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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 ($p \leq 0.01$) 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 ($p \leq 0.01$) with plaque size and surface irregularity. In follow-up, 5 SNPs in vWF were associated ($p \leq 0.05$) 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.

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).

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2. Methods

2.1. Study population

2.1.1. 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.

2.1.2. 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/m^2), 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 inform 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 [4].

The primary outcome of interest was the 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 plaque. 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) [9]. 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), PLAU (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 $p \leq 7.25 \times 10^{-04}$ [0.05/(23 * 3)]. A priori significance for follow-up analysis was $p \leq 0.01$.

A follow-up study of SNPs in moderate to high LD ($r^2 > 0.25$) with the SNPs implicated in the discovery analysis ($p \leq 0.01$) 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 [10]. 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 [11].

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 [12]. 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 [10].

Evaluation of the effect of haplotype combination and interaction between two genes for each carotid plaque phenotype was done with FAMHAP v16 [11]. This analysis was restricted to the discovery sample. Genes were chosen for interaction analysis if a SNP in the gene had $p \leq 0.01$ or a haplotype in the gene had $p \leq 0.01$. 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 [11].