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Research paper

Genetic association and gene-smoking interaction study of carotid intima-media thickness at five GWAS-indicated genes: The Bogalusa Heart Study

Changwei Li [a], Wei Chen [b], Fan Jiang [c], Jeannette Simino [d], Sathanur R. Srinivasan [b], Gerald S. Berenson [e,*], Hao Mei [c,d,**]

[a] Department of Epidemiology, Tulane University, New Orleans, LA, USA
[b] Tulane Center for Cardiovascular Health, Tulane University, New Orleans, LA, USA
[c] Shanghai Children's Medical Center, Shanghai Jiaotong University, Shanghai, China
[d] Center of Biostatistics and Bioinformatics, University of Mississippi Medical Center, Jackson, MS 39216-4505, USA
[e] Center for Cardiovascular Health, 1440 Canal St, Suite 1829, New Orleans, LA 70112, USA

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ABSTRACT

Objectives: To examine the associations of five GWAS-identified genes with carotid intima-media thickness (IMT) in a biracial sample from the Bogalusa Heart Study, and evaluate their participation in gene-smoking interactions.
Methods: Far wall IMTs of common carotid arteries were measured using high-resolution B-mode ultrasound. Both the gene-smoking interactions and single-marker associations were evaluated by linear models of carotid IMT levels, while the gene-based analyses were assessed through the truncated product method. A Bonferroni multiple testing correction was applied.
Results: Marker rs7840785 (*PINX1*) was significantly associated with right carotid IMT ($p = 0.0003$) using all participants; mean levels for the CC, TC, and TT genotypes were 0.74 (0.73 to 0.75), 0.76 (0.75 to 0.78), and 0.78 (0.75, 0.81), respectively. Similar trends were observed in blacks ($p = 0.0031$) and whites ($p = 0.0118$). Marker rs7844465 (*ZHX2*) was significantly associated with left carotid IMT in whites ($p = 0.0005$); mean IMT levels for the GG, TG, and TT genotypes were 0.73 (0.71 to 0.74), 0.75 (0.74 to 0.77) and 0.78 (0.75 to 0.81), respectively. Marker rs6841473 (*EDNRA*) modified the association between smoking and left carotid IMT in blacks ($p = 2.79 \times 10^{-5}$). In addition, gene-based analysis demonstrated that *EDNRA* and *ZHX2* were associated with left carotid IMT in the white and overall participants, respectively, while *PINX1* was associated with right carotid IMT in both blacks and whites.
Conclusion: We identified two novel markers that were associated with IMT in both blacks and whites. One gene-smoking interaction was identified in blacks only. Three genes showed gene-based associations with IMT levels. However, genetic markers with small effects may have been missed due to the limited number of black participants.

1. Introduction

Carotid intima-media thickness (IMT) is an important risk factor for cardiovascular disease (Bots et al., 1997; Ebrahim et al., 1999; O'Leary et al., 1999), the leading cause of death in the United States and worldwide (Mensah and Brown, 2007; Lloyd-Jones et al., 2010). Genetic factors play an important role in carotid IMT, which has an estimated heritability of 32%–59% across different populations (Xiang et al., 2002; Zhao et al., 2008; Medda et al., 2014). Several large genome-wide association studies (GWAS) have been conducted to delineate genetic factors underlying carotid IMT. Many genes and genetic polymorphisms have attained genome-wide significance levels ($p < 5 \times 10^{-8}$) in populations of European ancestry (O'Donnell et al., 2007; Shrestha et al., 2010; Bis et al., 2011). The effects of these genetic loci in populations of non-European ancestry remain unclear. Furthermore, very few studies have focused on the interaction between these genes and environmental factors. Therefore, we tested the associations of five GWAS-identified genes with left and right carotid IMT in a biracial (black and white) sample from the Bogalusa Heart Study, and evaluated the modulation of their effects by smoking status.

Abbreviations: GWAS, genome-wide association study; IMT, intima-media thickness; EDNRA, endothelin receptor type A; ZHX2, zinc fingers and homeoboxes 2; PINX1, PIN2/TERF1 interacting, telomerase inhibitor 1; SNP, single nucleotide polymorphism; TPM, truncated product methods.

* Corresponding author.
** Correspondence to: H. Mei, Center of Biostatistics and Bioinformatics, University of Mississippi Medical Center, 2500 North State Street, Jackson MS 39216-4505, USA.
E-mail addresses: cli8@tulane.edu (C. Li), wchen1@tulane.edu (W. Chen), riversailer@hotmail.com (F. Jiang), jsimino@umc.edu (J. Simino), ssriniv1@tulane.edu (S.R. Srinivasan), berenson@tulane.edu (G.S. Berenson), hmei@umc.edu (H. Mei).

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2. Materials and methods

2.1. Study participants

The goal of the longitudinal Bogalusa Heart Study was to investigate the natural history of cardiovascular disease from childhood through young adulthood in the biracial community of Bogalusa, Louisiana. The study design has been previously reported (Li et al., 2003). The study began in 1973, with cross-sectional surveys conducted every 3–4 years from childhood through young adulthood. During the 2000–2001 cross-sectional survey, carotid intima-media thickness (IMT) was measured in 1203 adults aged 18–44 years. Written informed consent was obtained from each participant, while standardized data collection protocols were approved by the Institutional Review Board of the Tulane University Health Sciences Center (Berenson, 1980; Berenson and Pickoff, 1995).

2.2. Demographics and anthropometric measurements

Demographic (age, gender, and race) information and smoking status were collected by questionnaires. Smoking status was a dichotomous (yes/no) indicator of whether a participant had ever smoked ≥1 cigarette per week. Height and weight were measured twice (to ±0.1 cm and to ±0.1 kg, respectively) during a physical examination; body mass index (BMI, kg/m^2) was calculated from the mean height and weight.

2.3. IMT measurement

Trained sonographers measured far wall IMTs of the left and right common carotids using high-resolution B-mode ultrasound and a previously reported protocol (Urbina et al., 2002). Briefly, images were scanned using a Toshiba SonoLayer SSH 160A ultrasound system (Toshiba Medical, Tokyo, Japan), a 7.5-MHz linear-array transducer that recorded images at both sides of the common carotid, carotid bulbs, and internal carotid arteries using protocols developed for the Atherosclerosis Risk in Communities Study (Li et al., 1996). Measurements of IMT were then performed by a single reader with the help of a semi-automated border-detection program. The measurements were reliable and comparable to that in other carotid IMT studies. The mean of the maximum carotid IMT readings was calculated for the left, right, and overall carotid arteries, respectively. The three means were used in the current analyses.

2.4. Single nucleotide polymorphism (SNP) genotyping of selected genes

This study focused on 5 genes (*PINX1*, *ZHX2*, *APOC1*, *PIK3CG* and *EDNRA*) previously associated with measures of subclinical atherosclerosis (Bis et al., 2011). Descriptions of the five genes are reported in Table 1. All SNPs located within ±5000 base pairs of these genes were genotyped with a chip-based hybridization assays (Illumina Human610 BeadChip and HumanCVD BeadChip) at the Scripps Research Institute in La Jolla, California. SNPs were filtered within each analysis set (black-only, white-only, or all participants) using the following criteria: Hardy–Weinberg equilibrium (HWE) test P-value > 0.05, call rate > 0.95, and minor allele frequency (MAF) > 0.01. Tag SNPs were extracted from this filtered set using Haploview (Barrett et al., 2005) and a linkage disequilibrium threshold of $r^2 = 0.80$. A total of 121, 83, and 74 tag SNPs were selected for the analysis set containing black, white, and all participants, respectively. For the gene-smoking analysis, we repeated the same filtering and tagging procedure using only participants with known smoking status. Restricting to those with known smoking status, 119, 84, and 85 tag SNPs were selected for the analysis set containing black, white, and all participants, respectively. Detailed information about the tag SNPs in each sample, including their genomic locations, MAFs, call rates, and HWE P-values, is presented in Supplemental Tables 1, 2, and 3.

2.5. Statistical analysis

2.5.1. Single-SNP association analysis

We fit one overall and two race-specific (black and white) linear regression models to examine the additive effect of each SNP on the left and right carotid IMTs. We adjusted for age, gender, and continuous BMI in all models; we also adjusted for race in the model containing all participants. The analysis was performed using SAS software (version 9.2; SAS Institute, Inc., Cary, North Carolina). After accounting for the number of SNPs tested in each analysis group, the significance thresholds were 6.76×10^{-4} (0.05/74) for the overall, 4.13×10^{-4} (0.05/121) for the black-specific, and 6.02×10^{-4} (0.05/83) for the white-specific models.

2.5.2. Gene-based analysis

The association of a candidate gene with carotid IMT was assessed by combining P-values from the single-SNP association analyses of main effects. This was done via the truncated product method (TPM) assuming constant correlation (Zaykin et al., 2002; Sheng and Yang, 2013). The truncation point, τ, was set to 0.1, which is quite robust under the assumption of constant correlation (Yang et al., 2012). The p-value from TPM was estimated by 10,000 simulations; however, up to 10,000,000 simulations were conducted when the first 10,000 failed to produce a p-value. After correcting for the multiple testing burden of 5 genes, a significance threshold of 0.01 (0.05/5) was used. In addition, we performed sensitivity analyses by excluding significant SNPs from the gene-based analyses. Gene-based analyses were conducted using R software (Version 2.15.2; http://www.r-project.org).

Table 1
Characteristics of the *APOC1*, *PIK3CG*, *PINX1*, *ZHX2* and *APOC1* genes.

Gene symbol	Gene name	Locus	Physical position ±5000 bp	SNPs	Function
EDNRA	Endothelin receptor type A	4q31.22	(148397069, 148471106)	38	Encodes the receptor for endothelin-1 that plays a role in potent and long-lasting vasoconstriction.
PIK3CG	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma	7q22.3	(106500924, 106552592)	7	Encodes an enzyme that phosphorylates phosphinositides on the 3-hydroxyl group of the inositol ring. It is involved in E-cadherin-mediated cell-cell adhesion, plays an important role in the maintenance of the structural and functional integrity of epithelia, and regulates cytotoxicity in NK cells.
PINX1	PIN2/TERF1 interacting, telomerase inhibitor 1	8p23	(10617884, 10702299)	31	Telomerase inhibitor
ZHX2	Zinc fingers and homeoboxes 2	8q24.13	(123788901, 123991755)	53	Encodes member 2 of the zinc fingers and homeobox gene family which forms homodimers and heterodimerizes with member 1 of the family.
APOC1	Apolipoprotein C-I	19q13.2	(45412921, 45427606)	9	Encodes a member of the apolipoprotein C1 family.

SNP, single nucleotide polymorphism.