Homocysteine and Carotid Intima-Media Thickness in a German Population
Lack of Clinical Relevance

Michael Linnebank, MD; Susanna Moskau, MD; Susan Farmand; Klaus Fliessbach, MD; Heike Kölsch, PhD; Monika Bös, MD; Christoph Grothe, MD; Dietmar Becker, MD; Ursula Harbrecht, MD; Christoph Pohl, MD; Ullrich Wüllner, MD, PhD; Thomas Klockgether, MD

Background and Purpose—Common carotid artery intima-media thickness (CCA IMT) is a predictor of stroke. This study aimed to analyze whether homocysteine (Hcys) metabolism influences CCA IMT.

Methods—We analyzed the association of personal, clinical, and biochemical data (multivariate analysis) and of 9 polymorphisms involved in Hcys metabolism (ANOVA) with CCA IMT in 714 individuals of 187 families.

Results—CCA IMT was significantly predicted by age, sex, creatinine levels, lipoprotein(a) levels, pack-years of smoking, the presence of hypertension, and the presence of diabetes mellitus but not by Hcys levels. Homozygosity for the T allele of the polymorphism methylenetetrahydrofolate reductase c.677>C was significantly associated with higher Hcys levels but not with a higher CCA IMT.

Conclusions—These data do not support the thesis that elevated Hcys levels are causally involved in cerebrovascular disease. (Stroke. 2006;37:2840-2842.)

Key Words: carotid intima-media thickness ■ carotid ultrasound ■ genetics ■ homocysteinemia ■ stroke

Within an ongoing study, we have previously reported that common carotid artery intima-media thickness (CCA IMT) is strongly influenced by genetic determinants other than the known classic risk factors.1 Because CCA IMT is a good indicator of early systemic atherosclerosis and of future stroke as well as myocardial infarction, CCA IMT is a promising candidate for genetic studies that aim to identify susceptibility genes for atherosclerosis.1 This study aimed to analyze the impact of plasma homocysteine (Hcys) levels and of 9 polymorphisms influencing Hcys metabolism for CCA IMT.

Subjects and Methods
From the ultrasound division of the Department of Neurology, we recruited 187 (mean±SD age, 64.3±8.8 years; 28% female) of 323 consecutive white patients who had been referred owing to vascular events like stroke (n=101), transient ischemic attack (n=101), coronary artery disease (n=64), myocardial infarction (n=37), or arterial occlusive disease (n=28). The inclusion criterion was at least unilateral carotid stenosis of 30% or higher. In addition, we recruited the included patients’ partners if they had no history of vascular events (n=139); mean±SD age, 61.5±8.8 years; 81% female) and all common children (n=388; mean±SD age, 36.2±8.1 years, 52% female). In summary, this study included 714 individuals. Personal data, CCA IMT, and additional clinical and laboratory parameters, including fasting plasma total Hcys, were determined as previously described.1

Genomic DNA was prepared from peripheral leukocytes to analyze the following polymorphisms: cystathionine β-synthase c.837>T>c (I278T) and c.844_855ins68 (change of transcript levels); dihydrofolate reductase c.594+59del19bp (change of transcript levels); glutathione S-transferase Ω-1 c.428C>A (A140D); methylenetetrahydrofolate reductase (MTHFR) c.677>C>T (A222V) and c.1298>A>C (E429A); methyltetrahydrofolate homocysteine S-methyltransferase c.2756>A>G (D919G); reduced folate carrier 1 c.80G>A (R27H); and transcobalamin 2 (Tc2) c.776C>G (P259R).2–6

The prediction of CCA IMT by vascular risk factors was analyzed by linear-regression analysis. The association of polymorphisms with CCA IMT and Hcys levels was analyzed by ANOVA with threshold of α=0.001 for multiple testing. The study was approved by the local ethics committee, and all subjects gave informed, written consent.

Results
CCA IMT was significantly predicted by age, plasma levels of creatinine and lipoprotein(a), pack-years of smoking, male sex, and the presence of hypertension and of diabetes but not by Hcys levels (Table 1). Also, in separate analyses of patients, partners, and children and in subgroups of patients divided by type of vascular event, Hcys levels did not predict CCA IMT. Furthermore, in ANOVA analysis, CCA IMT did not significantly differ between individuals (all/patients/partners/children) with Hcys levels of the highest quartile in comparison with those individuals with Hcys levels of the...
TABLE 1. Factors Predicting CCA IMT

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.120</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Sex</td>
<td>0.050</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of hypertension</td>
<td>0.060</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of diabetes</td>
<td>0.048</td>
<td>0.014</td>
</tr>
<tr>
<td>Hcy</td>
<td>&lt;0.001</td>
<td>0.747</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>&lt;0.001</td>
<td>0.013</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>&lt;0.001</td>
<td>0.498</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>&lt;0.001</td>
<td>0.378</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>&lt;0.001</td>
<td>0.903</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;0.001</td>
<td>0.957</td>
</tr>
</tbody>
</table>

Table 1 shows the results of linear-regression analysis of all listed covariables for all individuals. Hcy levels were also not significantly predictive of CCA IMT when analyzed for the subgroups of patients, partners, or children (not shown). The lacking influence of cholesterol levels on CCA IMT might in part be explained by the fact that persons with known atherosclerosis or a history of vascular events might more often be taking cholesterol-lowering therapy, which was not evaluated for the subjects included in this study.

Discussion

The present study aimed to analyze whether Hcy levels or polymorphisms involved in Hcy metabolism independently influence CAA IMT, which is an established early indicator of systemic atherosclerosis preceding stroke and myocardial infarction. We investigated 714 individuals of 187 families ascertained by an index patient with proven carotid atherosclerosis. Careful data stratification was performed in multivariate analysis. We did not find a significant prediction of CCA IMT by Hcy level either in all individuals or in the subgroups of patients, partners, and children. Hcy levels did not significantly predict vascular events (represented by membership in the group of patients versus partners), although such comparison was limited owing to differences between those groups and clinical heterogeneity within the patient group. Concerning the polymorphisms, the MTHFR c.677(T) genotype was significantly associated with higher Hcy levels, as expected from the literature, but in contrast, not with higher CCA IMT.

Current studies controversially discuss whether elevated Hcy levels are only a secondary phenomenon of vascular events like stroke. In the Vitamin Intervention for Stroke Prevention (VISP) study, elevated baseline Hcy levels at the time of first stroke contributed a significant risk for recurrent stroke. However, multivitamin treatment to lower plasma Hcy did not prevent recurrent stroke. Taken together with our data, indicating that Hcy levels and polymorphisms were without significant effects, and there were no significant differences between patients, partners, and children (not shown). Furthermore, logistic-regression analysis with all vascular risk factors as covariables did not reveal an independent predictive character of Hcy levels for the dependent variable “patient” versus “partner” (B = 0.004, P = 0.444).

TABLE 2. Polymorphisms and CCA IMT

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>T/T (0.98)</th>
<th>C/T (0.02)</th>
<th>C/C (0)</th>
<th>F = 0.000; P = 0.997</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS c.833T&gt;C</td>
<td>0.76±0.19</td>
<td>0.76±0.16</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>MTHFR c.677C&gt;T</td>
<td>0.76±0.19</td>
<td>0.76±0.16</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>MTHFR c.1298A&gt;C</td>
<td>0.76±0.19</td>
<td>0.76±0.16</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>MTR c.2756A&gt;G</td>
<td>0.76±0.19</td>
<td>0.76±0.16</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>DHFR c.594+59del119bp</td>
<td>0.76±0.19</td>
<td>0.76±0.16</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>RFC1 c.80G&gt;A</td>
<td>0.76±0.19</td>
<td>0.76±0.16</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

CBS indicates cystathionine β-synthase; MTR, methylenetetrahydrofolate homocysteine S-methyltransferase; DHFR, dihydrofolate reductase; Tc2, transcobalamin 2; and RFC1, reduced folate carrier 1. Other abbreviations are as defined in text. Table 2 shows the relative frequency of the different genotypes together with the respective CCA IMT (in mm; mean ± SD). The last column shows results from ANOVA.
involved in homocysteine metabolism also have no significant causal effect on early atherosclerosis preceding stroke and myocardial infarction, this supports the contention that plasma Hcys levels are confounded with other stroke risk factors, although it remains uncertain what these factors are. The intake of folate, which is important for Hcys levels, may be a candidate.9 The influence of the MTHFR c.677TT genotype remains undefined, but any putative effect seems considerably weaker than those of the classic risk factors. Thus, screening for increased Hcys levels or the MTHFR genotype in clinical routine may not be warranted.

Acknowledgments
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Disclosures
None.

References
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