The Association of Apolipoprotein E Allele 4 Polymorphism with the Recovery of Sleep Disturbance after Mild Traumatic Brain Injury

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Abstract -

Purpose: Mild traumatic brain injury (mTBI) is a major public health concern. The apolipoprotein E (APOE) gene contains three polymorphisms, and the APOE4 polymorphism may affect several physiological states, such as the recovery from mTBI as well as sleep. This study aims to investigate the association between APOE4 with the recovery of sleep disturbance after mTBI.

Methods: From May 2012 to Aug 2015, 189 mTBI patients completed baseline (1st week post-mTBI) and follow-up (6th week post-mTBI) sleep assessments that involved using the Pittsburgh Sleep Quality Index (PSQI). APOE genotypes were determined by sequencing the products of polymerase chain reaction from genomic DNA. Statistical analyses were performed using the Wilcoxon signed-rank or chi-square test.

Results: Thirty-five (18.5%) participants were APOE4 carriers. At baseline, the demographic data and the severity of sleep disturbance were similar in both groups. APOE4 carriers demonstrated significant improvement in the overall PSQI score (8.34±3.9 at baseline and 7.43±3.99 at follow-up, p = 0.05) and scores of several PSQI subscales, including sleep disturbance, sleep latency, daytime dysfunction caused by sleepiness, and overall sleep quality, which was similar to APOE4 noncarriers.

Conclusion: APOE4 is not associated with the recovery of sleep disturbance after mTBI.

Key Words: mild traumatic brain injury, sleep, Apolipoprotein E4

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INTRODUCTION

Mild traumatic brain injury (mTBI) is a major public health concern affecting people globally. According to the survey conducted by World Health Organization, the incidence of hospital-treated mTBI is approximately 100–300/100,000 people; however, if mTBI cases not treated at hospitals are considered, the real incidence would be more than 600/100,000 people (1). A latest population-based study conducted in New Zealand demonstrated that the incidence may be up to 749/100,000 people (2). mTBI is defined as minimal changes in consciousness (Glasgow Coma Scale, 13–15) and full neurological recovery or minimal neurological deficit. More than direct damage, mTBI probably results in indirect and delayed effects to the brain, including inflammation and breakdown of the blood–brain barrier, thus triggering brain oedema and swelling, leukocyte infiltration, lactic acid accumulation, immunocompetent cell recruitment, and apoptosis in the damaged brain (reviewed by Laskowski et al (3)). These pathological events can be long-lasting and explain why patients sustaining repetitive mTBIs typically develop long-term neuropathological changes similar to those observed in neurodegenerative diseases (4).

Clinically, mTBI may cause neurological sequelae in many aspects, including changes in cognition and personality, psychiatric disorders, memory impairment, and sleep disturbance (5). Posttraumatic hypersomnia, excessive daytime sleepiness, fatigue, insomnia, sleep architecture changes or reduced sleep quality, increased wake after sleep onset, decreased sleep efficiency, and alteration in sleep stage proportion are common presentations of sleep disturbance in patients with mTBI and affect their quality of life (6-8).

The gene encoding Apolipoprotein E (APOE) is located at chromosome 19q13.2. APOE is a component of plasma lipoproteins and synthesised in the liver, brain, spleen, kidneys, gonads, adrenals, and macrophages (9,10). The Apolipoprotein E (APOE) gene is polymorphic and has three alleles: APOE ε2, ε3, and ε4, which produce three isoforms: APOE2, E3, and E4, respectively (11,12). APOE ε4 (APOE4) is a crucial genetic risk factor for Alzheimer disease (AD) and modulates APOE levels and regulates pathological amyloid-β (Aβ) accumulation, and it is involved in neuronal repair and remodelling (13).

APOE4 has been identified as a risk factor for the poor outcome of traumatic brain injury (TBI) (14-16). Sleep disturbance is one of the common sequelae of TBI and in our previous studies, we have demonstrated significant recovery from sleep disturbance 6 weeks after mTBI (17), and that a genetic polymorphism is associated with the recovery of sleep disturbance (18). Meanwhile, APOE genotypes are associated with the progression of sleep/wake disturbances in patients with AD (19) and the association between APOE4 allele is significantly associated with obstructive sleep apnoea/hypopnoea (20,21). However, the association between APOE polymorphisms and the recovery of mTBI-related sleep disturbance is still unknown. We hypothesise that APOE polymorphism, which is associated with the outcome of TBI and sleep disturbance, respectively, is associated with the recovery of sleep disturbance after mTBI.

METHODS

Participants and study design

This study was approved by the Taipei Medical University-Joint Institutional Review Board (TMU-JIRB). From May 2012 to Aug 2015, the participants in the current study were recruited from the emergency department of three hospitals: Taipei Medical University Hospital, Taipei Municipal Wan Fang Hospital (Managed by Taipei Medical University), and Taipei Medical University Shuang Ho Hospital. The inclusion criteria were age between 20 and 70 years, Glasgow Coma Scale of 13–15 and full neurological recovery or minimal neurological deficit, and sleep disturbance (5). Posttraumatic hypersomnia, excessive daytime sleepiness, fatigue, insomnia, sleep architecture changes or reduced sleep quality, increased wake after sleep onset, decreased sleep efficiency, and alteration in sleep stage proportion are common presentations of sleep disturbance in patients with mTBI and affect their quality of life (6-8).

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APOE genotyping

Genomic DNA was extracted using the Blood Genomic DNA Extraction Midiprep System (VIOGENE, Sunnyvale, CA, USA). An APOE DNA fragment containing rs429358 and rs7412 single-nucleotide polymorphisms (SNPs) was amplified using polymerase chain reaction (PCR) in the DNA Thermal Cycler (Perkin Elmer Cetus, Foster City, CA, USA) with forwards and reverse primers 5'-TCCAAGGACCTGAGGGCGGCGCA-3' and 5'-ACAGAATTCGCCCCGGCTGTAACGCTGCA-3', respectively. The PCR reaction mixture (25 µL) contained 1 µg of gDNA, 100 ng of primers, 0.4 mM each dNTP, 1X PCR buffer, and 1 unit of Taq polymerase (TOYOBO, Osaka, Japan). The reaction mixture was subjected to 35 cycles of 0.5 min at 94 °C for denaturation, 0.5 min at 61 °C for annealing, and 30 min at 72 °C for extension. All PCR products were sequenced at Tri-I Biotech (Tri-I Biotech Inc., New Taipei City, Taiwan), and the genotypes were determined using Sequencher 5.4® (Gene Codes Corporation, Ann Arbor, MI USA).

Outcome measure

Subjective sleep quality over the previous month was evaluated using the Pittsburgh Sleep Quality Index (PSQI), a 19-item, self-rated questionnaire. The 19 items of the PSQI are categorised into seven subscales: sleep latency, sleep duration, daytime dysfunction caused by sleepiness, habitual sleep efficiency, sleep disturbance, subjective sleep quality, and sleeping medication. Each subscale is scored from 0 (no problem) to 3 (severe), and the scores are summed into an overall score ranging from 0 to 21. A higher score indicates a worse sleep problem. The Chinese version of the PSQI was administered to the study participants. At both baseline and 6-week follow up, the written-form of PSQI was filled out by patients themselves.

Statistical analyses

Descriptive statistics (e.g. mean ± standard deviations, frequencies with percentage) were calculated for all variables. Nonparametric statistics were calculated using Wilcoxon signed-rank and chi-square tests for comparison of APOE4 carriers with APOE4 noncarriers. Statistical significance was set at $p < 0.05$, and statistical analyses were performed using R, Version 3.2.2. (Copyright© 2015, The R Foundation for Statistical Computing).

RESULTS

Table 1 presents the comparison of baseline demographic characteristics between groups. One hundred and thirteen (59.8%) were women. Most mTBIs were caused by traffic accident (47.6%), followed by falls (35.4%). Thirty-five (18.5%) participants were APOE4 carriers. The APOE4 carrier and noncarrier groups were similar in gender (women: 54.3% and 61.0%, respectively), age (42.2±14.7 and 40.1±15.2 years, respectively), and the rest of demographic data.

The average overall PSQI scores of all participants were 8.3±3.9 and 8.5±4.3 among APOE4 carrier and noncarrier at baseline, respectively. There was no significant difference observed in the overall score and all subscale scores of the PSQI at baseline between the

<table>
<thead>
<tr>
<th>Table 1. Demographic data of all participants and APOE4 carriers and noncarriers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample size</strong></td>
</tr>
<tr>
<td><strong>Female (percentage)</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
</tr>
<tr>
<td><strong>Smoker (%)</strong></td>
</tr>
<tr>
<td><strong>GCS</strong></td>
</tr>
<tr>
<td><strong>Mode of injury</strong></td>
</tr>
<tr>
<td><strong>Traffic accident</strong></td>
</tr>
<tr>
<td><strong>Falls</strong></td>
</tr>
<tr>
<td><strong>Other</strong></td>
</tr>
</tbody>
</table>

GCS: Glasgow Cowa Scale
**Table 2.** Overall and subscale scores of Pittsburgh Sleep Quality Index (PSQI) at baseline between APOE4 carriers and noncarriers

<table>
<thead>
<tr>
<th></th>
<th>APOE4 carriers</th>
<th>APOE4 noncarriers</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall PSQI</td>
<td>8.34±3.9</td>
<td>8.51±4.38</td>
<td>0.97</td>
</tr>
<tr>
<td>PSQI-1 (Duration of sleep)</td>
<td>1.26±1.22</td>
<td>1.29±1.23</td>
<td>0.83</td>
</tr>
<tr>
<td>PSQI-2 (Sleep disturbance)</td>
<td>1.49±0.66</td>
<td>1.34±0.65</td>
<td>0.33</td>
</tr>
<tr>
<td>PSQI-3 (Sleep latency)</td>
<td>1.54±0.78</td>
<td>1.40±1.02</td>
<td>0.34</td>
</tr>
<tr>
<td>PSQI-4 (Daytime dysfunction caused by sleepiness)</td>
<td>1.14±0.85</td>
<td>1.00±0.89</td>
<td>0.33</td>
</tr>
<tr>
<td>PSQI-5 (Sleep efficiency)</td>
<td>0.91±1.04</td>
<td>1.20±1.29</td>
<td>0.45</td>
</tr>
<tr>
<td>PSQI-6 (Overall sleep quality)</td>
<td>1.86±0.73</td>
<td>1.77±0.81</td>
<td>0.55</td>
</tr>
<tr>
<td>PSQI-7 (Use of sleep medication)</td>
<td>0.14±0.60</td>
<td>0.51±1.07</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

* p-value <0.05

**Table 3.** Differences in overall and subscale scores of Pittsburgh Sleep Quality Index (PSQI) between baseline and the sixth week for APOE4 carriers and noncarriers.

<table>
<thead>
<tr>
<th></th>
<th>APOE4 carriers (n = 35)</th>
<th>APOE4 noncarriers (n = 154)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall PSQI</td>
<td>Baseline</td>
<td>8.34±3.9</td>
<td>8.51±4.38</td>
</tr>
<tr>
<td>PSQI-1 (Duration of sleep)</td>
<td>Baseline</td>
<td>1.26±1.22</td>
<td>1.29±1.23</td>
</tr>
<tr>
<td>PSQI-2 (Sleep disturbance)</td>
<td>Baseline</td>
<td>1.49±0.66</td>
<td>1.34±0.65</td>
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<tr>
<td>PSQI-4 (Daytime dysfunction caused by sleepiness)</td>
<td>Baseline</td>
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<tr>
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<td>0.51±1.07</td>
</tr>
</tbody>
</table>

* p-value <0.05

**Table 4.** Differences of Pittsburgh Sleep Quality Index (PSQI) at baseline and the sixth week between APOE4 carriers and noncarriers.

<table>
<thead>
<tr>
<th>Difference</th>
<th>APOE4 carriers</th>
<th>APOE4 noncarriers</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall PSQI</td>
<td>-0.91±3.11</td>
<td>-0.45±3.44</td>
<td>0.41</td>
</tr>
<tr>
<td>PSQI-1 (Duration of sleep)</td>
<td>0.23±0.91</td>
<td>0.00±1.24</td>
<td>0.22</td>
</tr>
<tr>
<td>PSQI-2 (Sleep disturbance)</td>
<td>-0.31±0.68</td>
<td>-0.15±0.69</td>
<td>0.24</td>
</tr>
<tr>
<td>PSQI-3 (Sleep latency)</td>
<td>-0.23±0.84</td>
<td>-0.06±0.98</td>
<td>0.16</td>
</tr>
<tr>
<td>PSQI-4</td>
<td>-0.37±1.00</td>
<td>-0.06±0.93</td>
<td>0.10</td>
</tr>
<tr>
<td>PSQI-5 (Sleep efficiency)</td>
<td>0.23±1.11</td>
<td>-0.05±1.32</td>
<td>0.20</td>
</tr>
<tr>
<td>PSQI-6 (Overall sleep quality)</td>
<td>-0.43±0.81</td>
<td>-0.19±0.79</td>
<td>0.10</td>
</tr>
<tr>
<td>PSQI-7 (Use of sleep medication)</td>
<td>-0.03±0.38</td>
<td>0.05±0.79</td>
<td>0.96</td>
</tr>
</tbody>
</table>

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APOE4 carrier and noncarrier groups except the use of sleep medicine, which is significantly higher among noncarrier (Table 2).

Both APOE4 carriers and noncarriers exhibited improvement in overall PSQI scores between baseline and 6th weeks follow-up (carrier: baseline 8.34±3.9, 6th week: 7.43±3.99, p=0.05; noncarrier: baseline 8.51±4.38, 6th week: 8.05±3.80, p=0.03). In the subscale of PSQI, both group demonstrated significant improvement in the aspect of “Sleep disturbance” and “Overall sleep quality”, while carrier also significantly improved in “Daytime dysfunction caused by sleepiness” (Table 3). Comparing the differences of PSQI at baseline and the 6th week between APOE4 carriers and noncarriers also showed no significant different (Table 4).

**DISCUSSION**

The current study demonstrated that the APOE4 polymorphism is not associated with the recovery of sleep disturbance after mTBI. Both APOE4 carriers and noncarriers exhibited significant improvement in sleep quality at the 6th week follow-up. To the best of our knowledge, it is the first report to investigate the association between APOE4 with the recovery of sleep disturbance after mTBI.

The association between sleep disturbance and APOE4 has been investigated. Some studies claim that APOE4 carriers exhibit a higher probability of developing moderate-to-severe sleep-disordered breathing (20,24). Besides, compared with noncarriers, APOE4 carriers with mild cognitive impairment demonstrated a reduction in rapid eye movement sleep, which is associated with memory consolidation (25). However, the underlying mechanism is ambiguous and the association was not consistent. Saarelainen et al. suggested the lack of association between APOE4 and obstructive sleep apnea (26). A meta-analysis pooled 8 studies together also fail to demonstrate the association (27). The present study revealed that the quality of sleep measured by PSQI was identical between carriers and noncarriers 1-week and 6-week after mTBI, which also indicated the lack of association between APOE4 and sleep disturbance after mTBI.

APOE4 has been found to be associated with overall recovery from TBI (28). APOE4 carriers have a twofold risk of an unfavourable outcome 6 months after head injury (29). Considerably few APOE4 carriers achieve favourable functional recovery (30), and APOE4 negatively affects the outcome of rehabilitation and memory performance in patients with TBI (31,32). For mTBI, Sundström et al found that APOE4 affected the neuropsychological outcome after mild head injury, but Liberman et al argued that APOE4 only affected the severity of acute injury but not the recovery pattern (28,33). In addition, late cognitive decline to occur after TBI is not associated with the APOE genotype and TBI increased the risk for AD in APOE4 noncarriers only (34,35), which hint APOE4 is not consistently associated with the outcome after TBI. The present study revealed that the recovery of sleep disturbance was identical in both APOE4 carrier and noncarrier groups, and there was no significant difference in all subscale of PSQI. It speculates that APOE4 is not associated with the recovery of sleep disturbance after mTBI.

The current study has some limitations. The sample size is only 189 and only 18.5% were APOE4 carriers, thus limiting the possibility of reaching high statistical power. Moreover, the drop-out rate is high, because mTBI is not a severe disease; hence, participants may be unwilling to revisit for follow-up after recovery from symptoms. Fortunately, there was no significant difference among the demographic data between drop-out and retained group. The present study recruited more female participants, which may be in contrast with the fact that male were in majority of mTBI patients. The use of the self-reported PSQI questionnaire may also have disadvantages such as overestimation, recall bias, social desirability bias, and errors in self-observation.

In conclusion, the current study showed that APOE4 allele was not associated with the recovery of sleep disturbance 6 weeks after mTBI. A longer follow-up period may be required for further investigation about the APOE4-associated consequence after mTBI, such as cognitive decline and the risk of AD.

**CONTRIBUTORSHIP STATEMENT**

Hsun-Hua Lee: study design, data collection, drafting of the manuscript and its final approval

Chun-Ting Yeh: study design, data collection, drafting of...
the manuscript and its final approval  
Ju-Chi Ou: study design, data analysis, drafting of the manuscript and its final approval  
Hon-Ping Ma: acquisition of data and its final approval  
Kai-Yun Chen: acquisition of data and its final approval  
Cheng-Fu Chang: acquisition of data and its final approval  
Jing Huei Lai: acquisition of data and its final approval  
Kuo-Hsing Liao: acquisition of data and its final approval  
Chien-Min Lin: acquisition of data and its final approval  
Dean Wu: acquisition of data and its final approval  
Yao-Hsien Huang: acquisition of data and its final approval  
Chaur-Jong Hu: study design, data collection, drafting of the manuscript and its final approval  
Chien-Tai Hong: study design, data collection, drafting of the manuscript and its final approval  

Statement of competing interests  
All the authors have no conflict of interest to declare  

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Data sharing statement  
There is no additional data available  

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