Heritability of Carotid Artery Atherosclerotic Lesions
An Ultrasound Study in 154 Families

Susanna Moskau, MD; Astrid Golla, PhD; Christoph Grothe, MD; Monika Boes, MD; Christoph Pohl, MD; Thomas Klockgether, MD

Background and Purpose—Ultrasound examination of the carotid arteries yields several quantitative measures that may serve as intermediate phenotypes in genetic studies. This study was undertaken to compare the heritabilities of 3 ultrasound measures: intima-media thickness (IMT), plaque score, and maximal stenosis.

Methods—We studied 565 individuals from 154 families ascertained by an affected parent with carotid artery atherosclerosis. IMT, plaque score, and maximal stenosis of the carotid arteries were examined by B-mode ultrasound and analyzed quantitatively. Heritability estimates were obtained by variance component analysis as implemented in the program SOLAR (sequential oligogenic linkage analysis routines). Covariates were age, sex, weight, height, body mass index (BMI), arterial hypertension, diabetes mellitus, amount of nicotine consumed, and plasma levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, LDL/HDL ratio, lipoprotein(a) [Lp(a)], triglycerides, factor VIII, factor XIII, fibrinogen, and von Willebrand factor (vWF).

Results—After accounting for the covariables age, sex, hypertension, diabetes mellitus, and Lp(a), heritability of IMT was estimated as $h^2 = 0.61 \pm 0.17 \ (P = 0.001)$. Variation of plaque score was influenced by age, sex, hypertension, diabetes mellitus, hypercholesterolemia, amount of nicotine consumed, factor VIII, and vWF. When these were considered, no significant heritability could be detected. Heritability of stenosis was estimated as $h^2 = 0.47 \pm 0.07 \ (P = 0.006)$, with age, sex, BMI, hypertension, diabetes mellitus, amount of nicotine consumed, and LDL/HDL ratio as covariates.

Conclusions—Among the 3 ultrasound measures studied, IMT had the highest heritability. IMT was strongly influenced by genetic determinants other than those influencing known risk factors. This makes IMT a promising candidate for use as an intermediate phenotype in genetic studies aiming to identify novel genes for atherosclerosis. (Stroke. 2005;36:000-000.)

Key Words: atherosclerosis ■ carotid arteries ■ genetics ■ ultrasonography

Atherosclerosis, with its major clinical manifestations myocardial infarction and stroke, is the leading cause of death and disability in western countries. Numerous studies have identified risk factors for development of atherosclerosis. Whereas some of these risk factors such as smoking and nutritional habits are mainly exogenous, others such as arterial hypertension and hypercholesterolemia are at least partly under genetic control. In addition, a positive family history for myocardial infarction or stroke confers an independent risk, suggesting that there are additional susceptibility genes for atherosclerosis.1–3

Ultrasound examination of the carotid arteries yields a number of quantitative measures that provide information beyond which can be inferred from conventional risk factors. In addition, abnormal carotid structure is a strong predictor of myocardial infarction and stroke.4,5 Because carotid artery ultrasound measures can be noninvasively obtained in large populations, they are promising candidates for use as intermediate phenotypes in epidemiological and genetic studies. Indeed, a number of studies found that carotid artery intima-media thickness (IMT) is under genetic control.6–11 However, published estimates of heritability varied considerably ranging from <0.21 to 0.92. Other carotid artery ultrasound measures such as presence of plaques, degree of stenosis, or diastolic diameter have been studied less frequently. In particular, studies comparing the heritability of different measures are almost completely lacking.

The present study was therefore designed to determine and compare the heritability of carotid artery IMT, presence and extent of plaque formation measured by a dimensionless plaque score, and maximal degree of stenosis. In contrast to earlier studies, we ascertained our families by a parent affected with manifest atherosclerosis.

Subjects and Methods

Subjects
For identification of index cases, patients treated for stroke, transient ischemic attack, coronary heart disease, or other clinical manifesta-
tions of atherosclerosis at the University Hospital Bonn were screened by high-resolution B-mode ultrasonography of the carotid arteries. Inclusion criteria were: (1) age >30 years, and (2) plaque score beyond the sex- and age-adjusted 90th quantile of our own normative data or stenosis of the carotid arteries. Definitions of plaque score and stenosis are given below. Exclusion criteria were: (1) severe renal failure requiring hemodialysis; (2) suspected autosomal dominant familial hypercholesterolemia (serum homocystein >9.05 mmol/L in 2 subsequent measurements after 12-hour fasting); (3) suspected autosomal recessive familial hyperhomocysteinemia (serum homocystein >0.74 μmol/L in 2 subsequent measurements after 12-hour fasting); (4) severe arterial hypertension resistant to standard therapy (systolic arterial blood pressure >180 mm Hg or diastolic arterial blood pressure >110 mm Hg under medication); (5) diabetes mellitus type 1/MODY (manifestation of an insulin-dependent hyperglycemic metabolism <45 years); and (6) rare vessel diseases, such as CADASIL, Moyamoya syndrome, fibromuscular dysplasia, collagen disorders (Marfan syndrome, Ehlers-Danlos syndrome), and previous carotid artery dissection.

In a second step, partners of index cases and all available common children were recruited for the study.

The study was approved by the ethics committee of the Medical Faculty of the University of Bonn. Informed and written consent was obtained from all subjects.

Ultrasound Methods
B-Mode ultrasound examination was performed using a Sonosite 180 (SonoSite) with a linear 5- to 10-MHz broad spectrum probe. The ultrasound examination was recorded by a digital video recorder, and the data were transferred to a personal computer. Evaluation of the data were performed using SigmaScan Pro 5.0 (SPSS) based on the evaluation protocol of the Atherosclerosis Risk in Communities (ARIC) study.12,13 In the presence of plaques creating a focal decrease in lumen area of >30%, stenosis quantification was performed with a stationary ultrasound appliance (HDI 3000; ATL) combining morphological and hemodynamic (Doppler ultrasound) parameters.

IMT was measured with focus on the far common carotid artery (CCA) wall proximal of the CCA bifurcation with a 45° anterolateral and 45° posteralateral angulation of the probe as well as on the far wall of the mid-CCA from 45° anterolateral. IMT measurements were done by manually outlining the IMT over a distance of 10 mm at the respective sites. Average IMT was then automatically calculated by computer software (SigmaScan Pro 5.0). The final IMT measurement was obtained by averaging these results of the respective sites. A plaque was defined as a local wall thickening of >50% compared with the adjacent arterial wall. Presence of plaque was separately documented for 4 localizations on both sides (CCA, bifurcation, internal carotid artery [ICA], and external carotid artery), yielding a dimensionless plaque score ranging from 0 to 8.

If plaques were present, maximal degree of stenosis was determined according to European Carotid Surgery Trial (ECST)14 criteria (ie, the lumen narrowing at the narrowest site was divided by the estimated original width of the artery at that point). Stenosis measurements were done at the site of maximal stenosis, irrespective of whether it was at the CCA, the bifurcation, or the ICA.

Additional Phenotyping
All participants underwent a standardized interview asking for the presence of arterial hypertension, diabetes mellitus, hypercholesterolemia, use of medications, and amount of nicotine consumed in pack years. Weight and height were measured, and the body mass index (BMI) was calculated. Arterial blood pressure was measured 5× in supine position using an automatic oscillometric blood pressure measuring device (TM 2430; Boso-Busch).

After fasting for a minimum of 12 hours, blood samples were taken, and serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, lipoprotein(a) [Lp(a)], triglycerides, factor VIII, factor XIII, fibrinogen, and von Willebrand factor (vWF) were determined.

A participant was considered to have arterial hypertension if the average systolic blood pressure of 5 measurements was ≥160 mm Hg or the average diastolic blood pressure was ≥90 mm Hg, or if the participant currently used antihypertensive medication. Diabetes mellitus was assumed if the fasting glucose level was ≥6.66 mmol/L or if the participant reported a previous diagnosis of diabetes mellitus or use of antidiabetic medication. Hypercholesterolemia was assumed if the LDL cholesterol level was ≥4.14 mmol/L or if the participant used cholesterol-lowering medication.

Statistical Analysis
Heritabilities of the IMT, plaque score, and maximal degree of stenosis were estimated using a variance component analysis as implemented in the program SOLAR.15 IMT, plaque score, and degree of stenosis were analyzed as quantitative continuous traits. Age, sex, weight, height, BMI, arterial hypertension, diabetes mellitus, amount of nicotine consumed, plasma levels of LDL and HDL cholesterol, LDL/HDL ratio, Lp(a), triglycerides, factor VIII, factor XIII, fibrinogen, and vWF were taken into consideration as covariates.

Arterial hypertension and diabetes mellitus were regarded as qualitative traits.

Results
Study Sample
The study population consisted of 154 families comprising 565 individuals (154 index cases, 106 spouses, and 305 offspring). The number of children in each family ranged from 1 to 8 with a median of 2. Age, sex, and prevalence of major risk factors in the study sample are given in Table 1. Prevalence and degree of plaques and stenoses are given in Table 2.

Influence of Risk Factors and Heritability
IMT variation was influenced by age, sex, arterial hypertension, diabetes mellitus, and Lp(a). Together, these factors accounted for 55% of the IMT variation. When these covariates were taken into account, heritability of IMT was estimated as $h^2=0.61±0.17$ ($P=0.001$). Without Lp(a) as a covariate, heritability was $h^2=0.71±0.17$ ($P=0.001$).

TABLE 2. Prevalence and Degree of Plaques and Stenoses in the Study Sample

<table>
<thead>
<tr>
<th></th>
<th>Index Patients</th>
<th>Spouses</th>
<th>Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of plaques (%)</td>
<td>100</td>
<td>44.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Plaque score, range and median</td>
<td>1–8 (6)</td>
<td>0–8 (1.5)</td>
<td>0–6 (0.2)</td>
</tr>
<tr>
<td>Prevalence of stenosis</td>
<td>100</td>
<td>44.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Degree of stenosis (range)</td>
<td>1–100%</td>
<td>0–99%</td>
<td>0–100%</td>
</tr>
</tbody>
</table>
Heritability of Risk Factors

We also estimated the heritabilities of the different measured traits used as covariates in the heritability estimation of carotid artery ultrasound measures. For Lp(a), which is known to be under strong genetic control of the apolipoprotein(a) gene,16,17 a high heritability ($h^2=0.86±0.07; P<0.0001$) was found, with no influence of other covariates. Arterial hypertension showed a high heritability as well, with $h^2=0.62±0.18 (P=0.001)$, and was further influenced by age and weight, which explained 24% of its variability. LDL and HDL cholesterol, factor VIII, factor XIII, and vWF were all partly determined by heritable factors (Table 3).

Smoking behavior (total pack years and number of cigarettes smoked currently) was not heritable but a familial influence was found because a common household (estimated as household effect $c^2$) explained 17% of the current and 25% of the total amount of nicotine consumed.

Discussion

The present study was undertaken to determine and compare the heritabilities of 3 different ultrasound measures: CCA IMT, plaque score, and maximal degree of carotid artery stenosis, each of which reflect atherosclerosis of the carotid arteries. Of the 3 parameters, IMT showed the strongest heritability, with more than half of its variability determined by heritable factors. There was also a high heritability of stenosis, with about half of its variance determined by genetic factors. However, heritability of stenosis was only partly attributable to genes, which act independently of genes influencing IMT. In contrast, no significant heritability of plaque score could be demonstrated. In addition, our study confirmed that a number of conventional risk factors, particularly arterial hypertension and serum Lp(a) concentrations, are under strong genetic control.

The present study is part of an ongoing study that aims to identify susceptibility genes for atherosclerosis. To recruit a family sample that is enriched with regard to atherosclerotic lesions of the carotid arteries, we ascertained our families by a parent affected with manifest atherosclerosis. The present results are therefore not directly comparable to results obtained in unselected, healthy populations.

An increase of IMT is thought to reflect reorganization of the arterial wall because of proliferation of smooth muscle cells and deposition of extracellular matrix material. IMT increase is an early event in development of atherosclerosis, making it an attractive candidate for use as an intermediate phenotype in genetic studies. Unfortunately, carotid IMT is not uniformly defined, and different studies used varying IMT measures. The major differences concern the carotid artery site at which the measurement is made. Whereas some studies examined the internal carotid IMT, others focused on the CCA or used composite measures. All measures are correlated with the prevalence of atherosclerotic disease and vascular risk factors, but the strongest relationships were observed for composite measures considering ICA and CCA.18 On the other hand, the CCA is more easily accessible for ultrasound evaluation than the ICA, so that CCA IMT measurements are more reproducible and yield more valuable results. This was confirmed in our own preliminary study of 80 carotid arteries, in which we found an intraobserver reliability of IMT measurements of 0.85 for the CCA and of only 0.70 for the ICA. The rate of nonevaluable measurements was 8% for the CCA and 20% for ICA because of thick neck, high bifurcation, ultrasound extinction from plaques. A previous study that compared the heritabilities of CCA and ICA IMT found a slightly higher heritability of CCA IMT.6 We therefore decided to use CCA IMT in the present study.

Heritability of IMT was determined as 0.61, suggesting that more than half of the IMT variability is determined by genetic factors. Further factors that influenced IMT were age, sex, arterial hypertension, diabetes mellitus, Lp(a). Interestingly, the heritability of IMT was estimated considerably lower without Lp(a) as a covariate, although Lp(a) itself accounted for <5% of IMT variability. This may be explained by the mode of inheritance of Lp(a) itself, which is strongly determined by the kringle-IV repeat-length polymorphism of the apolipoprotein(a) gene, which is inversely related to the Lp(a) plasma concentrations and explains between 30% and 70% of the variability in Lp(a) levels. The approximately autosomal dominant mode of inheritance may disturb the estimation of additive genetic effects on IMT by variance component analysis. By taking Lp(a) into

### Table 3. Heritability Estimates of Measured Risk Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Heritability</th>
</tr>
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<tbody>
<tr>
<td>Arterial Hypertension</td>
<td>0.62±0.18 (P=0.001)</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>0.34±0.07 (P&lt;0.0001)</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>0.49±0.07 (P&lt;0.0001)</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.86±0.07 (P&lt;0.0001)</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>0.49±0.10 (P&lt;0.0001)</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>0.48±0.13 (P&lt;0.0001)</td>
</tr>
<tr>
<td>vWF</td>
<td>0.41±0.11 (P&lt;0.0001)</td>
</tr>
</tbody>
</table>
account as a covariable, additive genetic effects may appear more clearly.

Previous studies reported heritabilities of IMT ranging from <0.21 to 0.92.1,6,9 The variation is most probably attributable to selection of the study populations. There is a trend that studies enriched with individuals with cardiovascular disease or risk factors yielded higher estimates of IMT heritability. This was confirmed by the present study, which was performed in families with manifest atherosclerosis and found a comparatively high estimate of heritability. Despite the variations, the available data on IMT heritability clearly show that IMT in families at high risk for atherosclerosis is strongly determined by genetic factors other than known risk factors, which themselves are partly under genetic control.

The influence of heritable factors on stenosis is also strong, with about half of its variability determined by genes. Stenosis heritability was estimated only half as high when accounting for IMT as a cofactor, which may indicate that a considerable part of the heritable factors influencing stenosis seem to influence IMT as well, whereas others appear to be specific for stenosis. To our knowledge, heritability of carotid artery stenosis has not been studied previously. However, heritability of carotid artery luminal diameter was estimated as 0.44 in the Strong Heart Family Study performed in 950 individuals of American Indian origin.9 This estimate is close to that of carotid artery stenosis determined in the present study.

Despite a considerable correlation between IMT and plaque score, plaque score was not found to be determined by heritable factors beyond those influencing arterial hypertension, diabetes mellitus, and factor VIII. This is in line with the results of the Strong Heart Family Study, which similarly found no significant heritability of plaque. In contrast, the San Antonio Family Heart study performed in 750 individuals from 29 extended Mexican American pedigrees found a low but significant heritability of 0.28.19 Both studies considered the presence or absence of plaques, whereas we used a semiquantitative score considering the number of plaques in each patient. Nevertheless, all available data suggest that heritability of carotid artery plaque is considerably lower than that of IMT.

The reasons responsible for the different heritabilities of the ultrasound measures under study remain speculative. Smooth muscle cell proliferation and deposition of extracellular matrix material resulting in increased IMT are early events in the development of atherosclerosis, whereas formation of plaques and stenosis are signs of advanced atherosclerosis. Determination of IMT may thus facilitate detection of incipient atherosclerosis in offspring of affected individuals with manifest disease, resulting in higher estimates of heritability. This makes IMT a promising candidate for genetic studies that aim to identify susceptibility genes for atherosclerosis. Results of a genomewide linkage analysis for carotid IMT were reported recently showing significant linkage for ICA IMT on chromosome 12 but no significant linkage for CCA IMT. This analysis was performed in a general population in which IMT heritability was 0.35 and 0.38, respectively.11,12 Therefore, the present sample, with a higher heritability of 0.61, appears promising for further genetic studies.

Acknowledgments

This study was supported by a University of Bonn research grant (BONFOR). We gratefully acknowledge the participation of the families of patients who volunteered to participate in this study.

References


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Stroke. published online November 29, 2004;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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