MMP-9 Polymorphisms Are Not Associated With Spontaneous Cervical Artery Dissection

Simone Wagner, MD; Britta Kluge; James A. Koziol, PhD; Armin J. Grau, MD; Caspar Grond-Ginsbach, PhD

Background and Purpose—Cervical artery dissection (CAD) is a common cause of ischemic stroke in young adults. Alteration in the structure of the vascular extracellular matrix has been described in CAD. Matrix metalloproteinases (MMPs) degrade extracellular matrix proteins and can lead to vascular damage.

Methods—We tested 2 different MMP-9 DNA polymorphisms, a CA repeat and a cytosine to thymidine transition in the promotor sequence, for frequency in 52 patients with CAD. We compared the results with those of 52 healthy controls.

Results—No differences were found in the allelic distribution of either polymorphism.

Conclusions—Alleles of these well-characterized functional polymorphisms of MMP-9 gene are not associated with structural alterations in the matrix of vessels of patients with CAD. (Stroke. 2004;35:e62-e64.)

Key Words: dissection ■ metalloproteinases ■ polymorphism

Cervical artery dissection (CAD) is a connective tissue disorder that is a common cause of cerebral ischemia in young adults. Structural alterations of the extracellular matrix (ECM) in the vascular wall and also in the skin of these patients have been described.1 Hereditary connective tissue disorders, in particular the vascular Ehlers-Danlos syndrome (EDS), are known to be risk factors for spontaneous dissections.2 Therefore, a genetic cause for non-EDS–associated CAD is plausible. The matrix metalloproteinases (MMPs) can degrade ECM proteins. Increased expression of MMPs may be based on polymorphisms in the MMP gene. We tested the hypothesis that the DNA polymorphisms in MMP genes that are associated with protein expression3,4 are a risk factor for CAD.

Subjects and Methods
Blood was sampled from 52 CAD patients and 52 control subjects. The control subjects were randomly selected from healthy students and staff members of the Department of Neurology, living in the same area as the patients. None of the control subjects showed signs of any known connective tissue disorder. All individuals were white and German.

DNA was isolated from EDTA blood samples after SDS-proteinase-K digestion and phenol-chloroform extraction following standard procedures. For the amplification of the CA repeat in the MMP-9 promotor sequence, we used the primers CT-GAGGCGCTGCGGTTTCCT and (FAM-labeled) CCTTGACCCAGGCGCCCATC. Microsatellite amplicons were analyzed with the GeneScan program after electrophoresis on POP6 gel on a ABI 310 Genetic Analyzer (Applied Biosystems) sequencer, as previously described.5 For restriction fragment length polymorphism analysis, the 435-bp polymerase chain reaction product containing the C-1562T SNP was digested with NspI restriction enzyme and run on a 2% agarose gel stained with ethidium bromide (Figure 1). Sequencing was performed in only a few individuals to confirm the nature of each band seen on the gels.

The study was approved by the local ethics committee.
We used $\chi^2$ analysis to test for deviations of genotype distributions from Hardy-Weinberg equilibrium and to assess differences in allele frequencies between cases and controls. Fisher exact tests were implemented in StatXact 5 (Cytel Software).

Results
The CAD patients comprised 30 men and 22 women (mean±SD age, 43.3±8.7 years). Of the 52 CAD patients, 22 had unilateral carotid artery dissections, 16 unilateral vertebral artery dissections, 6 bilateral carotid artery dissections, and 4 bilateral vertebral artery dissections. Four patients suffered from multiple dissections of both a carotid and a vertebral artery. There were 30 male and 22 female controls, with a mean±SD age of 41.8±11.5 years.

The microsatellite fragment analysis (Figure 2) revealed a fragment of 176 bp to be the most common, both in patients (62.5%) and in controls (56.7%). There was no statistically significant difference in the frequencies of the
have been negative. An alternative pathophysiological mechanism could be vessel wall alteration by MMPs.

Recently, naturally occurring sequence variations have been detected in the promoter region of MMP genes. These sporadic polymorphisms have been shown to have specific effects on the transcriptional activities of the relevant MMP gene promoters and to be associated with aneurysms. By analogy with functional and genetic studies on cerebral aneurysms and arteriosclerosis and in view of the increasing amount of literature on the association between MMP-9 and stroke as well as connective tissue diseases, we therefore have used the candidate gene approach to investigate the association between MMP-9 alleles and CAD.

We found no statistically significant differences in overall frequencies of the DNA fragments of the dinucleotide repeat length polymorphism and the allelic distribution of the SNP between the controls and patients. Nevertheless, the possibility of a type II error exists. In this regard, we have calculated that prospectively we would need a total sample size of 450 for a power of 0.90 to detect the difference in genotype frequencies observed in the Table. With a standard 0.05 level of significance, we would need a total sample size of 1800 for a power of 0.82; Fisher exact test). In conclusion, we did not find any evidence that MMP-9 gene is a marker of susceptibility for CAD, by both microsatellite and SNP analysis. Factors that are able to induce MMP-9, such as cytokines, may be better candidates as markers for susceptibility of CAD.

Acknowledgments

We thank Brunhilde Schagen for excellent technical assistance and Stefan Lehnert for help in preparing the figures. We thank Professor Werner Hacke for excellent working conditions.

Discussion

CAD is a disease with an underlying alteration in the structure of the vascular wall. In this condition, ultrastructural changes of the ECM have been described in the vessel wall and in the skin, suggesting a genetic predisposing cause. This is also suggested by the fact that hereditary connective tissue disorders such as EDS predispose to spontaneous CAD. Thus far, the tested candidate genes for mutations in ECM molecules, for example, collagen V, have been negative. An alternative pathophysiological mechanism could be vessel wall alteration by MMPs. Recently, naturally occurring sequence variations have been detected in the promoter region of MMP genes. These sporadic polymorphisms have been shown to have specific effects on the transcriptional activities of the relevant MMP gene promoters and to be associated with aneurysms. By analogy with functional and genetic studies on cerebral aneurysms and arteriosclerosis and in view of the increasing amount of literature on the association between MMP-9 and stroke as well as connective tissue

Figure 1. Agarose gel after digestion with restriction enzyme. Lane 1 is an example of a T/T genotype, lane 5 shows a C/T genotype, and the remaining lanes show C/C genotypes.

<table>
<thead>
<tr>
<th>Allele Distribution: Healthy Controls Compared With Patients With Dissection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>C/C</td>
</tr>
<tr>
<td>C/T</td>
</tr>
<tr>
<td>T/T</td>
</tr>
</tbody>
</table>

Length of Alleles (bp) (alleles):

<table>
<thead>
<tr>
<th>Length of Alleles (bp) (alleles)</th>
<th>Controls (n=104)</th>
<th>Mild Disease (n=76)</th>
<th>Severe Disease (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>176</td>
<td>59 (56.7%)</td>
<td>65 (62.5%)</td>
<td>46 (60.5%)</td>
</tr>
<tr>
<td>178</td>
<td>3 (2.8%)</td>
<td>4 (3.8%)</td>
<td>3 (3.9%)</td>
</tr>
<tr>
<td>186</td>
<td>2 (1.9%)</td>
<td>1 (0.9%)</td>
<td>0</td>
</tr>
<tr>
<td>188</td>
<td>2 (1.9%)</td>
<td>2 (1.9%)</td>
<td>2 (2.6%)</td>
</tr>
<tr>
<td>190</td>
<td>15 (14.4%)</td>
<td>14 (13.5%)</td>
<td>10 (13.1%)</td>
</tr>
<tr>
<td>192</td>
<td>13 (12.5%)</td>
<td>14 (13.5%)</td>
<td>12 (15.7%)</td>
</tr>
<tr>
<td>194</td>
<td>8 (7.7%)</td>
<td>4 (3.8%)</td>
<td>3 (3.9%)</td>
</tr>
<tr>
<td>196</td>
<td>2 (1.9%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>198</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Mild disease refers to single-vessel dissection, severe disease to bilateral or multiple dissections. There is no statistically significant difference between mild and severe disease with respect to either genotype (P=0.51; Fisher exact test) or length of alleles (P=0.66; Fisher).

The Table with a standard 0.05 level of significance, we would need a total sample size of 1800 for a power of 0.82; Fisher exact test). In conclusion, we did not find any evidence that MMP-9 gene is a marker of susceptibility for CAD, by both microsatellite and SNP analysis. Factors that are able to induce MMP-9, such as cytokines, may be better candidates as markers for susceptibility of CAD.

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