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Genetic contribution to brachial artery flow-mediated dilation: The Northern Manhattan Family Study

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Abstract

Background—Brachial artery flow-mediated dilation (FMD) is a non-invasive measure of endothelial function. Endothelial dysfunction has been associated with traditional vascular risk factors and increased risk of cardiovascular disease. The importance of genetic contribution to FMD and baseline brachial artery diameter has not been shown in Hispanic populations. The purpose of this study was to estimate the heritability of FMD.

Methods—Flow mediated dilation and brachial artery diameter were measured in a subset of Caribbean Hispanic families from the ongoing Northern Manhattan Family Study (NOMAFS), which studies the contribution of genetics to stroke and cardiovascular risk factors. The age- and sex-adjusted heritability of FMD was estimated using variance component methods.

Results—The current data include 620 subjects (97 probands and 523 relatives) from 97 families. The age and sex-adjusted heritability of brachial artery diameter was 0.57 ($p < 0.01$). The age- and sex-adjusted heritability of FMD was 0.20 ($p = 0.01$). After additional adjustment for systolic and diastolic blood pressure, body mass index, smoking, lipid, diabetes mellitus, medication, and baseline brachial artery diameter, the heritability of FMD was 0.17 ($p = 0.01$).

Conclusions—We found modest heritability of FMD. FMD might be a reasonable phenotype for further investigation of genetic contribution to atherosclerosis.

Keywords

Flow-mediated dilation; Endothelial function; Atherosclerosis; Heritability; Genetics

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1. Introduction

Flow-mediated dilation (FMD) of the brachial artery, which is assessed by high-resolution ultrasonography, is widely used for the study of endothelial function [1,2]. Experimental and clinical studies suggest that endothelial dysfunction contributes to atherosclerosis and to the pathogenesis of cardiovascular disease [3,4]. FMD is diminished in patients with atherosclerosis and patients with coronary risk factors [3–5]. The endothelium regulates vascular homeostasis and is, therefore, essential for vasodilation in response to increases in blood flow-associated shear stress [6]. In response to shear stress, nitric oxide (NO) is released by the endothelium and the vessel dilates [7,8]. Lower FMD is associated with aging, smoking, increased systolic blood pressure, body mass index, dyslipidemia, and diabetes mellitus [3,4, 9].

Much of the variability of endothelium-dependent FMD is unexplained, and genetic factors likely play a role. In particular, the genetic contribution to variation in FMD among Caribbean Hispanics is unknown. Our aims were to estimate the heritabilities of brachial artery diameter and FMD in Caribbean Hispanic families.

2. Methods

2.1. Subjects

The subjects in the present study were drawn from the ongoing Northern Manhattan Family Study (NOMAFS). NOMAFS was designed to investigate the genetic contribution to quantitative traits associated with stroke and cardiovascular disease among Caribbean Hispanic families. The selected probands were high-risk members enrolled in a prospective community-based stroke free cohort, the Northern Manhattan Study (NOMAS). At the time of selection of probands, the NOMAS cohort was comprised of 3298 individuals of whom 1730 were Hispanic [10]. A high-risk proband was defined as someone who either: (1) reported having a sibling with history of myocardial infarction or stroke; or (2) was above the 75th percentile of the distribution of the cohort in two of three quantitative risk phenotypes: maximal carotid plaque thickness assessed by high-resolution B-mode ultrasonography, left ventricle mass divided by the body surface area, or homocysteine level. Families of the eligible probands were considered for enrollment if the probands were able to provide their family histories, obtain permission from their family members to be contacted by the research staff, and had at least three first-degree relatives who were able to participate. After the proband made the first contact, staff members contacted the relatives to explain the study and solicit participation. All participants gave informed consent. This study was approved by the Columbia University Medical Center Institutional Review Board.

2.2. Data collection

Baseline data were collected through interviews of the subjects by trained bilingual research assistants using standardized data collection instruments. Standardized questions were adapted from the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System. Brachial BP recordings were obtained after at least 5 min of rest in a supine position, and blood specimens were drawn after fasting. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, a diastolic blood pressure ≥ 90 mmHg, the patient's self-report of a history of hypertension or antihypertensive medication use. Current tobacco use was defined as smoking within the past year, while past tobacco use was defined as smoking in the past but having quit smoking.

2.3. Flow-mediated dilation assessment

Participants fasted for 12 h prior to the ultrasound assessment of brachial artery endothelial function, and were asked to avoid vasoactive medications for 24 h prior to the study. Subjects were also asked to avoid exercise for at least 4–6 h prior to the FMD examination, not to ingest substances that might affect FMD such as caffeine, or use tobacco at least 6 h prior to the appointment. Subjects were evaluated in a quiet, temperature-controlled room in the morning before 11 a.m. The brachial artery diameter was measured 6 cm proximal to the antecubital fossa using a 7–15 MHz linear array transducer (Philips 5500, Andover, MA). FMD was measured as the dilator response to reactive hyperemia induced by 5-min blood pressure cuff occlusion of the upper arm. The cuff was inflated to 50 mmHg above systolic BP when baseline systolic BP was less than 150 mmHg, and to 200 mmHg when baseline systolic BP was more than 150 mmHg. End-diastolic images were acquired at baseline and 1 min after cuff deflation. A blinded reader analyzed brachial artery diameters off-line using analysis software. Measurement of the brachial arterial diameter is expressed in millimeters up to one decimal place. The percent diameter change for FMD was calculated as follows:

$$\text{FMD} = ((\text{brachial artery diameter at peak hyperemia} - \text{diameter at rest}) / \text{diameter at rest}) \times 100.$$

Intra-observer variability for FMD measurements was 1.3% ($n = 15$) [11].

2.4. Statistical analysis

The mean and standard deviations of the quantitative phenotypes were evaluated. Log transformations were used for abnormally distributed variables. We used the Sequential Oligogenic Linkage Analysis Routines package to fit a variance-components model and estimate heritability [12]. The heritability analyses were adjusted for the sex effect. The maximum-likelihood estimation was applied to a mixed effects model, which incorporates fixed-covariate effects, random-additive genetic effects, and residual error. The random-additive genetic effects and residual error were assumed to be normally distributed and to be mutually independent.

3. Results

A total of 620 subjects (97 probands and 523 relatives) from 97 families participated in the study. The characteristics of the participants are shown in Table 1. Men accounted for 38% of the study subjects. The mean age of the subjects was 46.4 years, with a range of 18–100 years, and the mean family size was 11 members, with a range of 1–55 family members. A number of major pair-wise relationships are shown in Table 2.

The heritability of baseline brachial artery diameter and FMD, after adjusting for age and sex, were 0.57 ($p < 0.01$) and 0.20 ($p = 0.01$), respectively. Significant correlation was found between FMD and baseline brachial artery diameter ($r = -0.414$, $p < 0.001$). After adjusting for age, sex, systolic and diastolic blood pressure, smoking, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, BMI, and medications such as antihypertensive, lipid lowering, aspirin, insulin and oral hypoglycemic, the heritability of brachial artery diameter was 0.53 ($p < 0.01$). The heritability of FMD was 0.17 ($p = 0.01$) after adjusting for baseline brachial artery diameter and the same variables (Table 3).

4. Discussion

We observed modest heritability of FMD among Caribbean Hispanic families. In addition, baseline brachial artery diameter had a significant heritability. To our knowledge, this is the first report of heritability of FMD and baseline brachial artery diameter in Caribbean Hispanics. Our results suggest that there are likely genetic determinants of this quantitative trait. The

heritability of arterial diameter in our study was greater than that of FMD. This observation is not surprising because diameter is an intrinsic anatomical trait of an artery, while endothelial function is a functional parameter that may be more affected by environmental influences, such as hemodynamic variables and atherosclerotic risk factors.

The diagnostic and prognostic value of baseline brachial artery diameter has not been clearly established. In the Framingham study, a strong association between brachial artery diameter and cardiovascular disease risk was reported [4]. A strong relationship between large artery diameter and angiographic CAD was observed in one study [13], while the baseline brachial artery diameter was not an independent predictor of prognosis in several other studies [14, 15]. Our FMD heritability estimate of 0.17 suggests that genetic factors contribute modestly to the variability in endothelial function. Heritability of brachial artery diameter was 0.53. These results are similar to those observed in the Framingham study, which reported heritability of 0.14 for FMD and 0.33 for brachial artery baseline diameter [4]. In the Framingham study, the majority of the subjects studied were Caucasian. Some differences between the studies may also be attributed to race-ethnic variability of the trait.

Brachial artery FMD reflects NO-dependent endothelial function [3,7,8]. A reduction in NO bioavailability may play a role in the pathogenesis of vascular disease [6,16]. The synthesis of endothelial NO from L-arginine is regulated by the endogenous nitric oxide synthase (eNOS) [17]. One of the mechanisms that increases NO in response to changes in shear stress is the activation of eNOS gene transcription [3]. A number of polymorphisms in the eNOS gene sequence have been identified and the associations between eNOS polymorphisms and vascular disease such as coronary artery disease, myocardial infarction, and stroke were reported [17]. Due to the regulation of NO synthesis by the eNOS gene, genetic determinants can contribute to the measure of NO dependent FMD. A genetic contribution to endothelial function has also been supported by several studies demonstrating that genetic polymorphisms of eNOS may influence endothelial function [18–20].

FMD is affected by not only the presence of traditional cardiovascular risk factors, but also by other factors, such as sleep deprivation, diurnal variation, glucose and fat load, caffeine, exercise, mental stress, vasoactive medication and hormonal factors [1,3]. The difficulty of simultaneously controlling all these potential confounders may partially explain the modest effect of genetic factors on endothelial function. Furthermore, some recent evidence indicates that FMD response to various stimuli does not always reflect NO bioavailability [6,21]. Impaired FMD may occasionally not be due to the impaired release of NO from the vascular endothelium, but to an impaired microvascular response to reactive hyperemia, which would result in decreased flow velocity and shear stress during reactive hyperemia and would therefore result in a less potent stimulus to NO release [3,22].

We found a significant negative correlation between FMD and baseline brachial artery diameter, as found in several previous studies [4,13,14]. The reason for this negative correlation is not clear. In the presence of atherosclerosis, the active process of vascular remodeling is more likely to result in larger but less reactive vessels [23], which might help explain this result.

All subjects in our Northern Manhattan Family Study were Caribbean Hispanics; therefore, the extrapolation of our results to other cohorts with a different race-ethnic composition should be considered with caution. Race-ethnic differences in the pattern of atherosclerosis have been reported. Intracranial atherosclerosis is more frequent in blacks and Hispanics [24]. Hispanics have significantly lower prevalence of carotid plaque [25], coronary artery calcification [26, 27]. However, Hispanics had high ischemic stroke risk incidence compared to whites living in the same community [28]. Genetic determinants of vascular disease and quantitative traits are likely to vary by race-ethnicity.

Our study has several limitations. Although we attempted to control for temperature, foods, medications, and sympathetic stimuli to the greatest extent possible during subject preparation, these factors depend on patient cooperation, and any aberration may have influenced the FMD results. Furthermore, the inflammatory markers such as high sensitive C-reactive protein, which are known to influence cardiovascular events, were not available in our study. Also we did not measure FMD in certain menstrual phases among pre-menopausal women.

In conclusion, we found significant heritability of baseline brachial artery diameter and modest heritability of FMD. Because it is associated with atherosclerosis and is heritable, FMD is a reasonable expected phenotype for those harboring genes associated with atherosclerosis.

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Table 1

Characteristics of study participants

Characteristic	Mean $\pm$ S.D.
Family size	11 $\pm$ 8
Proband, male/female	20/77
Age (years)	46.4 $\pm$ 17.7
Gender (% male)	38.0
Hypertension (%)	43.6
Current smoking (%)	15.3
Past smoking (%)	23.6
Body mass index (kg/m ²)	28.8 $\pm$ 5.6
Systolic BP (mmHg)	123 $\pm$ 20
Diastolic BP (mmHg)	76 $\pm$ 10
Total cholesterol (mg/dl)	185.8 $\pm$ 39.8
HDL cholesterol (mg/dl)	49.3 $\pm$ 13.9
LDL cholesterol (mg/dl)	110.9 $\pm$ 34.9
Triglycerides (mg/dl)	127.0 $\pm$ 97.9
Diabetes mellitus (%)	5.5
Baseline arterial diameter (mm)	3.6 $\pm$ 0.6
FMD (%)	6.1 $\pm$ 4.5
Medication (%)	
Antihypertensive agents	28.7
Lipid lowering agents	12.3
Aspirin	32.7
Insulin	2.2
Oral hypoglycemic	13.2
History of cardiovascular disease, probands (%)	
Coronary artery disease	40.3
Myocardial infarction	18.2
Stroke	9.1
History of cardiovascular disease, relatives (%)	
Coronary artery disease	18.1
Myocardial infarction	3.5
Stroke	1.9

Table 2

Number of pair-wise relationships among participants

Pair-wise relationship	No.
Parent-offspring	272
Siblings	282
Grandparent-grandchild	58
Avuncular	327
Half siblings	68
Grand avuncular	31
First cousins	196
First cousins, once removed	144
First cousins, twice removed	26
Half-first cousins	48
Second cousins	98
Second cousins, once removed	47
Third cousins	2

Once removed means there is a difference of one generation. Twice removed means that there is a two-generation difference.

Table 3
Heritability of baseline artery diameter and flow-mediated dilation

	<i>n</i>	Mean ± S.D.	Heritability	<i>p</i>	Covariates	Covariate variance
Baseline (mm)	620	3.54 ± 0.64	0.57	<0.01	Age, sex	0.40
Baseline (mm)	612	3.54 ± 0.64	0.53	<0.01	Age, sex, BP, BMI, lipid, smoking, DM, medication	0.44
FMD (%)	620	6.29 ± 4.59	0.20	0.01	Age, sex	0.12
FMD (%)	612	6.26 ± 4.60	0.17	0.01	Age, sex, BP, BMI, lipid, smoking, DM, medication	0.29

BP: systolic and diastolic blood pressure; lipid: total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides; DM: diabetes mellitus; medication: antihypertensive agents, lipid-lowering agents, aspirin, insulin and oral hypoglycemic agents.