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Specific P-Selectin and P-Selectin Glycoprotein Ligand–1 Genotypes/Haplotypes are Associated with Risk of Incident CHD and Ischemic Stroke: The Atherosclerosis Risk in Communities (ARIC) Study

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Abstract

Objective—P-selectin (PSEL) and its ligand, P-selectin glycoprotein ligand-1 (PSGL-1), play key roles in both the inflammatory response and the atherosclerotic process, but there are conflicting results regarding the affect of PSEL and PSGL-1 gene variation on risk for cardiovascular and cerebrovascular disease. We tested the association of four PSEL and two PSGL-1 polymorphisms with incident coronary heart disease (CHD) and ischemic stroke among 13,875 participants in the prospective Atherosclerosis Risk in Communities (ARIC) study. We also tested common haplotypes in the PSEL and PSGL-1 genes to assess associations with incident CHD and ischemic stroke.

Methods and Results—Incident ischemic stroke and CHD were identified through annual telephone calls and hospital and death certificate surveillance. Five hundred twenty-five validated ischemic stroke and 1,654 CHD events were identified. Allele frequencies for all PSEL and PSGL-1 polymorphisms were markedly different between whites and African Americans; therefore, all analyses were performed race-specific. Independent analyses showed the PSEL 290NN genotype to be a significant predictor of CHD in whites (HRR 1.30, 95%CI 1.00-1.70, P=0.05). PSGL-1 genotypes carrying the 62I allele were significantly protective for incident CHD (HRR 0.53, 95%CI 0.31-0.92, P=0.02) and ischemic stroke (HRR 0.73, 95%CI 0.55-0.97, P=0.03) in African Americans. Haplotype analyses showed the PSEL NNVP haplotype to be a significant predictor of incident CHD in whites (HRR 2.09, 95%CI 1.23-3.55, P=0.006). No significant haplotype findings were observed in African-Americans.

Conclusions—PSEL S290N, in single polymorphism analysis and in the haplotypic background with T715P, was associated with increased risk of incident CHD in whites. The PSGL-1 M62I polymorphism was associated with decreased risk of both incident CHD and stroke in African

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Americans. These findings illustrate the complex relationship between genetic variation and disease in different racial groups.

Keywords

Cell adhesion molecules; Coronary Heart Disease; Stroke; P-Selectin; PSGL-1

Introduction

The pathogenesis of atherosclerosis is known to contain an important inflammatory component, involving the recruitment and adhesion of circulating leukocytes to the vascular endothelium [1,2]. P-selectin (PSEL), a member of the selectin family of adhesion molecules, initiates leukocyte rolling and mediates interactions of leukocytes with the endothelium, platelets with the endothelium, and leukocytes with platelets [3-5]. Multiple studies provide evidence supporting a key role for PSEL in atherosclerotic lesion formation, thrombosis and arterial wall changes [3,5-11]. Leukocyte-endothelium and leukocyte-platelet interactions require the presence of a counter-ligand on the leukocyte surface, P-selectin glycoprotein ligand-1 (PSGL-1) [12-14]. By acting as the primary ligand for PSEL, PSGL-1 plays a pivotal role in both the inflammatory response and the atherosclerotic process [15-18].

Several studies have investigated associations between genetic variation in the PSEL and PSGL-1 genes and cardiovascular disease, but results of these studies have been inconsistent [14,19-28]. All of these studies have focused on independent associations with single genetic variants, with only one examining PSEL haplotypes and one examining PSGL-1 haplotypes [14,23]. The most commonly studied PSEL polymorphism is T715P in relation to CHD, with two studies showing no association with CHD [19,20], three studies showing a protective effect of the 715P allele against CHD [21-23], and one study showing the 715P allele to be associated with increased risk of CHD [24]. Each of these was a case-control study comprised of European populations. The most commonly studied genetic variation in the PSGL-1 gene with regards to cardiovascular disease is a VNTR located in the coding region of the gene [14,25-27]. Studies investigating the PSGL-1 VNTR have been limited to Spanish and German populations, with results showing this polymorphism to have no association with CHD [14,25,26], to be associated with decreased risk of CHD [27], or to be associated with decreased risk of cerebrovascular disease [25]. We have genotyped the large prospective Atherosclerosis Risk in Communities (ARIC) cohort, comprised of both whites and African Americans, for six polymorphisms in the PSEL and PSGL-1 genes and analyzed their association, both independently and as haplotypes, with incident coronary heart disease (CHD) and ischemic stroke.

Methods

The ARIC Study

Study participants were selected from the ARIC study, a prospective investigation of atherosclerosis and its clinical sequelae involving 15,792 individuals aged 45 to 64 years at recruitment (1987-1989). Institutional review boards approved the ARIC study, and all participants provided their written informed consent. A detailed description of the ARIC study design and methods, as well as details on quality assurance for ascertainment and classification of CHD and stroke events, have been published elsewhere [29-31]. Briefly, subjects were selected by probability sampling from four communities: Forsyth County, North Carolina; Jackson, Mississippi; northwestern suburbs of Minneapolis, Minnesota; and Washington County, Maryland. Incidence of CHD and ischemic stroke were determined by contacting participants annually to identify hospitalizations during the previous year and by surveying discharge lists from local hospitals and death certificates from state vital statistics offices for

potential cardiovascular and cerebrovascular events [29-31]. Participants were excluded from analyses (n=1917) if they had 1) a positive or unknown history of prevalent stroke or CHD or a history of transient ischemic attack (TIA)/stroke symptoms at the initial clinic visit, 2) prohibited use of their DNA for research purposes, 3) an ethnic background other than white or African-American, or 4) missing genotype information for all six PSEL and PSGL-1 polymorphisms. Incident CHD cases were defined as a definite or probable myocardial infarction (MI), a silent MI between examinations by ECG, a definite CHD death, or a coronary revascularization. Ischemic stroke cases were defined as validated definite or probable hospitalized embolic or thrombotic brain infarctions. Following exclusions, a total of 1,654 CHD cases and 525 ischemic stroke cases were identified.

Baseline Examination and Laboratory Measures

Seated blood pressure was measured three times with a random-zero sphygmomanometer and the last two measurements were averaged. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or current use of antihypertensive medications. Questionnaires and in-person interviews were used to assess use of antihypertensive medications. Diabetes was defined by a fasting glucose level ≥ 126 mg/dL, a non-fasting glucose level ≥ 200 mg/dL, and/or history of or treatment for diabetes. Cigarette-smoking status was analyzed by comparing current smokers to individuals who had formerly or never smoked. Body mass index (BMI, kg/m^2) was calculated from height and weight measurements. Plasma total cholesterol was measured by an enzymatic method [32]. High-density lipoprotein (HDL) cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins [33].

Genotype Determination

Polymorphisms were selected based on being missense mutations in the PSEL or PSGL genes or previously reported to be associated with CHD and/or stroke. The polymorphisms included: PSEL S290N (rs6131), PSEL N562D (rs6127), PSEL V599L (rs6133), PSEL T715P (rs6136), PSGL-1 5′UTR (rs8179131), PSGL-1 M62I (rs2228315). Genotyping was carried out using the SNPlex or TaqMan Assays (Applied Biosystems, Inc, Foster City, CA). Primers and probes are available from the authors upon request.

Statistical Analysis

Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium expectations were tested using a χ^2 goodness-of-fit test. All statistical analyses were conducted utilizing STATA version 9.2 (College Station, TX). Because allele frequencies were different between whites and African Americans for all polymorphisms studied, all analyses were done separately by race. The variant alleles were identified as the low frequency allele in whites, and homozygous wildtype (non-variant) genotypes were designated as the referent group in the statistical analyses. The PSGL-1 5′UTR polymorphism was not polymorphic in whites, therefore results for were provided for African Americans only. Only one African American person was homozygous for the PSEL 715P allele. Therefore, the heterozygous and rare homozygous genotypes at this locus were combined for analyses in this racial group. Haplotypes were inferred using PHASE (version 2.1) [34,35]. For analyses, haplotypes were coded as indicators and compared to the most frequent (referent) haplotype.

Cox proportional hazards (PH) models were used to estimate the hazard rate ratios (HRRs) of incident CHD and ischemic stroke. For analyses of incident CHD and stroke, follow-up time intervals were defined as the time between the initial clinical visit and the date of the first event. For non-cases, follow-up continued until December 31, 2003, the date of death, or the date of last contact if lost to follow-up, whichever came first. For the analyses of incident CHD, the covariates included age, gender, field center, BMI, HDL and total cholesterol, smoking,

diabetes and hypertension status. For stroke, the covariates (identified by the National Institute of Neurological Disorders and Stroke, www.ninds.nih.gov) included age, gender, field center, smoking, diabetes and hypertension status. Covariates were assessed for statistical significance in the models by the Wald χ^2 statistic.

Results

The ARIC cohort was genotyped for six polymorphisms in the PSEL gene and three polymorphisms in the PSGL-1 gene. PSEL V168M and T233I, as well as PSGL-1 P236S, had a very low allele frequency in both whites and African Americans, did not have different frequencies between cases and non-cases, and were observed to be "tagged" by another polymorphism presented in the current study. Therefore, no additional analyses were conducted for these three polymorphisms; the current study presents results for four PSEL polymorphisms (S290N, N562D, V599L, T715P) and two PSGL-1 polymorphisms (5′UTR, M62I).

All PSEL and PSGL-1 genotype distributions were in accordance with Hardy-Weinberg equilibrium expectations. Due to the marked allele frequency differences between whites and African Americans for each polymorphism studied, analyses were performed separately by racial group. Only one African American person was homozygous for the PSEL 715P allele. Therefore, 715TP and 715PP genotypes were combined for analyses in this racial group. The PSGL-1 5′UTR polymorphism was not polymorphic in whites and therefore results were presented for African Americans only.

Results from Cox PH models used to estimate the HRRs of incident CHD for each PSEL and PSGL-1 polymorphism are presented in Table 1 by racial group. After adjustment for CHD risk factors and covariates (Model 2), the PSEL 290NN homozygous genotype was a significant predictor of incident CHD in whites (HRR 1.30, 95%CI 1.00-1.70), and the PSGL-1 62II homozygous genotype was a significant predictor of incident CHD in African Americans (HRR 0.53, 95%CI 0.31-0.92). Results from Cox PH models used to estimate the HRRs for ischemic stroke for each PSEL and PSGL-1 polymorphism are presented in Table 2 by racial group. After adjustment for established stroke risk factors and other covariates (Model 2), the PSGL-1 62MI heterozygous genotype was a significant predictor of incident ischemic stroke in African Americans (HRR 0.73, 95%CI 0.55-0.97). No significant associations were observed in whites.

Table 3 provides genotype frequencies for the PSEL S290N polymorphism in white CHD cases and non-cases, as well as genotype frequencies for the PSGL-1 M62I polymorphism in African American ischemic stroke cases, CHD cases and non-cases. The frequency of the PSEL 290NN homozygous genotype was significantly higher in CHD cases compared to non-cases (P=0.03). The frequency of the PSGL-1 62II homozygous genotype was significantly lower in CHD cases compared to non-cases (P=0.01), and the PSGL-1 62MI heterozygous genotype was significantly lower in ischemic stroke cases compared to non-cases (P=0.05).

Haplotypes were inferred using PHASE (version 2.1) [34,35]. All four PSEL polymorphisms were included in the haplotype construction for whites, resulting in eight common haplotypes that accounted for 99.8% of all haplotypes. In African Americans, the PSEL T715P polymorphism was not included in the haplotype construction due to the low frequency of the 715P allele in this racial group (f[P]=0.02). There were six common haplotypes that accounted for 99.8% of all haplotypes in African Americans. Haplotype construction for the two PSGL-1 polymorphisms were conducted only in African Americans due to the PSGL-1 5′UTR polymorphism not being polymorphic in whites; four haplotypes resulted from this analysis.

Results from Cox PH models used to estimate the HRRs of incident CHD for PSEL haplotypes are presented in Table 4a for whites only. After adjustment for age, gender and center (Model 1), the PSEL NNVP haplotype (for the S290N, N562D, V599L and T715P polymorphisms)

was a significant predictor of incident CHD in whites (HRR 1.90, 95%CI 1.12-3.23), with significance remaining after adjustment for additional covariates (HRR 2.09, 95%CI 1.23-3.55). No significant PSEL haplotype associations were observed for ischemic stroke in whites, or for incident CHD or ischemic stroke in African Americans (data not shown). Results from Cox PH models used to estimate the HRRs of incident ischemic stroke for PSGL-1 haplotypes in African Americans showed borderline significance for both haplotypes carrying the 62I allele (GI: HRR 0.74, 95%CI 0.54-1.01, and AI: HRR 0.63, 95%CI 0.39-1.00). No significant PSGL-1 haplotype associations were observed for incident CHD in African Americans (data not shown).

PSEL haplotype frequencies in white CHD cases and non-cases are presented in Table 4b. The frequency of the PSEL NNVP haplotype was significantly higher in CHD cases compared to non-cases (P=0.008). PSGL-1 haplotype frequencies were not significantly different between ischemic stroke cases and non-cases in African Americans (data not shown).

Discussion

In separate analyses of each PSEL polymorphism, we found the 290NN homozygous genotype to be a significant predictor of CHD in whites, but not in African-Americans. No significant findings were observed for ischemic stroke in either whites or African Americans. PSEL haplotype analyses in whites resulted in eight common haplotypes that accounted for 99.8% of all haplotypes observed. The NNVP haplotype was associated with increased risk of incident CHD in whites. No significant haplotype findings were observed in African-Americans.

Tregouet and colleagues performed haplotype analyses using a maximum likelihood method in two European populations (Belfast and France) comprised of the same four PSEL polymorphisms investigated in the current study [23]. They found the simultaneous presence of the 290N and N562 alleles to be associated with a significantly increased risk of MI in both the Belfast and French populations (OR=2.84 and 2.09, respectively). However, they found this to be significant only on the NNVT haplotype. In the current study, we found the NNVT haplotype to be associated, non-significantly, with an increased risk of CHD (HRR=1.15). Furthermore, in Tregouet's study, the NNVP haplotype was only observed in the French population and was associated, non-significantly, with an increased risk of MI (OR=1.59). It is hypothesized that the S290N and N562D polymorphisms, both located within the consensus repeat domain of the PSEL protein, may affect the binding of PSEL to PSGL-1 on leukocytes, resulting in a protein that is more efficient at recruiting leukocytes to the endothelium [23]. We further hypothesize that this effect on PSEL/PSGL-1 binding is additionally influenced by the presence of the T715P polymorphism, also located in the consensus repeat domain of PSEL. Functional studies investigating the implications of these polymorphisms on PSEL/PSGL-1 binding have not been performed.

For independent analyses of each PSGL-1 polymorphism, we found the 62II homozygous genotype to be a significant predictor of incident CHD, while the 62MI heterozygous genotype was a significant predictor of ischemic stroke in African Americans, but not in whites. Borderline significance was observed for both PSGL-1 haplotypes carrying the 62I allele (GI and AI haplotypes) for ischemic stroke in African Americans. These results suggest the M62I polymorphism to independently contribute to disease risk regardless of the haplotype on which it is carried.

The most commonly studied genetic variation in the PSGL-1 gene is a variable number of tandem repeats (VNTR) shown to affect the binding efficacy of PSEL to PSGL-1 and leukocyte-platelet interaction [25]. Lozano and colleagues demonstrated that the shorter VNTR alleles resulted in less efficient binding and were associated with a two-fold lower risk for

cerebrovascular disease, but not CHD [25]. The PSGL-1 M62I polymorphism is positioned upstream of this VNTR, just bordering the PSEL binding domain. Although the functional consequence of the M62I polymorphism is unknown, the proximity of this variant to the binding domain suggests that it too may have an effect on PSEL binding [14,36]. To our knowledge, the PSGL-1 M62I polymorphism has not been studied with regards to stroke, and only two case-control studies have investigated the PSGL-1 M62I polymorphism with regards to CHD: one study showed no association [14] while the other study showed the 62I allele to be protective against CHD [24]. Our finding of an inverse relationship between the number of 62I alleles and risk of incident CHD and ischemic stroke is consistent with the suggestion that this polymorphism results in lower PSEL/PSGL-1 binding capacity, thus resulting in fewer leukocyte-endothelium and leukocyte-platelet complexes. Although we did not find a significant association of the 62II homozygous genotype in whites, the HRRs for both incident CHD and ischemic stroke showed a similar trend in the inverse direction (HRR 0.72 and 0.64, respectively). The lack of a significant association in whites may be due to the small number of persons with the 62II homozygous genotype in this racial group (N=43).

As is commonly observed in genetic association studies investigating different racial/ethnic groups, we detected associations with disease status in one racial group and not another. In the current study, the PSEL S290N polymorphism was associated with incident CHD in whites but not African Americans, while the PSGL-1 M62I polymorphism was associated with both incident CHD and stroke in African Americans but not whites. Possible explanations for these race-specific findings include the marked variant allele frequency difference between whites and African Americans for PSEL S290N (0.33 and 0.18, respectively) and PSGL-1 M62I (0.06 and 0.27, respectively) may relate to discrepancies in underlying genetic susceptibility to disease with regards to cell adhesion molecules. Additionally, cell adhesion molecules may be more important in the development of disease in one racial group, with other factors acting through different mechanisms being more important in the other group [37]. Moreover, we cannot determine by our study if the polymorphisms studied have a direct causative effect; they may be in linkage disequilibrium with the true functional variant(s). Owing to the fact that linkage disequilibrium patterns are different between whites and African Americans, if we are not investigating the true functional variant, we may detect associations with ‘marker’ polymorphisms in one race and not another. We must also acknowledge that our results may be due to chance findings when one considers multiple testing and that significant findings were not consistent between racial groups. Therefore, results should be taken in context and note that all findings have been stated to be suggestive.

Results from the current study suggest that the PSEL S290N polymorphism, most notably on a haplotypic background with the N562, V599 and 715P alleles, to be a predictor of incident CHD in whites, while the PSGL-1 M62I polymorphism was determined to be a predictor of both incident CHD and ischemic stroke in African Americans. Possible mechanism by which these polymorphisms may affect disease risk is by altering the binding affinity of PSEL to PSGL-1, thus attenuating/increasing the recruitment and adhesion of leukocytes and platelets to the endothelium wall and/or reducing/enhancing leukocyte-platelet aggregates. Further studies are warranted to replicate the current findings in other large US populations of whites and African Americans and to determine the functional consequence of the PSGL-1 M62I polymorphism, the PSEL S290N polymorphism and the PSEL NNVP haplotype.

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Table 1

Hazard Rate Ratio (HRR) for PSEL and PSGL-1 Genotypes and Incident CHD

Incident CHD Genotype*	Genotype Counts**	Model 1[a]	Model 2[b]
		HRR (95%CI), *P*	HRR (95%CI), *P*
PSEL S290N			
SN (white)	6562 / 2930	1.04 (0.92-1.18), *0.5*	1.06 (0.94-1.20), *0.3*
NN (white)	6562 / 349	1.29 (0.99-1.68), *0.06*	1.30 (1.00-1.70), *0.05*
SN (African-Am)	1525 / 1419	1.07 (0.86-1.34), *0.5*	0.98 (0.78-1.23), *0.8*
NN (African-Am)	1525 / 410	0.77 (0.53-1.13), *0.2*	0.72 (0.49-1.06), *0.09*
PSEL N562D			
ND (white)	2912 / 4374	1.05 (0.93-1.20), *0.4*	1.07 (0.94-1.22), *0.3*
DD (white)	2912 / 1746	0.94 (0.79-1.11), *0.5*	0.92 (0.77-1.08), *0.3*
ND (African-Am)	2120 / 884	0.86 (0.67-1.10), *0.2*	0.90 (0.70-1.15), *0.4*
DD (African-Am)	2120 / 108	0.94 (0.51-1.72), *0.8*	1.04 (0.56-1.90), *0.9*
PSEL V599L			
VL (white)	7662 / 2051	1.02 (0.89-1.16), *0.8*	1.05 (0.92-1.20), *0.5*
LL (white)	7662 / 115	1.14 (0.69-1.86), *0.6*	1.02 (0.62-1.67), *0.9*
VL (African-Am)	672 / 1632	1.11 (0.83-1.50), *0.5*	1.02 (0.76-1.37), *0.9*
LL (African-Am)	672 / 1042	1.22 (0.89-1.67), *0.2*	1.07 (0.78-1.46), *0.7*
PSEL T715P			
TP (white)	7888 / 1952	1.01 (0.88-1.16), *0.9*	0.99 (0.86-1.14), *0.9*
PP (white)	7888 / 121	1.03 (0.64-1.67), *0.9*	1.00 (0.62-1.62), *1.0*
TP+PP (African-Am)	3094 / 140	0.96 (0.57-1.60), *0.9*	1.03 (0.61-1.72), *0.9*
PSGL-1 5′UTR			
AG (African-Am)	2748 / 605	1.06 (0.80-1.39), *0.7*	0.96 (0.72-1.26), *0.8*
AA (African-Am)	2748 / 42	0.66 (0.21-2.07), *0.5*	0.71 (0.23-2.24), *0.6*
PSGL-1 M62I			
MI (white)	8559 / 1184	1.13 (0.96-1.33), *0.1*	1.09 (0.93-1.29), *0.3*
II (white)	8559 / 43	0.74 (0.28-1.98), *0.5*	0.72 (0.27-1.92), *0.5*
MI (African-Am)	1749 / 1338	0.93 (0.75-1.16), *0.5*	0.94 (0.76-1.18), *0.6*
II (African-Am)	1749 / 243	0.53 (0.31-0.91), *0.02*	0.53 (0.31-0.92), *0.02*

[a] Adjusted for age, gender, center

[b] Adjusted for age, gender, center, BMI, HDL and total cholesterol, smoking, diabetes, hypertension status

* Referent group: wild-type genotype

** Genotype counts presented as (# referent genotype) / (# variant genotype); PSGL-1 5′UTR was not polymorphic in whites and therefore not presented

Table 2

Hazard Rate Ratio (HRR) for PSEL and PSGL-1 Genotypes and Incident Ischemic Stroke (ISC)

Incident ISC Genotype*	Genotype Counts**	Model 1[a]	Model 2[b]
		HRR (95%CI), *P*	HRR (95%CI), *P*
PSEL S290N			
SN (white)	6545 / 2927	1.05 (0.81-1.35), *0.7*	1.03 (0.80-1.33), *0.8*
NN (white)	6545 / 349	0.94 (0.50-1.78), *0.9*	0.92 (0.48-1.73), *0.8*
SN (African-Am)	1559 / 1421	0.86 (0.64-1.15), *0.3*	0.83 (0.62-1.11), *0.2*
NN (African-Am)	1559 / 415	1.27 (0.86-1.87), *0.2*	1.29 (0.87-1.90), *0.2*
PSEL N562D			
ND (white)	2908 / 4364	1.01 (0.76-1.33), *0.9*	1.00 (0.76-1.32), *1.0*
DD (white)	2908 / 1743	1.07 (0.76-1.51), *0.7*	1.01 (0.72-1.43), *0.9*
ND (African-Am)	2135 / 904	0.93 (0.68-1.27), *0.6*	0.97 (0.71-1.32), *0.9*
DD (African-Am)	2135 / 109	0.69 (0.28-1.68), *0.4*	0.73 (0.30-1.78), *0.5*
PSEL V599L			
VL (white)	7649 / 2047	0.87 (0.65-1.17), *0.4*	0.91 (0.68-1.23), *0.5*
LL (white)	7649 / 113	1.71 (0.71-4.16), *0.2*	1.57 (0.64-3.81), *0.3*
VL (African-Am)	683 / 1651	1.22 (0.84-1.78), *0.3*	1.11 (0.76-1.62), *0.6*
LL (African-Am)	683 / 1051	1.27 (0.85-1.90), *0.2*	1.08 (0.73-1.62), *0.7*
PSEL T715P			
TP (white)	7871 / 1950	1.01 (0.76-1.34), *1.0*	1.01 (0.76-1.34), *0.9*
PP (white)	7871 / 121	0.75 (0.24-2.33), *0.6*	0.73 (0.23-2.29), *0.6*
TP+PP (African-Am)	3121 / 144	0.67 (0.31-1.41), *0.3*	0.68 (0.32-1.44), *0.3*
PSGL-1 5′UTR			
AG (African-Am)	2775 / 617	0.80 (0.55-1.16), *0.2*	0.77 (0.53-1.13), *0.2*
AA (African-Am)	2775 / 42	0.71 (0.18-2.86), *0.6*	0.73 (0.18-2.95), *0.7*
PSGL-1 M62I			
MI (white)	8538 / 1186	0.94 (0.65-1.36), *0.7*	0.87 (0.60-1.26), *0.5*
II (white)	8538 / 43	0.72 (0.10-5.17), *0.7*	0.64 (0.09-4.61), *0.7*
MI (African-Am)	1764 / 1359	0.73 (0.55-0.98), *0.03*	0.73 (0.55-0.97), *0.03*
II (African-Am)	1764 / 245	0.74 (0.42-1.31), *0.3*	0.75 (0.42-1.33), *0.3*

[a] Adjusted for age, gender, center

[b] Adjusted for age, gender, center, smoking, diabetes, hypertension status

* Referent group: wild-type genotype

** Genotype counts presented as (# referent genotype) / (# variant genotype); PSGL-1 5′UTR was not polymorphic in whites and therefore not presented

Table 3

PSEL S290N and PSGL-1 M62I Genotype Frequencies in Whites by Incident CHD Status and in African Americans by Incident CHD and Ischemic Stroke (ISC) Status

Whites						
PSEL S290N Genotype	**CHD Cases**			**Non-Cases**		**P***
	n (%)			**n (%)**		
SS	825 (65)			5737 (67)		Ref
SN	375 (30)			2555 (30)		0.8
NN	58 (5)			291 (3)		0.03
African Americans						
PSGL-1 M62I Genotype	CHD Cases	Non-Cases	P*	ISC Cases	Non-Cases	P*
	n (%)	n (%)		n (%)	n (%)	
MM	190 (55)	1559 (52)	Ref	128 (59)	1636 (52)	Ref
MI	139 (41)	1199 (40)	0.7	75 (35)	1284 (41)	0.05
II	14 (4)	229 (8)	0.01	13 (6)	232 (7)	0.3

*P-value comparing genotype frequencies between cases and non-cases, with the SS genotype (for PSEL S290N) and MM genotype (for PSGL-1 M62I) serving as the referent group

Table 4a. Hazard Rate Ratio (HRR) for PSEL Haplotypes and Time to Incident CHD in Whites

Incident CHD Haplotype	Haplotype Counts	Model 1[a]	Model 2[b]
		HRR (95%CI), *P*	HRR (95%CI), *P*
SDVT	4693	Ref	Ref
NNVT	1544	1.15 (0.98-1.34), *0.09*	1.15 (0.98-1.34), *0.08*
NDVT	1182	0.99 (0.83-1.18), *0.9*	1.00 (0.84-1.20), *1.0*
SNLT	708	1.01 (0.81-1.26), *0.9*	1.05 (0.84-1.30), *0.7*
SNVT	675	0.97 (0.77-1.22), *0.8*	1.00 (0.79-1.26), *1.0*
SNVP	552	0.94 (0.73-1.21), *0.7*	0.90 (0.70-1.16), *0.4*
NNLT	479	0.95 (0.72-1.25), *0.7*	1.01 (0.77-1.32), *1.0*
NNVP	58	1.90 (1.12-3.23), *0.02*	2.09 (1.23-3.55), *0.006*

Table 4b. PSEL Haplotype Frequencies in Whites, by Incident CHD Status

PSEL Haplotype	CHD Cases	Non-Cases	P[*]
	n (%)	n (%)	
SDVT	587 (46)	4106 (48)	Ref
NNVT	212 (17)	1332 (15)	0.2
NDVT	149 (12)	1033 (12)	0.9
SNLT	92 (7)	616 (7)	0.7
SNVT	84 (7)	591 (7)	1.0
SNVP	67 (5)	485 (6)	0.8
NNLT	57 (5)	422 (5)	0.7
NNVP	14 (1)	44 (0.5)	0.008

[a] Adjusted for age, gender, center

[b] Adjusted for age, gender, center, BMI, HDL and total cholesterol, smoking, diabetes, hypertension status

[*] P-value comparing genotype frequencies between cases and non-cases, with the SNVT haplotype serving as the referent group