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Heritability of Carotid Intima-Media Thickness: A Twin Study

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Abstract

Objective—To estimate the heritability of carotid intima-media thickness (IMT), a surrogate marker for atherosclerosis, independent of traditional coronary risk factors.

Methods and Results—We performed a classical twin study of carotid IMT using 98 middle-aged male twin pairs, 58 monozygotic (MZ) and 40 dizygotic (DZ) pairs, from the Vietnam Era Twin Registry. All twins were free of overt cardiovascular disease. Carotid IMT was measured by ultrasound. Bivariate and multivariate analyses were used to determine the association between traditional cardiovascular risk factors and carotid IMT. Intraclass correlation coefficients and genetic modeling techniques were used to determine the relative contributions of genes and environment to the variation in carotid IMT. In our sample, the mean of the maximum carotid IMT was 0.75 ± 0.11 . Age, systolic blood pressure and HDL were significantly associated with carotid IMT. The intraclass correlation coefficient for carotid IMT was larger in MZ (0.66; 95% confidence interval [CI], 0.62–0.69) than in DZ twins (0.37; 95% CI, 0.29–0.44), and the unadjusted heritability was 0.69 (95% CI, 0.54–0.79). After adjusting for traditional coronary risk factors, the heritability of carotid IMT was slightly reduced but still of considerable magnitude (0.59; 95% CI, 0.39–0.73).

Conclusion—Genetic factors have a substantial influence on the variation of carotid IMT. Most of this genetic effect occurs through pathways independent of traditional coronary risk factors.

Keywords

heritability; carotid intima-media thickness; twin study; atherosclerosis

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Disclosures None.

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1. Introduction

Atherosclerosis is a multifactorial metabolic disorder that underlies various clinical cardiovascular diseases which are known to aggregate within families, such as coronary heart disease [1–4], myocardial infarction [2,3], and stroke [5]. Investigation of whether genetic factors influence a significant proportion of the interindividual variability in atherosclerosis is important in the search for susceptibility genes for these common disorders [6]. Although there is a consensus that genetic factors play a role in atherogenesis [6], the precise magnitude of the genetic influence is poorly described.

The arterial wall consists of three layers: the intima, the media, and the adventitia. Carotid IMT refers to a measurement of the first two layers of the artery (intima and media). During the progression of atherosclerosis, arterial wall vessel changes are characterized by gradual increase in IMT, which can be measured noninvasively using B-mode ultrasound. Carotid IMT is one of the best established and most commonly used surrogate markers of atherosclerosis [7,8]. It correlates with severity of coronary artery disease and is a predictor of future cardiovascular events [9–12]. Carotid IMT has been shown to be strongly determined by genetic factors [13,14]. However, the precise extent to which genetic predisposition explains the variance of carotid IMT is unclear [13,15–22]. In addition, it is not clear to what extent the heritability of IMT reflects genetic influences on traditional coronary risk factors, many of which are strongly heritable [23–28], or pathways independent of such risk factors.

Thus far, heritability estimates of carotid IMT range from 0.24 to 0.92, with most studies reporting heritability between 0.30 and 0.65 [15–19,21,22]. These estimates, however, were primarily derived from family studies. Compared with family designs, twin studies are preferable for heritability estimation, because they allow a more precise separation of common environmental influences from genetic effects [29]. To date, only two twin studies reported heritability estimates for carotid IMT [20,30]. Neither of these two studies did a thorough adjustment for coronary heart disease risk factors. In addition, only one of these studies statistically tested the genetic contribution to carotid IMT, and found it to be not statistically significant from zero [20]. This result is in contrast with family studies which found high heritability for carotid IMT [16,17,22].

The goal of this investigation, therefore, was to assess the relative contribution of genetic and environmental influences on interindividual differences in carotid IMT using a sample of U.S. adult male twins, before and after adjusting for known coronary risk factors.

2. Subjects and Methods

2.1. Study population

The Twins Heart Study (THS) is an investigation of psychological, behavioral and biological risk factors for subclinical cardiovascular disease in twins. Twins included in this study are members of the Vietnam Era Twin (VET) Registry [31]. This registry is composed of 7,369 middle-aged male-male twin pairs both of whom served in the United States 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 U.S. Details on the registry construction and composition have been published [31].

As part of THS, we studied 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 [32]. 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. The present analyses included 98 twin pairs (58 MZ and 40 DZ) in which both members were free of 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 [32], which in our sample had an accuracy of 94%.

2.2. 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 and a physical exam were obtained from all twin subjects. Weight and height were used to calculate body mass index (BMI) as weight in kilograms divided by height in meters squared. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by mercury sphygmomanometer on the right arm with the subject in sitting position after 10 minutes of rest. The average of two measurements 5-minute apart was used in the statistical analyses. Venous blood samples were drawn for the measurements of glucose and lipid profile after an overnight fast. The Emory Lipid Research Laboratory, a participant in the Centers for Disease Control/National Heart, Lung and Blood Institute Lipid Standardization Program, performed all analyses from freshly isolated EDTA plasma. Total triglycerides and cholesterol were determined by enzymatic methods (Beckman Coulter Diagnostics, Fullerton, CA). Direct high-density lipoprotein (HDL) and direct low-density lipoprotein (LDL) cholesterol were obtained using homogeneous assays (Equal Diagnostics, Exton, PA). The presence/absence of high levels of remnant lipoproteins was assessed by non-denaturing gradient electrophoresis using plasma that had been prestained for lipids as previously described [33], given the reported association between remnant lipoproteins and carotid IMT [34]. Glucose levels were measured on the Beckman CX7 chemistry autoanalyzer. Physical activity was assessed by means of a modified version of the Baecke Questionnaire of Habitual Physical Activity used in the Atherosclerosis Risk in Communities (ARIC) Study [35], a 16-question instrument documenting level of physical activity at work, during sports and non-sports activities. The total physical activity score ranging from 3.43 to 13.04 was used in the analysis. Cigarette smoking was classified into current smoker (any number of cigarettes) versus never or past smoker. Pack-years of smoking were calculated as the number of packs of cigarettes smoked per day times the number of years smoked. Because the results were similar when the analyses were run using current smoking versus pack-years, only the results with current smoking are presented.

2.3. IMT Measurements

Common carotid artery IMT was measured using high resolution B-mode ultrasonography with standard techniques [7,36,37]. Briefly, IMT was quantified both on the near and far wall at the distal 1.0 cm of the left and right common carotid arteries proximal to the bifurcation. For each segment, the sonographer used multiple different scanning angles to identify the longitudinal image of IMT showing the maximum IMT. At least 10 pictures for each segment were stored digitally, and measurements were made off-line using semi-automated computerized analytical software (Carotid Tools, MIA Inc., Iowa City, Iowa) by one observer blinded to other twin data. Of the stored images, the one with maximum thickness was selected, and IMT measured, for each segment. Average values of the IMT of each of the four segments (right near and far walls, and left near and far walls) were used as the IMT values for each twin in the analysis (total mean of maximum IMT). In order to minimize error, the same investigator (F.A.C.) did IMT measurements throughout the study, and the same equipment and analytical software was used to measure IMT for all the twin participants. In our lab, the mean absolute difference in IMT measured in 7 subjects in whom 2 carotid artery examinations were performed 3 days apart, was 0.03 (± 0.02) mm. The mean difference in 2 successive readings

of the same 10 segments of common carotid IMT was 0.02 (± 0.02) mm with a Pearson correlation coefficient of 0.93.

2.4. Statistical Analysis

2.4.1. Risk factor assessment—Initial descriptive analysis examined means and percents for all risk factors and the carotid IMT values in MZ and DZ twins. The IMT values were not normally distributed and were logarithmically transformed. We tested for differences between MZ and DZ pairs using generalized estimating equations (GEE) to account for the lack of independence of twins within pairs. We next assessed the correlation between each of the cardiovascular risk factors with carotid IMT and obtained corrected p-values using bivariate GEE analysis. Multivariate GEE analyses were performed by including all the significant risk factors. All the analyses used SAS 9.1 for Windows. Differences were considered as significant at an alpha value of 0.05.

2.4.2. Estimating genetic influence on carotid IMT—Estimating the genetic influence on carotid IMT is based on the biologic difference in shared genetic material in MZ and DZ twins. MZ twins share 100% of their DNA while DZ twins share, on average, 50% of their DNA. If the within pair similarity for a phenotype, such as carotid IMT, is greater in MZ than DZ pairs this provides evidence for genetic influence. A descriptive estimate of the genetic influence on carotid IMT was calculated using the intraclass correlation in MZ (r_{MZ}) and DZ (r_{DZ}) pairs. Intraclass correlations for carotid IMT in MZ and DZ twin pairs were calculated from a random-effects one-way analysis of variance [38]. The corresponding 95% confidence intervals for r_{MZ} and r_{DZ} were calculated according to the methods described by McGraw and Wong [39].

Formal statistical analysis is based on the multi-factorial model that assumes that the phenotypic variance of carotid IMT is function of additive genetic (A), common environmental (C), and unique environmental (E) effects [40]. The model assumes no gene-gene interaction and gene-environment interaction. The additive genetic component measures the effects due to genes at multiple loci or multiple alleles at one locus. The common environmental component estimates the contribution of the shared family environment by both twins, whereas the unique environmental component estimates the effects that apply only to each individual twin, and includes measurement error.

Structural equation modeling, implemented in Mx software, was used to obtain estimates of the relevant genetic and environmental parameters [41]. This method compares the difference in covariance of carotid IMT between MZ and DZ twins. The process involves initially estimating the so-called ACE model that includes additive genetic, common environmental and unique environmental effects. Following this, reduced models are constructed, such as AE and CE, to assess the influence of deleting parameters on the overall model fit. The best fitting model is determined based on chi-square statistics. The model with the smallest Akaike information criterion (AIC) reflects the most parsimonious model.

We first fit a model to the carotid IMT raw data without adjusting for any risk variables to obtain the crude heritability for IMT. Since we are interested in the heritability of IMT independent of the influence of traditional risk factors, we next fit a model to obtain multivariable-adjusted estimates of additive genetic and environmental effects. In the multivariable-adjusted model, we only included significant risk factors, including age, HDL and SBP. For both the crude and multivariable-adjusted analyses we obtained estimates of genetic and environmental effects from the best fitting model. To further determine the combined effects of traditional cardiovascular risk factors on IMT variance, we have also constructed a multivariate Cholesky model [41] to estimate the genetic and environmental

correlations between IMT and those significant risk factors including age, HDL and SBP (according to Table 2).

3. Results

The zygosity-specific means or frequencies of cardiovascular risk factors are presented in Table 1. The current age of the twins ranged from 47 to 59 years with a mean of 54. There were no significant differences between MZ and DZ twins for all the known cardiovascular risk factors.

Table 2 shows the correlation between traditional cardiovascular risk factors and carotid IMT. SBP showed the largest correlation with carotid IMT ($r = 0.32$, $p = 0.0001$). In bivariate analysis, age, blood pressure (both SBP and DBP) and HDL were significantly correlated with IMT. In a separate multivariate analysis including age, HDL and SBP, we found that age ($p = 0.006$), HDL ($p = 0.01$) and SBP ($p < 0.001$) remained significantly associated with IMT.

The intraclass correlation for carotid IMT was higher in MZ (0.66; 95% CI, 0.62 – 0.69) than in DZ twin pairs (0.37; 95% CI, 0.29 – 0.44) which is suggestive of genetic influence. Table 3 presents the crude and multivariable-adjusted heritability estimates for carotid IMT using structural equation modeling. In the crude model, AE was the best fitting model for carotid IMT; heritability was estimated as 0.69 (95% CI, 0.54–0.79). In multivariable analysis, after adjusting for age, SBP and HDL, the best fitting model remained AE (goodness of fit p -value = 0.83). The magnitude of the genetic effects decreased slightly to 0.59 (95% CI, 0.39–0.73) in this model. The multivariate Cholesky model indicated that 17% of the IMT variance is explained by the combined effects of traditional risk factors including age, SBP and HDL.

4. Discussion

This is the first twin study to investigate the relative contribution of genetic and environmental factors to carotid IMT in a U.S. twin sample, before and after adjusting for known coronary risk factors. We found that additive genetic influences explain a significant proportion of the inter-individual variation in carotid IMT. A portion of the overall genetic influence on carotid IMT was due to traditional cardiovascular risk factors. The genetic influence on IMT, not shared by traditional cardiovascular risk factors, however, remained substantial (59%). This heritability estimate represents the contribution of the genes that are acting independently of traditional cardiovascular risk factors. In addition, our results indicate that shared environmental factors do not contribute significantly to carotid IMT in this population of adult twins.

Our heritability estimates of carotid IMT are consistent with previous findings. Most studies reported that genetic factors account for 0.30 to 0.65 of carotid IMT variation in families after adjustment for traditional cardiovascular risk factors [11,16,18–22]. A very high heritability estimate of carotid IMT (0.92) was reported by Duggirala et al. [22] in a Mexico population, but the sample size of this study was very small (only 46 sibships). Most previous studies included sib-pairs or families ascertained on the basis of disease status. Only two studies reported the estimates of heritability for carotid IMT using a twin design [20,30]. One twin study by Swan et al. [20] estimated the heritability of IMT in 132 twin pairs presumably healthy middle-aged twins in Scotland, yielding a non-significant heritability estimate of 0.31. The other study by Jartti et al. [30] reported a modest heritability of IMT (0.36) using 74 male Finnish twin pairs, but did not report statistical significance testing for this estimate. Using a larger sample of twins and appropriate statistical methodology, our study demonstrates a significant heritability for carotid IMT, which withstands adjustment for conventional coronary risk factors.

Though carotid IMT and clinical atherosclerosis may have distinct genetic and biological determinants [42,43], heritability estimation of IMT provides important information for the identification of genetic variants in atherosclerosis and its risk factors. Several studies have investigated genetic polymorphisms in candidate pathways implicated in the pathogenesis of atherosclerosis [43–53]. For instance, functional variants in the genes APO A–E [44,45,50], eNOS [47], PCK1 [48], PON1 [49], OPG [53], PPARG [52], and GPx-1 [51] are associated with carotid IMT or its risk factors. However, association studies of genetic polymorphisms with carotid IMT are inconsistent. For example, Bednarska-Makaruk et al.[45] reported that subjects with APO E epsilon4 allele were more prone to develop atherosclerotic lesions in carotid arteries compared with subjects with homozygous epsilon3 genotype; conversely, Fernandez-Miranda et al.[54] found no difference between APO E genotype and atherosclerosis in patients with coronary artery disease.

In addition to genetic factors, our study also suggests a strong influence of unique environmental factors on IMT variation. Although we do not know exactly what these environmental risk factors are and their role in atherosclerotic process, it is safe to hypothesize that the environment is important for the development of atherosclerosis. The interaction of these environmental factors with genes, coupled with the interactions among multiple genes, may be central to the underlying pathogenesis of atherosclerosis [55,56].

There are some limitations to our study. First, the sample is derived from a twin registry of military veterans; therefore, the generalizability to other populations is not known. Second, since our analyses included only male twin pairs, generalization to female populations is problematic because of differences in carotid IMT levels between men and women [11,15]. Third, our results were derived from healthy middle-aged twins, and therefore may not extend to younger subjects or populations with clinically manifest cardiovascular disease. Fourth, our modeling of heritability assumes equal common environmental influences on both MZ and DZ pairs. If the equal environment assumption does not hold, then our estimate of heritability may be biased upwards. Fifth, our study has limited power to detect the influences of common environment on IMT variance due to relatively small sample size, which may bias our estimation. However, the CE model was significantly worse than the AE model and the C parameter was estimated at zero in the adjusted model, indicating that C had a very small contribution, if any, to the model.

In summary, the heritability of carotid IMT in U.S. middle-aged males is substantial, and much of this genetic influence occurs independently of common coronary risk factors. Equally noteworthy is that the remaining variation is attributable to unique environmental factors which are potentially modifiable. These findings highlight the complex genetic and environmental etiology of atherosclerosis.

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Table 1
Demographic, clinical and laboratory characteristics of twins

Characteristic	MZ (58 pairs)	DZ (40 pairs)	P-value [*]
	mean $\pm$ SD or %	mean $\pm$ SD or %	
Age (years)	54.8 $\pm$ 2.8	55.1 $\pm$ 2.7	0.4918
White race (%)	95.3	96.1	0.7021
Type 2 diabetes (%)	3.4	9.2	0.1020
Current smoking (%)	57.3	55.3	0.8407
Physical activity score	7.5 $\pm$ 1.4	7.6 $\pm$ 1.5	0.8311
BMI (kg/m ²)	28.8 $\pm$ 4.0	29.5 $\pm$ 5.2	0.3104
SBP (mmHg)	131.4 $\pm$ 14.6	129.1 $\pm$ 16.5	0.4262
DBP (mmHg)	82.7 $\pm$ 11.3	79.8 $\pm$ 10.4	0.1480
HDL (mg/dL)	30.8 $\pm$ 8.9	40.3 $\pm$ 9.4	0.1981
LDL (mg/dL)	127.8 $\pm$ 32.3	123.3 $\pm$ 33.2	0.1683
Total cholesterol (mg/dL)	191.1 $\pm$ 35.7	184.7 $\pm$ 40.3	0.3000
Total triglyceride (mg/dL)	186.7 $\pm$ 104.3	177.9 $\pm$ 105.3	0.6215
Blood glucose (mg/dL)	99.1 $\pm$ 14.1	103.4 $\pm$ 19.1	0.1453
Presence of plasma remnants (%)	33.6	32.4	0.9104
Max_RFW	0.73 $\pm$ 0.13	0.71 $\pm$ 0.14	0.3854
Max_LFW	0.77 $\pm$ 0.15	0.75 $\pm$ 0.15	0.4718
Max_RNW	0.77 $\pm$ 0.14	0.73 $\pm$ 0.14	0.1417
Max_LNW	0.77 $\pm$ 0.13	0.73 $\pm$ 0.11	0.0533
Total mean of maximum IMT	0.76 $\pm$ 0.10	0.73 $\pm$ 0.11	0.1107

* P-values were obtained with generalized estimating equations (GEE) taking into account intra-pair correlations.

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL = high density lipoprotein; LDL = low density lipoprotein; IMT = intima-media thickness; Max RFW = maximum IMT for right far wall; Max LFW = maximum IMT for left far wall; Max RNW = maximum IMT for right near wall; Max LNW = maximum IMT for left near wall.

Table 2
Association of carotid IMT with traditional cardiovascular risk factors

Covariates	Correlation	Corrected p-values using GEE
Age (years)	0.27	0.0063
Type 2 diabetes	0.02	0.9180
Body mass index (kg/m ²)	0.11	0.0811
Current smoking	0.05	0.2190
Physical activity	−0.05	0.3752
SBP (mmHg)	0.32	0.0001
DBP (mmHg)	0.25	0.0019
LDL (mg/dl)	0.11	0.4293
HDL (mg/dl)	−0.26	0.0172
Total triglycerides (mg/dl)	0.16	0.0984
Total cholesterol (mg/dl)	0.10	0.5809
Blood glucose (mg/dl)	0.11	0.3901
Plasma remnants (%)	0.06	0.9870

GEE: generalized estimating equations. Other abbreviations are the same as in Table 1

Table 3
Parameter estimates for additive genetics, common environment and unique environmental influences on carotid IMT by structural equation modeling

Model	AIC	-2LL	DF	χ^2	P Value	Parameter estimates		
						a ² (95% CI)	c ² (95% CI)	e ² (95% CI)
Model 1								
ACE	-605.6	-245.6	180			0.47 (0.00-0.78)	0.21 (0-0.61)	0.32(0.21-0.48)
AE	-607.0	-245.0	181	0.59	0.44	0.69 (0.54-0.79)		0.31 (0.21-0.46)
CE	-603.9	-241.9	181	3.72	0.05		0.58 (0.43-0.70)	0.42 (0.30-0.57)
Model 2								
ACE	-638.7	-278.7	180			0.59 (0.18-0.73)	0.00 (0-0.33)	0.41 (0.27-0.61)
AE	-640.7	-278.7	181	0.00	1.000	0.59 (0.39-0.73)		0.41 (0.27-0.61)
CE	-634.0	-272.0	181	6.65	0.010		0.42(0.23-0.57)	0.58 (0.43-0.77)

Model 1: crude model

Model 2: adjust for age, HDL and SBP.

a^2 = additive genes; c^2 = common environment; e^2 = unique environment

CI = confidence interval; -2LL = -2 Log Likelihood; AIC = Akaike Information Criterion