>
<td>SBP, mean (SD), mm Hg</td>
<td>148.7±17.5</td>
<td>148.1±18.6</td>
<td>172.6±18.9</td>
</tr>
<tr>
<td>DBP, mean (SD), mm Hg</td>
<td>81.7±10.4</td>
<td>85.1±10.1</td>
<td>103.1±7.2</td>
</tr>
<tr>
<td>Heart rate, mean (SD), bpm</td>
<td>75.2±9.3</td>
<td>73.6±9.1</td>
<td>N/A</td>
</tr>
<tr>
<td>History of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>153 (19.2)</td>
<td>90 (23.7)</td>
<td>342 (8.2)</td>
</tr>
<tr>
<td>Heart failure (class I to II)</td>
<td>50 (6.3)</td>
<td>18 (4.7)</td>
<td>N/A</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>280 (35.2)</td>
<td>53 (13.9)</td>
<td>101 (2.4)</td>
</tr>
</tbody>
</table>

Values are presented as number (percentage) unless otherwise noted. BMI indicates body mass index; DBP, diastolic blood pressure; INVEST, INternational VErapamil SR-Trandolapril STudy; N/A, characteristic not available in NORDIL genetic study; NORDIL, NORdic DILtiazem; and SBP, systolic blood pressure.

### Table 2. SNP–Treatment Interaction in INVEST

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Position</th>
<th>Gene</th>
<th>Amino Acid Change</th>
<th>Minor Allele</th>
<th>Race</th>
<th>MAF</th>
<th>Interaction P Value</th>
<th>Combined Interaction P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs16982743</td>
<td>19</td>
<td>566967615</td>
<td>SIGLEC12</td>
<td>Q29X</td>
<td>A (Stop)</td>
<td>White</td>
<td>0.179</td>
<td>0.0329</td>
<td>0.0038</td>
</tr>
<tr>
<td>rs893184</td>
<td>19</td>
<td>635556291</td>
<td>A1BG</td>
<td>H52R</td>
<td>A (His)</td>
<td>White</td>
<td>0.046</td>
<td>0.0248</td>
<td>0.0036</td>
</tr>
</tbody>
</table>

Position denotes NCBI build 36 position. Chr indicates chromosome; INVEST, INternational VErapamil SR-Trandolapril STudy; and MAF, minor allele frequency.
(interaction $P=0.0004$), with subjects with a low-risk score (0 or 1) being more neutral to treatment strategy or trending toward favoring the CCB strategy (combined OR [95% CI]=0.78 [0.50–1.22]; $P=0.2791$) and subjects with a high-risk score (2) experiencing significant risk in the CCB strategy (combined OR [95% CI]=2.19 [1.45–3.33]; $P=0.0002$). Because we also identified a region on chromosome 1 that showed consistent evidence of association in both INVEST whites and Hispanics, we evaluated a 3-nSNP risk score, including the 2 nSNPs on chromosome 19 and adding each of the significant SNPs on chromosome 1. We also evaluated a model including the 2 nSNPs on chromosome 19, with the significant haplotype from the chromosome 1 region. Four of the five 3-SNP models (including the chromosome 19 nSNPs and then rs9332701, rs4525, rs6131, or rs6125) and the haplotype model showed equal or stronger evidence of association compared with the 2-SNP model (combined interaction $P<0.0004$).

Next, we sought to replicate the genetic risk score models in NORDIL. Three of the four 3-SNP models above were tested (the model with rs9332701 was not tested because of low rs9332701 minor allele frequency in NORDIL, and the haplotype model was not tested because of the fact that NORDIL data used imputed SNPs, thus construction of haplotypes was not appropriate). These data suggested that rs4525 (His865Arg) in $F5$ was the most informative nSNP for the $F5$-SEL-P-SELL-SELE region across studies as part of a 3-SNP model (Table S5). The results of the treatment interaction analysis with the 3-SNP model (rs16982743, rs933184, and rs4525) genetic risk score relative to the PO for each of the 3 cohorts are shown in the Figure. We observed consistent associations with the risk score in INVEST whites and Hispanics, with subjects with the low-risk score (0 or 1) being neutral to treatment strategy or trending toward favoring the CCB strategy and subjects with the high-risk score (2 or 3) being at significant risk in the CCB strategy (favoring the $\beta B$ strategy). In NORDIL, these results replicated, with a significant risk score*treatment interaction (NORDIL interaction $P=0.0315$, 1-sided $P$ value). When all 3 studies were combined in meta-analysis, the interaction $P$ value was more significant (interaction $P=2.39 \times 10^{-5}$). In those with a low genetic risk score, CCB treatment was associated with lower risk of the PO (OR [95% CI]=0.60 [0.42–0.86]; $P=6.51 \times 10^{-3}$), and for those with a high genetic risk score, CCB treatment was associated with higher risk of the PO compared with $\beta B$ treatment (OR [95% CI]=1.31 [1.08–1.59]; $P=6.55 \times 10^{-4}$; Figure).

Despite limited power, additional analyses of the risk score on secondary outcomes by genetic risk score in INVEST suggested that each element of the PO contributes (Figure S4). Both nonfatal MI and all-cause deaths seemed to contribute to the PO pharmacogenomic effect (white/Hispanic combined interaction $P=0.0457$ and 0.0026, respectively). In addition, nonfatal stroke contributed to the PO pharmacogenomic effect in Hispanics (interaction $P=0.0404$).

**Discussion**

We performed a genome-spanning analysis of nSNPs in cardiovascular, metabolic, and inflammatory candidate genes to investigate genetic variants associated with differential treatment-related outcomes in high-risk patients with hypertension. Through this analysis, we identified 3 regions with a pharmacogenetic association with treatment-related outcomes in white and Hispanic patients with hypertension and coronary artery disease treated with a CCB versus $\beta B$ strategy. In 2 of these regions, a single SNP was associated in treatment interaction analysis: $SIGLEC12$ rs16982743 (Gln29Stop) and $A1BG$ rs893184 (His54Arg). The third region on chromosome 1, containing $F5$, $SEL-P$, $SELL$, and $SELE$, showed evidence of association at multiple SNPs across INVEST whites and Hispanics. A risk score accounting for all 3 regions was significantly associated with treatment outcomes in INVEST whites, INVEST Hispanics, and replicated in NORDIL.

This study represents the first pharmacogenetics study in hypertension to identify and replicate genetic markers for differential treatment-related outcomes across large clinical trials. This was likely aided by selecting SNPs that had associations in independent race/ethnic groups within INVEST for testing in a different clinical trial population.
In addition, the use of a genetic risk score enhanced the ability to replicate the finding in NORDIL. Risk scores have been more commonly used in disease genetics,\textsuperscript{10,11} but their use in pharmacogenomics is valuable for future clinical translation. This is especially true for hypertension pharmacogenomics because often multiple signals are likely involved, as demonstrated in this study. Risk scores are typically constructed from prior association signals and tested in an independent population.\textsuperscript{10,11} In this study, we constructed the score in the discovery population, but we have replicated the score in an independent population.

SIGLEC12, located at 19q13.4, encodes the sialic acid-binding Ig-like lectin 12 gene. Siglecs are a family of single-pass, type 1 transmembrane proteins belonging to the immunoglobulin superfamily. Most siglecs are expressed on cells of the immune system or the hematopoietic system.\textsuperscript{12} SIGLEC12 is expressed on the epithelial cell surface and on some macrophages.\textsuperscript{12} It has been suggested that SIGLEC12 could be involved in the negative regulation of macrophage signaling by functioning as an inhibitory receptor.\textsuperscript{13} In addition, other members of the siglec family have been shown to be expressed abundantly on macrophages recruited during the pathogenesis of atherosclerosis.\textsuperscript{14} Although there are no published data on the function of the Gln29Stop SNP, premature stop codons are essentially always functional, and one this early in the protein would particularly be expected to be functional.

The α-1-B glycoprotein precursor (\(\alpha\)1B) is also located at 19q13.4 (6.85 Mb away from SIGLEC12) and encodes a plasma glycoprotein with unknown function. The α1B protein is a member of the immunoglobulin superfamily and has been shown to form a complex with cysteine-rich secretory protein 3, a protein present in neutrophilic granulocytes that is thought to play a role in innate immunity.\textsuperscript{15}

The last region identified from the treatment interaction analyses contained signals in 3 genes: \(F5\), coagulation factor V; \(SELP\), selectin P; and \(SELE\), selectin E. Because we did not have a consistent signal across all 3 populations, it is likely we have not found the causal variant in this region. However, we did observe an association or trend in each group, and overall, we have not found the causal variant in this region. However, we have a consistent signal across all 3 populations, it is likely we have found the causal variant. In addition, we observed evidence of an association with treatment-related cardiovascular outcomes with variants in \(A1BG\) and SIGLEC12. Although the exact functions of these genes are unknown, our results may hint that their functions are also related to the progression of the atherosclerotic environment in the vasculature. From our results, it is unclear whether the CCB strategy advanced this progression, leading to increased risk in the genotypes identified, or whether the βB strategy reduced this risk. We hypothesize that the latter would seem more plausible because it is more likely that one of the drugs is protective in a given genotype, versus a drug adding risk in a specific genotype. Our study is not without limitations. We did not reach a Bonferroni significance level in our initial treatment interaction analysis of nsSNPs on the HumanCVD Beadchip, and we cannot discount the possibility of reaching these results by chance: with a \(p<0.00125\) for 1313 nsSNPs analyzed in INVEST whites, we would expect 1.6 significant hits, and we found 2. However, our analysis only focused on nonsynonymous SNPs, giving them a higher probability of being functional variants. In addition, consistent associations in both whites and Hispanics in INVEST reduces the risk of these representing a false positive. Replication of the risk score constructed from these SNPs in NORDIL further reduces the likelihood of this representing a chance finding. We also had reduced power in by race analyses. Still, we were able to identify 3 regions with evidence of association, and our genetic risk score constructed from these 3 regions was significant in INVEST whites, INVEST Hispanics, and NORDIL. Finally, we may have missed true signals by the design of our study because one of our replication cohorts had a different racial/ethnic background. Nevertheless, true functional variants should be functional across all populations, and there are numerous examples for this within the pharmacogenomics literature.

Perspectives

There is great interpatient variability in antihypertensive drug response, with only \(\approx50\%\) of patients responding to any 1 drug,\textsuperscript{3} and limited data are available to guide treatment selection. Our study strived to identify novel genetic markers associated with treatment-related outcomes in patients with hypertension and coronary artery disease. We found that nsSNPs in \(A1BG\), SIGLEC12, and the selectin region are statistically associated with treatment-related adverse cardiovascular outcomes in 3 independent hypertensive populations. By constructing a genetic risk score to account for all 3 regions, we were able
to provide a potential approach for identifying which patients
would benefit from a CCB strategy versus a β-blocker strategy with
good treatment-related adverse cardiovascular outcomes.
These results require further investigation to clarify their role in
hypertension treatment and to establish the mechanism through which they elicit their effect. However, the specific
association and our risk score method provide insight into a potential approach to personalized antihypertensive
treatment selection.

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18. Ehret GB, O’Connor AA, Weder A, Cooper RS, Chakravarti A. Follow-up of a major linkage peak on chromosome 1 reveals suggestive QT traits asso-


### Novelty and Significance

**What Is New?**

- Nonsynonymous SNPs in *SIGLEC12*, *A1BG*, and the selectin region are associated with antihypertensive treatment-related adverse cardiovascular events.

**What Is Relevant?**

- There is great interpatient variability in adverse outcomes related to antihypertensive treatment. This research identifies SNPs that may contribute to this variability and may help explain differential outcomes with a given hypertension treatment based on genotype.

**Summary**

Nonsynonymous SNPs in *SIGLEC12*, *A1BG*, and the selectin region are associated with treatment-related adverse cardiovascular outcomes in patients with hypertension and coronary artery disease. A risk score accounting for the 3 regions provides a potential approach for identifying which patients would benefit from a calcium channel blocker strategy versus a βB strategy.
Pharmacogenomic Association of Nonsynonymous SNPs in SIGLEC12, A1BG, and the Selectin Region and Cardiovascular Outcomes
Caitrin W. McDonough, Yan Gong, Sandosh Padmanabhan, Ben Burkley, Taimour Y. Langae, Olle Melander, Carl J. Pepine, Anna F. Dominiczak, Rhonda M. Cooper-DeHoff and Julie A. Johnson

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PHARMACOGENOMIC ASSOCIATION OF NON-SYNONYMOUS SNPS IN SIGLEC12, A1BG AND THE SELECTIN REGION AND CARDIOVASCULAR OUTCOMES

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Short title: Pharmacogenetics & hypertension treatment outcomes

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SUPPLEMENTAL METHODS

Study design and participants.

INVEST. INVEST (www.clinicaltrials.gov, NCT00133692) was a prospective, randomized, open-label, blinded-end point trial in 22,576 patients aged 50 or older with HTN and coronary artery disease (CAD). Patients were randomized to a verapamil-SR (calcium channel blocker, CCB) or an atenolol (β-blocker, βB) based treatment strategy. Hydrochlorothiazide (HCTZ) and trandolapril were available as add-on treatment in either strategy for BP control, with trandolapril added first to verapamil-SR and HCTZ added first to atenolol. The primary outcome (PO) was the first occurrence of all-cause death, nonfatal myocardial infarction (MI) or nonfatal stroke. Secondary outcomes included the individual components of the PO. Events were adjudicated by an independent committee that was blinded to treatment strategy. The methods and results of INVEST have been previously published.1

NORDIL. NORDIL was a HTN outcomes trial including 10,881 subjects in Norway and Sweden comparing a CCB strategy (diltiazem), to conventional antihypertensive treatment utilizing diuretics, βBs, or both. Patients were 50-74 years old, with an untreated DBP ≥100 mmHg. The primary endpoint was fatal or nonfatal stroke, fatal or nonfatal MI, or other CV death, and information is available on all death events. The methods and results of NORDIL have been previously published.2

Genotyping and Quality Control

INVEST-GENES. Genomic DNA was extracted from buccal cells obtained by mouthwash using the Gentra Systems PureGene kit. Patients were genotyped on the Illumina HumanCVD (Cardiovascular Disease) Beadchip, a gene-centric array containing ~50,000 SNPs in ~2,100 genes involved in cardiovascular, inflammatory, and metabolic processes.3 Genotyping was performed on Illumina’s iScan System using the Infinium II Assay (Illumina, San Diego, CA). Genotypes were called using GenomeStudio Software version 2011.1 and the Genotyping Module version 1.9 calling algorithm (Illumina, San Diego, CA). Patients were excluded if sample genotype call rates were below 95% and SNPs were excluded if genotype call rates were below 90%. Eighty-seven blind duplicates were included in genotyping and had a concordance rate of 99.997%. Gender was confirmed from X chromosome genotype data, and those who were discordant were excluded. Cryptic relatedness was estimated by pairwise identity-by-descent (IBD) analysis implemented using PLINK4 (http://pngu.mgh.harvard.edu/purcell/plink/). Ten pairs of samples were identified as first degree relatives; these individuals were kept for the analysis and sensitivity analyses were performed without these subjects. Heterozygosity was assessed using PLINK, by estimating the inbreeding coefficient, F. Six subjects had F values > 4 standard deviations from the mean. One of these subjects also had a high missing genotype rate of > 4% and this subject was excluded. Outliers in the by race/ethnic group Principal Component Analysis were also removed (n=4). The final case-control cohort consisted of 1,345 subjects.

NORDIL. 4,196 subjects were successfully genotyped on the Illumina 610Quad genome-wide array (Illumina, San Diego, CA) and the technology described above.
Standard quality control procedures were applied. Genotypes were imputed to 2.4 million SNPs using the CEU panel from HapMap 2.

**Principal Component Analysis in INVEST**. To address the issue of population substructure and admixture in our racially and ethnically diverse population, a Principal Component Analysis (PCA) was performed in all subjects on a linkage disequilibrium (LD) pruned dataset using the EIGENSTRAT method implemented through JMP Genomics version 5.0 (SAS, Cary, NC). Race/ethnic groups were confirmed with PCA clustering results. If race/ethnic category disagreed strongly with the race/ethnicity information recorded during the trial, patients were re-categorized to reflect the PCA result, considered to better reflect genetic ancestry. Then, PCA was performed in each genetically determined race/ethnic group. The top principal components that provided the best separation of ancestry clusters were selected to be included as covariates for analysis: PCs 1-3 in the by race analyses, and PCs 1-2 in the combined cohort.

**Statistical Analysis**

**Hardy-Weinberg equilibrium**. Hardy-Weinberg equilibrium was evaluated by race/ethnic group using an exact test, implemented through PLINK.

**Haplotype Analysis and Linkage Disequilibrium**. Haplotypes were reconstructed in each race/ethnic group in INVEST from raw genotype data for **SELP** rs6125 and **SELE** rs5361 using PHASE version 2. Each haplotype was coded according to the number of copies (zero, one or two). Haplotype x treatment interaction analysis was performed by race/ethnic group using SAS version 9.2 (Cary, NC). Pairwise measure of $r^2$ and D were assessed using Haploview version 4.2.

**Initial Genetic Risk Score Calculations**. Genetic risk scores were first calculated in INVEST (whites and Hispanics) based on a two SNP model, including rs16982743 and rs893184; and then on a three SNP model, testing each of the significant SNPs on chromosome 1 with the chromosome 19 SNPs above. The genetic risk score was tested for replication in NORDIL, testing the three 3 SNP models that performed better than the two SNP model in INVEST (the chromosome 19 SNPs plus rs4525, rs6125, or rs6131), and had a MAF >1% in NORDIL (rs9332701 model was excluded for low MAF). Additionally, the haplotype model was not tested in NORDIL as the genotype data was imputed and haplotypes were unable to be phased.

**Supplemental References.**


4. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. Plink: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559-575.


Table S1. Baseline Demographics and Characteristics stratified by treatment strategy.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>INVEST Whites (n=795)</th>
<th>INVEST Hispanics (n=380)</th>
<th>NORDIL (n=4196)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCB n=398</td>
<td>βB n=397</td>
<td>CCB n=199</td>
</tr>
<tr>
<td>Age, mean (SD) y</td>
<td>69.9 ± 9.6</td>
<td>71.7 ± 9.3</td>
<td>72.2 ± 8.8</td>
</tr>
<tr>
<td>Male</td>
<td>213 (53.5)</td>
<td>202 (50.9)</td>
<td>87 (43.7)</td>
</tr>
<tr>
<td>BMI, mean (SD) kg/m²</td>
<td>28.8 ± 5.3</td>
<td>28.4 ± 5.6</td>
<td>27.4 ± 4.7</td>
</tr>
<tr>
<td>SBP, mean (SD), mm Hg</td>
<td>147.4 ± 18.9</td>
<td>149.9 ± 16.0</td>
<td>148.3 ± 18.3</td>
</tr>
<tr>
<td>DBP, mean (SD), mm Hg</td>
<td>81.3 ± 10.6</td>
<td>82.0 ± 10.1</td>
<td>85.2 ± 9.8</td>
</tr>
<tr>
<td>Heart Rate, mean (SD), beats/min</td>
<td>75.2 ± 9.5</td>
<td>75.2 ± 9.2</td>
<td>74.2 ± 8.9</td>
</tr>
<tr>
<td>History of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>75 (18.8)</td>
<td>78 (19.6)</td>
<td>44 (22.1)</td>
</tr>
<tr>
<td>Heart Failure (class I to III)</td>
<td>25 (6.3)</td>
<td>25 (6.3)</td>
<td>12 (6.0)</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>138 (34.7)</td>
<td>142 (35.8)</td>
<td>24 (12.1)</td>
</tr>
</tbody>
</table>

Values are presented as number (percentage) unless otherwise noted. SD: Standard Deviation. kg: kilograms. m: meters. SBP: systolic blood pressure. DBP: diastolic blood pressure. mm Hg: millimeters of Mercury. min: minute. N/A: characteristic not available in NORDIL genetic study. CCB: calcium channel blocker. βB: β-blocker.
Table S2. Genotype Frequencies and Hardy-Weinberg Equilibrium $P$-values in INVEST and NORDIL.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Alleles</th>
<th>Minor Allele Frequency</th>
<th>Genotype Frequencies</th>
<th>Hardy-Weinberg Equilibrium</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>minor(m) Major(M)</td>
<td>mm/mM/ MM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INVEST White</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16982743</td>
<td>A G</td>
<td>0.179</td>
<td>26/233/536</td>
<td></td>
<td>0.9041</td>
</tr>
<tr>
<td>rs893184</td>
<td>A G</td>
<td>0.046</td>
<td>4/65/723</td>
<td></td>
<td>0.0781</td>
</tr>
<tr>
<td>rs9332701</td>
<td>G A</td>
<td>0.038</td>
<td>1/59/735</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>rs4525</td>
<td>G A</td>
<td>0.260</td>
<td>49/316/430</td>
<td></td>
<td>0.4076</td>
</tr>
<tr>
<td>rs6131</td>
<td>A G</td>
<td>0.180</td>
<td>26/234/535</td>
<td></td>
<td>0.9047</td>
</tr>
<tr>
<td>rs6125</td>
<td>A G</td>
<td>0.057</td>
<td>1/88/705</td>
<td></td>
<td>0.5057</td>
</tr>
<tr>
<td>rs5361</td>
<td>C A</td>
<td>0.096</td>
<td>7/139/647</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INVEST Hispanic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16982743</td>
<td>A G</td>
<td>0.195</td>
<td>17/114/248</td>
<td></td>
<td>0.4135</td>
</tr>
<tr>
<td>rs893184</td>
<td>A G</td>
<td>0.101</td>
<td>4/68/304</td>
<td></td>
<td>0.7816</td>
</tr>
<tr>
<td>rs9332701</td>
<td>G A</td>
<td>0.012</td>
<td>0/9/371</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>rs4525</td>
<td>G A</td>
<td>0.362</td>
<td>48/179/153</td>
<td></td>
<td>0.7398</td>
</tr>
<tr>
<td>rs6131</td>
<td>A G</td>
<td>0.201</td>
<td>15/122/242</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>rs6125</td>
<td>A G</td>
<td>0.070</td>
<td>2/49/329</td>
<td></td>
<td>0.7010</td>
</tr>
<tr>
<td>rs5361</td>
<td>C A</td>
<td>0.070</td>
<td>2/49/329</td>
<td></td>
<td>0.7010</td>
</tr>
<tr>
<td>NORDIL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16982743</td>
<td>A G</td>
<td>0.191</td>
<td>148/1254/2657</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>rs893184</td>
<td>A G</td>
<td>0.045</td>
<td>8/359/3828</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>rs9332701</td>
<td>G A</td>
<td>0.005</td>
<td>0/32/2918</td>
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<td>1</td>
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<tr>
<td>rs4525</td>
<td>G A</td>
<td>0.255</td>
<td>263/1613/2319</td>
<td></td>
<td>0.4644</td>
</tr>
<tr>
<td>rs6131</td>
<td>A G</td>
<td>0.209</td>
<td>185/1383/2627</td>
<td></td>
<td>0.8519</td>
</tr>
<tr>
<td>rs6125</td>
<td>A G</td>
<td>0.073</td>
<td>28/559/3609</td>
<td></td>
<td>0.2107</td>
</tr>
<tr>
<td>rs5361</td>
<td>C A</td>
<td>0.111</td>
<td>49/832/3314</td>
<td></td>
<td>0.7541</td>
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</table>
Table S3. Pairwise $r^2$ and $D'$ between rs16982743 and rs893184 on chromosome 19 in INVEST whites and Hispanics.

<table>
<thead>
<tr>
<th></th>
<th>SNP 1</th>
<th>SNP 2</th>
<th>D'</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whites</td>
<td>rs16982743</td>
<td>rs893184</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>Hispanics</td>
<td>rs16982743</td>
<td>rs893184</td>
<td>0.24</td>
<td>0.02</td>
</tr>
</tbody>
</table>
**Table S4.** Chromosome 1 rs6125-rs5361 Haplotype-Treatment Interaction in INVEST.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Chr</th>
<th>Positions</th>
<th>Genes</th>
<th>Amino Acid Changes</th>
<th>Coded Haplotype</th>
<th>Race</th>
<th>Haplotype Frequency</th>
<th>Interaction P-value</th>
<th>Combined Interaction P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6125,</td>
<td>1</td>
<td>167,848,941,</td>
<td>SELP,</td>
<td>V209M,</td>
<td>GA</td>
<td>White</td>
<td>0.8472</td>
<td>0.0097</td>
<td></td>
</tr>
<tr>
<td>rs5361</td>
<td></td>
<td>167,967,684</td>
<td>SELE</td>
<td>S149R (Val-Ser)</td>
<td></td>
<td>Hisp</td>
<td>0.8605</td>
<td>0.0028</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Chr: Chromosome.  Position: NCBI build 36 position.
<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>Gene</th>
<th>A1</th>
<th>INVEST White</th>
<th>INVEST Hispanic</th>
<th>NORDIL</th>
<th>Meta-Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MAF</td>
<td>OR</td>
<td>P value</td>
<td>MAF</td>
</tr>
<tr>
<td>19</td>
<td>rs16982743</td>
<td>SIGLEC12</td>
<td>A</td>
<td>0.179</td>
<td>1.98</td>
<td>0.0329</td>
<td>0.195</td>
</tr>
<tr>
<td>19</td>
<td>rs893184</td>
<td>A1BG</td>
<td>A</td>
<td>0.046</td>
<td>5.08</td>
<td>0.0248</td>
<td>0.101</td>
</tr>
<tr>
<td>1</td>
<td>rs9332701</td>
<td>F5</td>
<td>G</td>
<td>0.038</td>
<td>8.66</td>
<td>0.0110</td>
<td>0.012</td>
</tr>
<tr>
<td>1</td>
<td>rs4525</td>
<td>F5</td>
<td>G</td>
<td>0.260</td>
<td>0.83</td>
<td>0.5554</td>
<td>0.362</td>
</tr>
<tr>
<td>1</td>
<td>rs6131</td>
<td>SELP</td>
<td>A</td>
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<tr>
<td>1</td>
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<td>SELP</td>
<td>A</td>
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<td>0.1001</td>
<td>0.070</td>
</tr>
<tr>
<td>1</td>
<td>rs5361</td>
<td>SELE</td>
<td>C</td>
<td>0.096</td>
<td>0.43</td>
<td>0.0486</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Figure S1. Pharmacogenomic association with the primary outcome. A) SIGLEC12 rs16982743. B) A1BG rs893184. n: number.
Figure S2. Regional plot of the F5-SELP-SELL-SELE region on chromosome 1 in INVEST whites. $P$-value displayed is the SNPxTreatment interaction $P$-value.
Figure S3. Regional plot of the F5-SELP-SELL-SELE region on chromosome 1 in INVEST Hispanics. $P$-value displayed is the SNPxTreatment interaction $P$-value.
**Figure S4.** Risk-Score x Treatment Interaction analysis for secondary outcomes in INVEST. A) Nonfatal Myocardial Infarction (MI). B) Nonfatal Stroke. C) All-Cause Death.

CCB: Calcium Channel Blocker. βB: β-Blocker. Low Risk defined as risk score = 0 or 1; High Risk defined as risk score = 2 or 3.