Association of Apolipoprotein E Polymorphism With Outcome After Aneurysmal Subarachnoid Hemorrhage
A Preliminary Study

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Background and Purpose—Variation in the outcome after aneurysmal subarachnoid hemorrhage (SAH) is not fully explained by known prognostic factors. APOE genotype is the most important genetic determinant of susceptibility to Alzheimer’s disease, and it is also shown to be associated with the outcome after traumatic brain injury. We studied the association of apolipoprotein E polymorphism with the outcome after aneurysmal SAH.

Methods—A total of 160 consecutive patients were admitted after SAH to a neurosurgical unit. The clinical assessment after the SAH was performed with the Hunt and Hess grading scale. The severity of the bleeding as visualized on CT was assessed by Fisher’s grading system. Outcome was assessed with the Glasgow Outcome Scale. APOE genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism.

Results—126 patients had aneurysmatic SAH, and detailed information on outcome and APOE genotype was available for 108 patients (86%). Sixteen (40%) of 40 patients with APOE e4 had an unfavorable outcome compared with 13 (19%) of 68 without the APOE e4 allele (OR 2.8, 95% CI 1.18 to 6.77). Association was more significant after adjustment for age, rebleeding, clinical status on admission, and CT scan findings (OR 7.1, 95% CI 1.9 to 26.3; P=0.0035).

Conclusions—Our findings show a significant genetic association of APOE polymorphism with outcome after spontaneous aneurysmal SAH. Genetic factors thus seem to explain a part of individual differences in the recovery of SAH. (Stroke. 2001;32:1181-1184.)

Key Words: apolipoproteins ■ genetics ■ outcome ■ subarachnoid hemorrhage

Aneurysmal subarachnoid hemorrhage (SAH) is a major cause of neurological disability and death, with an annual incidence of up to 18 per 100,000 in Finland. Approximately 12% of patients die of the initial hemorrhage before receiving medical attention, and a total of 40% of the hospitalized patients die within 1 month after the hemorrhage. More than one third of the survivors have major neurological deficits. Rebleeding is the most important preventable cause of death in hospitalized patients and accounts for approximately 20% of the 30-day mortality, whereas delayed ischemic deterioration accounts for 6% to 25% of all deaths.

It is known that the most important factors predicting poor outcome from aneurysmal SAH are severity of initial bleeding as seen on CT scan, patient’s clinical condition on admission, incidence and severity of delayed ischemia, and possible surgical complications. Although known risk factors predict much of the patient’s outcome after aneurysmal SAH, there are individual differences in the recovery from hemorrhage that cannot be fully explained by the known prognostic factors.

Apolipoprotein E (apoE) is a polymorphic protein associated with plasma lipoproteins having a special relevance to nervous tissue, especially in nervous system injury. In humans there are 3 common alleles of the APOE gene (e2, e3, and e4), which encode 3 isoforms of the protein (E2, E3, and E4), with differences in action. APOE genotype is the most important genetic determinant of susceptibility to Alzheimer’s disease, with the APOE e4 allele in particular being overrepresented in groups of patients with Alzheimer’s disease. The APOE e4 allele has been reported to be associated with a fatal course after head injury and also, in a clinical study, with poor outcome after traumatic brain injury.

We report the results of a retrospective clinical study to assess the association of APOE genotype on outcome after aneurysmal SAH in patients treated at a neurosurgical center with population responsibility.

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Subjects and Methods
From April 1, 1995, to March 30, 1996, a total of 160 consecutive patients with spontaneous SAH were admitted to the Department of Neurosurgery of Helsinki University Central Hospital (catchment area of approximately 1.6 million inhabitants). Of these patients, a total of 126 had an intracranial aneurysm as a cause of SAH and were included in the study. The nonaneurysmatic patients were not included, because of known better prognosis.20

All patients underwent cranial CT on admission. The CT findings were classified according to the grading system of Fisher et al.21 Bilateral carotid angiography and (if clinically or radiologically indicated) vertebral angiography were performed. Patients’ clinical condition was classified according to the grading system of Hunt and Hess (H&H).22 Surgery for aneurysm clipping was performed within the first 3 days in patients with H&H grades I–III. Patients with expanding intracerebral hematoma were operated on immediately after angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography. To prevent vasospasm, the calcium channel blocker nimodipine (Nimotop, Bayer AG) was started after the angiography.

Outcome was assessed without a knowledge of the patient’s APOE genotype. If the patient was not able to come to the routine follow-up examination for outcome measurement, data were collected from other hospitals involved or from the patients or relatives by a questionnaire or by phone. The study end point was the outcome assessed by the Glasgow Outcome Scale23 at the follow-up examination, by the data from other hospitals, or by questionnaire.

The study was approved by the ethical committee of The Department of Neurosurgery at Helsinki University Central Hospital. Either written or oral informed consent was obtained from the patients or, when impossible because of the patients’ poor clinical condition, from the relatives.

APOE Genotyping
Samples for DNA analysis were collected either at the arrival or afterward during the follow-up period. DNA analysis was performed at the Department of Forensic Medicine, University of Tampere. DNA was isolated either from peripheral venous blood samples24 or from buccal epithelial cells of the patients.25 APOE genotypes were determined by polymerase chain reaction,26 followed by HhaI restriction enzyme digestion. Finally, the digested fragments were run on 12% polyacrylamide gel electrophoresis and visualized by silver staining. According to the APOE genotype, patients were divided into the 2 groups whether they had 1 or 2 e4 alleles or not.

Statistical Analysis
Comparison of the outcome between the groups (with or without the e4 allele) was done with the Fisher exact test. A logistic regression analysis supported by odds ratios with 95% confidence intervals was calculated with SPSS/win (version 8.0; SPSS, Inc). Adjustment was made for age, primary H&H, rebleeding, and CT findings. Age was included in the model as a continuous variable. Rebleeding was classified as yes/no according to clinical and radiological verification. CT findings were allocated to 1 of the 4 categories of Fisher’s classification21 and included as a categorical variable. The 5 categories of H&H classification were used as a assessment of primary clinical condition and included also as a categorical variable.

Results
Clinical data and APOE genotype were available for 108 (86%) of the 126 aneurysmal SAH patients. Of the 18 missing, 4 (3%) were dead, and 14 (11%) were known to be alive but could not be contacted.

There was a slight (60%) female predominance. Eighty-eight patients were treated by microsurgery and 5 by endovascular coiling; 9 patients were treated conservatively because of poor initial neurological condition or advanced age.

| TABLE 1. Demographic Data of Patients With and Without an ApoE e4 allele |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Total Series    | ApoE e4 Allele  | With ApoE e4 Allele | P                |
| Age, y (range)                  | 51.5 (19–79)    | 52 (19–79)      | 51 (31–74)         | (n=108)          |
| Gender (male/female)            | 43/65           | 26/42           | 17/23             | (n=68)           |
| Delay in admission to neurosurgical unit, h (range) | 39 (0.5–312)     | 38 (0.5–240)    | 40 (1–312)        | (n=40)           |
| Hypertension                    | 42 (39%)        | 25 (36%)        | 17 (45%)          | 0.098            |
| Current smoker                  | 46 (43%)        | 31 (44%)        | 15 (39%)          | 0.0975           |
| Operation                       | 93 (86%)        | 61 (90%)        | 32 (80%)          | 0.073            |
| Microsurgical clipping          | 88 (81%)        | 58 (85%)        | 30 (75%)          | (86%) of the 126 aneurysmal SAH patients. Of the 18 missing, 4 (3%) were dead, and 14 (11%) were known to be alive but could not be contacted. | (n=40)           |
| Endovascular coiling            | 5 (5%)          | 3 (4%)          | 2 (5%)            | (n=40)           |
| Follow-up time, mo              | 7.5 (0–57)      | 7.1 (0–49)      | 8.2 (0–57)        | (n=40)           |
|                                      | (n=108)         | (n=68)          | (n=40)            | (n=68)          |

| TABLE 2. Comparison of Aneurysmal SAH Patients With and Without e4 Allele on Admission |
|---------------------------------|-----------------|-----------------|
| Patients, n (%)                 | Without         | With            |
|                                | ApoE e4         | ApoE e4         |
|                                | (n=68)          | (n=40)          |
|                                | P                |
| Initial H&H score              |                  |
| I                               | 1 (1)           | 2 (1)           | 0.029            |
| II                              | 27 (40)         | 17 (42.5)       | 0.098            |
| III                             | 19 (28)         | 11 (27.5)       | 0.089            |
| IV                              | 15 (22)         | 3 (7.5)         | 0.065            |
| V                               | 6 (9)           | 8 (20)          | 0.072            |
|                                |                  |
| Initial CT finding (Fisher)     |                  |
| 0, no blood                     | 2 (3)           | 1 (3)           | 0.030            |
| 1, thin                         | 7 (10)          | 6 (15)          | 0.067            |
| 2, moderate                     | 28 (41)         | 18 (45)         | 0.099            |
| 3, thick blood                  | 18 (26)         | 9 (23)          | 0.085            |
| 4, intracerebral or intraventricular blood | 13 (19)        | 6 (15)          | 0.074            |
| Rebleeding before clipping      | 9 (13)          | 1 (3)           | 0.048            |
occurrence of rebleeding the association of APOE genotype and outcome from hemorrhage was even more strong. The risk of an unfavorable outcome was 7.1 times higher among carriers of the e4 allele than patients without the e4 allele.

The methodology in our study merits some consideration. It should be emphasized that the size of the study is limited, and there were only 40 patients with APOE e4 allele, thus enabling a chance finding. Despite of the robust nature of the outcome measure (Glasgow Outcome Scale), it should give quite good assessment of the functional survival. In addition, some patients died soon after arrival at the hospital and thus could not be reached for the study. It could also be argued that patients with the APOE e4 allele have had previous age-related neuropathological changes and because of that are more likely to recover poorly.

Despite the above considerations, our findings support previous studies that have shown a trend toward worsened survival after hemorrhagic stroke in the patients with the APOE e4 allele. In a postmortem study, it has been shown that the presence of plaque-like deposits of amyloid β-protein in the cerebral cortex after fatal head injury was strongly associated with the possession of an APOE e4 allele. In clinical studies, patients with APOE e4 allele have had a poorer prognosis after traumatic brain injury. In addition, APOE genotype is related to cognitive dysfunction after cardiopulmonary bypass. However, APOE e4 genotype was not found to be a poor prognostic factor in ischemic stroke.

APOE seems to have an active role in response to acute brain injury. The protein is synthesized by reactive astrocytes and is responsible for transporting lipids to regenerating neurons, promoting repair and construction of new cell membranes, neurites, and synapses. Three APOE isoforms have differences in action. apoE E4 being associated with reduced growth and branching of neurites in cell culture. According to earlier findings, apoE has an antioxidant effect, with E3 being more efficient than E4, which may protect neurons. ApoE E4 binds more avidly than E3 to amyloid β-protein and promotes more rapid aggregation of amyloid β-protein into amyloid fibrils, which is also known to damage endothelial cells by producing superoxide radicals. ApoE immunoreactivity of neurons is greatly increased within a few hours of experimental ischemic injury, which is consistent with upregulation of lipid transport. Ischemia or hypoxia could so activate the process that leads to the accumulation of amyloid β-protein in the brain as a component of the neuritic plaque leading to neuronal damage and dysfunction.

Our study is the first to report a significant association between APOE polymorphism and recovery after aneurysmal SAH. This finding gives further confirmation of the influence of APOE genotype on outcome from acute brain damage. Together with earlier findings considering acute brain trauma, intracerebral hemorrhage, and recovery from post-traumatic coma, it is likely that the APOE e4 allele is a significant determinant to the prognosis after acute central nervous system injury with different etiologies.

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**TABLE 3. Outcome After Aneurysmal SAH With and Without e4 Allele**

<table>
<thead>
<tr>
<th>Glasgow Outcome Scale Score</th>
<th>Without ApoE e4 (n=68)</th>
<th>With ApoE e4 (n=40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 (36)</td>
<td>11 (28)</td>
<td>0.095</td>
</tr>
<tr>
<td>2</td>
<td>29 (43)</td>
<td>13 (33)</td>
<td>0.095</td>
</tr>
<tr>
<td>3</td>
<td>6 (9)</td>
<td>7 (18)</td>
<td>0.069</td>
</tr>
<tr>
<td>4</td>
<td>1 (1)</td>
<td>2 (5)</td>
<td>0.037</td>
</tr>
<tr>
<td>5</td>
<td>6 (9)</td>
<td>7 (18)</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Hypodense area consistent with infarction: 21 (31) 15 (38) 0.095
References


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