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Heritability of flow-mediated dilation: a twin study

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Summary

Background—Endothelial dysfunction assessed by brachial artery flow-mediated dilation (FMD) is a marker for early atherosclerotic vascular disease and future cardiovascular events.

Objective—To estimate the heritability of brachial artery FMD using a twin design.

Methods—We estimated the heritability of FMD using 94 middle-aged male twin pairs. FMD was measured by ultrasound, and traditional coronary heart disease risk factors were measured. Genetic modeling techniques were used to determine the relative contributions of genes and environment to the variation in FMD.

Results—The mean age of the twin participants was 54.9 ± 2.8 years. The mean FMD was 0.047 ± 0.030 . The intraclass correlation coefficient was higher in MZ twins [0.38, 95% confidence interval (CI) 0.32–0.43] than in DZ twins (0.19, 95% CI 0.11–0.26), suggesting a role of genetic influence in FMD variation. Structural equation modeling showed that both genetic and unique environmental factors contributed significantly to the variation in FMD. The crude FMD heritability was 0.37 (95% CI 0.15–0.54). After adjustment for traditional cardiovascular risk factors, including age, total cholesterol, blood pressure, and body mass index, the heritability of FMD was 39% (95% CI 0.18–0.56). The remaining variation in FMD could be explained by individual-specific environment.

Conclusion—This is the first study using twins to estimate the relative contributions of genetics and environment to the variation in FMD in a US population. Our results demonstrate a moderate genetic effect on brachial artery FMD, independent of traditional coronary risk factors. Our data also highlight the importance of unique environment on the variability in FMD.

Keywords

atherosclerosis; brachial artery flow-mediated dilation; heritability; twin study

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Introduction

Atherosclerotic vascular disease is the leading cause of death and disability worldwide [1]. The vascular endothelium is important in maintaining normal vascular tone, limiting vascular inflammation and smooth muscle cell proliferation [2]. When individuals are exposed to risk factors for atherosclerosis, the vascular endothelium is central in mediating damage to the vessel wall [2]. Impaired endothelial function, characterized by a reduction in the bioavailability of vasodilators, especially nitric oxide [3], is observed in patients with symptomatic vascular diseases [4], as well as in asymptomatic individuals with elevated cardiovascular risk factors [2]. Brachial artery flow-mediated dilation (FMD), a non-invasive technique using high-resolution ultrasound, is a reliable tool for assessing endothelial function in the brachial artery [5]. Many studies have used FMD of the brachial artery to evaluate the effect of cardiovascular risk factors on endothelial function and the effect of risk-reduction interventions such as medical or lifestyle interventions [6,7]. Brachial artery FMD is also a marker for future cardiovascular events [8].

Little is known about the familial or genetic basis of brachial artery FMD. A study reported a heritability of 0.14 for brachial artery FMD in the community, using parents and their offspring from the Framingham Heart Study [9]. However, twin studies are preferable for heritability estimation because they allow a more precise separation of common environmental influences from genetic effects [10]. To date, only one twin study in Finland has reported a heritability of 0.24 for brachial artery FMD [11]. There is no report on FMD heritability in the US population. The goal of this classic twin study is to estimate the genetic and environmental influences on brachial artery FMD in adult male twins.

Subjects and methods

Study population

The twins used in this study were drawn from the Twins Heart Study (THS), an investigation of psychological, behavioral and biological risk factors for subclinical cardiovascular disease in twins. The THS included 180 twin pairs drawn from the Vietnam Era Twin (VET) Registry [12]. This registry is composed of 7369 middle-aged male-male twin pairs, both of whom served in the US military during the time of the Vietnam War. It was originally developed to study the long-term health consequences of military service in Vietnam, and is one of the largest national twin registries in the USA. Details on the registry construction and composition have been published elsewhere [12].

The THS included a randomly selected sample of 112 twin pairs from the VET Registry, who were born between 1946 and 1956 and were without a history of symptomatic cardiovascular disease and major depression as of 1990, based on pre-existing survey data [13]. The THS also included a sample of pairs discordant for major depression, but these are not part of the current analysis. All twin pairs were examined at the Emory University General Clinical Research Center between March 2002 and March 2006, where their medical history was updated. Our final sample for analysis included 94 twin pairs (58 MZ and 36 DZ) in which both members were free of major depression and symptomatic cardiovascular disease (history of myocardial infarction, angina pectoris, stroke or heart failure) at the time of our examination. Zygosity information was determined by DNA analysis in all pairs except three, among whom it could not be obtained. The zygosity of these three twin pairs was assessed using questionnaires supplemented with blood group typing data abstracted from military records [13], which in our sample had an accuracy of 94%.

This study was approved by the institutional review board. Informed consent was obtained from all participants.

Measurements

All measurements were performed in the morning after an overnight fast, and both twins in a pair were tested at the same time. A medical history was obtained and a physical examination was conducted for all twins. Weight and height were used to calculate body mass index (BMI) as weight in kilograms divided by height in meters squared. Systolic blood pressure and diastolic blood pressure (DBP) were measured by mercury sphygmomanometer on the right arm with the subject in a sitting position after 10 min of rest. The average of two measurements 5 min apart was used in the statistical analyses. Venous blood samples were drawn for the measurements of fasting glucose and lipid profile according to standard procedures [14]. Plasma lipids were measured from freshly isolated EDTA plasma on the Beckman CX7 chemistry autoanalyzer. Total triglycerides and cholesterol were determined by enzymatic methods (Beckman Coulter Diagnostics, Fullerton, CA, USA). Direct high-density lipoprotein cholesterol and direct low-density lipoprotein (LDL) cholesterol levels were obtained using homogeneous assays (Equal Diagnostics, Exton, PA, USA). Cigarette smoking was classified into current, past and never.

Brachial artery FMD assessment

Endothelium-dependent brachial artery FMD was determined using bi-mode ultrasound according to standardized procedures [5]. Images were obtained with an Acuson 10-mHz linear array transducer and ultrasound system (Mountain View, CA, USA). We performed imaging with the subject resting supine for at least 10 min on a hospital bed in a quiet setting. Optimal brachial artery images were obtained between 2 and 10 cm above the antecubital crease. After baseline measurements, a blood pressure cuff was inflated to 200 mmHg over the proximal portion of the right forearm for 5 min. Endothelium-dependent function was determined during the first 2 min of release of the cuff [15]. After a 15-min period to re-establish baseline conditions, endothelium-independent dilation was assessed with similar procedures before and 3 min after administration of 0.4 mg of sublingual nitroglycerin. Images were digitized online, and arterial diameters were measured with edge-detection software (Medical Imaging Applications, Coralville, IA, USA) by an individual blinded to other subject data. Arterial diameter was measured in millimeters from the leading edge of the intima–lumen interface of the near wall (echo zone 3) to the leading edge of the lumen–intima interface of the far wall (echo zone 5), coincident with the R-wave on the electrocardiogram (i.e. end-diastole). The brachial artery vasodilator response was quantified as percentage change in vessel diameter from baseline. In order to minimize error, the same investigator (F. A. C.) did FMD measurements throughout the study, and the same equipment and analytic software was used to measure FMD for all the twin participants. In our laboratory, the mean difference in FMD (%) between two consecutive assessments performed in 11 subjects an average of 8 days apart was 1.26% ($\pm 0.76\%$), with a Pearson's correlation of 0.75. The mean difference in the FMD (%) between two readings of the same 11 measurements was 0.82% ($\pm 0.48\%$), with a Pearson's correlation of 0.97.

Methods of analysis

Risk factor assessment—Initial descriptive analysis examined potential differences for all risk factors and FMD between MZ and DZ twins. We tested differences between MZ and DZ pairs using generalized estimating equations (GEEs) to account for the dependency of twins within pairs. Similarly, the association between each of the cardiovascular risk factors and FMD was assessed by GEE regression analysis. FMD was log-transformed to obtain

normality. All the analyses were done with SAS 9.1 for Windows (SAS Institute Inc., Cary, NC, USA). The criterion for statistical significance was $P < 0.05$.

Classic twin analyses—The classic twin analysis is based on the fact that MZ twins share identical genes, whereas DZ twins share, on average, 50% of their segregating genes. A higher intraclass correlation coefficient (r) in MZ than in DZ twins provides a first indication of genetic influence on a trait. We calculated the MZ and DZ twin pair intraclass correlations for FMD using a random-effects one-way ANOVA [16]. This approach compares the expected value of the within-pair mean-square (WMS) and the between-pair mean-square (BMS) in MZ to that in DZ twin pairs. These values contain different contributions from genetic and environmental factors. For instance, the WMS in MZ twins contains no genetic variance, whereas the WMS in DZ twins contains half of the additive genetic variance. Information about shared environmental factors is included in the BMS for both MZ and DZ twin pairs. We calculated the intraclass correlation coefficient between twins using the formula: $(BMS - WMS)/(BMS + WMS)$. Its corresponding 95% confidence interval (CI) was calculated according to the methods described by McGraw and Wong [17].

Genetic model-fitting—We used genetic modeling techniques to estimate the relative contributions of genetic and environmental factors to the variation in FMD. This approach decomposes the observed phenotypic variance into genetic and environmental components: additive genetic variance (A), shared (or common) environmental variance, and specific (or unique) environmental variance, which also contains measurement error [18]. The heritability is then calculated as the ratio of additive genetic variance to total phenotypic variance. The genetic modeling approach has advantages over the classic twin methods in the study of heritability estimation because it uses both variance (i.e. difference between twins) and covariance (i.e. common characteristics between twins) information from the twin data, and allows for more extensive separation of the different variance components [18]. For instance, among the interindividual variation in FMD, the genetic modeling can determine what proportion of the variation is due to genes, and how much is due to environment. The environmental factor can be further decomposed into components that are shared by twin pairs and unique to twin pairs. In addition, genetic model-fitting allows for controlling for other risk factors.

The significance of additive genetic variance (A) and common environmental variance (C) was tested by removing each component sequentially in specific reduced models (i.e. the AE or CE or E model), eventually leading to a model that gives the most parsimonious fit to the data, i.e. a model in which the pattern of variance and covariance is explained by as few parameters as possible. Each reduced model was compared with the full model (i.e. the ACE model) by a chi-squared test. The difference between minus twice the log-likelihood ($-2 \ln L$) for a reduced model and that for the full model is approximately distributed as a chi-squared test with degrees of freedom (d.f.) equal to the difference of the number of estimated parameters in the full model and the number of estimated parameters in the reduced model. Akaike's information criterion ($AIC = \chi^2 - 2 \text{ d.f.}$) was used to evaluate the fit of the genetic models. The model with the lowest AIC reflects the best balance between goodness of fit and parsimony. Heritability estimates and their 95% CIs were obtained from the best-fitting model [18]. All genetic model fitting procedures were carried out using Mx [18].

Results

The zygosity-specific distribution of cardiovascular risk factors is presented in Table 1. The age of the twins ranged from 47 to 59 years, with a mean of 55 years. Over 95% of the twins

were Caucasians. There was no significant difference between MZ and DZ twins in any of the risk factors examined.

Table 2 shows the unadjusted correlations of FMD with traditional cardiovascular risk factors and the *P*-values after accounting for the dependency among twin pairs using GEEs. LDL (*P* = 0.004) and total cholesterol (*P* = 0.0006) showed significant inverse correlations with FMD, whereas DBP (*P* = 0.08) and BMI (*P* = 0.07) showed borderline associations with FMD.

By classic twin analysis, the intraclass correlation for FMD was 0.38 (95% CI 0.32–0.43) in MZ pairs and 0.19 (95% CI 0.11–0.26) in DZ pairs. The higher intraclass correlation in MZ than in DZ twins suggests that genes play a role in FMD variation.

Using a genetic modeling approach, we first fitted a model to estimate the crude FMD heritability. In order to determine the heritability that is independent of conventional cardiovascular risk factors, we also fitted a multivariable genetic model to adjust for all the risk factors that showed significant associations with FMD according to Table 2. These included age, total plasma cholesterol, DBP and BMI. Although both total cholesterol and LDL showed significant associations with FMD, we only included total cholesterol in the multivariate model, because of the high correlation between these two factors. Furthermore, total cholesterol was a more significant predictor of FMD in our data (Table 2). DBP and BMI were included because they are known to be highly heritable, and showed borderline associations with FMD in our sample. Although age was not significantly correlated with FMD, it was included in the multivariable analysis because other studies showed that FMD declines with age [9,19] and failure to account for age may produce spurious shared environmental effects [20]. Table 3 presents the crude and multivariable-adjusted FMD heritability obtained by the genetic modeling approach. The two reduced models, AE and CE, were not significantly different from the full ACE model; that is, we could not discriminate statistically between shared family environment or genes as the source of familial resemblance. However, the E-only model was statistically different from the ACE model. Therefore, we first removed the E-only model. Among the three models that did not show statistically significant differences (i.e. the ACE, AE and CE models), we chose the two reduced models on the basis of the theory of parsimony of twin modeling. Among the two reduced models (AE and CE), which fit the data almost equally well, we chose the AE model rather than the CE model because the AIC of the AE model was slightly lower than for the CE model. In addition, the higher intraclass correlation in MZ than in DZ twins (0.38 vs. 0.19) is more in line with heritability than with familial environment. Therefore, the AE model was chosen as the best-fitting model for both the crude and adjusted models. The crude FMD heritability was 0.37 (95% CI 0.15–0.54). After adjusting for age, total cholesterol, DBP and BMI, the FMD heritability remained similar, with a value of 0.39 (95% CI 0.18–0.56). Individual-specific environment accounted for about 60% of the variation in FMD in both the crude and multivariable-adjusted models.

Discussion

Twins are a true subset of the population and representative of the target population from which they are drawn. Studies of mortality and a number of diseases such as diabetes in the Danish Registry have shown that twins have the same incidence and mortality for several diseases as the general population. Results from twin studies can be generalized and compared to those from the general population [21]. In particular, twin studies may be very helpful in studies of phenotypes with variable age of onset such as FMD, as they control exactly for age effects. In a twin study of middle-aged men, we found that about 40% of the variation in FMD was due to additive genetic factors. Unique environmental factors

accounted for a large proportion of variability in FMD; in contrast, common environmental factors showed little influence on the FMD variation.

Our heritability estimate is higher than that found in the Finnish twin study by Jartti *et al.* [11], who reported a heritability of 0.24, and also higher than the heritability reported in the family study by Benjamin *et al.* [9], where it was 0.14. However, our heritability estimate is in line with, and actually more modest than, the heritability of other biological measures, such as blood pressure [22] and carotid intima-media thickness [23,24]. One possible reason why previous studies obtained lower estimates for FMD heritability may be that FMD was measured with more measurement error in previous studies. A lower precision increases E and, consequently, yields a lower heritability. In our laboratory, the same investigator performed all the FMD measurements, and the same equipment and analytic software was used in the analysis throughout the study. These procedures may have contributed to less measurement error as compared with previous investigations. Thus, we believe that our heritability estimate is more accurate than those previously reported. Similarly, we believe that it is unlikely that the large contribution of unique environment (E) in our results is due to FMD measurement error, as high error would have yielded lower heritability estimates as compared with previous literature. Although it is likely that our estimate of E includes some measurement error, the E component should truly reflect, for the most part, the influence of the unique environment on interindividual variations in FMD.

Among the coronary risk factors studied, LDL and total cholesterol showed a significant inverse correlation with FMD, which was consistent with findings from previous studies [25,26]. DBP and BMI showed borderline associations with FMD. Age and smoking were not associated with FMD in this study. One possible explanation for why age was not associated with FMD in our study is that our participants were homogeneous in age, with a range of only 10 years. Nonetheless, by using a same-sex twin design, we were able to control exactly for the confounding effects of demographic factors.

The functional and structural pathologic changes of atherosclerosis occur several years or even decades before its clinical end-point [27]. Endothelial dysfunction is an early feature of atherosclerosis and plays an important role in the development of atherosclerotic diseases as well as their risk factors [28]. Many studies have provided evidence that endothelial function is influenced by genetic factors [29]. For example, studies have shown that individuals with a family history of preclinical or overt atherosclerotic diseases have impaired or diminished endothelial function [30]. The possible role of single-gene polymorphisms in some of the candidate genes for atherosclerotic diseases has been investigated. For instance, polymorphism Glu298 → Asp in the endothelial nitric oxide synthase gene is related to endothelial dysfunction and cardiovascular diseases [31]. Arcaro *et al.* [32] reported that the D allele of the angiotensin-converting enzyme gene was associated with FMD in 92 young normal non-smoking individuals. A significant association between the α_{2B} adrenergic receptor gene and FMD was also reported [33]. Although the causal role of these polymorphisms with respect to endothelial dysfunction remains to be determined, it is clear that genetic factors contribute to the variation in endothelial dysfunction. In addition, environmental factors such as medication [34], smoking [9,35], physical exercise [9,36], diet [37] and geographic origin [38] have also been reported to be associated with FMD variation.

This study has some limitations that are worth mentioning. Firstly, our results were derived from middle-aged male twins, and it is difficult to generalize these findings to women or younger individuals. Secondly, our sample size is modest and our ability to discern differences between the genetic and common environmental models was limited. Nonetheless, by using 58 MZ and 36 DZ pairs, we should have ~80% power to detect a C

component of 28% at the 0.05 significance level. We chose to report the AE model as the best model, but we cannot exclude the CE model entirely as an alternative explanation of the data. Thirdly, our modeling of heritability assumes equal common environmental influences on both MZ and DZ pairs. If this assumption does not hold, then our estimate of heritability may be biased. However, several empirical studies support the validity of the equal environment assumption [39]. For instance, studies in twins correctly and incorrectly classified in terms of zygosity have shown no difference between the two groups on the studied phenotypes [21]. In addition, MZ twins reared apart yielded very similar intraclass correlations to those of twins reared together, suggesting that the similarity of MZ twins was not related to how closely they shared the environment [40].

In summary, the present study represents the first comprehensive investigation of the relative contributions of genetic and environmental factors to the variation in FMD, a measure of endothelial function and a marker of early atherosclerosis, using twins in the US male population. While lending support to the notion of heredity in the etiology of atherosclerotic vascular disease, our results also emphasize the important role of environmental influences, which may be modifiable. These findings highlight the complex genetic and environmental etiology of atherosclerotic vascular disease.

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Table 1

Demographic characteristics of the twin subjects

Covariates	MZ (58 pairs) Mean $\pm$ SD	DZ (36 pairs) Mean $\pm$ SD	<i>P</i> -value*
Age (years)	54.8 $\pm$ 2.8	55.1 $\pm$ 2.7	0.60
White (%)	95.3	96.1	0.70
Type 2 diabetes (%)	3.4	9.2	0.10
Current smoker (%)	57.3%	55.3%	0.66
BMI (kg m ⁻²)	28.5 $\pm$ 4.0	29.8 $\pm$ 5.0	0.10
Mean SBP (mmHg)	131.2 $\pm$ 14.5	128.7 $\pm$ 16.5	0.37
Mean DBP (mmHg)	82.5 $\pm$ 11.2	79.4 $\pm$ 10.5	0.127
HDL cholesterol (mg dL ⁻¹)	38.0 $\pm$ 9.0	40.7 $\pm$ 9.4	0.10
LDL cholesterol (mg dL ⁻¹)	128.3 $\pm$ 32.1	123.5 $\pm$ 34.1	0.12
Total cholesterol (mg dL ⁻¹)	191.1 $\pm$ 35.8	184.9 $\pm$ 41.2	0.34
Total triglyceride (mg dL ⁻¹)	182.8 $\pm$ 95.8	174.1 $\pm$ 99.7	0.60
Blood glucose (mg dL ⁻¹)	99.1 $\pm$ 14.2	102.8 $\pm$ 18.5	0.22
Brachial artery FMD	0.0501 $\pm$ 0.0328	0.0416 $\pm$ 0.0258	0.10

* Corrected for the dependency of twins using generalized estimating equations (GEEs).

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FMD, flow-mediated dilation.

Table 2

Unadjusted correlation of flow-mediated dilation with traditional cardiovascular risk factors

Covariates	Correlation	Corrected <i>P</i> -values using GEE
Age (years)	−0.03	0.34
Type 2 diabetes (%)	−0.02	0.93
Current smoking (yes/no)	−0.03	0.98
Body mass index (kg m ^{−2})	−0.13	0.07
SBP (mmHg)	−0.05	0.15
DBP (mmHg)	−0.08	0.08
LDL cholesterol (mg dL ^{−1})	−0.15	0.004
HDL cholesterol (mg dL ^{−1})	0.08	0.32
Total triglycerides (mg dL ^{−1})	−0.10	0.17
Total cholesterol (mg dL ^{−1})	−0.15	0.0006
Blood glucose (mg dL ^{−1})	−0.05	0.71

GEE, generalized estimating equation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Parameter estimates for additive genetics, common environment and unique environmental influences on flow-mediated dilation by structural equation modeling

Table 3

Parameter estimates								
	AIC	-2LL	d.f.	χ^2	P-value	a^2 (95% CI)	c^2 (95% CI)	e^2 (95% CI)
Model 1								
ACE	56.8	462.8	203			0.26 (0–0.54)	0.10 (0–0.47)	0.64 (0.46–0.86)
AE	54.9	462.9	204	0.08	0.778	0.37 (0.15–0.54)		0.63 (0.46–0.85)
CE	55.3	463.3	204	0.52	0.473		0.31 (0.13–0.48)	0.69 (0.52–0.87)
E	63.5	473.5	205	10.7	0.005			1.00
Model 2								
ACE	47.7	451.7	202			0.34 (0–0.56)	0.05 (0–0.48)	0.61 (0.44–0.83)
AE	45.7	451.7	203	0.02	0.889	0.39 (0.18–0.56)		0.61 (0.44–0.82)
CE	46.6	452.6	203	0.86	0.353		0.33 (0.14–0.49)	0.67 (0.51–0.86)
E	55.9	463.9	204	12.2	0.002			1.00

Model 1: crude model.

Model 2: adjusted for age, total cholesterol, diastolic blood pressure and body mass index.

a^2 , additive genetics; c^2 , common environment; e^2 , unique environment; -2LL, -2 log likelihood; CI, confidence interval; AIC, Akaike Information Criterion.