Influence of APOE polymorphism on cognitive and behavioural outcome in moderate and severe traumatic brain injury

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ABSTRACT

Objective: The APOE ε4 allele is associated with unfavourable outcome after traumatic brain injury (TBI). To date, the influence of APOE genotype on behaviour and neuropsychological functions usually impaired after TBI in moderate and severe chronic survivors has only been partially demonstrated. The aim of the current study was to analyze the influence of APOE ε4 status on the cognitive and behavioural functions usually impaired after moderate and severe TBI.

Methods: Seventy-seven TBI patients selected from 140 consecutive admissions were genotyped for APOE. Each patient underwent neuropsychological and neurobehavioral assessment at least six months post injury.

Results: Subjects carrying the ε4 allele performed significantly worse on verbal memory (Auditory Verbal Learning Test), motor speed, fine motor coordination, visual scanning, attention and mental flexibility (Grooved Pegboard, Symbol Digit Modalities Test and part B of the Trail Making Test) and showed significantly more neurobehavioral disturbances (Neurobehavioral Rating Scale-Revised) than the group without the ε4 allele.

Conclusions: There is an association between APOE isoforms and typical impairment after moderate and severe TBI. In particular, we found performance on neuropsychological tasks that are presumed to be related to temporal lobe, frontal lobe and white matter integrity to be worse in patients with the APOE ε4 allele than in those without it. Moreover, APOE ε4 carriers showed more neurobehavioral disturbances than did APOE ε2 and ε3 carriers.
INTRODUCTION
APOE is the gene responsible for the production of apolipoprotein E (apoE) and has been widely studied in relation to outcome after traumatic brain injury (TBI). In humans, there are three common isoforms of apoE, encoded by the alleles $\varepsilon_2$, $\varepsilon_3$ and $\varepsilon_4$. Clinical and experimental studies suggest that APOE $\varepsilon_4$ is associated with an unfavourable functional outcome after TBI, [1][2][3][4][5] in some cases in association with other factors such as age.[6] The inheritance of APOE $\varepsilon_4$ allele has even been mentioned as a risk factor for Alzheimer’s disease after TBI, although this has not yet been conclusively demonstrated.[7]

The relationship between inheritance of APOE $\varepsilon_4$ and cognitive outcome in humans after TBI has been addressed in some studies.[8][9][10][11][12] Some degree of neuropsychological functions impairment has been demonstrated after mild head injury in individuals with the APOE $\varepsilon_4$ allele.[9][11] In a group of more severe TBI, possession of at least one APOE $\varepsilon_4$ allele has been related to memory impairment within 6 months of injury.[8] In this study, frontal lobe involvement was only assessed by means of verbal fluency and there were no differences between subjects with APOE-$\varepsilon_4$ and those without. However, in a study of mainly severe TBI, cognitive decline after 15 to 25 years of injury was not related to APOE genotype.[10]

Since moderate to severe TBI usually induces disseminated injury throughout fronto-temporal regions and white matter, these areas will be significantly affected in any TBI patient.[13][14][15] Therefore, impairment of memory and executive function, mental flexibility, attention, speed, motor function and visual scanning is expected. To date, the influence of APOE genotype on behaviour and neuropsychological functions usually impaired after TBI in moderate and severe chronic survivors has only been partially demonstrated.

The aim of the present study was to analyze the influence of APOE $\varepsilon_4$ status on the neuropsychological and behavioural disturbances usually impaired after moderate and severe TBI.

METHODS
Subjects
Patients were selected from a cohort of 140 consecutive cases admitted to the Neurotraumatology Unit of the Vall d’Hebron University Hospital in Barcelona between January 2000 and December 2001, and who had a Glasgow Coma Scale (GCS) score of 12 or less. GCS was estimated initially (at the place of injury) and on arrival at hospital. The worst GCS value was used. Head injury was moderate (GCS from 9 to 12) in 50 patients and severe (GCS $\leq 8$) in 90. Twenty-five (27%) severe and 7 (14%) moderate patients died as a consequence of the injury. Of the 108 TBI survivors, 8 patients could not be contacted and 6 refused to participate in the neuropsychological study. Six patients were too severely impaired to undergo neuropsychological testing, 5 did not have native language proficiency in Spanish and 5 had psychiatric history. This left 78 patients for the neuropsychological study, aged between 16 and 65 years old. All patients included were literate and had no aphasia, dysarthria or motor impairment that would preclude neuropsychological evaluation; none of them had a history of previous TBI or neurological or psychiatric diseases. Verbal informed consent was obtained from the patients or parents (underage patients) in all cases.
APOE genotype
Genomic DNA was extracted using the phenol/chloroform method from the leukocyte fraction. PCR was used to amplify the common alleles of APOE genes following the protocol published elsewhere.[16]

Neuropsychological and neurobehavioural assessment
Each TBI patient underwent cognitive and behavioural assessment at least six months post injury (mean: 215±23 days, range: 182-272 days). A modified version of Rey’s Auditory Verbal Learning Test was used to measure verbal learning and memory.[17] Visual memory was assessed by short term recall (3-minute) of the Rey-Osterrieth Complex Figure Test.[18] Verbal fluency was evaluated using the Controlled Oral Word Association Test. Speed and fine motor coordination were assessed with the Lafayette Grooved Pegboard Test. Visual scanning, tracking and motor speed were also assessed by the Symbol Digit Modalities Test. Parts A and B of the Trail Making Test were given to measure visual scanning, motor speed and attention and mental flexibility.

Behaviour was assessed with the five-factor model of the Neurobehavioral Rating Scale—Revised.[19]

Global adjustment to activities of daily living and general outcome was assessed using the extended Glasgow Outcome Scale (GOS).

Statistical analyses
All statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc, Chicago, IL, USA). Chi-square or Fisher’s exact probability test were used to compare categorical variables between the genetic groups. The continuous variables were compared by means of the Student’s t-test for independent samples. Allele group comparisons were performed using ANCOVA to control for the effects of age on cognitive and behavioural performance among TBI individuals.

RESULTS
APOE genotype was determined in the whole cohort except in one case; the final sample thus included 77 patients.

In the whole cohort, inheritance of APOE ε4 allele was not related to mortality (20% of ε4 and 23.5% of non-ε4 died; p=1.00) or to suitability for neuropsychological testing in survivors (62.5% of ε4 and 73.6% of non-ε4 underwent neuropsychological testing; p=0.38). Survivors who underwent neuropsychological testing versus those that did not were also comparable in sex (χ²=0.00; p=1.00) and CT initial findings (χ²=2.67; p=0.11) but differed in age (t=2.23; p=0.03), GCS (t=2.66; p=0.01) and GOS (χ²=15.08; p=0.001).

Demographic and clinical variables of subjects with and without the APOE ε4 allele were compared in the final sample. Sex (ε4=7 males and 3 females; non-ε4=53 males and 14 females; p=0.68), years of formal education (ε4=9.6±2.63; non-ε4=10.25±2.86; t=0.68; p=0.50), GCS (ε4=7.10±2.81; non-ε4=7.82±2.24; t= 0.92; p=0.36), duration of coma, finishing with eyes opening (ε4=13.50±8.16; non-ε4=11.15±7.08; t=0.95 ; p=0.34), post-traumatic amnesia measured by means of the Galveston Orientation and Amnesia Test (ε4=38.44±16.29; non-ε4=31.93±17.48; t=1.05; p=0.30) and CT initial
findings coded with a regrouping of the Traumatic Coma Data Bank categories (ε4=7 with diffuse injury and 3 with focal mass lesion; non-ε4=50 with diffuse injury and 17 with focal mass lesion; p=0.71) did not differ between the TBI genetic groups. However, the differences in age were significant (ε4=37.70±18.31; non-ε4=28.87±11.47; t=2.08; p=0.04). In a recent study, Teasdale et al. (2005) confirmed the interaction between age and APOE genotype.[6] We therefore performed an analysis of covariance, entering age as a covariable to rule out its effect on neuropsychological and neurobehavioral outcome.

Subjects carrying the ε4 allele performed significantly worse on almost all neuropsychological and behavioural measures than did the group without the ε4 allele, as is shown in table 1.

Table 1. Neuropsychological and neurobehavioural performance for the genetic groups

<table>
<thead>
<tr>
<th></th>
<th>ε4 present (N=10)</th>
<th>ε4 absent (N=67)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVLT Immediate Recall</td>
<td>31.20 (20.88 to 41.52)</td>
<td>37.66 (34.94 to 40.38)</td>
<td>5.86</td>
<td>0.005</td>
</tr>
<tr>
<td>AVLT Long Term Recall</td>
<td>5.00 (3.09 to 6.91)</td>
<td>7.50 (6.63 to 8.37)</td>
<td>5.66</td>
<td>0.005</td>
</tr>
<tr>
<td>CFT Short Term Recall</td>
<td>18.10 (11.48 to 24.72)</td>
<td>21.28 (19.3 to 23.26)</td>
<td>2.16</td>
<td>0.12</td>
</tr>
<tr>
<td>Grooved Peg Right</td>
<td>147.90 (89.81 to 205.99)</td>
<td>93.85 (78.53 to 109.17)</td>
<td>11.15 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Grooved Peg Left</td>
<td>149.40 (98.30 to 199.97)</td>
<td>99.00 (84.01 to 113.99)</td>
<td>7.33</td>
<td>0.001</td>
</tr>
<tr>
<td>SDMT</td>
<td>33.78 (16.11 to 51.44)</td>
<td>47.36 (43.56 to 51.15)</td>
<td>11.37 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>TMT A</td>
<td>71.00 (29.45 to 112.55)</td>
<td>53.67 (44.25 to 63.09)</td>
<td>2.82</td>
<td>0.066</td>
</tr>
<tr>
<td>TMT B</td>
<td>137.00 (39.15 to 234.85)</td>
<td>105.12 (88.85 to 121.38)</td>
<td>5.16</td>
<td>0.008</td>
</tr>
<tr>
<td>COWAT (FAS)</td>
<td>15.80 (7.65 to 23.95)</td>
<td>21.98 (19.34 to 24.63)</td>
<td>2.87</td>
<td>0.063</td>
</tr>
<tr>
<td>NRS-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Executive/Cognition</td>
<td>15.00 (9.80 to 20.20)</td>
<td>9.79 (7.77 to 11.82)</td>
<td>4.68</td>
<td>0.012</td>
</tr>
<tr>
<td>Positive Symptoms</td>
<td>13.60 (10.11 to 17.09)</td>
<td>8.37 (6.73 to 10.01)</td>
<td>2.89</td>
<td>0.062</td>
</tr>
<tr>
<td>Negative Symptoms</td>
<td>9.50 (5.98 to 13.02)</td>
<td>4.12 (2.99 to 5.25)</td>
<td>7.81</td>
<td>0.001</td>
</tr>
<tr>
<td>Mood/Affect</td>
<td>12.30 (8.94 to 15.66)</td>
<td>6.54 (5.24 to 7.83)</td>
<td>6.98</td>
<td>0.002</td>
</tr>
<tr>
<td>Oral/Motor</td>
<td>8.80 (5.55 to 12.05)</td>
<td>6.37 (5.02 to 7.73)</td>
<td>1.56</td>
<td>0.22</td>
</tr>
<tr>
<td>Global score</td>
<td>59.20 (44.04 to 74.36)</td>
<td>35.19 (28.78 to 41.61)</td>
<td>5.60</td>
<td>0.005</td>
</tr>
<tr>
<td>GOS extended</td>
<td>6.00 (4.93 to 7.07)</td>
<td>6.61 (6.21 to 7.01)</td>
<td>2.85</td>
<td>0.064</td>
</tr>
</tbody>
</table>
DISCUSSION
This APOE study in a cohort of moderate and severe TBI survivors is based on a broad neuropsychological and neurobehavioral assessment. Subjects with the ε4 allele showed poorer learning and long term memory. Outcomes on tests involving frontal lobe participation such as speed, motor coordination, visual scanning, and executive function or mental flexibility were also worse in the ε4 allele carriers than among non-carriers.

Furthermore, the neurobehavioral assessment showed a significant poor score for the global score of the NRS-R, as well as on some of its components for the ε4 allele carriers. Lack of differences in oral/motor component may be related to the exclusion of patients with severe aphasia, dysarthria or motor impairment. The exclusion of patients whose GCS and GOS scores differed from those in the final sample, could explain the homogeneity in coma duration and length of PTA between the APOE genotype groups studied.

Our data support and extend the results of previous studies that examined cognitive dysfunction after mild TBI. Liberman et al. showed that an APOE ε4-carrier group performed worse on almost all neuropsychological tests, the differences being significant for some measures of frontal lobe involvement. In a study of preinjury and postinjury within-person comparisons of neuropsychological measures, Sundstrom et al. found that participants with the APOE ε4 allele had poorer postinjury performance compared with their preinjury performance on memory and divided attention tests, whereas the performance of participants without the ε4 allele remained unchanged.

Our results are also in agreement with a study of mainly severe TBI. Crawford et al., in a sample of 110 TBI patients, demonstrated that subjects with APOE ε4 performed worse on verbal memory than did those without this allele. As in our study, they found no relationship between the presence of the ε4 allele and certain measures that presumably involve the frontal lobe (verbal fluency).

In a recent study, Chamelian et al. analyzed the relationship between possession of the APOE ε4 allele and both cognitive and behavioural measures in a predominantly mild TBI sample at six months postinjury. However, they found no association between the presence of the APOE ε4 allele and poor cognitive or behavioural outcome. We did observe a relationship between APOE ε4 carriers and both poor neuropsychological measures and an unfavourable neurobehavioral outcome and these differences may be due to the greater severity of our TBI sample.

The study by Millar et al. (2003) did not find differences either. The most important difference between the present report and Millar et al. 2003 is the significantly longer follow up after injury in their study: 15 to 25 years. This may mean that APOE ε4 possession is related more to acute than to chronic cognitive decline after severe TBI. It may also be related to the lack of consensus on the relationship between APOE genotype, TBI and later cognitive decline such as Alzheimer disease.
The mechanisms underlying the modulating effect of APOE in the acute response to brain injury remain unclear. ApoE is involved in synaptic repair, remodeling and regeneration in an isoform-specific way.[3][20] The apoE E4 isoform is believed to provide less neuroprotection and a lower capability for brain issue recovery and functional restoration than apoE E2 and E3 isoforms.[3] Therefore, it may be that APOE ε4 carriers are less able to avoid secondary damage, remove injury-induced degeneration products, or repair damaged tissue than those without this allele. The combined effect of these mechanisms may result in poorer neuropsychological performance and greater behavioural disturbances in the sub-acute phase.

In addition to the small sample size, other possible limitations should be borne in mind, such as the fact that premorbid ability was not well controlled. Years of education may not be a very good proxy for premorbid ability and the differences found may have been related to pre-existing cognitive differences.

In summary, we demonstrated an association between APOE isoforms and typical impairment after moderate and severe TBI. The poor cognitive and behavioural outcome of the APOE-ε4 carriers in moderate and severe TBI may be due to the worsening of the initial brain injury and the poor effectiveness of recovery in the presence of the apoE E4 isoform.

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