Original Contribution

Heritability of the Ankle-Brachial Index

The Framingham Offspring Study

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The ankle-brachial blood pressure index (ABI) is a widely utilized measure for detecting peripheral arterial disease. Genetic contributions to variation in ABI are largely unknown. The authors sought to estimate ABI heritability in a community-based sample. From 1995 to 1998, ABI was measured in 1,097 men and 1,189 women (mean age = 57 years; range, 29–85 years) from 999 families in the Framingham Offspring cohort. Correlation coefficients for sibling pairs were calculated using the family correlations (FCOR) procedure in S.A.G.E. (Case Western Reserve University, Cleveland, Ohio). The heritability of ABI was estimated using variance-components methods in SOLAR (Southwest Foundation for Biomedical Research, San Antonio, Texas). Analyses were performed on normalized crude ABI and on normalized residuals from multiple linear regression analyses in SAS (SAS Institute, Inc., Cary, North Carolina) that adjusted for age, sex, smoking, diabetes, hypertension, ratio of total cholesterol to high density lipoprotein cholesterol, log triglyceride level, and body mass index. The mean ABI was 1.1 (range, 0.4–1.4). The age- and sex-adjusted and multivariable-adjusted sibling-pair correlation coefficients for normalized ABI were 0.15 and 0.11, respectively, resulting in heritability estimates of 0.30 and 0.22. Crude, age- and sex-adjusted, and multivariable-adjusted heritabilities for normalized ABI estimated using variance-components analysis were 0.27 (standard error, 0.06), 0.30 (standard error, 0.06), and 0.21 (standard error, 0.06), respectively (all p values < 0.0001). A modest proportion of the variability in ABI is explained by genetic factors.

blood pressure; genetic predisposition to disease; peripheral vascular diseases

Peripheral arterial disease is a highly prevalent condition affecting more than five million persons in the United States (1). The ankle-brachial blood pressure index (ABI) is a measure of subclinical peripheral arterial disease that is correlated with cardiovascular disease risk factors (2–7) and measures of coronary and carotid atherosclerosis (8–10)

and is predictive of an increased risk of coronary and cardiovascular mortality (11–18). Thus, ABI is a useful clinical measure of generalized atherosclerotic burden and risk of future cardiovascular disease events.

Small studies have suggested that a family history of premature onset of peripheral arterial disease is associated with

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subclinical atherosclerotic disease and cardiovascular events in young adults (19, 20). In one twin study, Carmelli et al. (21) estimated heritability for ABI to be nearly 50 percent. However, ABI heritability has not yet been confirmed in other populations, and the prior study was limited to elderly male twins, raising concern about selective mortality bias. If heritability of ABI can be established in a general population sample, this noninvasive measure of atherosclerosis might prove useful in subsequent genetic studies. We hypothesized that ABI is a heritable phenotype, and we sought to estimate ABI heritability among participants in the community-based Framingham Offspring Study.

MATERIALS AND METHODS

Study sample

The Framingham Offspring Study is a prospective epidemiologic study of cardiovascular and other chronic diseases initiated in 1971 when 5,124 offspring (and offspring spouses) of the original Framingham Heart Study cohort were recruited (22, 23). Offspring participants have been examined approximately every 4 years since the study's inception. Written informed consent was obtained at the time of each examination. The institutional review board of the Boston Medical Center approved the content of each examination.

Offspring participants who were members of one of the 1,643 Framingham Heart Study families and who attended the sixth research examination in 1995–1998 were eligible for this study. The research examination included a standardized medical history and physical examination, an electrocardiogram, noninvasive cardiovascular testing, and phlebotomy for measurement of fasting levels of lipids and glucose. Of the 3,489 participants attending the clinic examination, 86 had incomplete or missing data on ABI and seven were excluded because of prior lower-extremity bypass surgery. We excluded additional participants for not being a biologic part of a Framingham Study family (e.g., spouses) ($n = 1,062$), having an ABI greater than 1.4 ($n = 11$), or having incomplete data on cardiovascular disease risk factors ($n = 37$). Thus, our final study sample comprised 2,286 offspring participants from 999 extended families. Participants were contained in sibships with the following distribution: 42 percent had one member, 28 percent had two members, 14 percent had three members, 10 percent had four or five members, and 6 percent had at least six members. Our sample included 1,599 sibling pairs, four parent-offspring pairs, 102 avuncular pairs, 45 half-sibling pairs, 855 first-cousin pairs, 88 first-cousin-once-removed pairs, 18 second-cousin pairs, and three double-first-cousin pairs.

Measurement of ankle and arm blood pressures

As part of the research examination, ankle-brachial systolic blood pressure measurements were routinely obtained by trained technicians according to a standard protocol (2). Systolic blood pressure was measured using an 8-MHz Doppler pen probe and an ultrasonic Doppler flow detector (Parks Medical Electronics, Inc., Aloha, Oregon). For each

limb (right arm, left arm, right ankle, left ankle), the cuff was inflated quickly to the maximal inflation level and was deflated at a rate of 2 mmHg per second until systolic blood pressure became audible. All limb blood pressure measurements were repeated in reverse order. ABI was calculated for each leg as the ratio of average systolic blood pressure in the ankle divided by average systolic blood pressure in the higher arm.

Measurement of cardiovascular disease risk factors and events

Risk factors were measured at the time of the examination. Height and weight were obtained by trained technicians using standardized protocols, and body mass index was calculated as weight in kilograms divided by height in meters squared. Two blood pressure measurements were taken at rest by the examining physician, and the mean of the two readings was used to determine the presence of hypertension. Hypertension was defined as a blood pressure of 140/90 mmHg or greater or the use of antihypertensive medication. A current smoker was defined as someone who had smoked one or more cigarettes per day during the year preceding examination. Blood was obtained in the fasting state, and levels of total cholesterol, high density lipoprotein cholesterol, triglyceride, and glucose were measured. The presence of diabetes was defined by a fasting glucose level of 126 mg/dl or greater or use of insulin or an oral hypoglycemic agent.

Cardiovascular events were identified at the time of each research clinic examination, from hospital surveillance, and from health history updates on participants who did not attend an examination. Cardiovascular events included angina pectoris, coronary insufficiency, myocardial infarction, stroke or transient ischemic attack, and intermittent claudication. All cardiovascular disease events were adjudicated by a panel of three senior investigators (or a panel of study neurologists for cerebrovascular disease events) using standardized criteria previously reported (24).

Statistical analysis

ABI was examined as a continuous measure. The lower of the two ABIs calculated for each lower extremity was used for analysis. If ABI was missing for one lower extremity, we used data from the nonmissing extremity. Since the distribution of ABIs was skewed and estimation of heritability requires the phenotype to be normally distributed, normalization of ABI was performed using the SAS procedure (25) PROC RANK, which computes normal scores from the ranks of original ABI values. The resulting ABI values appeared to be normally distributed by Blom's (26) formula $y_i = \Phi^{-1}(r_i - 3/8)/(n + 1/4)$, where Φ^{-1} is the inverse cumulative normal (PROBIT) function, r_i is the rank of the ith observation, and n is the number of nonmissing observations for the ranking of ABI. For age- and sex-adjusted ABI and multivariable-adjusted ABI, we performed multiple linear regression analysis on log-transformed ABI that adjusted for covariates of interest and obtained studentized residuals (residuals divided by their standard errors), which were also

normalized because of their skewed distribution. Covariates entered into the multivariable models included age, sex, hypertension, smoking status, diabetes, ratio of total cholesterol to high density lipoprotein cholesterol, log triglyceride level, and body mass index. Thus, normalized crude, age- and sex-adjusted, and multivariable-adjusted ABIs were used as phenotypes in subsequent analyses.

Heritability was estimated using two methods (27). First, we calculated intraclass correlation coefficients for sibling pairs using the family correlations (FCOR) procedure in S.A.G.E. (Statistical Applications for Genetic Epidemiology) (28, 29). The FCOR procedure can estimate multivariate family correlations for all types of pairs in a set of pedigrees. For our data, the primary relationship was sibling pairs. We specified equal weights for each pedigree as the weights to be used to compute the correlations, since this is a uniform weighting scheme such that the contributions from each pedigree have the same weights regardless of the number of pairs in the pedigree. A simple estimate of heritability is obtained by doubling the sibling-pair intraclass correlation using the equation $h^2 = 2r$ (h^2 indicates heritability and r indicates correlation among sibling pairs). Next, heritability was estimated using variance-components methods as implemented in the SOLAR (Sequential Oligogenic Linkage Analysis Routines) computer package, which simultaneously utilizes data on all family relationships (30, 31). The variance-components model assumes that variation in the trait, normalized ABI, can be partitioned into genetic and environmental components. This method applies maximum likelihood estimation to a mixed-effects model that incorporates fixed effects for known covariates and variance components for genetic effects. Heritability is estimated as the ratio of genetic variance to total phenotypic variance using this maximum likelihood method.

RESULTS

Clinical characteristics

The baseline characteristics of our study sample are shown in table 1. Among the 2,286 participants (mean age = 57.4 years; 48 percent men), the mean ABI was 1.12 (range, 0.4–1.4). Prevalent cardiovascular disease was present in 11.9 percent of men and 7.4 percent of women. The prevalences of hypertension, diabetes, and current smoking were 38 percent, 10.4 percent, and 16 percent, respectively, in the overall sample, with no important differences between men and women.

Correlation coefficients

Intraclass correlation coefficients for sibling pairs were calculated using the FCOR procedure in S.A.G.E. and are presented in table 2. For age- and sex-adjusted and multivariable-adjusted analyses, the correlation coefficients for normalized ABI were 0.15 and 0.11, respectively. Using the multivariable-adjusted sibling correlation coefficients, the estimate of heritability ($h^2 = 2r$) was 0.22.

TABLE 1.   Baseline characteristics of the study sample, Framingham Offspring Study, Framingham, Massachusetts, 1995–1998

Characteristic	Men ($n = 1,097$)	Women ($n = 1,189$)
Mean age (years)	57.0 (9.6)*	57.9 (9.95)
Mean ankle-brachial blood pressure index	1.16 (0.1)	1.09 (0.10)
Cigarette smoking (%)		
Current smoker	17	16
Former smoker	51	45
Hypertension† (%)	40	37
Diabetes‡ (%)	11.8	9.2
Mean total:high density lipoprotein cholesterol ratio	5.0 (2.1)	3.9 (1.4)
Mean triglyceride level (mg/dl)	148.8 (187.6)	132.6 (79.8)
Mean body mass index§ (kg/m²)	28 (4.4)	27 (5.8)
Intermittent claudication (%)	2.8	2.5
Cardiovascular disease (%)	11.9	7.4

* Numbers in parentheses, standard deviation.

† Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive medication.

‡ Diabetes was defined as fasting blood glucose level ≥126 mg/dl or use of insulin or oral hypoglycemic agents.

§ Weight (kg)/height (m)².

Heritability estimates

Heritability estimates derived from SOLAR are presented in table 3. Heritabilities for normalized crude, age- and sex-adjusted, and multivariable-adjusted ABI were 0.27 (standard error, 0.06), 0.30 (standard error, 0.06), and 0.21 (standard error, 0.06), respectively. Therefore, 21 percent of the interindividual variability in normalized ABI was attributable to genetic effects. Multivariable-adjusted heritability estimates were slightly higher in men (0.29, $p = 0.006$) than in women (0.23, $p = 0.02$) and were similar in younger (age <60 years) and older (age ≥60 years) persons.

TABLE 2.   Intraclass correlation coefficients for ankle-brachial blood pressure index (1,599 sibling pairs) obtained using the family correlations (FCOR) procedure in S.A.G.E.,* Framingham Offspring Study, Framingham, Massachusetts, 1995–1998

Model	Coefficient	Heritability ($h^2 = 2r$)
Age- and sex-adjusted	0.15	0.30
Multivariable-adjusted†	0.11	0.22

* S.A.G.E., Statistical Applications for Genetic Epidemiology (Case Western Reserve University, Cleveland, Ohio).

† Multivariable model adjusted for age, sex, current smoking, diabetes, hypertension, ratio of total cholesterol to high density lipoprotein cholesterol, log triglyceride level, and body mass index.

TABLE 3.   Estimated variance-components heritabilities for normalized ankle-brachial blood pressure index ($n = 2,286$), Framingham Offspring Study, Framingham, Massachusetts, 1995–1998*

Ankle-brachial index phenotype	Heritability	
	Mean	Standard error
Crude	0.27	0.06
Age- and sex-adjusted	0.30	0.06
Multivariable-adjusted†	0.21	0.06

* All *p* values < 0.0001.

† Multivariable model adjusted for age, sex, current smoking, diabetes, hypertension, ratio of total cholesterol to high density lipoprotein cholesterol, log triglyceride level, and body mass index.

Components of variance analysis

The overall contributions of genetic factors and covariates to ABI variability were examined. The contribution of genetic factors to overall variation in ABI was 21 percent (heritability), and the contribution of covariates was 14 percent; this left a residual of 65 percent.

DISCUSSION

In our community-based sample of middle-aged adults, we found that ABI is heritable, with a magnitude of heritability suggesting that genetic effects are significant but modest. In the only prior study estimating ABI heritability, Carmelli et al. (21) examined elderly male monozygotic ($n = 94$) and dizygotic ($n = 90$) twin pairs and found that 48 percent of the variability in ABI values could be attributed to genetic effects. Carmelli et al. acknowledged that the estimation of genetic effects may have been biased by selective mortality and loss to follow-up of participants at high risk for peripheral arterial disease (21). In that study, both twins had to participate in the follow-up examination; however, risk factors for peripheral arterial disease, such as smoking and hypertension, were more common among those lost to follow-up. Heritability may also be more evident in younger persons, while traditional risk factors may play a greater role in atherosclerosis as people age, as has been suggested by a study of subclinical atherosclerosis defined using measures of carotid intimal-medial thickness (32). Finally, our results may be more modest than those of the prior twin study (21) because twin studies may overestimate heritability. Heritability estimates cannot effectively distinguish between genetic factors and early environmental influences, which are more common among twins (33).

Carotid intimal-medial thickness, another measure of subclinical peripheral atherosclerosis, has been definitively demonstrated to be heritable. Genetic factors accounted for a greater contribution to variation in carotid intimal-medial thickness than our estimates of heritability for ABI (32, 34–37). The differences in the genetic contribution to these two subclinical disease measures may be partly related to differences in measurement variability and measurement error. Carotid intimal-medial thickness measures are obtained by

ultrasound, and the images are read by a single trained reader (35) or at central reading centers (38) with high correlations between readers. Whereas Doppler systolic blood pressure measurements have previously been reported to be reliable (13), the ABI is a cruder measure than carotid intimal-medial thickness, as it is calculated from the ratio of the ankle and arm Doppler systolic blood pressure readings. Prior studies have demonstrated that genetic factors contribute to coronary disease death and stroke (39, 40), as well as variation in levels of cardiovascular disease-related variables, including hypertension (41, 42), diabetes (43–45), cholesterol levels (46), and measures of obesity (47, 48). To our knowledge, our findings represent the first large, population-based estimate of heritability for ABI. Our results suggest that ABI may be a useful noninvasive measure with which to further explore the genetics of atherosclerosis and clinical cardiovascular disease. ABI is easily obtainable, inexpensive, and clinically accessible, as opposed to other measures of subclinical atherosclerosis that require expensive equipment and may not be available for clinical use.

ABI may share common genetic determinants with individual risk factors for cardiovascular disease. For example, apolipoprotein E genotype is associated with an adverse lipid profile and is linked to an increased risk of cardiovascular disease. Investigators from the Honolulu-Asia Aging Study demonstrated an association between apolipoprotein E genotype and the prevalence of a low ABI in elderly nonsmoking Japanese-American men (49). Confirmation of the apolipoprotein E-low ABI association in other populations, as well as clarification of the role of diabetes in this association, requires further investigation. Reports of an association between fibrinogen and hemostatic factor genotypes and risk of peripheral arterial disease have yielded inconsistent results (50, 51). While an initial report demonstrated an increased risk of peripheral arterial disease associated with the beta fibrinogen gene (-455G/A) (50), a subsequent report could not identify an association between the fibrinogen T/G (+1689) polymorphism and peripheral arterial disease (51).

Our study had several important limitations that merit comment. Our sample was primarily Caucasian, limiting the generalizability of our findings to other racial and ethnic groups. Studies of racially diverse samples have observed a higher prevalence of peripheral arterial disease among non-Hispanic Blacks as compared with Whites (1). A single ABI measurement was used in our study; however, several ABI measurements taken over time may provide a more stable estimate. Long-term blood pressure estimates have been shown to be highly heritable, with significant linkage to chromosome 17 (41). Finally, our results are limited to the definition of ABI used in our study. Other investigators have calculated ABI on the basis of right arm blood pressure alone (1, 12, 14) or blood pressure in only one leg (52), and a recent study examined the higher of two ABIs in addition to lowest-leg ABI (53).

In conclusion, a modest proportion of the variability in ABI is explained by genetic factors. Further studies of genetic linkage and candidate gene association are warranted to identify specific genetic variants associated with this important predictor of cardiovascular disease events and mortality.

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