Early Carotid Atherosclerosis and Family History of Vascular Disease

Specific Effects on Arterial Sites Have Implications for Genetic Studies

Paula Jerrard-Dunne, Hugh S. Markus, Donata A. Steckel, Alexandra Buehler, Stefan von Kegler, Matthias Sitzer

Objective—Carotid artery intima-media thickness (IMT) is an intermediate phenotype for atherosclerosis. In a community population (n=5400), we determined whether a parental history of myocardial infarction (MI) or stroke is associated with increased IMT and whether associations differ at specific sites in the carotid arterial tree.

Methods and Results—Using regression modeling, the proportion of IMT that remains unexplained after controlling for vascular risk factors was determined. A parental history of stroke was associated with both increased common carotid artery (CCA) and increased internal carotid artery (ICA)-IMT, but in young individuals (≥60 years of age), the association was stronger with ICA-IMT, with an odds ratio (95% CI) for ICA-IMT in the highest quartile of 2.31 (1.67 to 3.21), P<0.001, compared with 1.53 (1.07 to 2.20), P=0.019, for CCA-IMT. In contrast, a parental history of MI was associated with increased CCA-IMT both overall and in young individuals but not with ICA-IMT, with an odds ratio (95% CI) for increased CCA-IMT of 2.51 (1.94 to 3.25), P<0.001, compared with 1.03 (0.78 to 1.35), P=0.861, for ICA-IMT.

Conclusions—IMT has a significant familial component that is independent of conventional risk factors. Associations for stroke and MI differ at specific sites in the carotid arterial tree. Although commonly used aggregate CCA/ICA-IMT measures may be appropriate for candidate gene studies investigating stroke risk, these results suggest that CCA-IMT alone may be a better marker for MI risk. (Arterioscler Thromb Vasc Biol. 2003;23:302-306.)

Key Words: atherosclerosis genes ultrasound carotid arteries family history

Both twin and family history studies suggest that genetic factors play an important role in the risk of developing atherosclerosis.1,2 One intermediate phenotype that has been widely used to study genetic and other novel risk factors for atherosclerosis is carotid artery intima-media thickness (IMT). This can be estimated noninvasively using ultrasound and seems to reflect subclinical atherosclerosis. Cross-sectional studies have shown that increased common carotid IMT is both a marker of atherosclerosis elsewhere in the arterial system3 and an independent predictor of future stroke and myocardial infarction (MI) risk.4-6

Carotid IMT measurements are increasingly being used as an intermediate phenotype for genetic studies of vascular disease.7-9 Their use has several advantages. First, the number of genes involved in this intermediate phenotype are likely to be considerably less than those involved in ischemic stroke or MI. Second, the technique can be readily applied to large-scale community-based populations, reducing selection bias. Third, the use of a continuous index of risk rather than a dichotomous variable such as stroke and the related avoidance of nonpenetrance of asymptomatic disease markedly increase power.

IMT measurements are strongly influenced by conventional risk factors.10 This may complicate and reduce the power of studies to investigate genes that independently increase IMT rather than those that merely predispose to conventional risk factors. Spence et al11 devised a novel method of combining quantitative measures of carotid plaque area measured by ultrasound and linear regression modeling to determine unexplained atherosclerosis, ie, the proportion of atherosclerosis that remains unexplained after controlling for conventional vascular risk factors. This approach was then used to identify subjects with novel risk factors for atherosclerosis in this population, elevated homocysteine.11 A drawback of this method is that measurements can only be obtained in subjects with established carotid plaque, and it is therefore not applicable to a general community population. We have applied this method to carotid IMT measurements instead of plaque area, using similar regression modeling, to determine the extent of IMT that remains unexplained by conventional risk factors.
risk factors. We hypothesized that this measure of unexplained atherosclerosis might be a useful way of identifying individuals with a predisposition to early carotid atherosclerosis, independent of conventional cardiovascular risk factors.

Using a positive family history as a marker of increased genetic risk, the aim of this study was to determine whether familial aggregation of vascular risk relates to IMT measurements. In a large community population, we have determined whether a family history of MI or stroke is associated with increased IMT at specific sites in the carotid arterial tree in view of the predilection for carotid plaque to occur at the carotid bifurcation. In addition, we have determined associations between a family history of stroke or MI and the degree of unexplained atherosclerosis to determine whether familial associations were independent of conventional cardiovascular risk factors.

Methods

Study Population
The study sample was drawn from participants in the Carotid Atherosclerosis Progression Study (CAPS), details of which have been published elsewhere. All members of a German primary health care service population aged 40 years or older (n = 8795) who within a radius of 50 kilometres from 5 study sites in Western Germany were invited to participate. Within a predefined time limit, 5400 subjects were enrolled. Vascular risk factors were assessed using a standardized computer-assisted interview technique performed by a physician experienced in vascular medicine. Risk factors determined included the following: pack-years of cigarette smoking, duration of diagnosed hypertension, duration of antihypertensive treatment, history of diabetes mellitus, and body mass index. Every subject was asked whether a first-degree relative had suffered from MI or stroke diagnosed by a physician. Cases with an unknown or uncertain family history were classified as having a negative family history. In all cases with a positive family history, the age of the affected relative at the time of the event and the relationship between the proband and the affected relative was determined. The mean value of 3 blood pressure measurements (ASM 1000, Elmed), each determined in the supine position after 10 minutes of rest, was taken as the actual arterial blood pressure. Fasting blood samples (>10 hours) were drawn from each subject, and serum total cholesterol and glycated hemoglobin A1c (HbA1c) were determined using standard methods. The study was approved by the local institutional review committee, and all participants gave informed written consent.

Ultrasound Imaging
For ultrasonic examinations, a 7.5- to 10-MHz linear-array transducer was used (P700SE, Philips Medical System). Preprocessing configurations (log gain compensation [60 dB] and image persistence) were held constant during all examinations. The gain was adjusted so that the least-dense arterial wall interface was just visible. Using antero-oblique sononation, far-wall carotid IMT was visualized within the common carotid artery (CCA) 20 to 60 mm proximally from the flow divider, and the internal carotid artery IMT (ICA-IMT) 0 to 20 mm distally from the flow divider on both sides. The images were digitally captured during the systole of a single heartbeat on a personal computer using S-VHS PC-EYE 2-frame grabber (ELTEC Elektronik GmbH, Mainz, Germany) in 16-bit R-G-B packing mode (748×576 pixels) for offline measurements. Vertical and horizontal calibration measurements were performed every 100th measurement using an ultrasound assurance phantom.

The method used for IMT measurements and interobserver and intraobserver reliabilities have been described in detail previously. The mean length of the arterial segment in which IMT was determined was 14.35 mm for the left CCA-IMT, 12.85 mm for the right CCA-IMT, and 3.45 mm for the ICA-IMT on both sides.

Statistical Analyses
There were no differences between associations with left or right IMT, and therefore mean IMT values were used for both the CCA and ICA-IMT. Both the CCA and ICA data were log transformed before analysis to normalize the distributions. Associations between family history of both MI and stroke and IMT were stronger throughout for parental history alone compared with family history in either parents or siblings, and therefore only associations with parental history are presented in the results. Genetic factors are likely to be stronger in individuals with young age of disease onset, and therefore we performed a prespecified analysis in which parental history was limited to those cases where the parent had suffered a first stroke or MI at age 60 years or younger.

We first determined associations between a parental history of stroke or MI with IMT values adjusted for age and sex. We then modified the methodology of Spence et al to develop an estimate of unexplained atherosclerosis (IMT unexplained by conventional risk factors) for each individual. The measured IMT values were adjusted for the following conventional risk factors: age, sex, systolic and diastolic blood pressure, duration of treatment of arterial hypertension, pack-years of cigarette smoking, low-density lipoprotein cholesterol, body mass index, HbA1c, and a history of diabetes mellitus using multiple linear regression analysis. A measure of unexplained IMT, namely the unstandardized residual IMT value (observed IMT value minus predicted IMT value), was derived from this analysis both for the CCA and ICA-IMT.

Relationships were determined both with IMT values across the whole range and with an IMT in the upper quartile as a measure of more advanced atherosclerosis. Means were compared using t testing between groups, and odds ratios with 95% confidence intervals for having an IMT in the highest quartile were calculated using logistic regression. Where several consecutive analyses were performed, the level of significance was α-adjusted using the modified Bonferroni procedure. All statistical analyses were performed using the SPSS (10.0.7) software package.

Results
A parental history of MI was present for 856 (15.8%) subjects, and a parental history of young MI (≤60 years) was present for 372 (6.9%) subjects. A parental history of stroke was present for 856 (15.8%) subjects, and a parental history of young stroke (≥60 years) was present for 188 (3.5%) subjects.

Baseline demographic characteristics of the population are given in Table 1. Age, male sex, systolic and diastolic blood pressure, duration of antihypertensive treatment, pack-years of cigarette smoking, lipoprotein cholesterol, HbA1C concentration, and body mass index were all independently associated with CCA and ICA-IMT measures on multiple linear regression analysis (P<0.001 for all).

A positive parental history of stroke was associated with both increased CCA and ICA-IMT, depending on the family history variable applied (Table 2). The strongest relationship was seen with ICA-IMT in those with a parental history of stroke ≤60 years, in whom an age- and sex-adjusted odds ratio for ICA-IMT in the highest quartile of 2.31 (95% CI, 1.67 to 3.21; P<0.001) was seen. A weaker association was found between CCA-IMT and a parental history of stroke ≤60 years, with an odds ratio 1.53 (95% CI, 1.07 to 2.20; P=0.019).

A positive parental history of MI was also associated with increased CCA-IMT, with an age- and sex-adjusted odds ratio
of 2.51 (95% CI, 1.94 to 3.25; \( P<0.001 \)) for CCA-IMT in the highest quartile in those with a parental history of MI \( \leq 60 \) years (Table 2). In contrast to the strong association between ICA-IMT and family history of stroke, a parental history of MI was not associated with an elevated ICA-IMT either overall or in those presenting with young age of disease onset.

To determine whether the observed familial associations could be explained by conventional vascular risk factors, we then applied the unexplained atherosclerosis model and re-calculated the odds ratios (Table 3). The observed associations remained highly significant, suggesting the familial component to elevated IMT was largely independent of conventional risk factors.

### Discussion

In this study, a positive parental history of stroke was associated with both increased CCA and ICA-IMT. In contrast to the strong association between ICA-IMT and a parental history of stroke, a parental history of MI was associated with increased CCA but not ICA-IMT. This suggests that different, or additional, genetic influences may predispose to carotid ICA-IMT, compared with those conferring increased risk of generalized arterial thickening, as indicated by a raised CCA-IMT. Several of the large published IMT studies have used composite IMT scores that average measurements taken from both the CCA and ICA.\(^6,18,19\) The findings of this study suggest that although commonly used aggregate CCA/ICA-IMT measures may be appropriate for candidate gene studies investigating stroke risk, they may be inappropriate for candidate gene studies of MI, because measures of CCA-IMT alone seem to be a better marker for familial MI risk.

This specific relationship is likely to have a pathophysiological basis. Carotid atherosclerosis accounts for a significant proportion of ischemic stroke, and the internal carotid artery is a predilection site for atherosclerotic plaque.\(^9\) The risk of developing atherosclerosis at this site is governed by local hemodynamic and anatomical factors as well as systemic factors.\(^21\) Anatomical variants, such as the angle of origin of the internal carotid artery, influence the flow pattern within the carotid bifurcation\(^22\) and therefore may influence the local atherogenic process. Thus, ICA-IMT may be a more sensitive measure of a familial component to stroke risk and therefore a more appropriate measure to use when investigating candidate genes involved in large artery stroke risk. In contrast, CCA-IMT may better reflect the systemic arterial response and is strongly related to systemic factors and therefore is less specific for stroke.

The association of parental history with CCA-IMT was stronger for MI than for stroke. This may reflect the fact that stroke is more heterogeneous than MI. Differentiating stroke subtypes on the basis of reported family history is unreliable, and a positive parental history of stroke could therefore include individuals with hemorrhagic stroke. This heterogeneity may have weakened the association between both ICA and CCA-IMT and parental history of stroke. If one could reliably identify parental history of large artery disease stroke alone, it is likely the association would be much stronger, particularly for ICA-IMT.

The measure of unexplained IMT allows one to identify a group of patients in whom increased IMT occurs in the absence of conventional risk factors and may therefore represent a useful model for studying novel factors, including genes, that promote atherosclerosis independently of conventional risk factors. However, there is growing evidence that gene-environment interactions may be crucial in the pathogenesis of atherosclerosis. The unexplained IMT model as presented would not be suitable for testing such interactions.

### Table 1. Demographic and Risk Factor Profile of the Study Population

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>5400</td>
</tr>
<tr>
<td>Age, y</td>
<td>55.9±9.7</td>
</tr>
<tr>
<td>Female sex</td>
<td>2733 (50.6)</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>1497 (27.9)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>131.5±17.3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79.3±10.0</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>193 (3.6)</td>
</tr>
<tr>
<td>HbA1C, %</td>
<td>5.44±0.68</td>
</tr>
<tr>
<td>Current smokers</td>
<td>985 (18.3)</td>
</tr>
<tr>
<td>Pack years</td>
<td>18.1±6.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1±4.0</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.53±0.94</td>
</tr>
<tr>
<td>ICA stenosis &gt;50%</td>
<td>38 (0.01)</td>
</tr>
</tbody>
</table>

Values are n (%) or mean±SD.

### Table 2. Age- and Sex-Adjusted Odds Ratios (95% CI) for IMT in the Top Quartile According to Parental History of Stroke or MI

<table>
<thead>
<tr>
<th>Positive parental history of stroke</th>
<th>At Any Age</th>
<th>( P )</th>
<th>At Age ( \leq 60 ) Years</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA-</td>
<td>1.32 (1.10–1.58)</td>
<td>0.003</td>
<td>1.53 (1.07–2.20)</td>
<td>0.019</td>
</tr>
<tr>
<td>ICA-</td>
<td>1.24 (1.04–1.48)</td>
<td>0.018</td>
<td>2.31 (1.67–3.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive parental history of MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCA-</td>
<td>1.82 (1.52–2.19)</td>
<td>&lt;0.001</td>
<td>2.51 (1.94–3.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICA-</td>
<td>1.10 (0.92–1.32)</td>
<td>0.306</td>
<td>1.03 (0.78–1.35)</td>
<td>0.861</td>
</tr>
</tbody>
</table>

Because the eight consecutive statistical tests were performed for each block, \( \alpha \)-adjustment according to the modified Bonferroni procedure has been applied. Therefore, a \( P<0.05/8=0.00625 \) indicates statistically significant findings.
but could readily be adapted to test specific gene-interaction hypotheses.

Other mechanisms, including familial aggregation of stroke risk factors, cannot be excluded as a potential explanation for the findings of this study. Hypertension, dyslipidemia, and diabetes mellitus have all been shown to aggregate in families affected by vascular disease.23–25 However, after adjustment for conventional vascular risk factors using the unexplained atherosclerosis model, a positive parental history remained a strong independent predictor of IMT. This suggests that aggregation of risk factors cannot completely explain the relationship.

It must also be considered that a positive family history is not a synonym for a genetic background but may reflect lifestyle practices, dietary habits, or risk behaviors. There is, however, strong evidence for a genetic component to IMT variability. One twin study found significantly higher concordance rates for IMT in monozygotic compared with dizygotic twin pairs, giving a heritability estimate of 36%.1 In addition, several family studies using segregation analysis have suggested that genes account for 30% to 66% of CCA-IMT variability.1,2,7,27,28 Only one study looked specifically at measurements from the internal carotid artery, and it suggested that genes explained as much as 75% of phenotypic variation.2 Our findings of an association between family history of MI and CCA-IMT are consistent with the findings of several previous population studies.29–31 Only one of these studies looked at family history of stroke and found it to be an independent predictor of IMT in both sexes after controlling for traditional cardiovascular risk factors but did not differentiate between measures from the CCA and the ICA.32

In summary, this study has several important implications for future studies using carotid IMT to investigate the role of genetic factors in the pathogenesis of MI and ischemic stroke. First, IMT measures have a significant familial component that is largely independent of conventional risk factors, suggesting that IMT is a good intermediate phenotype for candidate gene studies of vascular disease. Second, associations for stroke and MI differ at specific sites in the carotid arterial tree. Although commonly used aggregate CCA/ICA-IMT measures may be appropriate for candidate gene studies investigating stroke risk, these results suggest that future candidate gene studies investigating associations between candidate genes for MI and IMT may be more successful if they determine associations with CCA-IMT alone. Third, the measure of unexplained IMT may represent a useful model for studying novel factors, including genes, that promote atherosclerosis independently of conventional risk factors.

References

| TABLE 3. Odds Ratios (95% CI) for “Unexplained IMT” (ie, IMT Residual) in the Highest Quartile According to Parental History of Stroke or MI |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Positive parental history of stroke | At Any Age | P | At Age ≥60 Years | P |
| CCA- | 1.32 (1.12–1.55) | 0.001 | 1.37 (0.99–1.87) | 0.054 |
| ICA- | 1.22 (1.03–1.43) | 0.020 | 2.12 (1.57–2.86) | <0.001 |

Positive parental history of MI

| CCA- | 1.93 (1.65–2.26) | <0.001 | 2.21 (1.78–2.74) | <0.001 |
| ICA- | 1.05 (0.89–1.25) | 0.545 | 0.96 (0.75–1.23) | 0.733 |

Because the eight consecutive statistical tests were performed for each block, α-adjustment according to the modified Bonferroni procedure has been applied. Therefore, a P<0.05/8=0.00625 indicates statistically significant findings.


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