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Novel genetic variants modify the effect of smoking on carotid plaque burden in Hispanics

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ABSTRACT

Background and purpose: Smoking greatly increases the risk of atherosclerotic plaque and the effect may vary from individual to individual. A genome-wide scan was performed for smoking × single nucleotide polymorphism (SNP) interactions on carotid plaque burden (CPB) to identify the potential genetic moderators in Hispanics.

Methods: Carotid B-mode ultrasonography and genotyping by the Affymetrix 6.0 chip were performed in a discovery sample of 665 Caribbean Hispanics, followed by replication analyses in 264 Caribbean Hispanics. CPB was expressed as the sum of plaque areas over the segments in common and internal carotid arteries and bifurcation. Smoking was classified as 0, <20, and ≥20 cigarette pack-years. Assuming an additive genetic model, regression analysis was conducted to test for smoking × SNP interaction on the cube root transformed CPB while controlling for age, sex, and the top 3 principal components of ancestry.

Results: Two SNPs showed a significant interaction with smoking on CPB with the similar effects in both discovery ($P < 1.0E-5$) and replication ($P < 0.05$) populations. Specifically, for SNP rs10205487 within MXD1, more smoking was significantly associated with greater CPB in A allele carriers (beta ± SE: 0.24 ± 0.08, $P = 0.005$ in AG carriers; beta ± SE: 0.48 ± 0.12, $P = 0.0002$ in AA carriers) but not in GG ($P = 0.06$). For SNP rs7001413 within LY96 and JPH1, more smoking was significantly associated with greater CPB in GG carriers (beta ± SE: 0.24 ± 0.06, $P = 6.8E-5$) but not in T carriers ($P = 0.06$).

Conclusions: Our study suggests that genetic variants may modulate the effect of smoking on CPB and highlights several genes for further investigation of their role in atherosclerosis, especially in smoking population.

1. Introduction

Atherosclerosis is a chronic and multifactorial process underlying most ischemic strokes (IS) and myocardial infarctions (MI), the leading causes of disability and death in the Western countries [1,2]. Numerous studies have clearly established that intermediate markers of subclinical atherosclerosis may be useful in risk prediction of clinical vascular events [3–5]. These markers reflecting biological and genetic different phenotypes of atherosclerosis [6] include artery flow-mediated dilation, arterial stiffness, carotid intima-media thickness (cIMT), coronary calcification, carotid plaque, and stenosis. Recently, the Tromsø Study, in a research conducted in 3240 men and 3344 women, demonstrated as total plaque area appears to be a stronger predictor than cIMT for IS [7]. Moreover, compared to other methods such as measurement of cIMT, carotid plaque burden (CPB), as the sum of plaque area, has been indicated as the strongest cross-sectional predictor of coronary artery calcium score suggesting its clinical utility as predictor of future cardiovascular events [8]. Given that CPB reflects distinct biological and genetic aspects of atherogenesis comparing to other markers of atherosclerosis [9], evaluation of these individuals may reduce heterogeneity and facilitate discovery of novel genetic variants that influence susceptibility to atherosclerosis.

Despite several decades of efforts, there were still 21.2% of men and 17.5% of women who continued to be cigarette smokers among US adults in 2010; and more importantly, overall, 26% of students in grades 9 through 12 reported current tobacco use [2]. Globally, the number of smokers even continues to increase and is estimated to be 1.7 billion by 2025 [10]. Experimental, epidemiological and clinical data strongly

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implicate that cigarette smoking is one of the major modifiable risk factors for cardiovascular disease (CVD) and IS, and impacts all phases of atherosclerosis [2,11]. Smoking can initiate and accelerate atherosclerosis either directly or indirectly by multiple mechanisms such as causing endothelial dysfunction and vascular smooth muscle cell (VSMC) proliferation in the artery wall, increasing lipid peroxidation and free-radical oxidation stress (ROS) and altering the procoagulant status [11].

Population, family and twin studies have demonstrated that atherosclerosis and stroke are under substantial genetic control [6]. However, the associated genetic variants found in candidate gene or genome-wide association studies (GWAS) just account for a small proportion of variation in the atherosclerosis phenotypes [6]. Part of the difficulty in identifying the associated genes could reflect biological interaction between risk alleles and exposure to environmental risk factors. Recently, in the Northern Manhattan Study (NOMAS) cohort population we demonstrated BTB (POZ) domain containing protein 1 (RCBTB1) gene as a modifier for smoking effect on cIMT, further supporting the hypothesis that including gene-environment interaction can help identify genes that may be missed in genome-wide association studies [12].

Given that cigarette smoking is one of the well-established risk factors, the degree of the cigarette smoking-induced damage varies from individual to individual [13], and carotid plaque is considered a genetic and biologic distinct subclinical phenotype of atherosclerosis compared to cIMT [14], we conducted a genome-wide interaction study (GWIS) to scan for smoking × single nucleotide polymorphism (SNP) interactions on CPB and identify the potential genetic moderators.

2. Materials and methods

2.1. Study population

Subjects used in this study are nested with the population-based NOMAS, which has been described extensively before [15,16]. In brief, NOMAS participants had never been diagnosed with a stroke, were at least 40 years of age, and resided for at least 3 months in a household with a telephone in Northern Manhattan. Within NOMAS, all Hispanic subjects who had high resolution B-mode ultrasound measurement of CPB and genotype available were used for the study (N = 929) (Table 1). Demographic, socioeconomic and risk factor data were collected through direct interview based on the NOMAS instruments. All subjects provided informed consent and the study was approved by the Institutional Review Boards of Columbia University and University of Miami.

2.2. Carotid plaque phenotypes

High-resolution B-mode 2-dimensional ultrasound was performed for the examination of carotid plaque according to the standard scanning and reading protocols [3]. Carotid bifurcation and internal and common carotid arteries were examined for plaque defined as an area of focal wall thickening >50% greater than surrounding wall thickness in millimeters. Once plaques were detected, in-depth imaging of plaques was performed in long axes and multiple angles. The optimized and normalized images were analyzed offline by automated computerized edge detection system M'Ath (Intelligence in Medical Technologies, Inc, Paris, France) and area of each plaque was measured. The sum of all plaque areas (mm^2) within each subject was calculated and expressed as a total carotid plaque area (CPB) [17].

Table 1
Characteristics of discovery and replication Hispanic samples.

	Hispanic	Hispanic	P
	Discovery (N = 665)	Replication (N = 264)	
Age, years, mean ± SD	68 ± 8	69 ± 8	0.032
Sex, n (%)			0.217
Male	248 (37.3)	110 (41.7)	
Female	417 (62.7)	154 (58.3)	
Smoking pack-years, n (%)			0.089
0	342 (51.4)	144 (54.5)	
1 − < 20	217 (32.6)	68 (25.8)	
≥20	106 (15.9)	52 (19.7)	
Carotid plaque area, mm^3, median (IQR)	2.9 (0–12.0)	3.3 (0–14.1)	0.519[a]
Carotid plaque area, cubic root transformed, mean ± SD	1.3 ± 1.3	1.3 ± 1.4	0.552

[a] Based on Wilcoxon–Mann–Whitney test.

2.3. Genotyping and quality control

Through the two waves of whole-genome genotyping (665 in the first wave and 264 in the second wave), all subjects included in the current study were genotyped using the Human SNP Array 6.0 chip (AffyMetrix) at the Genotyping Core of the Hussman Institute for Human Genomics (HIHG) at University of Miami following manufacturer's instruction. Extensive quality control at both sample and SNP levels were carried out to ensure the integrity of the genotype data. Samples were excluded if they had call rates below 95%, relatedness, gender discrepancies, or were outliers beyond six standard deviations from the mean based on Eigenstrat analysis [18]. SNPs with severe deviation from Hardy–Weinberg equilibrium ($p < 10^{-6}$) or a genotyping call rate less than 95% were also removed using PLINK 1.05 [19].

2.4. Data analysis

For sample characteristics, continuous variables were summarized as means with standard deviations and compared with t tests or with Mann–Whitney–Wilcoxon test if not normally distributed, whereas categorical variables were presented as percentages and compared with Chi-squared tests. In the genome-wide analysis of discovery Hispanic sample, the interaction between each SNP and smoking cigarette pack-years was evaluated using a linear regression model given in the following equation: $y = \beta_0 + \beta_g G + \beta_s S + \beta_{gs} GS + \sum_k \beta_k C_k$. Here, y is the level of CPB (after cubic root transformation), G ($G = 0$, 1 or 2) is the genotype of a SNP assuming an additive genetic effect, S ($S = 0$, 1, 2 for never smoking, cigarette pack-years <20 and ≥20, respectively) is the status of cigarette smoking pack-years, GS is the product of the genotype and category of smoking, and C_k is the kth covariate (including age, sex and the top 3 eigenvectors of ancestry derived from principal component analysis with EIGENSTRAT) [18]. In the model, we tested if $\beta gs = 0$ against a two-sided alternative hypothesis and performed the analysis using PLINK [19]. In the replication analysis, the interaction between each SNP showing an interaction with a P value less than 1×10^{-5} and smoking cigarette pack-years was evaluated using the same linear regression model as in the discovery analysis and was performed in Hispanic replication sample. To show the modification effects of the SNPs of interest in the combined Hispanic sample, linear regression model was used to estimate smoking effect on CPB stratified by the genotype and adjusted for the same covariates using SAS version 9.3 (SAS Institute Inc., Cary, NC).

3. Results

3.1. GWIS in the discovery sample

After QC, 722,379 SNPs were available in the discovery stage. The characteristics for discovery and replication Hispanic samples are reported in Table 1. Compared with the discovery sample, the replication sample had similar distribution of sex, smoking status and CPB, but was, on average, one year older. Fig. 1 is the Manhattan plot displaying the p values for the interaction effect between each SNP and cigarette smoking on CPB. The quantile–quantile (QQ) plot of the expected vs. observed genome-wide interaction P values suggests no inflated type I