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Association between Variations in Coagulation System Genes and Carotid Plaque

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Abstract

Objective—Genetic variation in coagulation and fibrinolysis may affect the development of subclinical atherosclerosis modifying the risk of stroke and cardiovascular disease. However, data on the relationship between subclinical atherosclerosis and genes involved in the coagulation system are sparse. The objective of this study is to examine the association between single nucleotide polymorphisms (SNPs) in coagulation system genes and subclinical carotid plaque phenotypes.

Methods—From the Genetic Determinants of Subclinical Carotid Disease study, 287 Dominicans were examined for carotid plaque presence, thickness, and surface irregularity by high-resolution B-mode carotid ultrasound. Logistic regression was used to test for association between 101 SNPs in 23 coagulation system genes and plaque phenotypes while controlling for age, sex, smoking, hypertension, dyslipidemia, and diabetes. Within gene haplotypes and interactions between genes were examined. A follow-up of SNPs in moderate to high ($r^2 > 0.25$) linkage disequilibrium (LD) with those implicated in the discovery analysis ($p < 0.01$) was performed in an independent sample of 301 Dominicans.

Results—The prevalence of carotid plaque (47% discovery; 46% follow-up) as well as the mean age (65 ± 8 discovery; 65 ± 9 follow-up) of the participants was similar in both datasets. Two genes (vWF and THBS1) were associated ($p < 0.01$) with plaque size and surface irregularity. In followup, 5 SNPs in vWF were associated ($p < 0.05$) with plaque size. SERPINE1 was an additional gene of interest in the haplotype and interaction analyses.

Conclusions—Variation in the vWF, THBS1, and SERPINE1 gene may play an important role in the pathogenesis of atherosclerotic plaque.

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Keywords

candidate genes; carotid plaque; coagulation system; single nucleotide polymorphism; subclinical atherosclerosis

1. Introduction

Atherosclerosis is a complex disorder and the underlying mechanism of cardiovascular diseases (CVD) and stroke, the most common causes of death in western countries [1]. Platelet activation, aggregation and thrombosis play pivotal roles in atherosclerotic plaque formation, progression and plaque rupture, and are accepted as the common pathogenetic pathways of ischemic thromboembolic events [2].

Small, non-stenotic carotid plaque represents a distinct phenotype of subclinical atherosclerosis and is an important marker of incident CVD and stroke [3]. Carotid plaque can be detected non-invasively with reasonable precision in population samples using high-resolution ultrasound imaging [3, 4]. Other carotid plaque phenotypes such as plaque size and plaque morphology are important predictors of increased vascular risk [4, 5]. Subclinical carotid atherosclerotic plaque is a highly heritable phenotype [6]. Much effort has been devoted to discovering and understanding genetic mechanisms regulating the hemostatic system and atherosclerosis [7], but data on the relationship between atherosclerotic plaque and variations in coagulation system genes are sparse. Further investigations in this field may lead to novel treatments for both atherosclerosis and thrombosis.

Therefore, the aim of this study was to examine the association between variation in select coagulation system genes and carotid plaque presence, size, and surface irregularity as part of the Genetic Determinants of Subclinical Carotid Disease Study. In addition to the analysis of single nucleotide polymorphisms (SNPs), haplotype analysis was performed to examine multi-SNP effects within genes, and interaction analysis for the multi-SNP effects between genes. A validation of genes implicated in the single SNP analysis was performed in an independent sample of 301 Dominicans with genotype data available from a genome-wide association study (GWAS).

2. Methods

2.1. Study population

Discovery Sample Set—Study participants (N=287 Dominicans) were part of the NINDS Genetic Determinants of Subclinical Carotid Disease Study (Gen-Carotid), a sub-study of the prospective, stroke-free, community-based Northern Manhattan Study (NOMAS) [3] who 1) self-identified to be of Dominican origin through a questionnaire modeled after the U.S. census, 2) had carotid ultrasound imaging, and 3) had a DNA sample available for genetic studies.

Follow-up Sample Set—A convenience sample (N=301 Dominicans) for follow-up was constructed from the remaining NOMAS cohort, with plaque and other risk factors measured by the same protocols. Participants were included in the follow-up analysis if they 1) had not previously been included in the Gen-Carotid study to ensure an independent validation set, 2) had carotid ultrasound and DNA samples, and 3) self-identified to be of Dominican origin.

All vascular risk factors were collected at baseline enrollment into NOMAS using structured questionnaires and examinations [3] including demographics and vascular risk factors, hypertension, dyslipidemia, diabetes, body mass index (BMI, weight over height square in kg/m²), and smoking. Cigarette smoking was assessed by self-report and categorized as ever versus never smoking; pack-years of smoking were also computed. Fasting blood samples were analyzed for blood sugar, high (HDL) and low (LDL) density lipoprotein cholesterol, total cholesterol, and triglycerides [8]. The study was approved by the University of Miami and Columbia University Institutional Review Boards. All participants signed written informed consent for participation in the study.

2.2. Carotid Ultrasonography

Carotid ultrasound was performed by high-resolution B-mode ultrasound using a GE LOGIQ 700 system with a multifrequency 9 to 13 MHz linear-array transducer according to standard scanning and reading protocols as previously described [4]. All measurements were performed by RVT technologists trained in ultrasound research protocols. The internal and common carotid arteries and the carotid bifurcations were examined for the presence of atherosclerotic plaque, defined as an area of focal wall thickening more than 50% greater than surrounding wall thickness. Maximal carotid plaque thickness (MCPT, in mm) was measured at the highest plaque prominence in any of the carotid arteries. Thick plaques were defined as an MCPT > 1.9 mm as these plaques were significantly associated with an increased stroke risk in our previous study [4]. Plaque surface regularity was recorded. In a sample of 88 stroke-free community subjects, the intraclass correlation coefficients for plaque thickness ranged from 0.87 to 0.94 and intra- and interrater correlations of plaque surface irregularity were greater than 0.90 [9].

The primary outcome of interest was presence of plaque, and secondary outcomes were plaque thickness and irregular plaque. Each of these outcomes was compared to the lack of plaque as the reference. Note that not all individuals with plaque had thick or irregular plaque, and some individuals could have both thick and irregular. Therefore, the secondary outcome analyses only incorporate a subset of the data from the primary analysis.

2.3. Gene and SNP Selection

SNPs from 23 genes with direct or indirect functional relevance to the coagulation system and atherosclerosis were available from the Illumina GoldenGate Assay in the Gen-Carotid study (genes and gene products are listed in Supplementary Table S1). The SNPs were selected if they met any one of the following five criteria: 1) a SNP with the minor allele frequency (MAF) > 0.05, submitted to dbSNP by more than one source (<http://www.ncbi.nlm.nih.gov/SNP>) and examined previously, 2) SNPs located at evolutionarily conserved sequence homology (<http://genome.lbl.gov/vista/index.shtml>), 3) tagging SNPs across different human populations (<http://pga.gs.washington.edu>), 4) functional SNPs, or 5) SNPs leading to amino acid changes. To reduce the effect of linkage disequilibrium (LD), SNPs were generally at least 3000 base pairs apart.

Genotyping of the discovery dataset was performed using the GoldenGate® Assay (Illumina Inc., San Diego, USA) [10]. After quality control checks (genotyping efficiency and Hardy-Weinberg equilibrium (HWE)), the final analysis set consisted of 101 SNPs from the 23 genes. These genes (chromosome, # of SNPs) included SERPINC1 (1,3), THPO (3,2), FGA (4,3), FGB (4,4), FGG (4,4), THBS4 (5,4), PLG (6,5), CD36 (7,4), FGL2 (7,3), SERPINE1 (7,4), CYP11B2 (8,6), FSBP (8,3), PLAT (8,4), PLA1 (10,3), vWF (12,12), CPB2 (13,4), THBS1 (15,6), GP1BA (17,2), ITGA2B (17,5), SERPINB2 (18,7), PLAUR (19,5), THBD (20,5), and PDGFB (22,4). The supplementary Table S1 reports the gene characteristics, the

protein products and functions. The median number of SNPs in each gene was 4, with a range of 2 through 12. The average gene size was 27 Kb.

2.4. Statistical analysis

Using an additive genetic model, multiple logistic regression was performed using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). Association between the 101 SNPs and plaque phenotypes was tested while controlling for age, sex, ever (vs. never) smoking, hypertension, dyslipidemia, and diabetes. Statistical significance was based on the number of genes and phenotypes tested and considered significant if $p < 7.25 \times 10^{-04}$ [$0.05/(23 \times 3)$]. A priori significance for follow-up analysis was $p < 0.01$.

A follow-up study of SNPs in moderate to high LD ($r^2 > 0.25$) with the SNPs implicated in the discovery analysis ($p < 0.01$) was performed in an independent set of 301 NOMAS Dominican participants with DNA genotyped by the Affymetrix 6.0 platform. A subset of the samples in the Dominican discovery set (153 of 287) was also genotyped on the Affymetrix 6.0 platform and used to compute the D-prime (D') and R-squared (r^2) between SNPs from the Gen-Carotid substudy and the follow-up study. The first principal component from Eigenstrat was used as a covariate in addition to covariates as mentioned previously [11]. Multiple logistic regression, using an additive genetic model, was conducted in PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>) with the major allele as the reference [12].

While the sample size was too small to achieve a high level of power for interactions, in an exploratory analysis, we employed Multifactor Dimensionality Reduction (MDR) as a filter (<http://www.epistasis.org/software.html>) to identify within gene risk haplotypes in the discovery sample only [13]. All SNPs in each of the 23 genes were used as candidates in MDR. For each carotid plaque phenotype and for each gene, up to a 5 SNP model was tested in order to find the best fit. These SNPs were then examined as a haplotype using the haplo.glm function of the haplo.stats package, adjusting for age, sex, smoking, hypertension, dyslipidemia, and diabetes [11].

Evaluation of the effect of haplotype combination and interaction between two genes for each carotid plaque phenotype was done with FAMHAP v16 [12]. This analysis was restricted to the discovery sample. Genes were chosen for interaction analysis if a SNP in the gene had $p < 0.01$ or a haplotype in the gene had $p < 0.01$. A gene region was defined as the single SNP or haplotype from a gene as chosen above. The FAMHAP algorithm applies multiple testing correction using the minP approach [12].

3. Results

Demographic and clinical characteristics of each dataset are presented in Table 1. The prevalence of carotid plaque (47% discovery; 46% follow-up) as well as the mean age (65 ± 8 discovery; 65 ± 9 follow-up) of the participants was similar in both datasets. However, the number of smokers (42% discovery; 47% follow-up) and sex distribution (58% female discovery; 70% female follow-up) were slightly different.

While no single SNP in our discovery analysis met the a priori multiple testing threshold of 7.25×10^{-04} SNPs in the 2 genes, von Willebrand factor (vWF) and thrombospondin 1 (THBS1), were associated ($p < 0.01$) with any carotid plaque phenotype (Table 2, full data in Supplementary Table S2). In vWF, rs216809 was associated with thick plaques ($p = 6.70 \times 10^{-03}$ OR=0.44), and trending towards association with plaque presence ($p = 3.22 \times 10^{-02}$) and irregular plaque ($p = 4.26 \times 10^{-02}$). Lastly, rs1478604 in THBS1 was associated with irregular plaques ($p = 6.20 \times 10^{-03}$ OR=2.70) but not associated with plaque presence ($p = 0.127$) or thick plaque ($p = 0.364$).

In the independent follow-up validation sample, data were available on a total of 208 SNPs in vWF (91) and THBS1 (117) from the Affymetrix 6.0 GWAS. All were in HWE ($p > 1.00 \times 10^{-3}$ in controls), had minor allele frequency (MAF) greater than 0.05, and had genotyping efficiency greater than 95%. As the associated SNPs identified in the discovery analysis (rs216809 and rs1478604) were not included in the Affymetrix 6.0 platform, analysis was instead conducted using those SNPs with an $r^2 > 0.25$ to the discovery SNPs (8 SNPs in vWF and 4 SNPs in THBS1). Of the 8 SNPs in vWF, 5 had evidence for association ($p < 0.05$) with thick plaque (Table 3).

Analysis of haplotypes in the discovery sample suggested an additional gene of interest, SERPINE1, as well as confirmed the associations with vWF (Table 4, full data in Supplementary Table S3). MDR selected 3 SNPs (rs6092, rs2227631, and rs1050813) in SERPINE1 for plaque presence. Haplotype analysis also suggested significant association ($p = 5.28 \times 10^{-3}$). MDR selected 2 SNPs (rs980130 and rs216809) in vWF for thick plaque, which were also significant as a haplotype ($p = 5.21 \times 10^{-3}$). Additionally, haplotype association ($p = 7.68 \times 10^{-3}$) was seen with the 3 SNPs (rs4764478, rs2885517, and rs12580343) in vWF, which were selected as the best model for irregular plaque by MDR.

The two-gene interaction analysis (Table 5) detected evidence for an interaction between THBS1 and vWF in association with irregular plaque (global $p = 1.50 \times 10^{-2}$). The odds ratio was 4.01 for the interaction of the risk allele of rs1478604 (G) in THBS1 and the risk haplotype of vWF (rs4764478=A, rs2885517=G, rs12580343=G).

4. Discussion

In the current study, we report on the suggestive associations between variations in the vWF and THBS1 genes and subclinical carotid plaque thickness, and irregularity. The follow-up validation analysis confirmed the association of the vWF gene with thick plaque. In addition, a SERPINE1 haplotype was associated with plaque presence, and vWF haplotypes were associated with both thick and irregular plaque surface. An interaction between THBS1 and vWF was seen for irregular plaque. These associations may be explained by the effects of the investigated genes on atherosclerosis through facilitation of coagulation and thrombosis at the site of vascular injury.

The levels of plasma coagulation proteins have been associated with the increased risks of CVD and stroke [7, 14]. However, the association between variation in coagulation system genes and CVD remains unclear because of important contributions of non-genetic factors. Similarly, the association with carotid atherosclerosis is controversial [15]. While an association between the genetic variant factor V Leiden and symptomatic carotid stenosis was previously reported [16], no evidence of association between variation in the hemostatic factor genes and carotid intima-media thickness (cIMT) was observed in the Framingham Heart Study [17]. In addition, in the ARIC (Atherosclerosis Risk in Communities) study, polymorphisms in the factor VII gene were not associated with cIMT [18]. As with Framingham and ARIC, we also did not find any association between subclinical carotid plaque phenotypes and variation in the factor V, factor VII, fibrinogen, plasminogen activator inhibitor-1, or glycoprotein IIIa genes. However, it is important to note that carotid plaque and cIMT have been identified as biologically and genetically different phenotypes of atherosclerosis [19, 20]. Therefore, different genes may play a role in their development.

vWF has been associated with endothelial dysfunction and with pathogenesis of atherosclerosis, likely due to its ability to mediate platelet adhesion [21]. We report evidence for association between one intronic vWF SNP (rs216809) and thick carotid plaque in the discovery analysis and 5 SNPs in moderate to high LD with rs216809 were replicated

($p < 0.05$) in an independent sample. The vWF glycoprotein, which functions as both an anti-hemophilic factor carrier and a platelet-vessel wall mediator in the blood coagulation system, is crucial to the hemostasis process [22]. Elevated plasma levels of vWF are associated with established cardiovascular risk factors such as age, smoking, cholesterol, diabetes mellitus, and hypertension [23]. Plasma vWF levels are genetically determined and numerous association studies have shown an effect of genetic variability in vWF on its activity levels and on the risk of arterial thrombosis [24]. A study conducted by the CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium identified 6 novel genetic associations with vWF antigen levels [25]. However, as studies frequently differ in design, study populations, and endpoints, and are often underpowered, the precise genetic impact on vWF plasma levels remains unclear.

Animal studies have supported the pivotal role of vWF variations in the atherosclerotic processes leading to vessel injury [26]. Recently, an *in vivo* study demonstrated that vWF either secreted from vascular endothelial cells or circulating in plasma facilitates platelets accumulation on injured vascular walls in thrombospondin type 1 (TSP1) transgenic mice [27]. A clinical study demonstrated that ARC1779 (vWF inhibitor) reduces thromboembolism in patients undergoing carotid endarterectomy [28]. The role of vWF in arterial thrombogenesis makes it a useful clinical marker of risk associated with atherosclerosis [23]. However, other findings reported a marginal role in atherogenesis of vWF [15]. Therefore, investigations of the associations between vWF genetic variations and atherosclerosis may help to better understanding of the vWF role in vascular disease.

We observed the association between the minor allele of rs1478604, located in the 5'UTR region of the thrombospondin type 1 gene (THBS1 or also known as TSP1) and increased risk of irregular plaque in the discovery sample. Additionally, in an independent validation sample, a non-synonymous coding SNP in moderate LD with rs1478604 was associated with irregular plaque. The protein encoded by this gene is a subunit of a disulfide-linked homotrimeric protein which binds to fibrinogen, fibronectin, laminin, type V collagen and integrins α -V/ β -1, thereby influencing platelet aggregation, angiogenesis, and tumorigenesis [29]. Previously, we found an association between the non-synonymous THBS1 SNP rs2292305 and increased cIMT [30]. Here, we extend this association to carotid plaque, where THBS1 likely plays a functional role in the process leading to neointimal hyperplasia, the adhesion molecules expression, and smooth muscle proliferation [31, 32].

In addition to the single SNP associations, haplotypes in SERPINE1 and vWF were associated with plaque presence, thick, and irregular plaque. SERPINE1 encodes plasminogen activator inhibitor-1 (PAI-1) that works as a specific inhibitor of t-PA, thereby attenuating fibrinolysis. Several SERPINE1 variants have been associated with increased risk of stroke [33]. Recently, multiple novel variants in the thrombospondin gene family have been associated with familial premature myocardial infarction [34]. The increased PAI-1 expression in the atherosclerotic plaque may inhibit tissue-type plasminogen activator (t-PA) and urokinase plasminogen activator (uPA) and therefore protect the fibrous cap against degradation by matrix metalloproteinases (MMPs) and subsequently against plaque rupture [35]. Moreover, genetic expression of PAI-1 has been shown to significantly increase carotid plaques instability in symptomatic patients [36]. Simultaneous increases in t-PA and vWF plasma concentration may lead to endothelial dysfunction, an ongoing prothrombotic state, and an increased risk of thromboembolic events [37].

The mechanism by which these genes are associated with various phenotypes of atherosclerotic plaque is unclear. Pleiotropy and interconnection between multiple traits and environmental risk factors may explain the associations with atherosclerosis phenotypes. A

recent meta-analysis of GWAS from the CHARGE consortium has revealed new loci for cIMT and plaque, which are implicated in low-density lipoprotein metabolism, endothelial dysfunction, telomere maintenance, and platelet biology. For plaque, the PIK3CG (phosphoinositide-3-kinase, catalytic, gamma polypeptide) gene was implicated in multiple mechanisms including associations with platelet volume and aggregation as well as with cell-adhesion [38].

Since different plaque phenotypes are under different genetic control [39], the identification of the genetic underpinnings of common complex phenotypes, such as carotid atherosclerosis therefore, likely requires the interrogation of multiple pathways, rather than single gene approaches. Our analysis suggested a potential gene-gene interaction between vWF and THBS1 in association with irregular plaque. In the thrombospondin type 1 transgenic mice model, THBS1 interacts with vWF in the mechanisms of endothelium injury [27]. Moreover, the exponential increase in thrombospondin type 1 that accompanies platelet activation may help regulate the rate of thrombus growth by controlling platelet vWF multimer size in the developing platelet thrombus [40]. Although interactions between genes in a complex cascade of the coagulation system are biologically plausible, further investigations need to determine whether these interactions are clinically relevant.

Strengths of our study include the examination of novel SNPs, use of standardized imaging technology, and availability of two independent datasets. The lack of replication of some previously reported gene findings in our study most likely is due to the complexity of the atherosclerotic process and coagulation system, and also to the specific study population, risk factor profile, and limited sample size [41]. Another potential weakness is our candidate gene approach limited by the used genotyping platform. Therefore, other potentially important known and unknown genes were not captured although we examined the role of selected genes known to be directly and indirectly involved in the coagulation system. Validation of our results is needed in other Hispanics/Latino groups and race-ethnic populations.

In conclusion, our study reports the association between variation in vWF, THBS1, and SERPINE1 and subclinical carotid atherosclerotic plaque. These genes, in particular vWF, may play a significant role in carotid plaque burden and rupture by modifying the coagulation activity and the inflammatory responses to the atherosclerosis processes. More studies are necessary to further elucidate the role of interactions among coagulation-related genes, and between these genes and environmental risk factors. These results could help understanding the differences in the biological mechanisms and variability in predisposition to the different phenotypes of atherosclerosis and to the risk of vascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the study populations: Demographics, vascular risk factors, and carotid plaque phenotypes

	Discovery N= 287		Validation N=301	
	n	%	n	%
Hypertension [†]	228	79.4	216	71.8
Diabetes [†]	53	18.5	71	23.6
Dyslipidemia [†]	126	43.9	147	48.8
Ever smoking	120	41.8	141	46.8
Sex				
Female	167	58.2	210	69.8
Male	120	41.8	91	30.2
Plaque Prevalence [*]	134	46.7	139	46.2
% Among those with Plaque				
Thick Plaque	54	40.3	70	50.4
Plaque Irregularity	31	23.1	61	43.9
Calcified Plaque	20	14.9	12	8.6
	Mean ± SD		Mean ± SD	
Age [*]	65.11 ± 7.52		64.63 ± 8.95	
BMI (kg/m ²)	28.05 ± 4.36		28.91 ± 4.81	
Weight (pounds)	161.82 ± 27.78		163.19 ± 29.59	
Height (inches)	63.78 ± 3.54		63.04 ± 3.46	
Total cholesterol (mg/dl)	198.16 ± 37.37		203.26 ± 38.54	
LDL (mg/dl)	127.76 ± 33.10		130.79 ± 35.34	
HDL (mg/dl)	42.10 ± 12.04		45.24 ± 12.51	
TG (mg/dl)	142.48 ± 72.10		136.15 ± 68.41	
SBP (mmHg)	144.08 ± 20.50		139.36 ± 19.88	
DBP (mmHg)	86.92 ± 11.62		83.46 ± 9.86	
Years between Ultrasound and Baseline	0.53 ± 1.36		2.10 ± 3.47	

^{*} At time of ultrasound. All other risk factors measured at baseline.

[†] Hypertension is defined as systolic blood pressure (SBP) ≥ 140, diastolic blood pressure (DBP) ≥ 90, history of hypertension or on anti-hypertensive medication. Diabetes is defined as fasting glucose ≥ 126, history, or use of insulin. Dyslipidemia is defined as total cholesterol ≥ 240, history, or on cholesterol medications.

Table 2

Top Associated SNPs (p 0.01) in Discovery Sample

Phenotype	Chr	Gene	SNP	Position (MB)	Location	Minor allele	MAF	P-value*	Odds ratio (95% CI) [†]
Thick	12	VWF	rs216809	5.985	intron	A	0.33	6.70E-03	0.44 (0.25,0.80)
Irregular	15	THBS1	rs1478604	37.661	UTR5	G	0.50	6.20E-03	2.70 (1.33,5.51)

* adjusted for age, sex, smoking, hypertension, dyslipidemia, and diabetes

[†] Odds ratio is measuring the risk of the minor allele

MAF = Minor Allele Frequency based on the whole sample for each phenotype

Full Results are in Supplementary Table S1

Table 3

Association of SNPs in Moderate to High LD with the top discovery SNPs ($R^2 > 0.25$) in an independent Dominican cohort of 301

Phenotype	Gene	Discovery SNP	Follow-Up SNP	Function	Position (MB)	D'	R ²	Minor Allele	MAF	Odds Ratio (95% CI) [†]	P-value [*]
Thick	VWF	rs216809	rs216887	intron	5.969	0.89	0.44	C	0.47	1.65 (1.03,2.64)	3.62E-02
										1.77	
			rs216888	intron	5.969	0.86	0.42	G	0.47	(1.09,2.88)	2.20E-02
			rs216891	intron	5.970	0.64	0.26	G	0.47	1.84 (1.15,2.94)	1.06E-02
			rs216896	intron	5.971	0.86	0.42	T	0.47	1.52 (0.96,2.41)	7.53E-02
Irregular	THBS1	rs1478604	rs216903	intron	5.976	0.83	0.25	T	0.41	0.59 (0.36,0.95)	2.98E-02
										1.54	
			rs216904	intron	5.976	0.91	0.80	C	0.33	(0.95,2.48)	7.66E-02
			rs216905	intron	5.977	0.95	0.27	T	0.15	1.91 (1.07,3.61)	2.99E-02
			rs216801	intron	5.979	0.94	0.84	A	0.35	1.33 (0.82,2.14)	2.44E-01
			rs11070217	upstream	37.609	0.68	0.36	C	0.43	0.93 (0.56,1.55)	7.80E-01
			rs2618162	upstream	37.655	0.99	0.97	C	0.48	1.29 (0.76,2.19)	3.41E-01
			rs1134485	intron	37.667	1.00	0.54	T	0.34	1.39 (0.80,2.42)	2.43E-01
			rs2228261	CDS	37.668	1.00	0.39	T	0.27	1.82 (1.03,3.22)	3.96E-02

^{*} adjusted for age, sex, smoking, hypertension, dyslipidemia, diabetes, and PCA1

[†] Odds ratio is measuring the risk of the minor allele

MAF = Minor Allele Frequency

D' , and R^2 are the measures of LD between the SNP from the discovery analysis and the SNP from the follow-up analysis

Associated Haplotypes in (p 0.01) the Discovery Sample

Table 4

Phenotype	Gene	Haplotype Candidate from MDR	Freq in Controls	Freq in Cases	Coeff	SE	P-Value*
Plaque	SERPINE1	SNP1=rs6092; SNP2=rs2227631; SNP3=rs1050813					
		ggg (ref)	0.49	0.50			
Thick	vWF	gaa	0.03	0.09	1.46	0.52	5.28E-03
		SNP1=rs980130; SNP2=rs216809					
Irregular	vWF	gc (ref)	0.47	0.63			
		ga	0.33	0.15	-1.074	0.38	5.21E-03
		SNP1=rs4764478; SNP2=rs2885517; SNP3=rs12580343					
		tgg (ref)	0.3	0.22			
		agg	0.03	0.22	2.46	0.91	7.68E-03

*

p-value adjusted for age, sex, smoking, hypertension, dyslipidemia, and diabetes - from haplo.glm

Coeff = estimated regression coefficient.

Note: e^coeff = odds ratio of this haplotype compared to the reference (ref) haplotype

Haplotypes with a frequency < 10 were grouped as 'Rare Haplotypes'

Note that each haplotype was compared to the most common haplotype as noted by (ref) for each gene, however only haplotypes with p 0.0125 are presented in this table. Full results are in Supplementary Table S3.

Table 5

Two Region Interaction Analysis

Irregular Plaque		Gene2	Freq in Controls	Freq in Cases	OR	P-value*
Gene1						
THBS1	vWF					1.50E-02*
region 1:						
A	region 2: A A A	0.04	0.01	0.21		
region 1:						
A	region 2: T A A	0.12	0.13	1.11		
region 1:						
A	region 2: A G A	0.06	0.01	0.11		
region 1:						
A	region 2: T G A	0.11	0.05	0.43		
region 1:						
A	region 2: T G G	0.17	0.09	0.50		
region 1:						
A	region 2: A G G	0.03	0.06	2.21		
region 1:						
A	region 2: T A G	0.01	0.00	0.13		
region 1:						
A	region 2: A A G	0.01	0.00	0.10		
region 1:						
G	region 2: A A A	0.03	0.04	1.37		
region 1:						
G	region 2: T A A	0.13	0.14	1.06		
region 1:						
G	region 2: A G A	0.06	0.05	0.86		
region 1:						
G	region 2: T G A	0.09	0.16	2.10		
region 1:						
G	region 2: T G G	0.12	0.19	1.65		

Irregular Plaque				
Gene1	Gene2	Freq in Controls	Freq in Cases	OR
region 1:				
G	region 2: A G G	0.02	0.07	4.01
region 1:				
G	region 2: A A G	0.01	0.00	0.53

* multiple testing done via the min P approach from FAMHAP
SNPs for THBS1 included rs1478604.
SNPs for vWF included rs4764478, rs2885517, and rs12580343.