Lack of association between the apolipoprotein E gene and aneurysmal subarachnoid hemorrhage in an Italian population

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Object. The results of genome-wide scan studies have suggested the presence of a genetic risk factor for aneurysmal subarachnoid hemorrhage (SAH) on chromosome 19 (at 19p13). The apolipoprotein E (APOE) gene is located in this chromosomal region and encodes a protein that exerts several neuroprotective and neurotrophic functions in the brain. The purpose of this study was to evaluate whether a particular allele or genotype of the APOE gene would modify the occurrence or the clinical features of SAH.

Methods. Genomic DNA was extracted from 146 patients with aneurysmal SAH and 222 age- and sex-matched healthy controls and genotyped for the triallelic polymorphism of the APOE gene (ɛ2, ɛ3, and ɛ4). Allele and genotype frequencies were compared between patients and controls. The clinical characteristics of the disease were compared according to the different APOE genotypes. Allele and genotype frequencies of the APOE gene polymorphism were nearly identical in cases and controls. Patients carrying the APOE ɛ4 allele had a significantly higher Hunt and Hess grade on admission (p = 0.0014). There was no significant relationship between any of the other clinical characteristics and the APOE genotype.

Conclusions. The authors’ data do not support the hypothesis that genetic variations within the APOE gene are associated with aneurysmal SAH. However, the APOE gene influences the disease phenotype and may be regarded as a disease modifier gene.

Key words • apolipoprotein E • aneurysmal subarachnoid hemorrhage • clinical outcome

Subarachnoid hemorrhage due to ruptured IAs is a devastating neurological disorder with an annual incidence of approximately 10 cases per 100,000 population in Europe.3,16 Nearly half of the affected persons die within the first 30 days after the rupture, and survivors are often left with a substantial neurological disability. A number of clinical factors may influence outcome after SAH, including advanced age, aneurysm location (posterior circulation), intracerebral extension of hemorrhage, rebleeding, and the development of cerebral vasospasm or cerebral infarcts.

Genetic factors play a major role in the formation and rupture of IAs but, at present, the number and the type of genes involved in the disease are still unclear.3,21,25 Investigators using genome-wide linkage analysis in familial IA have found suggestive linkages on several chromosomal regions (1p34.3–36.13, 2p13, 5q22–31, 7q11, 14q22, 19q13, and Xp22).21,22,29,30 To date, candidate regions that have been replicated in more than one study include 7q11 and 19q13. Such concordant regions should be considered high-priority loci for future studies to identify the genetic risk factors for IAs.

The APOE gene is located on 19q13 and encodes a protein that plays an important role in lipid transport and metabolism within the central nervous system.9,17 In humans, there are three common alleles of the APOE gene—ɛ2, ɛ3, and ɛ4—which encode three isoforms of the protein (E2, E3, and E4). These three isoforms differ only by a single amino acid. Several studies have shown that the APOE ɛ4 allele is a genetic risk factor for Alzheimer disease,11,15,24 poor clinical outcome after brain injury,25 and stroke.16,20 In
addition, allelic variants of the APOE gene may influence the course of several neurological diseases, like multiple sclerosis and motor neuron disease.\textsuperscript{13,19}

The role of the APOE gene as a genetic risk factor for aneurysmal SAH has not been adequately investigated. In a small prospective study, Kokubo et al.\textsuperscript{13} found that the risk of aneurysmal SAH was increased 2.5-fold in patients carrying the APOE \( \epsilon 4 \) allele. In contrast, the authors of two subsequent studies were unable to replicate these findings.\textsuperscript{2,20} Furthermore, researchers who have investigated the association between APOE genotypes and outcome after aneurysmal SAH have reported conflicting results. Results of some studies have shown that carrying the APOE \( \epsilon 4 \) allele is associated with significantly poorer clinical and neurological outcomes, whereas others have shown no significant gene effects.\textsuperscript{5,14,17,19,26}

To further evaluate whether a particular allele or genotype of the APOE gene affects the occurrence or the clinical features of aneurysmal SAH, we performed a case–control association study in a cohort of patients recruited from an Italian university–based neurosurgical clinic and in healthy controls.

Clinical Material and Methods

Patient Population

A total of 146 consecutive unrelated patients (46 men and 100 women; mean age at admission 53.5 ± 14.2 years, mean age at hemorrhage 52.2 ± 14.9 years [± SD]) admitted to the Division of Neurosurgery of the University of Turin in Italy between January 2002 and December 2004, were included in the study. The diagnosis of aneurysmal SAH was made according to the presence of symptoms suggestive of SAH combined with the finding of subarachnoid blood on CT and a proven aneurysm on conventional angiography. The CT findings were classified according to the grading system of Fisher et al.\textsuperscript{6} Patients with genetic defects (such as Turner syndrome, Marfan syndrome, Ehlers–Danlos syndrome Type IV) known to be associated with an increased risk of IA were excluded from this study. The patients’ clinical condition on admission was rated according to the Hunt and Hess grading scale.\textsuperscript{12}

For additional statistical analyses, patients were divided into the following three subgroups: single versus multiple aneurysms, according to the results of conventional angiography; anterior or posterior circulation aneurysms; and development of secondary cerebral ischemia, defined as a gradual decline in the level of consciousness or a gradual development of new focal deficits with the confirmation of a new hypodensity on CT.

Outcome was assessed 6 months after SAH with the GOS\textsuperscript{10} by a neurosurgeon blinded to the APOE genotype. A standardized record of all the clinical characteristics of the patients, suitable for computer analysis, was obtained.

A group of 222 geographically matched healthy individuals (88 men and 134 women; mean age 57.3 ± 13.5 years [± SD]) served as controls. Table 1 shows the demographic and clinical characteristics of aneurysmal SAH patients and healthy controls. The study was approved by the hospital ethics committee, and informed consent was obtained from all participants prior to the assessment.

### Results

The distribution of genotypes and alleles conform to the Hardy–Weinberg equilibrium. The genotype and allele frequencies of the APOE gene in our control group were remarkably similar to what has been reported in other Italian populations.\textsuperscript{4,5}

Table 2 shows the comparison of genotype and allele frequencies of the APOE gene in patients with aneurysmal SAH and healthy controls. There was no significant difference between cases and controls for either genotype or allele frequencies (\( \chi^2 = 9.41, p = 0.094 \) and \( \chi^2 = 0.3, p = 0.73 \), respectively). When the patients with SAH were stratified into clinical subgroups, multiple comparisons of allele and genotype frequencies showed no significant difference.

The clinical characteristics of patients with aneurysmal SAH according to APOE genotypes (\( \epsilon 2\epsilon 2, \epsilon 2\epsilon 3, \epsilon 3\epsilon 3, \epsilon 3\epsilon 4, \epsilon 4\epsilon 4 \)) are shown in Table 3. In comparison with the remaining genotypes, patients carrying the \( \epsilon 3\epsilon 4 \) geno-
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**TABLE 2**

Frequencies of APOE alleles and genotypes in patients with aneurysmal SAH and healthy controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Participants</th>
<th>Allele Frequency</th>
<th>Genotype Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>e2</td>
<td>e3</td>
<td>e4</td>
</tr>
<tr>
<td>total sample</td>
<td>368</td>
<td>0.08</td>
<td>0.85</td>
</tr>
<tr>
<td>controls</td>
<td>222</td>
<td>0.07</td>
<td>0.86</td>
</tr>
<tr>
<td>SAH patients</td>
<td>146</td>
<td>0.10</td>
<td>0.84</td>
</tr>
<tr>
<td>single IA</td>
<td>123</td>
<td>0.10</td>
<td>0.84</td>
</tr>
<tr>
<td>multiple IAs</td>
<td>23</td>
<td>0.11</td>
<td>0.87</td>
</tr>
<tr>
<td>aneurysm location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anterior</td>
<td>128</td>
<td>0.11</td>
<td>0.83</td>
</tr>
<tr>
<td>posterior</td>
<td>18</td>
<td>0.03</td>
<td>0.92</td>
</tr>
</tbody>
</table>

It is therefore unlikely that genetic variations within the APOE gene greatly contribute to aneurysmal SAH susceptibility.

The results of our study agree with those of two previous molecular genetic studies, in which no association was found between the APOE gene and aneurysmal SAH.23,26 and suggest that alternative genes located in the 19q13 region should be examined as potential candidate genes for aneurysmal SAH. In addition, when we analyzed the clinical features of patients with SAH with respect to APOE genotypes, we found a modest effect of the APOE genotypes on the clinical characteristics of the disease: only Hunt and Hess grade at admission differed significantly between patients carrying the e4 allele and noncarriers. Our data provide support for the idea that the APOE gene should be considered only a disease modifier gene and that it does not play a major role in aneurysmal SAH.

The neurobiological mechanisms underlying the effect of the APOE e4 allele on the Hunt and Hess grading found in our patients with SAH are, at present, unknown. Apolipoprotein E is produced mainly in astrocytes and is responsible for the transportation of lipids within the brain. The
discussion

In this study of an Italian population we found no evidence of a genetic association between APOE gene polymorphisms and the risk for aneurysmal SAH. Allele and genotype frequencies of APOE gene polymorphism were similarly distributed between patients and controls. Furthermore, when the patients were divided into different clinical subgroups, no significant difference in APOE allele and genotype frequencies was found in multiple comparisons.

**TABLE 3**

Clinical features of 146 patients with aneurysmal SAH according to APOE genotypes*

<table>
<thead>
<tr>
<th>Variable</th>
<th>e2e2</th>
<th>e2e3</th>
<th>e3e3</th>
<th>e3e4</th>
<th>e2e4</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of patients</td>
<td>6 (4.1%)</td>
<td>17 (11.6%)</td>
<td>107 (73.3%)</td>
<td>15 (10.3%)</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td>men</td>
<td>2 (4.2%)</td>
<td>6 (12.8%)</td>
<td>34 (72.3%)</td>
<td>3 (6.4%)</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>mean age at admission in yrs</td>
<td>52.7 ± 22.1</td>
<td>54.6 ± 11.8</td>
<td>52.6 ± 14.4</td>
<td>58.1 ± 12.4</td>
<td>67</td>
</tr>
<tr>
<td>mean age at hemorrhage in yrs</td>
<td>25.7 ± 22.1</td>
<td>54.0 ± 11.9</td>
<td>51.1 ± 15.3</td>
<td>56.8 ± 12.5</td>
<td>67</td>
</tr>
<tr>
<td>mean Fisher grade</td>
<td>3.0 ± 0.9</td>
<td>2.6 ± 0.7</td>
<td>2.8 ± 0.8</td>
<td>3.1 ± 0.8</td>
<td>3</td>
</tr>
<tr>
<td>mean Hunt &amp; Hess grade</td>
<td>2.0 ± 0.6</td>
<td>2.6 ± 0.9</td>
<td>2.7 ± 0.9</td>
<td>3.5 ± 1.1†</td>
<td>3</td>
</tr>
<tr>
<td>mean GOS score</td>
<td>4.5 ± 0.8</td>
<td>4.8 ± 0.6</td>
<td>4.2 ± 1.2</td>
<td>3.9 ± 1.4</td>
<td>3</td>
</tr>
<tr>
<td>no. of patients w/ single IA</td>
<td>5 (4.0%)</td>
<td>14 (11.4%)</td>
<td>89 (72.4%)</td>
<td>14 (11.4%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>w/ multiple IAs</td>
<td>1 (4.3%)</td>
<td>3 (13.0%)</td>
<td>18 (78.3%)</td>
<td>1 (4.3%)</td>
<td>0</td>
</tr>
<tr>
<td>aneurysm location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anterior</td>
<td>6 (4.7%)</td>
<td>16 (12.5%)</td>
<td>92 (71.9%)</td>
<td>13 (10.2%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>posterior</td>
<td>0</td>
<td>1 (5.6%)</td>
<td>15 (83.3%)</td>
<td>2 (11.1%)</td>
<td>0</td>
</tr>
<tr>
<td>ischemia</td>
<td>1 (3.2%)</td>
<td>5 (16.1%)</td>
<td>20 (64.5%)</td>
<td>4 (12.9%)</td>
<td>1 (3.2%)</td>
</tr>
</tbody>
</table>

* Mean values are reported as the means ± SDs.
† p < 0.01.
protein mediates neuronal protection, interactions with estrogens, and modulation of synaptic proteins. Possession of the APOE ε4 allele has been shown to result in a greater propensity to develop age-related cognitive impairment, a decrease in the synapse/neuron ratio, and increased susceptibility to exogenous neurotoxins.

Taken together, these data suggest that patients with SAH carrying an APOE ε4 allele are more sensitive to the neuronal and oxidative damage induced by SAH.

Genetic association studies are becoming increasingly frequent in the medical literature and they are considered particularly useful to the deciphering of the genetic bases of complex diseases like aneurysmal SAH. However, association studies are vulnerable to several biases involving factors such as phenotypic definition of the disease, sample size of cases and controls, and population stratification. In our study, there are several points that reassure us regarding these issues. The diagnosis of aneurysmal SAH depends on clinical and radiological criteria that are unambiguous and precise. The allele and genotype frequencies of APOE gene polymorphism that we found in our control group were similar to those reported previously in other Italian populations. So, it is likely that cases and controls were derived from the same homogenous population. In addition, according to recent guidelines, we defined the level for statistical significance at a probability value less than 0.01 in order to exclude spurious associations. Nevertheless, our study was hospital based, and it is well known that a percentage of patients with SAH die before admission to hospital. These patients represent a bias of our study, and excluding them may have led us to underestimate the role of APOE ε4 allele in the disease. Thus, considering the limits of genetic association studies and the clinical heterogeneity of aneurysmal SAH, additional data from different populations may be useful to definitely exclude a role for the APOE gene in aneurysmal SAH.

Conclusions

We found that, in an Italian population, the frequencies of APOE alleles and genotypes did not differ significantly between patients with aneurysmal SAH and healthy controls. In addition, we found that the ε4 allele of the APOE gene exerts a modest effect on the clinical characteristics of the disease. It is therefore unlikely that the APOE gene plays an important role in aneurysmal SAH.

References

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