Polymorphisms in Matrix Metalloproteinase-1, -3, -9, and -12 Genes in Relation to Subarachnoid Hemorrhage

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Background and Purpose—Intracranial aneurysm, which underlies the vast majority of subarachnoid hemorrhage incidences, has a multifactorial etiology, and the importance of genetic factors is increasingly recognized. Development and rupture of intracranial aneurysms involve degradation and remodeling of the vascular wall matrix in which the matrix metalloproteinases (MMPs) play an important role. The possible impact of MMP gene polymorphisms on susceptibility to intracranial aneurysms is still controversial, with conflicting data from different reported studies.

Methods—In this study we analyzed 5 different functional promoter polymorphisms in the MMP-1, MMP-3, MMP-9, and MMP-12 genes in a sample of 92 patients with aneurysmal subarachnoid hemorrhage and 158 healthy control subjects, all from southern England.

Results—No significant difference was detected between the patient and control groups in genotype distribution of any of the polymorphisms studied.

Conclusions—The data do not support the hypothesis that MMP gene variations influence the development of intracranial aneurysms in the population studied. (Stroke. 2001;32:2198-2202.)

Key Words: aneurysm, intracranial • matrix metalloproteinases • microsatellite repeats • polymorphism (genetics) • subarachnoid hemorrhage
aneurysmal subarachnoid hemorrhage and 158 healthy control subjects, all from southern England.

Subjects and Methods

Subjects

A cohort of 92 unrelated patients (32 male and 60 female; age range, 23 to 75 years; mean age, 50 years) of European white ancestry, who suffered from aneurysmal subarachnoid hemorrhage and received surgical treatment (removal or clipping of aneurysm in craniotomy or endovascular Guglielmi detachable coil embolization) in the Wessex Neurological Center, Southampton General Hospital, were recruited in this study. The aneurysms were located in the anterior communicating artery in 32 patients, posterior communicating artery in 23 patients, middle cerebral artery in 23 patients, posterior inferior cerebellar artery in 6 patients, basilar artery in 4 patients, and carotid artery in 4 patients. The study was approved by the South and West Local Research Ethics Committee (submission No. 170/98), and written consent was obtained from the participants. The control subjects (86 male and 72 female; age range, 3 to 63 years; mean age, 39 years) were renal or bone marrow donors from the same population in the Wessex region in England as the cases.

Determination of Genotypes

Blood DNA samples were prepared by the standard salt precipitation method.28 The methods used to type the MMP-1 1G/2G, MMP-3 5A/6A, MMP-9 C-1562T, and MMP-12 A-82G polymorphisms have been described previously.17,21,29,30 Briefly, the DNA sequence containing the relevant polymorphic site was amplified by polymerase chain reaction (PCR), and the amplicon was digested with an appropriate restriction enzyme that cleaves only 1 of the 2 alleles. The digests were then subjected to gel electrophoresis and visualized by Vistra green or ethidium bromide staining.

The method described by St Jean et al31 was adopted with modification to type the MMP-9 microsatellite polymorphism. Briefly, the PCR conditions and primer sequences were as described by St Jean et al, but in this study the forward primer was labeled with a fluorescent tag (Fam) and the PCR amplicons were sized by the GeneScan method with an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

The sequences of PCR primers used in the aforementioned assays are described in Table 1. All PCR reactions were performed in 96-well microplates, and 2 negative control reactions without template DNA were included in each microplate. No PCR product was detected from any of the negative control reactions. The assays were repeated in 10% of the samples, and the results were consistent.

Statistical Analyses

We performed χ² tests to examine differences in genotype and allele frequencies between patients and controls. For 2 by 2 tables, Fisher’s exact test was performed when the table had a cell with an expected frequency of <5. Yates corrected χ² was calculated for all other 2 by 2 tables. For 2 by k tables, Pearson χ² was calculated.32 A P-value of <0.05 was taken as statistically significant.

Power calculation was as follows: On the basis of the frequencies of the rarer allele in the control group being 0.472, 0.497, 0.168, and 0.05 was taken as statistically significant.

Results

To investigate whether sequence variation in MMP genes influenced susceptibility of intracranial aneurysm, we analyzed 5 polymorphisms in 4 different MMP genes in 92 patients with aneurysmal subarachnoid hemorrhage and 158 control subjects. These polymorphisms were as follows: (1) 1G/2G polymorphism located at nucleotide position -1607 relative to the transcription start site in the promoter region of the MMP-1 gene; (2) 5A/6A polymorphism at nucleotide position -1612 in the promoter of the MMP-3 gene; (3) C-1562T polymorphism in the MMP-9 gene promoter; (4) A-82G polymorphism in the promoter of the MMP-12 gene; and (5) (CA)n microsatellite polymorphism from position -90 in the MMP-9 gene promoter. The former 4 were single nucleotide polymorphisms, and the last was a microsatellite polymorphism. All of these polymorphisms had previously been shown to influence the transcriptional activity of their respective gene promoter in an allele-specific manner.16,17,21,23,27,33

The genotype and allele frequencies in patients and controls are presented in Tables 2 and 3. There was no statistically significant difference between the patient and control groups in genotype distribution of any of the polymorphisms studied (P=0.881 for the MMP-1 -1607 1G/2G polymorphism; P=0.871 for the MMP-3 -1612 5A/6A polymorphism; P=0.308 for the MMP-9 C-1562T polymorphism; P=0.847 for the MMP-9 microsatellite; and P=0.709 for the MMP-12 A-82G polymorphism). All genotype distributions were consistent with Hardy-Weinberg equilibrium.

Discussion

The development of aneurysms is associated with weakening of the blood vessel wall, which might result from inherent defects of structural molecules, although mutations in the type III collagen gene have been shown to be rare causes of aortic and intracranial aneurysms.34,35 There is also accumulating evidence indicating that overdegradation of vascular structural proteins by proteinases is an important mechanism involved in the development and rupture of aneurysms.7 The present study sought to ascertain whether polymorphisms in MMP genes influence the susceptibility of intracranial aneurysms. All polymorphisms analyzed in this study are located in the regulatory regions of the MMP genes and have been shown to influence the transcriptional activity of the respective MMP gene promoter. For example, the 5A allele of the MMP-3 5A/6A polymorphism possesses a greater transcriptional activity than the 6A allele, which appears to be due to

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### TABLE 1. Sequences of PCR Primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Forward Primer (5'-3')</th>
<th>Reverse Primer (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>1G/2G</td>
<td>TCGTGAGAATGTCTTCCCATT</td>
<td>TCTTGGATTGATTTGAGATAAGTGAAATC</td>
</tr>
<tr>
<td>MMP-3</td>
<td>5A/6A</td>
<td>GATTACAGACATGGGTCACA</td>
<td>TTTCAATCAGGACAAGTTCATC</td>
</tr>
<tr>
<td>MMP-9</td>
<td>C-1562T</td>
<td>GCTGTGACATGAATGCTTGTACC</td>
<td>CTGCTATAGCCACGCGCATC</td>
</tr>
<tr>
<td>Microsatellite</td>
<td>GACTGGGCAATGGAGAGACACTGCGGCA</td>
<td>GACCCACCCCGTCTTGGACGACCA</td>
<td></td>
</tr>
<tr>
<td>MMP-12</td>
<td>A-82G</td>
<td>GTCAAGGGATGATATCAGCTC</td>
<td>TTCTAAACGGATCATAGC</td>
</tr>
</tbody>
</table>

This study would allow detecting a relative risk by allele of 2.0 for MMP-1 and MMP-3 and a relative risk of 2.3 for MMP-9 and MMP-12, with a power of 70% at the 0.05 significance level.
preferential binding of a transcription repressor to the latter. Similarly, the insertion of a guanine nucleotide in the 2G allele of the MMP-1 1G/2G polymorphism creates a core binding site for transcription factor Ets, leading to a significantly higher promoter activity. Accordingly, ovarian and endometrial tumor tissues in individuals with the 2G/2G genotype express increased levels of MMP-1. Allelic effects on transcriptional activity have also been demonstrated for the MMP-9 C-1562T and MMP-12 A-82G polymorphisms, the latter being located immediately adjacent to an AP-1 consensus element bound by transcription factors c-Fos and c-Jun.

The multiallelic (CA)n microsatellite polymorphism in the MMP-9 gene has a bimodal distribution of allele frequencies, with the first peak at the (CA)14 allele (with 14 tandem repeats of CA dinucleotide) and the second peak at the (CA)21, (CA)22, and (CA)23 alleles. It has been shown by the reporter gene assays that in fibroblasts (HT1080), an MMP-9 promoter encompassing 14 CA repeats has only 60% of the transcriptional activity of a promoter with 23 repeats. Differences in promoter activity between alleles have also been detected in esophageal carcinoma cells, with a promoter containing 14 CA repeats having only 50% of the activity of a promoter with 21 CA repeats. The (CA)n region has also been shown to interact with a nuclear protein in electrophoretic mobility shift assays, with the strength of nuclear protein binding being dependent on the number of CA repeats.

### TABLE 2. Distribution of Genotypes and Alleles of 4 Single Nucleotide Polymorphisms in MMP Genes

<table>
<thead>
<tr>
<th>MMP-1 Genotype</th>
<th>Patients, No. (Frequency)</th>
<th>Controls, No. (Frequency)</th>
<th>P (χ² Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2G/2G</td>
<td>24 (0.261)</td>
<td>33 (0.232)</td>
<td>0.881</td>
</tr>
<tr>
<td>1G/2G</td>
<td>42 (0.457)</td>
<td>68 (0.479)</td>
<td></td>
</tr>
<tr>
<td>1G/1G</td>
<td>26 (0.283)</td>
<td>41 (0.289)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Allele 2G</td>
<td>90 (0.489)</td>
<td>134 (0.472)</td>
<td>0.786</td>
</tr>
<tr>
<td>Allele 1G</td>
<td>94 (0.511)</td>
<td>150 (0.528)</td>
<td></td>
</tr>
<tr>
<td>MMP-3 Genotype</td>
<td>5A/5A</td>
<td>20 (0.217)</td>
<td>33 (0.209)</td>
</tr>
<tr>
<td>Allele 5A</td>
<td>90 (0.489)</td>
<td>157 (0.497)</td>
<td>0.941</td>
</tr>
<tr>
<td>Allele 6A</td>
<td>94 (0.511)</td>
<td>159 (0.503)</td>
<td></td>
</tr>
<tr>
<td>MMP-9 Genotype</td>
<td>C/C</td>
<td>71 (0.780)</td>
<td>81 (0.698)</td>
</tr>
<tr>
<td>Allele C</td>
<td>161 (0.885)</td>
<td>193 (0.832)</td>
<td>0.170</td>
</tr>
<tr>
<td>Allele T</td>
<td>21 (0.115)</td>
<td>39 (0.168)</td>
<td></td>
</tr>
<tr>
<td>MMP-12 Genotype</td>
<td>A/A</td>
<td>73 (0.793)</td>
<td>106 (0.746)</td>
</tr>
<tr>
<td>Allele A</td>
<td>164 (0.887)</td>
<td>246 (0.866)</td>
<td>0.508</td>
</tr>
<tr>
<td>Allele G</td>
<td>20 (0.113)</td>
<td>38 (0.134)</td>
<td></td>
</tr>
</tbody>
</table>

In the χ² tests, 2 by 3 tables were used to compare genotype distributions in patients and controls, and 2 by 2 tables were used to assess the differences in allele frequency between the 2 groups.

### TABLE 3. Allele Frequencies of MMP-9 Microsatellite Polymorphism

<table>
<thead>
<tr>
<th>Amplicon Size, bp (No. of CA Repeats)</th>
<th>Patients, No. (Frequency)</th>
<th>Controls, No. (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>147 (14)</td>
<td>96 (0.539)</td>
</tr>
<tr>
<td>2</td>
<td>149 (15)</td>
<td>5 (0.028)</td>
</tr>
<tr>
<td>3</td>
<td>153 (17)</td>
<td>1 (0.006)</td>
</tr>
<tr>
<td>4</td>
<td>159 (20)</td>
<td>3 (0.017)</td>
</tr>
<tr>
<td>5</td>
<td>161 (21)</td>
<td>5 (0.028)</td>
</tr>
<tr>
<td>6</td>
<td>163 (22)</td>
<td>32 (0.180)</td>
</tr>
<tr>
<td>7</td>
<td>165 (23)</td>
<td>31 (0.174)</td>
</tr>
<tr>
<td>8</td>
<td>167 (24)</td>
<td>3 (0.017)</td>
</tr>
<tr>
<td>9</td>
<td>169 (25)</td>
<td>1 (0.006)</td>
</tr>
<tr>
<td>10</td>
<td>171 (26)</td>
<td>1 (0.006)</td>
</tr>
</tbody>
</table>

P = 0.847 in χ² test (2 by 10 table) for difference in allele frequency between patients and controls.
While some of the aforementioned polymorphisms have been consistently shown to be associated with susceptibility and progression of coronary artery disease or cancers in a number of studies, there have been only 2 reported studies on MMP gene polymorphisms in relation to intracranial aneurysm with conflicting results. In a case-control study of 76 patients and 93 controls from western Pennsylvania, the MMP-9 gene (CA) in microsatellite polymorphism was found to be associated with susceptibility of intracranial aneurysm, with lower frequencies of alleles (CA)14 and (CA)22 but higher of 76 patients and 93 controls from western Pennsylvania, the studies on MMP gene polymorphisms in relation to intracranial aneurysms have demonstrated that cigarette smoking increases elastase activities in ruptured aneurysms.48 Taken together, these studies suggest that elastase/α1-antitrypsin imbalance in cigarette smokers may also contribute to aneurysm formation and/or rupture. In conclusion, the results of the present study do not support an influence of MMP gene variations on susceptibility of intracranial aneurysms in the population studied. Further studies should focus on achieving power to detect possible smaller allelic relative risk (<2) and should use wider panels of polymorphisms to represent other possible haplotypes of these genes.

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References
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