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Genetic epidemiology and genome-wide linkage analysis of carotid artery ultrasound traits in multigenerational African ancestry families

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ABSTRACT

Objective: Intima-media thickness, adventitial diameter and lumen diameter are indicators of cardiovascular disease risk. The influence of genetic factors on these measures in African ancestry populations is not well defined. Therefore, we estimated heritability and performed genome-wide linkage analysis of carotid ultrasound traits in 7 multigenerational families of African ancestry.
Methods: A total of 395 individuals (7 pedigrees; mean family size = 56; 2392 relative pairs) aged ≥18 years had a common carotid artery ultrasound scan. Statistical analyses were conducted using pedigree-based maximum likelihood methods.
Results: Significant covariates included age, sex, body mass index or height and waist, and systolic blood pressure. Residual heritabilities ranged from 0.35 ± 0.10 to 0.64 ± 0.12 ($P < 0.0001$). We identified a novel quantitative trait locus for adventitial and lumen diameters on chromosome 11 (max LOD = 4.09, 133 cm).
Conclusion: Further fine mapping of this region may identify specific mutations predisposing to subclinical vascular disease among African ancestry individuals.

1. Introduction

Carotid intima-media thickness (IMT) is a non-invasive, reproducible measure of subclinical atherosclerosis [1] that predicts risk of stroke, myocardial infarction and mortality [1–3]. Larger carotid adventitial diameter (AD) and smaller lumen diameter (LD) also predict cardiovascular disease (CVD) independently of IMT [4–6]. Although IMT is a heritable trait [7], little is known about the heritability of other carotid ultrasound measures.

Previous genome-wide linkage analyses of carotid IMT have found evidence of linkage on chromosomes 2 [8], 7 [9], and 12 [10] in Caucasian families. Variation in the 12p region has also been replicated in an independent genome-wide association study [11]. However, quantitative trait loci (QTL) for IMT have not been reported in African ancestry families. Therefore, in order to extend these previous reports and to gain insight into the genetic basis of arterial diameter, we estimated the heritability and conducted genome-wide linkage analysis of carotid artery ultrasound measures in extended, multigenerational families of African ancestry.

2. Methods

2.1. Tobago family health study

Details of the study have been published previously [12]. Briefly, probands were recruited without regard to medical history from a cohort study of body composition on the Caribbean island of Tobago [13]. A total of 471 individuals belonging to 7 large multigenerational families were recruited. An ancillary study invited all participants to complete a carotid ultrasound scan. Ultrasound images were obtained from 395 individuals who form the basis for these analyses. The Tobago Division of Health and Social Services and the University of Pittsburgh Institutional Review Boards

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approved the study. Written informed consent was obtained from each participant.

2.2. Carotid ultrasound

Methodology used to image the common carotid artery (CCA) in this study is outlined in the Supplemental Data. Briefly, an Acuson Cypress portable machine (Siemens Medical Solutions, Malvern, PA) was used to capture images of the near and far walls of the right and left distal CCA 1 cm proximal the carotid bulb. Measures were calculated using a semi-automated reading software system (AMS system; Dr. Thomas Gustavsson, Sweden). All images were read centrally at the Department of Epidemiology's Ultrasound Research Laboratory (University of Pittsburgh, Pittsburgh, PA). Reproducibility analyses on 35 study participants yielded inter-sonographer intraclass correlations (ICC) of 0.97 for mean IMT and 0.95 for mean AD, and inter-reader ICC of 0.99 for mean IMT and mean AD.

2.3. Other measurements

Covariates included in this analysis included age, sex, height, waist circumference, body mass index (BMI), current smoking, alcohol intake, walking for exercise, diabetes, hypertension, menopausal status, parity, oral contraceptive use, fasting serum lipid and lipoprotein concentrations and systolic blood pressure (SBP). Details of each covariate are presented in the Supplemental Data. For analyses including SBP as a covariate, we excluded individuals on antihypertensive medication ($n = 31$).

2.4. Genotyping and multipoint identity-by-descent (IBD) calculation

Genomic DNA was isolated from whole blood extracted by Qiagen column procedure (Qiagen, Santa Clara, CA). Whole-genome genotyping by fluorescence-based methods was performed using the Infinium HumanLinkage-12 Genotyping BeadChip (Illumina, San Diego, CA). After excluding SNPs with correlation > 0.3, call rate $< 90\%$, Hardy–Weinberg equilibrium $P < 0.001$, minor allele frequency < 0.05 or multipoint IBD calculation incompatibility, we retained 1512 autosomal SNPs and used the Markov Chain Monte Carlo algorithm as implemented in Loki [14] to calculate multipoint IBD. The final SNP set had a median MAF of 0.325 with a median spacing of 1.92 cm based on the Kosambi map [15].

2.5. Statistical analysis

Statistical methodology is expanded upon in the Supplemental Data. Briefly, we tested each covariate separately in an age and sex adjusted model using the variance components framework implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program [16]. All potentially significant covariates from age and sex adjusted models were then assessed simultaneously for each trait. We also used SOLAR to estimate the residual heritability (h^2_r) and the variance attributable to the fixed covariate effects (r^2) for each ultrasound trait.

Multipoint IBD probabilities were used to assess IBD sharing along each chromosome. The significance of the theoretical QTL was tested with a likelihood ratio test at 1 cm intervals across each autosomal chromosome. Logarithm of the odds (LOD) scores, computed as the $\log_{10}$ of the likelihood ratio, were used to assess the significance of the test. LOD score thresholds of 3.3 and 2.0 were considered to represent genome-wide significant and suggestive evidence for QTLs, respectively [17].

3. Results

3.1. Family study characteristics

The mean age of participants was 42 years (range 18–86 years; Supplemental Table S1). The family members were overweight on average (mean BMI = 28.4 kg/m^2), with women having a higher BMI than men ($P < 0.0001$). Men had a higher frequency of current smoking and alcohol consumption than women. The prevalence of diabetes and hypertension were 8.3% and 26.6%, respectively, and didn't differ by sex. Among individuals not on antihypertensive medication, women had lower SBP than men ($P < 0.0001$).

3.2. Correlates of carotid ultrasound traits

The carotid walls were thin on average (mean IMT: 0.69 mm; range: 0.4–1.3 mm) and were similar in men and women (Table 1 and Supplemental Table S1). IMT and AD were positively correlated with age, while LD was negatively correlated with age, as expected. In full multivariable models, only age, sex, BMI and SBP were independent correlates of IMT; whereas, age, sex, height, waist circumference and SBP were independent correlates of AD and LD.

3.3. Variance components analysis of carotid ultrasound traits

Covariates explained more than twice the variance in IMT than in diameter traits (r^2: mean IMT = 0.552, mean AD = 0.242, mean LD = 0.169; Table 1). Residual heritability estimates (h^2_r) were statistically significant (h^2_r: mean IMT = 0.467; mean AD = 0.641; mean LD = 0.584; $P < 0.0001$ for all). Genetic correlation was very high between AD and LD traits ($\rho_G = 0.966$, $P < 0.0001$) but only moderate between diameter and IMT traits ($\rho_G = 0.401$, $P = 0.03$; data not shown).

3.4. Linkage analysis of carotid ultrasound traits

Significant evidence for linkage was identified at ~134 cm on chromosome 11 (Fig. 1 and Supplemental Table S2). The most significant evidence of linkage in this region (LOD = 4.09) was for max AD. A nearly identical peak was identified for mean LD (LOD = 4.06) and min LD (LOD = 3.82). Family-specific analysis suggested that the largest pedigree was the greatest source of linkage signal for max AD (pedigree 7, $n = 113$, LOD = 3.08). However, three other smaller pedigrees showed some evidence of linkage (LOD $\geq$ 2.0; pedigree 1, 2 and 3; $n = 78$, 21 and 27, respectively). No other ultrasound trait had LOD scores achieve genome-wide significance. However, there were an additional seven chromosomal regions suggestive of linkage (Supplemental Table S2).

4. Discussion

We found that carotid IMT was significantly heritable after adjustment for covariates, with estimates similar to previous reports [7]. Covariates explained only ~20–30% of the variance in arterial diameters, but more than 50% of the variance in IMT. However, heritability estimates were greater for arterial diameters than for IMT. Our findings suggest that there may be a stronger genetic component for arterial diameter than IMT.

Since genetic correlation was high between mean and maximum measures of AD and LD, we expected to have very similar linkage results for these traits. However, diameter traits and IMT have only moderate correlation and are distinct vascular phenotypes. Arterial diameter changes generally occur early in the atherosclerotic process in response to changes in hemodynamic forces, while IMT increases as plaque is deposited later in the