Association between the Apolipoprotein E4 and Postoperative Cognitive Dysfunction in Elderly Patients Undergoing Intravenous Anesthesia and Inhalation Anesthesia

Yingmin Cai, M.D.*, Haitao Hu, M.D.,† Pengbin Liu, M.D.,‡ Gaifeng Feng, M.D.,§ Weijiang Dong, M.D.,§ Bing Yu, M.D.,∥ Yulin Zhu, M.D.,# Jinxin Song, M.D.,** Minggang Zhao, M.D.††

ABSTRACT

Background: Intravenous and inhalation anesthesia are commonly used in the clinical setting. Recovery of cognitive function in elderly patients after surgery has received increased attention. In this study, the authors compared recovery of cognitive function in patients after different anesthesia techniques, and investigated which technique is safer. The authors also explored association between apolipoprotein E4 and postoperative cognitive dysfunction in patients undergoing general anesthesia.

Methods: A total of 2,000 patients were equally and randomly divided into intravenous and inhalation anesthesia groups. Total intravenous and inhalation anesthesia were used. Within 10 days after surgery, cognitive function was assessed daily using the Mini-Mental State Examination (MMSE). Restriction fragment

What We Already Know about This Topic

- Gene polymorphism of apolipoprotein E (ApoE) has been associated with development of dementia. Information on a possible role of ApoE in postoperative cognitive dysfunction is scarce.

What This Article Tells Us That Is New

- This study (single center, case-controlled) in 2,000 patients showed an association between ApoE4 and the development of transient postoperative dysfunction in elderly patients after inhalational anesthesia.

Results: MMSE score in inhalation preoperative baseline group significantly decreased at day 3 after surgery compared with the preoperative and intravenous anesthesia group. The proportion of patients scoring less than 25 points was significantly greater in the inhalation anesthesia group than in the intravenous anesthesia group at 3 days after surgery. In the inhalation anesthesia group, the decrease in MMSE score was closely related with apolipoprotein E e4 allele. In the intravenous anesthesia group, the decrease in MMSE score was not correlated with apolipoprotein E e4 allele.

Conclusions: There was a strong association between the apolipoprotein E e4 and postoperative cognitive dysfunction in elderly patients undergoing inhalation anesthetics.

TOTAL intravenous anesthesia (TIVA) has been used long before propofol was introduced. TIVA has seen several major developments since it was first introduced. Since the synthesis of the first intravenous anesthetics, TIVA has evolved until the development of TIVA with target-controlled infusion pumps. The first pharmacokinetic model for the use of target-controlled infusion pumps. The first pharmacokinetic model for the use of target-controlled infusion was described by Schwilden in 1981. By using propofol with the target-controlled infusion technique, it is possible to maintain constant concentration in blood. In addition, due to the ultra-short effect of propofol, the anesthesiologist can accurately estimate the time of recovery. Because the drug has a rapid onset of action, the plasma concentration ap-

Anesthesiology. V116 • No 1 January 2012 84

Copyright © 2011, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2012; 116:84–93

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/116/1/84/255712/0000542-201201000-00019.pdf by guest on 12 September 2020
proximates the target concentration and anesthesia can be fully controlled. Thus, TIVA became possible.

There have been many studies of cognitive function after anesthesia and surgery. Potential mechanisms for either cognitive disorder or neurodegeneration after general anesthesia are numerous, including N-methyl-D-aspartate-mediated excitotoxicity, oxidative stress, suppression of cholinergic signal transduction, and enhancement of protein oligomerization. Gene polymorphism of apolipoprotein E (ApoE) residing on chromosome 19 is associated with senile dementia. The ApoE polymorphism of apolipoprotein E (ApoE) residing on chromosome 19 is associated with senile dementia. The ApoE polymorphism of apolipoprotein E (ApoE) residing on chromosome 19 is associated with senile dementia.

The ApoE gene contains three allele genes, e2, e3, and e4, which can make up six genotypes of homozygote and heterozygote, and six types of genetic phenotypes. In normal elderly people, the occurrence frequency of ApoE e3 gene is the highest and the gene frequency of ApoE e2 and e4 is lower, thus the genotype of e3/3 is most common and e2/2, e4/4 are least common. The e4 allele gene frequency has been shown to be increased in patients with senile dementia, and the e4 allele genotype is correlated with the risk of senile dementia. However, little attention has been paid to the relationship between ApoE and outcome after anesthesia. We hypothesize that there is an association between the ApoE e4 and postoperative cognitive dysfunction in patients undergoing general anesthesia. In the current study, we tested the hypothesis that there was an association between transient postoperative cognitive dysfunction in elderly patients and general anesthesia, there was an association between the ApoE e4 and postoperative cognitive dysfunction, and there was an association between the ApoE e4 and transient postoperative cognitive dysfunction in patients undergoing intravenous or inhalation anesthesia. To test the hypothesis, we designed the clinical study presented here. In this study, we focused our investigation on the association between the ApoE e4 allele and postoperative cognitive dysfunction in patients undergoing intravenous and inhalation anesthesia.

Materials and Methods

This study received the Institutional Review Board approval from the Second Affiliated Hospital, Medical College, Xi’an Jiaotong University, and all patients signed an informed consent form before the start of any procedures.

Subjects

Because ApoE single nucleotide polymorphism varies among people with different ethnic backgrounds and living in different regions, only the data from the same ethnic group is of scientific significance. Although China has a total of 56 ethnic groups, Han represents more than 90% of the total Chinese population. In our hospital more than 95% patients are of Han ethnicity. Because research in gene polymorphism requires a large sample size and our hospital cannot supply enough cases for the minority ethnicities, the few non-Han patients were excluded from our study. There were a total of 5,026 elderly Han patients (approximately 70 yr old) who were scheduled to undergo general anesthesia and screened for the study.

Methodology

This is a single-site, case-controlled, 1:1 ratio, parallel-group, block-randomization study in the Second Affiliated Hospital, Medical College, Xi’an Jiaotong University. Eligible participants were patients undergoing surgery in the Second Affiliated Hospital. Participants were randomly assigned after block randomization procedures (computerized random numbers) to one of two treatment groups. From 2005 to 2010, there were a total of 5,026 elderly Han patients (approximately 70 yr old) who were scheduled to undergo general anesthesia and screened for the study. Among screened patients, 3,026 patients were excluded either because they did not meet the inclusion criteria, or did not consent to be enrolled in the study, or required postoperative intensive care (because of bleeding, inflammation, respiratory failure, heart failure, anastomotic leaks, etc.), or required postoperative sedation, and as a result, were also excluded in the final data analysis (fig. 1). The 2,000 patients described in this study represent only those who were enrolled and followed up for the entire duration of the study. A total of 2,000 patients, who were American Society of Anesthesiologists Physical Status I–II, without genetic connection, were enrolled in this study. There were 1,140 males and 860 females, with a mean age of 70.1 ± 4.6 yr, and weight of 57.3 ± 7.5 kg. Diagnoses for the enrolled patients included esophageal cancer, gastric cancer, renal carcinoma, and fracture (table 1). Patients with severe heart or lung diseases or hepatic or renal dysfunction were excluded. Elderly patients with symptoms of dementia were excluded according to diagnostic criteria for dementia of the third revised edition of the American Psychiatric Disease Diagnosis and Statistics Handbook, and Folstein MMSE. The rationale for the exclusion is that if elderly people with senile dementia were included, we would not be able to distinguish whether the postoperative cognitive deficit arose from preoperative senile dementia or the insult of general anesthesia. Patients with MMSE score ≥ 25 points and Hachinski ischemic score ≤ 3 points have, at most, minimal cognitive dysfunction before general anesthesia. The test methods were described to and accepted by the patients according to the 33rd item of The Hospital Manage Regulations of State Council of China in 2005. Written preoperative informed consent, regarding anesthesia, was obtained from all patients. The patients were randomized into an intravenous anesthesia group and an inhalation anesthesia group. No statistical difference in baseline data of patients in two groups was detected (P > 0.05) (table 2).

In our study, anesthesiologists treated the patients. The patients were blinded to the anesthetic technique. Psychiatrists did the postoperative mental state examination; both patients and psychiatrists were blinded to the anesthetic technique. The examinations were performed once a day for 10 days. The staff of forensic medicine analyzed ApoE gene. They were also blinded to the anesthetic technique.

The primary outcome was MMSE, frequency distribution of ApoE alleles and genotypes. The primary outcomes should be held to a conservative type I error rate, and three primary outcomes should be interpreted at the 0.05/3 = 0.0167 level. P < 0.01 was considered statistically significant.
This trial was overseen by the Department of Statistics, Medical College, Xi’an Jiaotong University.

**Extraction of Genome DNA**

Before anesthesia, a blood sample (3 ml) from each patient was placed in ethylenediamine tetraacetic acid (1 mg/ml) for 1 h, and centrifuged to obtain plasma. Genomic DNA from whole blood was extracted in accordance with instructions from the DNA extraction kit (Bocai, Shanghai, China). Genome DNA was dissolved in tris EDTA buffer at $-20^\circ C$ for analysis.15

**Amplification of ApoE Gene using Polymerase Chain Reaction**

The amplification method was the same as previously published.16 Polymerase chain reaction conditions were as follows: the amplified gene fragments were located in ApoE gene exon 4. ApoE gene primer was designed and synthesized as follows: upstream: 5'-ACA GAA TTC GCC CCG CCG GCC TGG TAC AC-3'; downstream: 5'-TAA GCT TGG CAC GGC TGT CCA AGG A-3'. The total volume of polymerase chain reaction was 50 μl, including 50–100 ng template DNA, 2 mM deoxynucleoside triphosphate, 20 pmol upstream primer, 20 pmol downstream primer, 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl, pH 8.3, 1.5 U TaqDNA polymerase. The cycle profile was as follows: 95°C for 3 min; 35 cycles of 94°C for 45 s, 62°C for 45 s, 72°C for 30 s; 72°C for 10 min. The reactions were electrophoresed through agarose gel. Products (244 base pairs) were generated.17

**Restriction Fragment Length Polymorphism of ApoE Gene**

In accordance with the previous study,16 5 μl amplified products were electrophoresed through a 2% agarose gel to detect target DNA fragment. After purification, polymerase chain re-

---

**Fig. 1.** Flow diagram of a single-site trial of intravenous anesthesia versus inhalation anesthesia. The diagram includes detailed information on the excluded participants.
action products were digested with 6 U Hha I endonuclease and buffer solution at 37°C for 4 or 5 h, and then electrophoresed through a 15% polyacrylamide gel. After ethidium bromide staining, DNA type was observed with an ultraviolet transreflectometer. ApoE genotyping was performed by restriction fragment length polymorphism analysis using DNA extracted from buffy coats of whole blood samples. DNA samples were amplified with polymerase chain reaction and digested with the appropriate restriction enzymes.18

Anesthetic Method
All patients received 10 mg diazepam and 0.5 mg atropine, intramuscularly, 30 min before general anesthesia. A three-lumen venous catheter was used for infusion of fentanyl, propofol, and muscle relaxant. A catheter was put into the left radial artery to collect blood samples. The three-lumen central venous catheter was used for measuring central venous pressure. In the intravenous anesthesia group, the fentanyl target level was 5.12 ng/ml.19 The loading dose of fentanyl was 4 μg/kg, followed by continuous infusion at a rate of 0.03 μg/kg/min; propofol target level was 3 μg/ml,19 the loading dose of propofol 3 mg/kg, followed by continuous infusion at a rate of 53.8 μg/kg/min, which was injected by 6 steps with gradual increase in concentration; the initial target level was 1 μg/ml, and increased by 0.4 μg/ml with an interval of 150 s; vecuronium bromide target level was 2.0–2.5 μg/ml, the loading dose of vecuronium bromide 0.08 mg/kg, followed by continuous infusion at a rate of 0.5 μg/kg/min. Neuromuscular monitoring was obtained with force displacement transducers attached to each adductor pollicis. Tracheal intubation was performed once adductor pollicis muscular response obtained with train-of-four at 0.05 Hz was abolished20 and the bispectral index reached 40–60.

In the inhalation anesthesia group, the loading dose of fentanyl 4 μg/kg, followed by continuous infusion at a rate of 0.03 μg/kg/min; the loading dose of propofol 3 mg/kg, followed by continuous inhalation 2–3% end-tidal concentration of isoflurane, which was used for maintenance of anesthesia. The end-tidal concentration of isoflurane was administered to maintain a normal depth of anesthesia using the BIS™ monitor (Aspect, Newton, MA) and traditional signs of anesthetic depth. The loading dose of vecuronium was 0.08 mg/kg, followed by continuous infusion at a rate of 0.5 μg/kg/min. Neuromuscular monitoring was obtained with force displacement transducers attached to each adductor pollicis. Tracheal intubation was performed once adductor pollicis muscular response obtained with train-of-four at 0.05 Hz was abolished and the bispectral index reached 40–60. Lactated Ringer’s solution and hetastarch were used during surgery.

Respiration was assisted by ventilation via mask in case of increasing respiratory depression, and mechanical ventilation maintained to achieve a specific end-tidal carbon dioxide after intubation. Ventilation was adjusted to maintain the end-tidal carbon dioxide at 35 ± 5 mmHg.

Patient Monitoring
The Model 883 Multi-function Monitor (Hewlett-Packard, Palo Alto, CA) was used to monitor heart rate, electrocardiogram, blood pressure, oxygen saturation, and neuromuscular monitoring. Model A-2000 Monitor (Aspect, Newton, MA) was used to measure bispectral index. Within 10 days after surgery, cognitive function was assessed using MMSE daily.

Main Outcome Measures
Correlation of ApoE gene frequency and ApoEε4 allele to MMSE score was measured.

Statistical Analysis
Two-tailed tests were used. The data of physiologic and biochemical index were compared by the Z test. The data of

### Table 1. Diagnosis and Frequencies in Intravenous and Inhalation Groups

<table>
<thead>
<tr>
<th>Operations</th>
<th>Intravenous Anesthesia Group</th>
<th>Inhalation Anesthesia Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagectomy</td>
<td>23.2</td>
<td>23.5</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>12.3</td>
<td>12.1</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>3.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Fracture reduction</td>
<td>59.3</td>
<td>58.9</td>
</tr>
<tr>
<td>Others</td>
<td>1.6</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*P* value is greater than 0.05; no statistical difference between the two groups.

### Table 2. The Baseline Data of Patients in Two Groups

<table>
<thead>
<tr>
<th>Item</th>
<th>Intravenous Anesthesia Group (n = 1,000)</th>
<th>Inhalation Anesthesia Group (n = 1,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>71.2 ± 3.8</td>
<td>69.3 ± 5.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.1 ± 6.9</td>
<td>60.3 ± 5.7</td>
</tr>
<tr>
<td>Sex (n)</td>
<td>Male 570</td>
<td>570</td>
</tr>
<tr>
<td></td>
<td>Female 430</td>
<td>430</td>
</tr>
<tr>
<td>MMSE (point)</td>
<td>27.25 ± 1.13</td>
<td>7.45 ± 1.08</td>
</tr>
<tr>
<td>HIS (point)</td>
<td>2.39 ± 0.31</td>
<td>2.41 ± 0.29</td>
</tr>
<tr>
<td>Past medical history</td>
<td>No serious heart, lung diseases, hepatic or renal dysfunction</td>
<td>No serious heart, lung diseases, hepatic or renal dysfunction</td>
</tr>
<tr>
<td>Living area</td>
<td>Northwest China</td>
<td>Northwest China</td>
</tr>
<tr>
<td>Nation</td>
<td>Han</td>
<td>Han</td>
</tr>
<tr>
<td>Operations</td>
<td>Esophagectomy</td>
<td>Esophagectomy</td>
</tr>
<tr>
<td></td>
<td>Gastrectomy</td>
<td>Gastrectomy</td>
</tr>
<tr>
<td></td>
<td>Nephrectomy</td>
<td>Nephrectomy</td>
</tr>
<tr>
<td></td>
<td>Fracture reduction</td>
<td>Fracture reduction</td>
</tr>
<tr>
<td>Length of anesthesia (h)</td>
<td>3.32 ± 0.12</td>
<td>3.26 ± 0.09</td>
</tr>
</tbody>
</table>

*P* value is greater than 0.05, thus no statistical difference between the two groups.

HIS = Hachinski ischemic score; MMSE = Mini-Mental State Examination.
MMSE examined across groups by day were analyzed by two-way ANOVA with repeated measures, and the multiple means of MMSE were compared with multiple comparison. P ≥ 0.05 was considered as no statistical difference between the two groups, whereas P < 0.01 was considered significant. Gene frequency was obtained by gene count. Genotype and gene frequency were analyzed using the chi-square test. In two groups, the patient whose MMSE score was less than 25 points at day 1 was selected to perform the statistical comparison. This study was powered to detect clinically significant differences in the primary outcome. An a priori power calculation revealed that to have power of 80%, with a two-tailed α level of 0.05, a sample size of n = 2,000 would allow detection of odds ratios (OR) as small as 1.4. Differences smaller than this magnitude are not likely to be clinically meaningful. The association of ApoE ε4 allele frequency and ApoE ε4 allele to MMSE score was measured through calculating OR and 95% CI. P ≥ 0.05 was considered as no statistical difference between the two groups, whereas P < 0.01 was considered significant. The STATA4.0 medical statistic software (Stata Corporation, College Station, TX) was supplied by Department of Statistics, Medical College, Xi’an Jiaotong University, China.

Results
Quantitative Analysis of Participants
A total of 2,000 patients were included in final analysis, of a total of 5,026 patients that were screened for the study.

MMSE Score in Patients of Both Groups
With an ANOVA test, two groups were found significantly different regarding time and group factor (P < 0.01). For that reason, two groups were analyzed with the Bonferroni correction post hoc test to address the three stages at different time points that made a significant difference inside a group and to address the three stages at the same time point that made a significant difference between the two groups (P < 0.01). Before the surgery, there was no difference in MMSE scores between inhalation group and intravenous group. After the surgery, the MMSE score in the intravenous group remained unchanged during the entire 10 days of monitoring. For patients with inhalation anesthesia, however, the MMSE score at days 1, 2, and 3 after surgery decreased significantly (P < 0.01) when compared with its own preoperative value, and also significantly (P < 0.01) when compared with the intravenous group value for the corresponding date. Interestingly, by day 10, MMSE score in the inhalation group recovered to preoperative level. P < 0.01, versus before surgery; P < 0.01, versus the intravenous anesthesia group (fig. 2).

Proportion of Patients of Various Scores in Both Groups
The proportion of patients scoring less than 25 points was significantly greater in the inhalation anesthesia group than in the intravenous anesthesia group 3 days after surgery (P < 0.01) (fig. 3). It is worth noting that more than 10% of patients in the inhalation anesthesia group received a MMSE score of less than 20 points, compared with 0% in the intravenous anesthesia group.

Frequency Distribution of ApoE Genotypes in Both Groups
ApoE alleles and gene frequency were calculated in accordance with the Hardy–Weinberg equilibrium law in patients of the intravenous anesthesia and inhalation anesthesia groups. As figure 4 displayed, the frequency of ε3/ε3 was the highest, followed by ε2/ε3, and ε2/ε4 was the lowest. No statistically significant effect in gene frequency was found between the two groups.

Frequency Distribution of ApoE Genotypes in Patients Scoring Various MMSE Scores of the Inhalation Anesthesia Group
As figure 5 shows, in patients scoring ≥ 25 points, ε3/ε3 frequency was the highest, followed by ε3/ε4, and ε4/ε4 was the
Frequency Distribution of ApoE Genotypes in Patients Scoring Various MMSE Scores in the Inhalation Anesthesia Group

As figure 6 exhibited, the frequency of ε3/ε3 was the highest, followed by ε3/ε4, and ε2/ε2 was the lowest in all patients, regardless whether the MMSE score is < or ≥ 25 points.

Association between ApoE ε4 Allele and MMSE Scores in Inhalation Anesthesia and Intravenous Anesthesia Groups

In the inhalation anesthesia group, 113 patients scoring less than 25 points and 146 patients scoring ≥ 25 points carried the ApoE ε4 allele, and the gene frequency was 25.69% and 9.36%, respectively. The decrease in MMSE score was closely related with the ApoE ε4 allele (OR = 3.31; 95% CI: 1.25–6.39, P < 0.05). In the intravenous anesthesia group,
Postoperative cognitive dysfunction (POCD) is related to the score was not associated with ApoE frequency was 16.3% and 15.72%. The decrease in MMSE scoring 15 patients scoring less than 25 points and 300 patients intravenous anesthesia group (n/%). The results show that the frequency of MMSE scores and ApoE alleles in patients of various MMSE scores in the intravenous anesthesia group (n/%). The results show that the frequency of e3 was the highest, followed by e4, and e2 was the lowest in all patients, regardless of whether the MMSE score is < or ≥ 25 points. The group of MMSE less than 25 points (n = 46) versus the group of MMSE ≥ 25 points (n = 954) and P value of e4 is greater than 0.05, thus no statistical difference between the two groups. The results suggest that there was no significant association between MMSE scores and ApoE e4 allele in the intravenous anesthesia group. (B) Frequency distribution of ApoE alleles in patients of various MMSE scores in the intravenous anesthesia group (n/%). The results show that the frequency of ε3/ε3 was the highest and the group of MMSE less than 25 points (n = 46) versus the ApoE ε4 allele was more likely to develop neuropsychologic dysfunction after general anesthesia. It was considered that the change of brain metabolism induced by anesthetics is one of the factors that induces POCD. To be sure of the safety of patients, the anesthetic used in the study should be commonly used or have a long history in clinical study. The two important anesthetics that were compared in the study were isoflurane in the inhaled anesthesia group and propofol in the intravenous anesthesia group, both of which have been widely used in China and around the world. As such, isoflurane and propofol were chosen for our study.

The results presented here are the first clinical study illustrating the association between the ApoE4 and POCD in elderly surgical patients undergoing either intravenous anesthesia or inhalation anesthesia. There were many basic or clinical research studies on about ApoE or POCD, but none of them focused on the association between them. This is the first study demonstrating that POCD is relevant to ApoE single nucleotide polymorphism.

Our results show that MMSE scores were significantly decreased day 1, 2, and 3 after surgery in the inhalation anesthesia group in comparison with the intravenous anesthesia group and before surgery. Decreased MMSE scores indicated deficits in cognitive function. The proportion of patients scoring less than 25 points was significantly greater in the inhalation anesthesia group, compared with the intravenous anesthesia group 3 days after surgery. This finding is similar to the reports by Tan et al. and Ruindshagen et al., although the latter study comprised clinical observational studies and did not offer any reasons for the observation.

Discussion

Postoperative cognitive dysfunction (POCD) is related to factors such as physical status, drugs, advanced age, Alzheimer disease, and the abnormality of brain function/metabolism. An early report indicated that isoflurane anesthesia causes heterogeneous changes in local cerebral blood flow and metabolism. A clinically relevant concentration of isoflurane has been reported to induce apoptosis and increase amyloid β generation and aggregation, which is a key event in the pathogenesis of Alzheimer disease. These findings may suggest that the use of this inhalational anesthetic agent in elderly patients could potentially increase the risk of postoperative cognitive dysfunction.

The ApoE4 allele is a known risk factor for Alzheimer disease and has variably been associated with worse outcome after hemorrhagic and ischemic stroke in humans. It also has been associated with delayed cognitive impairment after cerebral ischemia.

In clinical practice, we have observed that even if known factors had been excluded, there were still some patients who suffered from POCD. We hypothesized that there must be some other factors that we did not know about. In the current study, we designed the case-control study, excluding some complicating factors such as patients with severe heart or lung disease or hepatic or renal dysfunction. Elderly patients with symptom of dementia were also excluded. No statistically significant effect in baseline data of patients in the two groups was detected.

Given that an increasing number of elderly patients undergoing general anesthesia suffer from POCD, we focused our current study to test the hypothesis that patients harboring the ApoE-ε4 allele were more likely to develop neuropsychologic dysfunction after general anesthesia.

The ApoE4 allele in the intravenous anesthesia group. ApoE = Apolipoprotein E.

15 patients scoring less than 25 points and 300 patients scoring ≥ 25 points carried the ApoE ε4 allele, and the gene frequency was 16.3% and 15.72%. The decrease in MMSE score was not associated with ApoE ε4 allele (OR = 0.93; 95% CI: 0.37–2.39, P > 0.05).

**Fig. 6.** (A) Frequency distribution of ApoE alleles in patients of various Mini-Mental State Examination (MMSE) scores in the intravenous anesthesia group (n/%). (B) Frequency distribution of ApoE alleles in patients of various MMSE scores in the intravenous anesthesia group (n/%).
To determine the reason why some patients’ MMSE score were less than 25 in the inhalation anesthesia group whereas no patient with a score less than 25 was observed in the intravenous anesthesia group, we measured the ApoE genotypes for both groups of patients. Our results demonstrated that there were no statistically significant effects in frequency distribution of ApoE genotypes between the inhalation and intravenous anesthesia groups, suggesting that ApoE distribution itself is not a contributing factor. However, further analysis of ApoE distribution and MMSE score revealed that the frequency of the e4 allele (Z = 3.15, P < 0.01) and e4/e4 gene (Z = 2.61, P < 0.01) was greatest in patients with a score less than 25 points than in patients with a score ≥ 25 points. Therefore, the presence of the e4 allele and e4/e4 gene is of great importance and should receive more attention in the clinic. To further confirm the association between ApoE e4 and postoperative cognitive dysfunction, the OR and 95% CI were calculated to analyze the correlation between the ApoE e4 allele and MMSE scores. Results indicated that lower MMSE scores correlated with the presence of the ApoE e4 allele in the inhalation anesthesia group (OR = 3.31, P < 0.05). These results provide the direct evidence for a strong association between the ApoE4 and transient postoperative cognitive dysfunction in patients undergoing inhalation anesthesia. In contrast, presence of the ApoE e4 allele did not correlate with reduced MMSE scores in the intravenous anesthesia group (OR = 0.93, P > 0.05).

No statistically significant effect was found with regard to genotype and allele frequency in the inhalation and intravenous anesthesia groups. However, there was a statistically significant effect in the number of patients who developed cognitive dysfunction after surgery. In the intravenous anesthesia group, some patients suffered from cognitive dysfunction, and their MMSE scores ranged from 20 to 25 points. These patients recovered over 10 days. There was no statistically significant effect in genotype and allele frequency between the previously mentioned patients and patients who scored ≥ 25 points in the intravenous anesthesia group. There was no significant correlation between MMSE scores and ApoE single nucleotide polymorphism. However, there was a statistically significant effect in the number of cognitive dysfunction patients between the inhalation and intravenous anesthesia groups. In the inhalation anesthesia group, there was a statistically significant effect in ApoE genotype and gene frequency in patients with various MMSE scores. Specifically, the greatest differences were detected between the e4 allele and gene frequency. The correlation analysis between the ApoE e4 allele and MMSE scores indicated that the e4 allele was a risk factor for cognitive dysfunction in elderly patients who received an inhalation anesthetic. The incidence rate of cognitive dysfunction in patients who received inhalation anesthesia was greater compared with intravenous anesthesia. There is a strong association between the ApoE4 and transient postoperative cognitive dysfunction in patients undergoing inhalation anesthesia.

In our study, the cognitive decline was seen 3 days postoperatively in the inhalation anesthesia group, but this decline was reversed at 10 days. Neither anesthesia technique is associated with long-term cognitive dysfunction. The risk of short-term cognitive decline, however, as determined by MMSE evaluation, is higher with an inhalation anesthetic, particularly in patients with the E4 allele. Although such kind of “short-term cognitive decline” could be recovered in approximately 10 days, we could not ensure that there are any morphologic changes in certain areas of the patient’s brain, or no changes in the physiologic and biochemical index. A recent study 42 illustrates that exposure of naïve mice to less than 2% isoflurane for only 2 h can induce caspase activation and increase amyloid β levels. Therefore, there is the potential risk that use of inhalation anesthesia could cause detrimental effect to the brain, at least in elderly patients with certain genotype. Whether this detrimental effect could cause long-lasting changes requires further investigation.

In the current study, only isoflurane was investigated. However, there are other volatile anesthetics that are commonly used in the clinical setting, such as sevoflurane and desflurane. It has been reported that sevoflurane and isoflurane have similar anesthetic properties but different potencies. 41 Sevoflurane and desflurane were found to be less potent than isoflurane in altering intracellular calcium, and produced less apoptosis. 41 In addition, sevoflurane may promote Alzheimer disease neuropathogenesis. 42 From literature reports and our study here, we do not know whether all volatile anesthetics will have a similar effect in causing POCD in patients with certain genetic background, and whether any particular volatile anesthetics may be more suitable to elderly people than to others. Apparently, further research is warranted to answer these intriguing questions.

Our results presented here raise potential concerns regarding the use of isoflurane, a commonly used anesthetic. 43 In addition, to carry out the study to investigate under what conditions that isoflurane is safe to use, there are other alternatives. One alternative is to use those inhalation anesthetics that do not cause POCD, or design better and safer anesthetics. Another alternative is that, based on our study, total intravenous anesthesia is more suitable for elderly patients because it has no observable effect on cognitive function of elderly patients after surgery, especially those carrying the ApoE e4 allele. ApoE single nucleotide polymorphism varies among people with different ethnic background and living in different regions. The current study was conducted in patients who are of Han ethnicity residing in northwest China; thus, inevitable limitation exists in our research findings. The scientific results would be more universal if performed and verified in much more diversified territories and ethnic groups.

Conclusions

There is a strong association between ApoE4 and transient POCD in patients undergoing inhalation anesthesia. Compared with inhalation anesthesia, TIVA is more suitable for elderly patients because it has no observable effect on cogni-
itive function of elderly patients after surgery, especially those carrying the ApoE e4 allele.

References
15. Kang TJ, Yang MS: Rapid and reliable extraction of genomic DNA from various wild-type and transgenic plants. BMC Biotechnol 2004; 4:20
ANESTHESIOLOGY REFLECTIONS

Oxygenated Nitrous Oxide at Ruffner’s Dental Parlors

A lifelong resident of Indiana County, Pennsylvania, Howard E. Ruffner (1866–1916) forsook western Pennsylvania just long enough to earn his D.D.S. degree in 1890 from the Philadelphia Dental College. Aware of the hazards posed by hypoxic administration of nitrous oxide, Dr. Ruffner switched his S. S. White brand of anesthesia machines in each “dental parlor” for ones manufactured by Teter of Cleveland. Blanketing Pennsylvania with colorful 2-cent postage postcards (above), Dr. Ruffner advertised that his dental patients were “taking nitrous oxide and oxygen.” The aggressive marketing of his dental skills and of his safer anesthetics paid off handsomely for the dentist, grossing him over $120,000 monthly in today’s dollars. Sadly, Dr. Ruffner died at 50 years of age, leaving his widow Jennie to sell off all of his dental office furnishings and Teter anesthesia machines. (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the Anesthesiology Reflections online collection available at www.anesthesiology.org.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA’s Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UYOC@aol.com.