Apolipoprotein E Genotype and Outcome in Aneurysmal Subarachnoid Hemorrhage

Clarence H.S. Leung, MB, ChB; W.S. Poon, MB, ChB; L.M. Yu, MSc; George K.C. Wong, MB, ChB; H.K. Ng, MD

Background and Purpose—Active management of ruptured intracranial aneurysm in subarachnoid hemorrhage is indicated in patients with favorable prognosis. Outcome prediction is based on patient characteristics and clinical and radiological factors. Current clinical grading scales are imprecise, with low interobserver reproducibility. Therefore, outcome prediction remains inconsistent and decision making becomes difficult, especially for patients with poor clinical grade.

Methods—The possible relationship between apolipoprotein E genotype and the outcome of patients suffering spontaneous subarachnoid hemorrhage was investigated. A prospective study was conducted on all patients with spontaneous aneurysmal subarachnoid hemorrhage admitted to our unit during a 2-year period. All patients were managed according to standard protocol, and treatments were given according to their clinical grading. Patient characteristics, clinical grade, radiological grade, and apolipoprotein E genotype were documented. The focus of the study was the 6-month neurological outcome for this group of patients after they were discharged.

Results—Seventy-two patients with aneurysmal subarachnoid hemorrhage were admitted to the Prince of Wales Hospital in Shatin, Hong Kong, China, from February 1998 to February 2000. Their ages ranged from 24 to 95 years of age, with a mean (SD) age of 58.3 (15.0) years. Apolipoprotein E e4 was found in 15 patients (21%). At 6 months, Glasgow Outcome Scale score ≤3 was found in 29 patients (40%). Univariate analysis showed that older patients (odds ratio [OR], 1.03; 95% CI, 1.00 to 1.07; P=0.07) and patients with poor Fisher’s grade (OR, 4.5; 95% CI, 1.3 to 15.2; P=0.01), poor World Federation of Neurological Surgeons grade (OR, 5.8; 95% CI, 1.9 to 17.8; P=0.002), or apolipoprotein E e4 (OR, 6.0; 95% CI, 1.7 to 21.3; P=0.006) were more likely to attain unfavorable outcome at 6 months. The additional effect of apolipoprotein E e4 remained significant in the multiple logistic regression model (OR, 11.3; 95% CI, 2.2 to 57.0; P=0.003); the gain in predictive performance was not significant (P=0.26).

Conclusions—Apolipoprotein E e4 genotype is related to poor outcome in patients with subarachnoid hemorrhage.

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Key Words: apolipoproteins ■ outcome ■ subarachnoid hemorrhage

Subarachnoid hemorrhage (SAH) accounts for 25% of all cerebrovascular deaths. The case fatality rate of SAH is reported to be as high as 50%. Among the remaining survivors, 50% are left severely disabled. The etiology of 80% of the cases is ruptured intracranial aneurysm. Morbidity and mortality are largely due to rebleeding aneurysm and vasospasm. Thus, early aneurysm intervention will facilitate both treatment of vasospasm and prevention of rebleeding. Currently, prediction of outcome relies on demographic, clinical, and radiological factors. However, accurate outcome prediction in aneurysmal SAH remains imprecise despite use of currently established clinical grading scales.

Evidence from animal studies on apolipoprotein E genotype has suggested its important role in the response to nervous system injury. The 3 most common genetic alleles in humans are apolipoprotein E e2, e3, and e4 (APOE2, APOE3, and APOE4, respectively), which encode 3 isoforms of the apolipoprotein E2, E3, and E4, respectively. Several prospective clinical studies on the APOE4 allele have shown its association with poor outcome in patients with intracerebral hemorrhage and head injury.

In a recent prospective case-control study by Kokubo et al, an association was suggested between APOE4 and SAH. However, the sample size of patients with SAH in that study was small (n=37), and the association between apolipoprotein E genotype and outcome was not investigated. Our study was designed to test the hypothesis that APOE4 genotype is associated with unfavorable outcome in SAH patients. This
may supplement other established outcome predictors with a more accurate outcome prediction.

**Subjects and Methods**

**Patient Recruitment**

A prospective clinical study was performed on all patients admitted to the Prince of Wales Hospital in Shatin, Hong Kong, China, with spontaneous aneurysmal SAH during a 2-year period from February 1998 to February 2000. The study was approved by the Ethics Committee of the Chinese University of Hong Kong and conducted in accordance with the Helsinki Declaration. CT or lumbar puncture was used to confirm the diagnosis of SAH. All SAH patients directly admitted or transferred from another hospital were clinically assessed by the neurosurgical team. The patients’ characteristics, World Federation of Neurological Surgeons (WFNS) grading score, and Fisher’s grading score (quantifying the severity of SAH on CT) were recorded. Blood samples for apolipoprotein E genotyping were obtained via venipuncture for other routine blood tests. Informed consent from patients or their next of kin was obtained before blood samples were taken.

**Neurological Assessment**

Assessment and classification of SAH patients were based on the grading scale as defined by the WFNS, as follows: grade 1, Glasgow Coma Scale (GCS) score 15; grade 2, GCS score 13 to 14 with no focal neurological deficit; grade 3, GCS score 13 to 14 with focal neurological deficit; grade 4, GCS score 7 to 12; and grade 5, GCS score 3 to 6. Patients with WFNS grade 1 to 3 were classified as patients with good WFNS grade (with favorable prognosis), and patients with grade 4 to 5 were defined as patients with poor WFNS grade (with unfavorable prognosis).

**Management Protocol**

All patients were managed under a standard protocol in either the neurosurgical high dependency unit or the intensive care unit if intubation and mechanical ventilation were required. Intravenous nimodipine 1 to 2 mg/h (Nimotop, Bayer) and the prophylactic anticonvulsant sodium valproate 400 mg/8 h (Epilim Intravenous, Sanofi Winthrop Industries) were started on admission. Systolic blood pressure was maintained at <140 mm Hg unless there was a known history of hypertension or the patient developed clinical vasospasm, in which cases higher systolic blood pressure ranges were accepted.

All patients admitted with spontaneous SAH were investigated within 24 hours, initially with the use of CT angiography to screen for intracranial aneurysm. Patients with a good WFNS grade (WFNS grade 1 to 3) would proceed to digital subtraction angiography if CT angiography was negative. If the patient initially presented with a poor WFNS grade (grade 4 or 5) or had negative CT angiography and subsequently improved to good WFNS grade, digital subtraction angiography would then be performed. Patients with persistent poor WFNS grade and negative CT angiography were not included in this study.

**Treatment of Aneurysm**

The treatment options were either endovascular aneurysm occlusion with Guglielmi detachable coils (GDC, Boston Scientific Corp) or microsurgical clipping of aneurysm. The decision of whether to use endovascular or surgical treatment depended on the configuration of the aneurysm (site, size, and shape), the patient’s medical condition, and the family’s or patient’s preference. All definitive treatments were performed within 48 hours.

**Determination of Apolipoprotein E Genotype**

The salting out method was used to extract DNA from the venous blood sample. The apolipoprotein E genotype was determined by a polymerase chain reaction on an MJ Research PTC-200 thermal cycler followed by enzyme restriction and polyacrylamide gel electrophoresis, as described previously by 1 of the authors.12

**Follow-Up and Outcome Assessment**

Outcome assessment was based on the Glasgow Outcome Scale (GOS), as follows: grade 5, good recovery; grade 4, moderate disability; disabled but independent; grade 3, severe disability: conscious but disabled; grade 2, persistent vegetative state; and grade 1, death. Grades 4 and 5 were classified as favorable outcome, and grades 1 to 3 were classified as unfavorable outcome.13 A follow-up interview was arranged for 6 months after the patients were discharged to determine the clinical outcome according to the GOS. For those patients who were unable to attend the interview because of severe disability, consultation with their family or caregivers was made over the telephone. Throughout the study, the investigators were blinded to the patients’ apolipoprotein E genotype. The patients’ outcome assessment and apolipoprotein E genotyping results were submitted independently for statistical analysis.

**Statistical Analysis**

Univariate analysis was performed to determine the effect of age, sex, Fisher’s grade, WFNS grade, and APOE4 on the outcome at 6 months (defined by GOS score), after which 2 prognostic models were developed. The first model (model 1) comprised all of the aforementioned key factors except APOE4, which was included in the second model (model 2), ie, age, sex, Fisher’s grade, and WFNS grade. The model was performed by a stepwise multiple logistic regression analysis14 with the use of the Akaike information criterion15 as variable selection criterion. The second model (model 2) added the effect of APOE4 to model 1. The predictive ability of each model was assessed by the area under the receiver operating characteristic (ROC) curve.16 An area under the ROC curve value of 0.5 indicates no predictive power, whereas a value of 1.0 indicates excellence in prediction. The areas under the ROC curves for both models were then compared according to the method developed by Hanley and McNeil.17 The bootstrapping technique was used as an internal validation to correct for possible bias due to overfitting.18 All analyses were performed with the use of S-Plus 2000 statistical software (MathSoft, Inc.).

**Results**

**Patient Characteristics**

Seventy-two patients with aneurysmal SAH, confirmed by CT angiography or digital subtraction angiography, were recruited for analysis. Their ages ranged from 24 to 95 years, with a mean (SD) age of 58.3 (15.0) years. There were 47 women (65%) and 25 men (35%). The patients’ characteristics, WFNS grade, Fisher’s grade, and apolipoprotein E genotype are summarized in Table 1.

**Outcome Analysis**

Of the 72 patients, 8 patients died during the same admission, 54 patients attended the 6-month interview, 6 patients were unable to attend the interview in person but were represented by their caregivers over the telephone, and 4 patients required management in a convalescence hospital. At 6 months, 61 patients (84.7%) remained alive, 43 patients (59.7%) had a GOS score >3, and 29 patients (40.3%) had a GOS score ≤3. Results from the univariable analysis suggested that older patients were more likely to attain an unfavorable outcome (62.2 [17.2] versus 55.7 [12.9] years; odds ratio [OR], 1.03; 95% CI, 1.00 to 1.07). Twenty-five of 50 patients (50%) with poor Fisher’s grade had significantly higher unfavorable 6-month outcome compared with only 4 of 22 patients (18.2%) with Fisher’s grade 1 and 2 (OR, 4.5; 95% CI, 1.3 to 15.2). Similarly, more unfavorable outcomes were found in patients who presented with poor WFNS grades than those
TABLE 1. Clinical and Genetic Characteristics of Patients With Aneurysmal SAH

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>62.2±15.0</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>25/47</td>
</tr>
<tr>
<td>WFNS grade</td>
<td></td>
</tr>
<tr>
<td>≤3 (Good grade)</td>
<td>52</td>
</tr>
<tr>
<td>≥4 (Poor grade)</td>
<td>20</td>
</tr>
<tr>
<td>Fisher’s grade*</td>
<td></td>
</tr>
<tr>
<td>1/2 (Good grade)</td>
<td>22</td>
</tr>
<tr>
<td>3/4 (Poor grade)</td>
<td>50</td>
</tr>
<tr>
<td>Apolipoprotein E genotype</td>
<td></td>
</tr>
<tr>
<td>ε2/ε2</td>
<td>1</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>3</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>53</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>2</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>13</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are number of patients unless indicated otherwise.

*The Fisher’s grading system indicates the amount of subarachnoid blood on CT, with higher grades indicating larger amount of subarachnoid blood and thus a higher risk of developing vasospasm.

with good WFNS grades (70% versus 28%; OR, 5.8; 95% CI, 1.9 to 17.8) and for those with APOE4 compared with those without the allele (73.3% versus 31.6%; OR, 6.0; 95% CI, 1.7 to 21.3) (Table 2).

The multivariable relationships between the variables, without (model 1) and with (model 2) the effect of APOE4, and outcome at 6 months are shown in Table 3. The results indicate that APOE4 (P=0.003; OR, 11.3; 95% CI, 2.2 to 57.0) remained significant in predicting outcome, after adjustment for other known risk factors. Model 2 had a bootstrap-corrected area under the ROC curve greater than model 1 (83% versus 78%). The addition of APOE4 to the prognostic model improved the predictive performance by 5%. However, such an improvement was not statistically significant (P=0.26).

Discussion

The results of this study demonstrate a clear relationship between APOE4 and poor outcome in aneurysmal SAH. This is the first prospective clinical study that had shown such a relationship. In a similar study by Dunn et al,19 such an effect of APOE4 on the 6-month outcome of those SAH patients admitted to the regional neurosurgical unit was not shown. The authors stated that the negative findings were due to selection bias, and no definite conclusion regarding the effect of APOE4 on SAH patients could be drawn. Evidence from previous experimental and clinical studies on apolipoprotein E genotype supports this interpretation. Apolipoprotein E is a known “injury factor” in the central nervous system, and previous proposed mechanisms include isoform-specific immunomodulatory,20 neurotoxic,6 and oxidative effects.21

In the experimental study performed on microglial cells by Barger and Harmon,20 apolipoprotein E4 was shown to be less effective in suppressing microglia-mediated neurotoxicity than apolipoprotein E3. In the transgenic work of Buttini et al,6 apolipoprotein E3 was shown to be a neuroprotective factor, but, in contrast, apolipoprotein E4 was shown to inhibit this beneficial function of apolipoprotein E3. Prospective clinical studies by Teasdale et al8 and Alberts et al7 suggest that APOE4 is associated with higher mortality and morbidity in head injury and nonaneurysmal intracerebral hemorrhage, respectively. Their findings indicate that apolipoprotein E4 exerts an inhibitory effect on neural recovery after brain injury. These studies support our findings that the

TABLE 2. Distribution of Characteristics at 6-Month Outcome in Patients With SAH

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unfavorable (n=29)</th>
<th>Favorable (n=43)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62.2±17.2</td>
<td>55.7±12.9</td>
<td>1.03 (1.00–1.07)</td>
<td>0.07</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>12/17</td>
<td>13/30</td>
<td>1.6 (0.6–4.4)</td>
<td>0.33</td>
</tr>
<tr>
<td>WFNS grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3†</td>
<td>15</td>
<td>37</td>
<td>…</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>14</td>
<td>6</td>
<td>5.8 (1.9–17.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Fisher’s grade†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>4</td>
<td>18</td>
<td>…</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>25</td>
<td>25</td>
<td>4.5 (1.3–15.2)</td>
<td>0.015</td>
</tr>
<tr>
<td>APOE4 heterozygous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No†</td>
<td>18</td>
<td>39</td>
<td>…</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>4</td>
<td>6.0 (1.7–21.3)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

*Six-month outcome defined by GOS score: GOS 1, 2, and 3=unfavorable; GOS 4 and 5=favorable.

†Reference group.
patients in our study who possess the APOE4 allele are more susceptible to an unfavorable outcome. The exact mechanism of apolipoprotein E4 still remains unknown.

The brain damage of SAH results from both the initial hemorrhage and the subsequent ischemia secondary to vasospasm. Vasospasm is the main cause of secondary brain injury in SAH. The results of our study indicate that apolipoprotein E4 may act directly on the effect of brain ischemia, which accounts for the poorer outcome in SAH patients. However, several previous studies have shown no evidence of association between APOE4 and outcome in patients with ischemic stroke.\(^{22,23}\) To explain the adverse prognostic effect of APOE4 on SAH patients, apolipoprotein E4 must act either indirectly or through a mechanism other than ischemia.

One study on animals has shown that the products of hemolysis in the subarachnoid space after SAH may lead to widespread necrosis of the cortex.\(^24\) If apolipoprotein E4 exerts its effect on the mechanism via products of hemolysis rather than directly on ischemic brain, this may provide an explanation for the divergent effects of APOE4 on the outcome of patients with hemorrhagic and ischemic stroke. This may also explain the adverse effect of APOE4 on patients with intracerebral hemorrhage, head injury, and SAH because all these patients may have blood in either the subarachnoid space (spontaneous and traumatic SAH) or within the brain parenchyma (hematoma and contusions), whereas the effect of APOE4 may be absent in patients with ischemic stroke as long as hemorraghic transformation does not occur. The study by Motto et al\(^{25}\) addressed the importance of hemorrhagic transformation on the poorer outcome of patients with ischemic stroke. Their findings may support our interpretation with the caveat that the exact mechanism of APOE4 remains to be defined.

On the other hand, apolipoprotein E4 may have an indirect effect on causing vasospasm in SAH. Endothelin-1 is known to be one of the most potent vasoconstrictors in the brain. In the study by Fassbender et al,\(^{26}\) endothelin-1 was found to be released from cerebrospinal fluid leukocytes during the acute phase of SAH. The animal study by Paris et al\(^{27}\) suggested that there is a synergistic relation between apolipoprotein E and endothelin-1 in vasoconstriction. This synergistic effect was especially strong with apolipoprotein E4 compared with apolipoproteins E2 and E3. Therefore, the effect of apolipoprotein E4 on SAH patients may be due to its synergistic effect with endothelin-1 during the acute phase of hemorrhage in causing widespread and persistent vasospasm. This may also explain the insignificance of APOE4 in ischemic stroke since vasospasm does not form an important part of the pathogenesis of secondary brain damage in this situation.

The frequency of APOE4 allele varies across different ethnic groups, with the frequency of APOE4 allele in the Chinese population reported to be between 5% and 8%.\(^{12,28,29}\) This is much lower than the average white population figure of approximately 20%.\(^{30}\) In our study, after exclusion of 1 white patient, the frequency of 1 APOE4 allele was 21.1%. If we assume that the previously reported frequencies of APOE4 in the Chinese population are accurate, this implies a possible link between APOE4 allele and SAH. Similar findings have been found between APOE4 and SAH patients in the Japanese population.\(^9\)

Alternatively, the finding of comparatively high APOE4 allele frequency in SAH patients may be due to age bias in previous reports on the Chinese population, which mainly focused on elderly patients. There is evidence to suggest that the frequency of APOE4 allele may be less in the elderly group.\(^{31}\)

There were several drawbacks in our study. The frequency of APOE2 homozygote was reported to be approximately 0.8% in the white population.\(^{30}\) In our study the sample size was too small to gather enough patients with the APOE2 genotype. Evidence has shown that APOE2 has a neuroprotective effect, but in our study only 1 patient harbored 2 APOE2 alleles, and it was therefore difficult to draw any conclusions regarding its impact on outcome.\(^{32,33}\) In addition, none of the patients in our study were APOE4 homozygous, making it impossible to assess the gene dose effect on outcome prediction. The areas under the ROC curves of our prognostic models (78% excluding APOE4 and 83% including the genotype) indicated a good predictive performance in poor outcome prediction, although the addition of APOE4 effect did not show any statistically significant improvement in the prognostic model. This may be due to the small sample size of our study, which may also explain the wide CIs obtained in our results. Thus, to yield a better estimation and prediction of 6-month neurological outcome, a larger or possibly a multicenter study would need to be performed.

The results from this study offer a new perspective on outcome prediction for patients with SAH. With currently available technologies in DNA extraction and amplification, apolipoprotein E genotyping can be achieved within 20 hours. Determination of the apolipoprotein E genotype, in combination with other clinical data, might be clinically useful in determining the patient’s prognosis and facilitating subsequent decision making on the definitive management strategy in SAH patients. A multicenter prospective study with a larger sample size is necessary to investigate the gene dose effect of APOE4 and the neuroprotective effect of APOE2 in SAH patients. A large sample size will also enable us to further investigate the contributory effect of APOE4 to the outcome predictive model. Further studies on the Chinese population and other ethnic groups are required to confirm our findings of a higher frequency of APOE4 in patients with SAH.

References


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