ONLINE DATA SUPPLEMENT

PHARMACOGENOMIC ASSOCIATION OF NON-SYNONYMOUS SNPS IN *SIGLEC12*, *A1BG* AND THE SELECTIN REGION AND CARDIOVASCULAR 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 PLINK[4] (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[5] 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.[4]

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.[6] 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.[7]

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.

1. Pepine CJ, Handberg EM, Cooper-DeHoff RM, Marks RG, Kowey P, Messerli FH, Mancia G, Cangiano JL, Garcia-Barreto D, Keltai M, Erdine S, Bristol HA, Kolb HR, Bakris GL, Cohen JD, Parmley WW. A calcium antagonist vs a non-calcium antagonist hypertension treatment strategy for patients with coronary artery disease. The international verapamil-trandolapril study (invest): A randomized controlled trial. *JAMA*. 2003;290:2805-2816.
2. Hansson L, Hedner T, Lund-Johansen P, Kjeldsen SE, Lindholm LH, Syvertsen JO, Lanke J, de Faire U, Dahlof B, Karlberg BE. Randomised trial of effects of

calcium antagonists compared with diuretics and beta-blockers on cardiovascular morbidity and mortality in hypertension: The nordic diltiazem (nordil) study. *Lancet*. 2000;356:359-365.

3. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, Galver L, Barrett JC, Grant SF, Farlow DN, Chandrupatla HR, Hansen M, Ajmal S, Papanicolaou GJ, Guo Y, Li M, Derohannessian S, de Bakker PI, Bailey SD, Montpetit A, Edmondson AC, Taylor K, Gai X, Wang SS, Fornage M, Shaikh T, Groop L, Boehnke M, Hall AS, Hattersley AT, Frackelton E, Patterson N, Chiang CW, Kim CE, Fabsitz RR, Ouwehand W, Price AL, Munroe P, Caulfield M, Drake T, Boerwinkle E, Reich D, Whitehead AS, Cappola TP, Samani NJ, Lusis AJ, Schadt E, Wilson JG, Koenig W, McCarthy MI, Kathiresan S, Gabriel SB, Hakonarson H, Anand SS, Reilly M, Engert JC, Nickerson DA, Rader DJ, Hirschhorn JN, Fitzgerald GA. Concept, design and implementation of a cardiovascular gene-centric 50 k snp array for large-scale genomic association studies. *PLoS One*. 2008;3:e3583.
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.
5. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904-909.
6. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet*. 2003;73:1162-1169.
7. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of ld and haplotype maps. *Bioinformatics*. 2005;21:263-265.

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

Characteristic	INVEST Whites (n=795)		INVEST Hispanics (n=380)		NORDIL (n=4196)	
	CCB n=398	βB n=397	CCB n=199	βB n=181	CCB n=2093	βB/Diuretic n=2103
Age, mean (SD) y	69.9 ± 9.6	71.7 ± 9.3	72.2 ± 8.8	72.4 ± 8.9	60.2 ± 6.6	60.0 ± 6.6
Male	213 (53.5)	202 (50.9)	87 (43.7)	98 (54.2)	1049 (50.1)	1044 (49.6)
BMI, mean (SD) kg/m²	28.8 ± 5.3	28.4 ± 5.6	27.4 ± 4.7	28.2 ± 4.4	28.0 ± 4.4	28.3 ± 4.4
SBP, mean (SD), mm Hg	147.4 ± 18.9	149.9 ± 16.0	148.3 ± 18.3	147.8 ± 18.9	173.0 ± 18.8	172.1 ± 19.0
DBP, mean (SD), mm Hg	81.3 ± 10.6	82.0 ± 10.1	85.2 ± 9.8	84.9 ± 10.4	103.1 ± 7.2	103.1 ± 7.2
Heart Rate, mean (SD), beats/min	75.2 ± 9.5	75.2 ± 9.2	74.2 ± 8.9	73.0 ± 9.3	N/A	N/A
History of						
Diabetes	75 (18.8)	78 (19.6)	44 (22.1)	46 (25.4)	172 (8.2)	170 (8.1)
Heart Failure (class I to III)	25 (6.3)	25 (6.3)	12 (6.0)	6 (3.3)	N/A	N/A
Myocardial Infarction	138 (34.7)	142 (35.8)	24 (12.1)	29 (16.0)	48 (2.3)	53 (2.5)

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: characertistic 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.

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

Table S3. Pairwise r^2 and D' between rs16982743 and rs893184 on chromosome 19 in INVEST whites and Hispanics.

SNP 1	SNP 2	D'	r^2
Whites			
rs16982743	rs893184	0.03	0
Hispanics			
rs16982743	rs893184	0.24	0.02

Table S4. Chromosome 1 rs6125-rs5361 Haplotype-Treatment Interaction in INVEST.

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

Chr: Chromosome. Position: NCBI build 36 position.

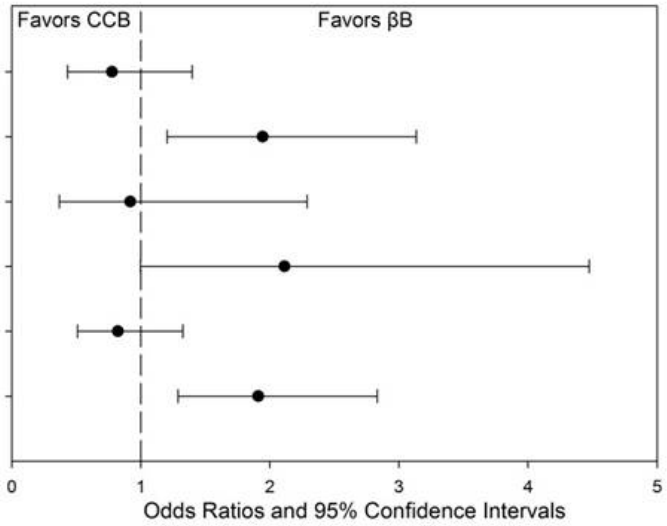
Table S5. SNP Treatment Interaction in INVEST and NORDIL

Chr	SNP	Gene	A1	INVEST White			INVEST Hispanic			NORDIL			Meta-Analysis	
				MAF	OR	*P* value	MAF	OR	*P* value	MAF	OR	*P* value	*P* value	Direction
19	rs16982743	SIGLEC12	A	0.179	1.98	0.0329	0.195	3.13	0.0215	0.191	1.16	0.4655	0.0032	+++
19	rs893184	A1BG	A	0.046	5.08	0.0248	0.101	5.12	0.0310	0.045	1.34	0.4004	0.0029	+++
1	rs9332701	F5	G	0.038	8.66	0.0110	0.012	4.78	0.3948	0.005	1.86	0.6200	0.0283	+++
1	rs4525	F5	G	0.260	0.83	0.5554	0.362	2.65	0.0242	0.255	1.32	0.1261	0.0509	-++
1	rs6131	SELP	A	0.180	0.46	0.0254	0.201	0.41	0.0686	0.209	1.36	0.1174	0.1349	--+
1	rs6125	SELP	A	0.057	0.39	0.1001	0.070	0.09	0.0023	0.073	1.61	0.1351	0.0556	--+
1	rs5361	SELE	C	0.096	0.43	0.0486	0.070	0.41	0.2659	0.111	1.16	0.5510	0.1638	--+

Chr: Chromosome. A1: Minor Allele. MAF: Minor Allele Frequency. OR: Odds Ratio.

A) *SIGLEC12* rs16982743

		Event, n (%) CCB	βB	OR (95% CI)	P-value
White	Stop Carrier	31 (23.5%)	36 (28.3%)	0.778 (0.432-1.40)	0.4015
	Gln/Gln	58 (21.8%)	34 (12.6%)	1.944 (1.205-3.136)	0.0065
Hisp	Stop Carrier	17 (22.1%)	15 (27.8%)	0.919 (0.369-2.289)	0.8553
	Gln/Gln	26 (21.3%)	17 (13.5%)	2.114 (0.999-4.475)	0.0503
All	Stop Carrier	48 (23.0%)	51 (28.2%)	0.823 (0.511-1.326)	0.4241
	Gln/Gln	84 (21.6%)	51 (12.9%)	1.911 (1.289-2.832)	0.0013



B) *A1BG* rs893184

		Event, n (%) CCB	βB	OR (95% CI)	P-value
White	His Carrier	3 (8.6%)	7 (20.6%)	0.068 (0.005-0.967)	0.0472
	Arg/Arg	86 (23.8%)	63 (17.4%)	1.527 (1.052-2.216)	0.026
Hisp	His Carrier	4 (11.4%)	9 (24.3%)	0.281 (0.063-1.249)	0.0954
	Arg/Arg	39 (23.9%)	23 (16.3%)	1.914 (1.012-3.622)	0.046
All	His Carrier	7 (10.0%)	16 (22.5%)	0.216 (0.067-0.698)	0.0105
	Arg/Arg	125 (23.9%)	86 (17.1%)	1.602 (1.168-2.196)	0.0034

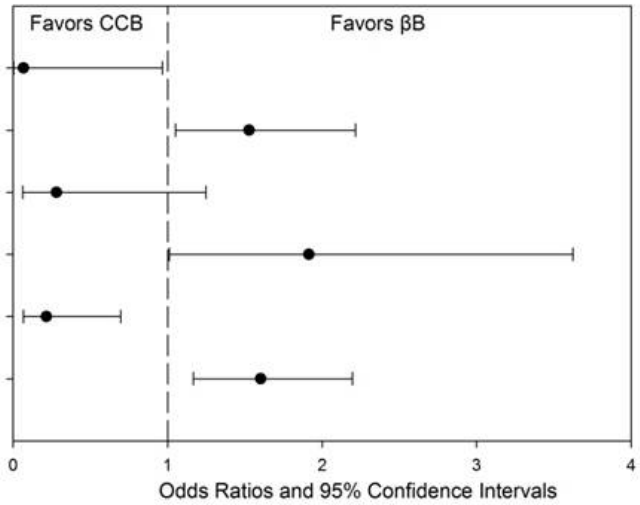


Figure S1. Pharmacogenomic association with the primary outcome. A) *SIGLEC12* rs16982743. B) *A1BG* rs893184. n: number.

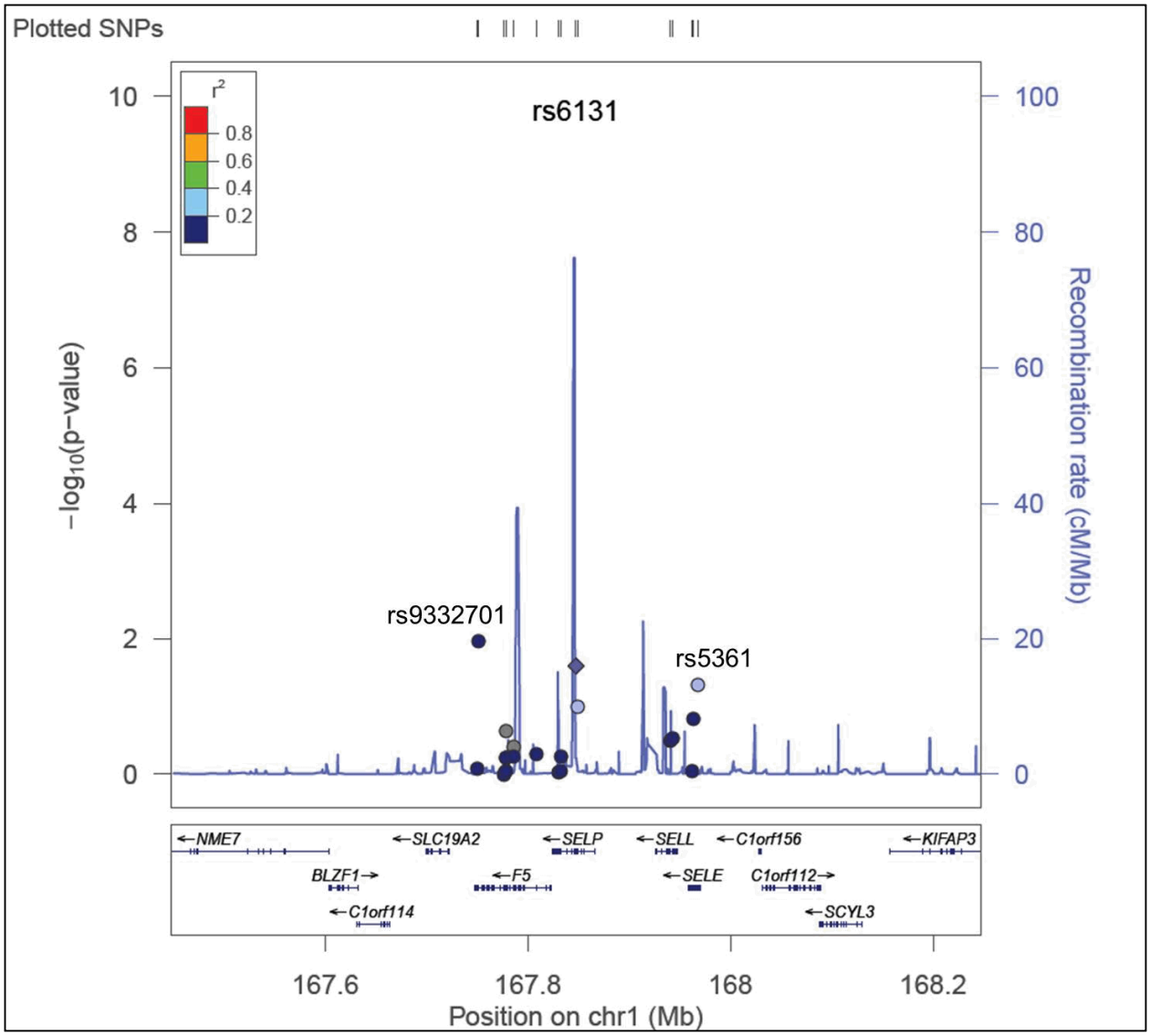


Figure S2. Regional plot of the *F5-SELP-SELL-SELE* region on chromosome 1 in INVEST whites. *P*-value displayed is the SNPxTreatment interaction *P*-value.

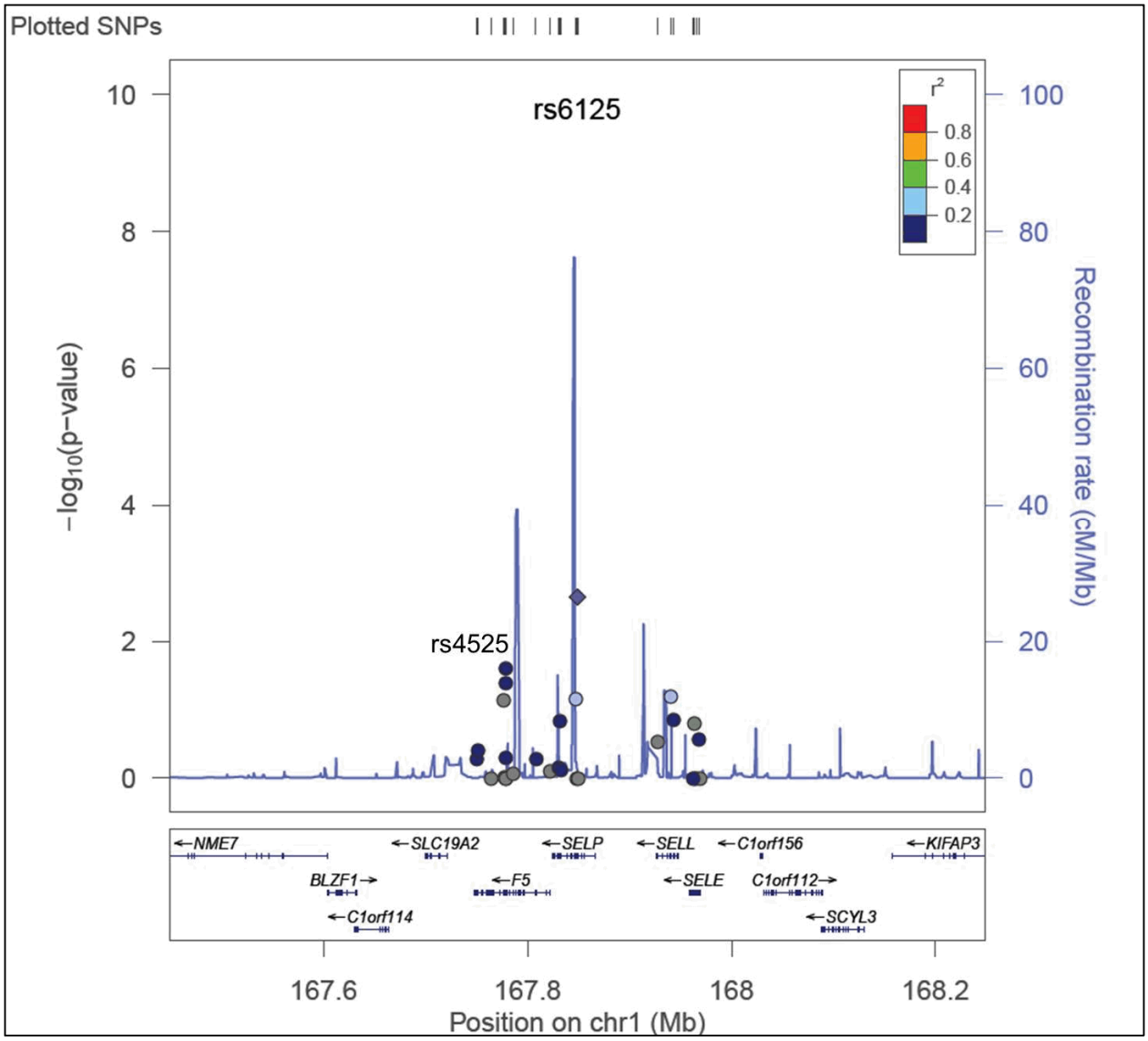
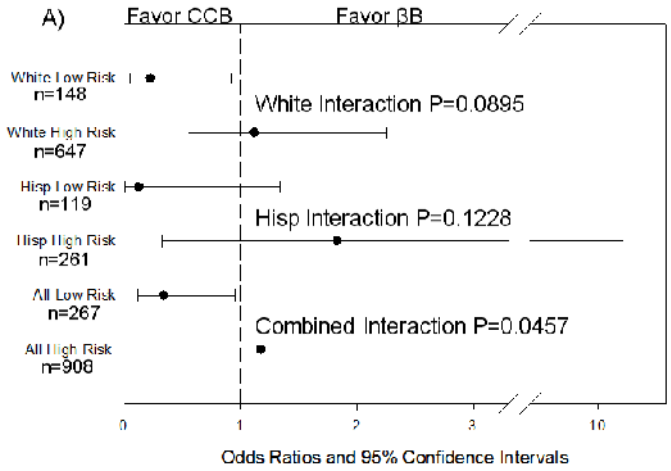
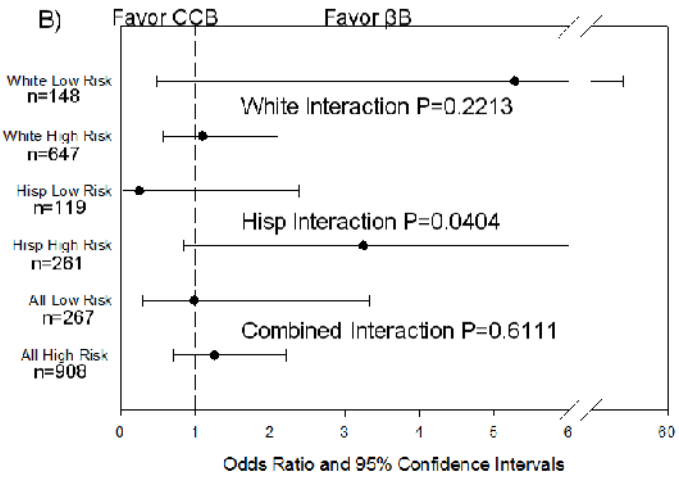


Figure S3. Regional plot of the *F5-SELP-SELL-SELE* region on chromosome 1 in INVEST Hispanics. *P*-value displayed is the SNPxTreatment interaction *P*-value.


A)
Favor CCB
Favor βB
White Low Risk
n=148
White High Risk
n=647
Hisp Low Risk
n=119
Hisp High Risk
n=261
All Low Risk
n=267
All High Risk
n=908
White Interaction P=0.0895
Hisp Interaction P=0.1228
Combined Interaction P=0.0457
0
1
2
3
10
Odds Ratios and 95% Confidence Intervals


B)
Favor CCB
Favor βB
White Low Risk
n=148
White High Risk
n=647
Hisp Low Risk
n=119
Hisp High Risk
n=261
All Low Risk
n=267
All High Risk
n=908
White Interaction P=0.2213
Hisp Interaction P=0.0404
Combined Interaction P=0.6111
0
1
2
3
4
6
60
Odds Ratio and 95% Confidence Intervals

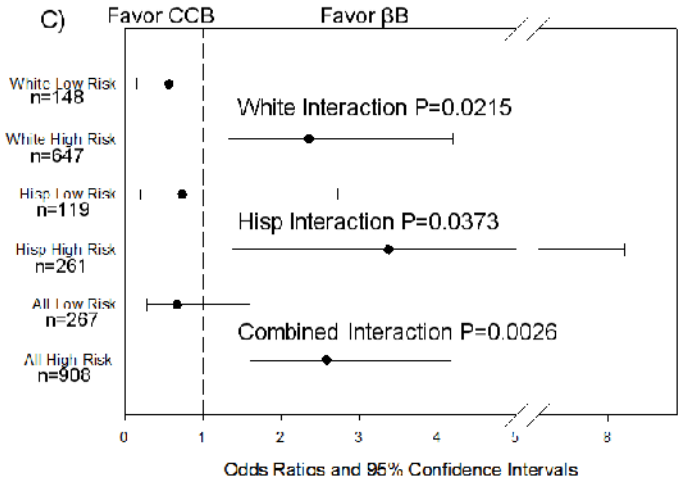


Figure S4. Risk-Score x Treatment Interaction analysis for secondary outcomes in INVEST. A) Nonfatal Myocardial Infarction (MI). B) Nonfatal Stroke. C) All-Cause Death.
CCB: Calcium Channel Blocker. βB: β-Blocker. Low Risk defined as risk score = 0 or 1; High Risk defined as risk score = 2 or 3.