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## Apolipoprotein E $\epsilon$ 4 and cognitive function after surgery in middle-aged and elderly Danish twins --Manuscript Draft--

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<b>Abstract:</b>	<p><b>BACKGROUND</b></p> <p>Transient cognitive impairment is common in adult patients of all ages following anaesthesia and surgery. Apolipoprotein E (APOE) <math>\epsilon</math>4 carriers may have a larger deterioration in short term cognitive function after major surgery compared to APOE <math>\epsilon</math>4 non-carriers.</p> <p><b>OBJECTIVES</b></p> <p>The aim was to examine the effect of APOE <math>\epsilon</math>4 on the association between exposure to surgery and anaesthesia, and subsequent cognitive functioning. A more pronounced deterioration in cognitive function in APOE <math>\epsilon</math>4 carriers was hypothesized.</p> <p><b>DESIGN</b></p> <p>An observational cross-sectional and a 6-10 years longitudinal twin cohort design.</p> <p><b>SETTING</b></p> <p>Survey and register study of 2,936 Danish twins aged 45-92 years.</p> <p><b>MAIN OUTCOME MEASURES</b></p>

	<p>Cognitive function was assessed using five age-sensitive cognitive tests. In the cross-sectional study, we compared twins exposed to surgery with a reference group (unexposed). Linear regression models were used adjusting for sex and age and stratified by APOE ε4 carrier status. In the longitudinal cognitive follow-up study 1671 twins participated. Intra-pair analyses were also performed using 70 same-sexed twin pairs concordant for APOE ε4 carrier status, but discordant for major surgery.</p> <p><b>RESULTS</b></p> <p>APOE ε4 carriers had lower cognitive scores compared with non-carriers, and this was statistically significant in elderly twins 70+ years of age (mean difference, -0.67; 95% CI, -1.14 to -0.17). There was no significant impact on cognitive function after surgery according to APOE ε4 carrier status in the cross-sectional study. Similarly, there was no APOE ε4 modification in the longitudinal study. Also, in the intra-pair analyses no evidence was found of lower cognitive score after major surgery compared with the non-exposed co-twins among APOE ε4 carriers.</p> <p><b>CONCLUSIONS</b></p> <p>No evidence was found of more pronounced long term deterioration in cognitive function after surgery among APOE ε4 carriers, but elderly APOE ε4 carriers in general performed worse on the cognitive tests than non-carriers.</p>
<b>Response to Reviewers:</b>	I have now corrected the typo in table 2 and accepted our modifications. Thanks for noticing it, so we had the chance to correct it.

## **Apolipoprotein E $\epsilon$ 4 and cognitive function after surgery in middle-aged and elderly Danish twins**

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## Abstract

**BACKGROUND:** Transient cognitive impairment is common in adult patients of all ages following anaesthesia and surgery. Apolipoprotein E (APOE)  $\epsilon$ 4 carriers may have a larger deterioration in short term cognitive function after major surgery compared to APOE  $\epsilon$ 4 non-carriers.

**OBJECTIVES:** The aim was to examine the effect of APOE  $\epsilon$ 4 on the association between exposure to surgery and anaesthesia, and subsequent cognitive functioning. A more pronounced deterioration in cognitive function in APOE  $\epsilon$ 4 carriers was hypothesized.

**DESIGN:** An observational cross-sectional and a 6-10 years longitudinal twin cohort design.

**SETTING:** Survey and register study of 2,936 Danish twins aged 45-92 years.

**MAIN OUTCOME MEASURES:** Cognitive function was assessed using five age-sensitive cognitive tests. In the cross-sectional study, we compared twins exposed to surgery with a reference group (unexposed). Linear regression models were used adjusting for sex and age and stratified by APOE  $\epsilon$ 4 carrier status. In the longitudinal cognitive follow-up study 1671 twins participated. Intra-pair analyses were also performed using 70 same-sexed twin pairs concordant for APOE  $\epsilon$ 4 carrier status, but discordant for major surgery.

**RESULTS:** APOE  $\epsilon$ 4 carriers had lower cognitive scores compared with non-carriers, and this was statistically significant in elderly twins 70+ years of age (mean difference,  $-0.67$ ; 95% CI,  $-1.14$  to  $-0.17$ ). There was no significant impact on cognitive function after surgery according to APOE  $\epsilon$ 4 carrier status in the cross-sectional study. Similarly, there was no APOE  $\epsilon$ 4 modification in the longitudinal study. Also, in the intra-pair analyses no evidence was found of lower cognitive score after major surgery compared with the non-exposed co-twins among APOE  $\epsilon$ 4 carriers.

**CONCLUSIONS:** No evidence was found of more pronounced long term deterioration in cognitive function after surgery among APOE ε4 carriers, but elderly APOE ε4 carriers in general performed worse on the cognitive tests than non-carriers.

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## Introduction

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3 Transient cognitive impairment is common in adult patients of all ages following surgery and  
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5 anaesthesia, but older patients ( $\geq 60$  years) are at a higher risk and are more likely to suffer  
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7 prolonged effects <sup>1</sup>. The aetiology of postoperative cognitive dysfunction (POCD) is not fully  
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9 understood, but there is general agreement in the literature that POCD is a multifactorial  
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11 condition in which both perioperative and patient-related factors are implicated; possible  
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13 interplays between surgery and anaesthesia and genetic factors have also been suggested <sup>2-5</sup>. The  
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15 majority of patients with POCD improve over months to years, but a small fraction seems to be  
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17 particularly susceptible as they suffer a more persistent cognitive impairment <sup>6</sup>. Recently we found  
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19 a negligibly lower cognitive functioning after major surgery in Danish twins that may lend support  
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21 for this differential susceptibility hypothesis <sup>7</sup>. Regarding long term longitudinal cognitive decline  
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23 no significant differences were observed<sup>8</sup>. However, there is large variation in cognitive function  
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25 and decline, and genetics partially influences both cognitive function and decline.  
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37 Although first established as a major determinant in lipoprotein metabolism and  
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39 cardiovascular disease, Apolipoprotein E (APOE) has emerged as an important factor in several  
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41 other biological processes including Alzheimer's disease and cognitive function <sup>9</sup>. Studies have  
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43 shown that carrying the  $\epsilon 4$  allele of APOE is associated with an increased risk of Alzheimer's  
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45 disease <sup>10-12</sup>, cognitive decline in healthy ageing individuals <sup>13, 14</sup>, and poor outcome after a number  
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47 of conditions including stroke <sup>15</sup> and head trauma <sup>16, 17</sup>. A possible association between the APOE  
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49  $\epsilon 4$  allele and postoperative cognitive dysfunction (POCD) after cardiac and non-cardiac surgery has  
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51 also been studied, but the literature is conflicting: A meta-analysis including both non-cardiac <sup>2, 4,</sup>  
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53 <sup>5, 18</sup> and cardiac surgical patients <sup>19-23</sup> reported a statistically significant association between the  
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1 presence of APOE ε4 and POCD about one week postoperatively, suggesting that carriers of the ε4  
2 allele may be at increased risk of POCD <sup>24</sup>.  
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5 Of four recent studies, not included in the meta-analysis, one showed a higher incidence of  
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7 POCD among APOE ε4 carriers compared with non-carriers three months postoperatively <sup>25</sup>,  
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9 whereas the three other studies found no association between APOE ε4 and POCD in a long-term  
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11 perspective (two to eight years postoperatively) <sup>26-28</sup>. However, one of the latter studies showed  
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13 less improvement in cognitive function five years after cardiac surgery among APOE ε4 carriers <sup>26</sup>,  
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15 and another study including both cardiac and non-cardiac patients showed a steeper cognitive  
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17 decline on the Mini–Mental State Examination (MMSE) among APOE ε4 carriers <sup>28</sup>.  
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23 Of the original sample of 8,503 middle-aged and elderly twins included in our recent study,  
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25 we sought to assess the importance of APOE genotype by including those with available  
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27 genotypes. Our aim was to examine the effect by APOE ε4 on the association between exposure to  
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29 surgery and anaesthesia, and subsequent cognitive functioning. We hypothesized that a more  
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31 pronounced deterioration in cognitive function would occur after surgery in APOE ε4 carriers  
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33 compared to non-carriers.  
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## Materials and methods

### Study population

The study sample consists of participants in two Danish population-based surveys, the Study of Middle-Aged Danish Twins (MADT) (n=4,314) and the Longitudinal Study of Aging Danish Twins (LSADT) (n=4,731), previously described in detail elsewhere<sup>29, 30</sup> (see table 1 for summery details of the combined MADT and LSADT sample). In brief, participants in the two studies were ascertained through the Danish Twin Registry, which comprises all twins born since 1870 and considered representative of the general population in Denmark<sup>29, 31</sup>. The MADT was initiated in 1998 and represented a random sample of 120 intact twin pairs from each birth cohort from 1931 to 1952. The LSADT was initiated in 1995 and included Danish twins aged 75 years or older and residing in Denmark by January 1995. Additional cohorts were added in 1997, 1999 and 2001 and the minimum inclusion age was progressively reduced to 70 years: Twins born between 1920 and 1923 (i.e. at least age 73 years at the beginning of 1997) were added in 1997; twins born 1924 to 1928 (i.e. at least age 70 years at the beginning of 1999) were added in 1999, and twins born between 1929 and 1930 (i.e. at least age 70 years at the beginning of 2001) were added in 2001. The MADT and the LSADT are comparable with regard to the design and data collection instruments, and both studies include assessment of cognitive function and a collection of blood samples. A total of 8,527 individuals (4,309 from the MADT (99.9%) and 4,218 from the LSADT (89.2%)), completed the cognitive assessment. Twin zygosity was classified using questionnaire-based zygosity assessment<sup>32</sup>. Participants who had undergone neurosurgery (10 from the MADT and 14 from the LSADT) were excluded reducing the sample sizes to 4,299 and 4,204, respectively; and of these, a total of 2,936 (1,914 from the MADT and 1,022 from the LSADT) were genotyped for APOE. This sample subset is referred to as the total genetic sample and those remaining from the combined sample of LSADT and MADT are referred to as the sample without genetic

1 information (see Table 1 for details). In the longitudinal analyses cognitive change was measured,  
2 individual-level change scores were calculated as the difference between the composite cognitive  
3 score at the first and the second assessment so that a negative change scores indicated cognitive  
4 decline. First assessments were for MADT participants in 1998, second assessment were for MADT  
5 participants performed in 2008-2011. For LSADT participants first assessment was in 1995-1999  
6 and second assessment 6 years later in either of the following assessments in 2001, 2003 or 2005.  
7 The calculated cognitive decline is named the slope in cognitive score (Table 1). All methods were  
8 carried out in accordance with relevant guidelines and regulations. Written informed consents  
9 were obtained from all participants, and all surveys and use of survey information were approved  
10 by The Regional Committees on Health Research Ethics for Southern Denmark (VF 20040241).  
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### 29 **Exposure assessment**

30 Information on surgical exposure was obtained from the Danish National Patient Register by  
31 linkage between the intake examination data of the MADT and the LSADT and the Danish National  
32 Patient Register using a unique civil registration number. The Danish National Patient Register was  
33 established in 1977 and contains data on all hospital admissions including data on surgeries and  
34 diagnoses and from 1995, data on outpatients and emergency patients were also added <sup>33</sup>.  
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45 The exposure group consisted of participants who had at least one surgical procedure in  
46 the period between 1977 and the date of intake cognitive examination, which is a period of up to  
47 24 years. The exposure group was stratified by type of surgical procedure according to our  
48 previous defined classifications <sup>7</sup>: (1) major surgeries, including cardiac, thoracic, laparotomy,  
49 central and peripheral vascular and major fracture surgeries, (2) knee and hip replacements, (3)  
50 minor surgeries, and (4) other surgeries. Minor surgeries included all outpatient procedures,  
51 surgeries followed by less than two days of hospitalization, and, independently of the first two  
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criteria, surgery codes representing eye, skin, endoscopic procedures along with biopsies and other small surgical procedures. The classification was performed by two experienced anaesthesiologists (T.G.H. and L.S.R.), who, independently of one another, went through the records of surgical codes in the study sample for the period from 1977 and until intake examination. If surgical codes were inconsistent a joint agreement was made.

Participants who had more than one surgical procedure were assigned to the groups using the following algorithm: (1) major surgery if any, (2) knee and hip replacement if any, but no major surgery, and (3) other surgeries if any, but neither major nor knee and hip replacement surgery. The reference group in all analyses comprised participants with no surgical procedures from 1977 and until intake examination.

### **Outcome assessment**

The outcome was cognitive function evaluated by a five-component test battery at study intake. The test battery consists of the following specific tasks selected to be sensitive to normative age changes and easily administered by lay interviewers: (1) a category fluency task, in which individuals were asked to name as many animals as they could during a one-minute interval, (2) forward and (3) backward digit span, and (4) immediate and (5) delayed recall of a 12-item list. Justified by correlations in the range of 0.33 to 0.46<sup>34</sup>, an overall composite measure of cognitive functioning was computed by adding the five standardized scores (using mean and SD from participants aged 45 to 49 years) of each of the five-component tasks<sup>35</sup>. The composite cognitive score has been used in a series of previous studies<sup>34-37</sup> and is shown to have a high internal consistency reliability (0.75) and to be moderately stable over a two-year interval (0.60)<sup>37</sup>.

## **Apolipoprotein**

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2 In the MADT, the APOE-genotyping was performed on two randomly selected samples from the  
3  
4 1998 wave one with age restriction of a maximum of 55 years used in previous aging studies  
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6 (n=736) and another sample from the remaining participants (n=1,185) otherwise they were  
7  
8 selected at random<sup>38</sup>. In the LSADT, the APOE-genotyping was performed on randomly selected  
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10 samples from the 1997 (n=703) and 1999 (n=349) waves. The figures did not strictly match the  
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12 equivalents in Table 1 due to missing cognitive scores (MADT: n=2, LSADT: n=28) and exclusion of  
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14 participants with neurosurgery (MADT: n=5, LSADT: n=2).  
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21 For some of the LSADT-participants, blood was donated two (n=406) and four years (n=34)  
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23 after measurement of intake cognitive abilities, making the inclusion of these participants in the  
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25 study conditional upon survival until time of blood collection. To investigate for selection bias, we  
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27 performed statistical analyses both including and discarding the 440 delayed blood donors. For  
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29 statistical analyses exclusion of LSADT-participants (N=440, aged 70+) cognitive abilities did not  
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31 change the results markedly from those obtained using the full sample thus the analyses were  
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33 made using the entire sample.  
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38 Genotyping of the APOE variants rs429358 and rs7412 was carried out using either custom-  
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40 made primers and probes (LSADT), or predesigned TaqMan<sup>®</sup> SNP Genotyping Assays (Applied  
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42 Biosystems, Foster City, CA, USA) (MADT)<sup>39</sup>.  
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## ***Statistical Analysis***

49 Descriptive statistics were expressed as means and standard deviations (SDs) for continuous  
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51 variables and as numbers and percentages or interquartile ranges (IQR) for categories.  
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1 To explore whether APOE  $\epsilon$ 4 modifies the association between surgery and anaesthesia  
2 and cognitive functioning, we stratified the study sample into two groups: APOE  $\epsilon$ 4+, including  
3 APOE  $\epsilon$ 2 $\epsilon$ 4,  $\epsilon$ 3 $\epsilon$ 4 and  $\epsilon$ 4 $\epsilon$ 4, and APOE  $\epsilon$ 4- (reference group), including APOE  $\epsilon$ 2 $\epsilon$ 2,  $\epsilon$ 2 $\epsilon$ 3 and  $\epsilon$ 3 $\epsilon$ 3.  
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5 Within the APOE  $\epsilon$ 4 strata, the composite cognitive scores were compared between twins exposed  
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7 to at least one surgery (major, minor surgery, knee and hip replacement surgery, and other  
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9 surgeries) before examination of cognitive functioning and twins not exposed, using multivariate  
10  
11 linear regression models adjusting for sex and age at examination. The effect estimates were  
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13 compared across the APOE  $\epsilon$ 4 strata, and if they appeared to be different, an interaction term  
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15 between APOE and surgery was introduced in a linear regression model including surgery,  
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17 cognitive score, sex and age, to test for statistical significance of the interaction. The within-pair  
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19 dependency of twin individuals was considered by estimating the standard errors using the cluster  
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21 option of STATA (release 13; STATA Corp., USA). For the longitudinal analyses the difference  
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23 between follow up and baseline was calculated. Model assumptions were checked by residual  
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25 plots and quantile-quantile plots of residuals.  
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### 38 *Intra-pair analysis*

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41 To take preoperative cognition and confounding from shared environmental and genetic factors  
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43 into account, we performed APOE  $\epsilon$ 4-stratified intra-pair analyses in twin pairs discordant for  
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45 major surgery, i.e. one twin in a pair had a history of major surgery and the other had no surgery.  
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48 Only same-sexed and APOE  $\epsilon$ 4-concordant pairs were included. Among the 2,936 participants with  
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50 genetic information, a total of 70 such pairs (35 monozygotic and 35 dizygotic) were identified and  
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52 included in the analyses. Stratifying on APOE  $\epsilon$ 4 status, 24 pairs were APOE  $\epsilon$ 4 carriers and 46  
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54 were APOE  $\epsilon$ 4 non-carriers.  
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1 In the intra-pair analyses, the proportion of pairs in which the co-twin who had been  
2 exposed to anaesthesia and major surgery did also have the lower cognitive score was calculated  
3 including 95% CI using the binominal distribution. In addition, we assessed the numerical  
4 differences in cognitive score within pairs using a Bland Altman plot and tested whether the mean  
5 difference in the cognitive score within pairs was equal to zero using a one-sample t-test.  
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## Results

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2 Characteristics of the middle-aged and elderly Danish twin sample are displayed in Table 1, in total  
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4 and by surgical exposure groups, along with characteristics of the samples without genetic  
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6 information. The cognitive score was higher for those who had genetic information but not when  
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8 adjusted for age (mean difference, 0.14; 95% CI, -0.02; 0.30). The distribution of APOE  $\epsilon$ 4 carrier  
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10 status was comparable across surgical exposure groups (Table 1), implying no potential  
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12 confounding of APOE  $\epsilon$ 4 carrier status on the association between surgery and cognitive  
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14 functioning. Also, no deviance in Hardy-Weinberg equilibrium was observed (supplementary table  
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23 1).

24 In the combined twin group APOE  $\epsilon$ 4 carriers had a slightly lower, but not significantly, cognitive  
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26 score compared with non-carriers (mean difference, -0.13; 95% CI, -0.38; 0.12). APOE  $\epsilon$ 4 carriers had  
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28 a significantly lower cognitive score compared with APOE  $\epsilon$ 4 non-carriers among elderly twins  
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30 aged 70+ (mean difference, -0.66; 95% CI, -1.08 ; -0.24), which is a moderate effect of only  
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32 approximately 1/5 of a standard deviation (SD=3.6), while among middle-aged twins no significant  
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34 difference was observed (mean difference, 0.16; 95% CI, -0.16; 0.47).  
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40 In participants exposed to surgery there were no indication of lower cognitive score than the non-  
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42 operated among APOE  $\epsilon$ 4 carriers or non-carriers. Moreover, no modifiable effects by APOE  $\epsilon$ 4  
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44 carrier were observed as indicated by the interaction (all p-values > 0.05) (Table 2), and when the  
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46 6 to 10 years longitudinal cognitive decline was investigated none of the reported cognitive slopes  
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48 reached statistical significance (Table 3).  
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55 The study uses twin cohorts, so we also performed an APOE  $\epsilon$ 4-stratified intra-pair twin analysis.

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57 Here one twin had a history of major surgery and the other had no surgery. In the APOE  $\epsilon$ 4 non-  
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1 carrier group, the intra-pair analysis of all 46 surgery-discordant pairs revealed that the surgery  
2 exposed twin had a lower cognitive score than the co-twin in 35 % (95% CI: 20 % to 49 %) of the  
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4 pairs, i.e. it was significantly more common to have the higher cognitive score when exposed to  
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6 surgery than it was to have the lower cognitive score. When twin pairs were restricted according  
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9 to the magnitude of the intra-pair differences in cognitive score to the top 75, 50, and 25%,  
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12 respectively, the proportion of surgically exposed twins having the lower score was reduced even  
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15 further. In the APOE ε4 carrier group, we found that the cognitive score was independent of  
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18 surgery, as the twin exposed to surgery had the lower cognitive score in 50% of the pairs (Figure  
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21 1).



## Discussion

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2 In this study, we examined effect modification by APOE  $\epsilon$ 4 carrier status on the association  
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5 between exposure to surgery and anaesthesia, and cognitive functioning among 2,936 middle-  
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7 aged and elderly Danish twins. Our findings did not support the hypothesis that being an APOE  $\epsilon$ 4  
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9 carrier would increase the negative impact on cognitive function after anaesthesia and surgery.  
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12 Furthermore, our recent finding of a negligibly lower cognitive score after major surgery  
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14 than after no surgery using the larger sample <sup>7</sup> could not be replicated in this genotyped  
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16 subsample. As expected, we found a significantly lower cognitive score among APOE  $\epsilon$ 4 carriers  
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18 compared with non-carriers in the group aged 70 years and above.  
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23 The possibilities to directly compare our findings with the findings from other studies are  
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25 restricted by the limited number of studies that include information about APOE status and  
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27 postoperative cognitive function as well as by the fact that the studies differed in several aspects,  
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29 i.e. by different designs, study population, length of follow up and cognitive measures. We  
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31 identified three other studies investigating effect modification by APOE  $\epsilon$ 4 carrier status on the  
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33 association between surgery and anaesthesia, and cognition functioning <sup>27, 28, 40</sup>. Schenning et al.  
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35 reported a modifying effect of APOE  $\epsilon$ 4 carrier status contributing to postsurgical cognitive decline  
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37 measured by the MMSE test in a study with a mean follow up of seven years after surgery <sup>28</sup>.  
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39 However, we did not find any such effect, and neither did Patel et al. <sup>27</sup> or Sprung et al. <sup>40</sup>.  
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46 In addition, a recent meta-analysis <sup>24</sup> including two studies <sup>2, 23</sup> found no association  
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48 between APOE  $\epsilon$ 4 and cognitive decline one year after surgery (OR 1.15, 95% CI 0.71 to 1.86), and  
49  
50 neither did six additional studies on cognitive impairment following one to eight years after non-  
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52 cardiac <sup>26, 41, 42</sup> and cardiac surgery <sup>43, 44</sup>.  
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56 The study by Bartels and co-workers did not include a reference group, but merely a  
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58 surgical group stratified on cardiac and non-cardiac surgery, showed less cognitive improvement  
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1 five years following cardiac surgery among patients carrying the  $\epsilon 4$  allele <sup>26</sup>. This might reflect the  
2 finding of a general APOE  $\epsilon 4$  adverse effect on cognition also present in the elderly participants in  
3 our study, although the Bartels study showed no association between APOE  $\epsilon 4$  allele status and  
4 change in cognition in the non-cardiac surgery group.  
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10 Our study population was in Hardy Weinberg equilibrium, and allele frequencies and  
11 distributions of APOE genotypes were in line with a previous report on allele frequencies in  
12 another Danish study <sup>45</sup>.  
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18 Major strengths of the present study were the representative nature of the study sample  
19 derived from population-based surveys retrieved from the Danish Twin Registry <sup>29</sup> and the  
20 nationwide coverage and completeness of the Danish National Patient Register <sup>33, 46</sup> that  
21 minimizes the risk of selection bias. Furthermore, the register-based information on exposure left  
22 no room for recall bias, which was stressed as a potential limitation in a recent study using self-  
23 reported exposure information <sup>28</sup>. Comparing twin pairs is on the other hand a very powerful  
24 analysis that can detect moderate environmental effects using few pairs of twin <sup>47</sup> as was done in  
25 the present work for APOE  $\epsilon 4$  carriers ( $n_{\text{pairs}}=24$ ) (Figure 1). However, we could not confirm our  
26 hypothesis that surgery is associated with lower cognitive function among the APOE  $\epsilon 4$  carriers. In  
27 contrast, the association between surgery and better cognitive function found among APOE  $\epsilon 4$   
28 non-carriers in the twin pair design is likely to be a chance finding ( $n_{\text{pairs}}=46$ ). A cautious alternative  
29 explanation might be selection, e.g. both twins in a pair needed to be alive and especially the  
30 twins who went through surgery are survivors of a major disease. Over all the twin paired analyses  
31 suggests that there are no strong or moderate environmental effects from major surgery or  
32 general anesthesia on subsequent long term cognitive function.  
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1 The large sample size of more than 2,900 genotyped participants was an additional  
2 strength, which enabled us to stratify our analysis on age and surgery making our exposure groups  
3 more homogeneous.  
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7 A potential limitation of our study is that the cognitive composite score primarily includes  
8 the memory component, and differences in other or more specific cognitive domains may thus  
9 have been overlooked; hence, alternative measures of cognitive ability should be considered,  
10 subjective cognitive function or capacity for activities of daily living in future studies <sup>48</sup>. It is  
11 noteworthy, though, that the cognitive composite score has shown to be very age-sensitive with a  
12 difference of 2.5 SD from age 45 to 90 years <sup>35</sup>. In addition, our notable finding of a significant  
13 association between APOE  $\epsilon$ 4 and cognitive function among participants aged 70 or above, but not  
14 in the younger group, was supported by a recent meta-analysis in which the observed differences  
15 in several cognitive measures between APOE  $\epsilon$ 4 carriers and APOE  $\epsilon$ 4 non-carriers increase at  
16 older ages <sup>49</sup>.  
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33 As preoperative cognitive trajectory is suggested as one of the most important  
34 determinants of postoperative cognitive trajectory <sup>50</sup>, the absence of preoperative cognitive  
35 assessments in the current study is a potential limitation. We cannot entirely exclude that a true  
36 association between surgery and anaesthesia, and cognitive function as well as an effect  
37 modification by APOE  $\epsilon$ 4 may have been masked by not taking preoperative cognitive function  
38 into consideration. However, the use of a co-twin design including 70 pairs takes to some extent  
39 preoperative cognition into account as the intra-pair similarity of cognitive function is high <sup>51</sup>. The  
40 co-twin design did also control for common environmental factors and genetics minimizing the risk  
41 of unmeasured confounding.  
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56 Even though we argued that APOE-genotyping was performed on randomly selected  
57 samples from two populations-based surveys minimizing the risk of selection bias, participants  
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with genetic information were generally younger (partly explained by the age restriction in one of the samples) in comparison with those without genetic information (Table 1), and in the group aged 70 years and above, participants had slightly higher age-adjusted cognitive score than non-participants.

An effect of APOE  $\epsilon$ 4 on the risk of Alzheimer's disease has been evident for decades<sup>10</sup> and is also associated with cognitive decline<sup>52, 53</sup>. We found no significant evidence on the longitudinal cognitive decline for the APOE  $\epsilon$ 4 carriers than for non carriers which may be due to lack of sufficient power.

## Conclusion

Our data revealed a general APOE  $\epsilon$ 4 adverse effect on cognition in the participants aged 70 and above, but this was independent of surgical exposure status. We found no evidence that APOE  $\epsilon$ 4 carrier status was associated with a more pronounced deterioration in cognitive function after surgery in middle-aged and elderly patients.

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11 Presentation: None declared.  
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**Table 1** Characteristics of participants from the Study of Middle-Aged and elderly Twins according to APOE ε4 genotyping status, in totals and by surgery exposure group

Characteristics	Totals	Surgical procedure groups				
		Major surgery*	Minor surgery	Knee/hip replacement	Other surgery	No surgery
n (%)						
Total genetic sample	2936(35)	549(36)	605(36)	46(27)	758(36)	978(33)
APOE ε4 non-carriers	2013(24)	370(24)	412(24)	32(19)	526(25)	673(23)
APOE ε4 carriers	923(11)	179(12)	193(11)	14(8)	232(11)	305(10)
Sample without genetic information	5567(65)	997(64)	1124(65)	125(73)	1334(64)	1987(67)
Female, n (% of all)						
APOE ε4 non-carriers	1148(25)	272(27)	219(26)	19(17)	312(27)	326(22)
APOE ε4 carriers	495(11)	135(13)	92(11)	10(9)	125(11)	133(9)
Sample without genetic information	2917(64)	604(60)	526(63)	83(74)	707(62)	997(68)
Age <sup>†</sup> , year						
APOE ε4 non-carriers	62.2(11.6)	61.8(11.6)	60.6(11)	69.3(10.1)	62(12.1)	63.1(11.5)
APOE ε4 carriers	61.9(11.4)	62.7(11.3)	59.5(10)	77.5(3.5)	61.1(11.8)	63(11.6)
Sample without genetic information	69.5(11)	70.8(10.7)	68(11.1)	74.1(7.7)	69.3(11.5)	69.4(10.8)
Cognitive score <sup>‡</sup>						
APOE ε4 non-carriers	-1.8(3.6)	-1.7(3.4)	-1.6(3.7)	-3(3.5)	-1.7(3.7)	-2(3.4)
APOE ε4 carriers	-1.9(3.7)	-1.8(3.9)	-1.8(3.5)	-3.7(2.8)	-1.8(3.8)	-2(3.5)
Sample without genetic information	-3.2(3.9)	-3.7(4)	-2.9(4.1)	-3.4(3.9)	-3.2(4)	-3.1(3.8)
Slope in cognitive score <sup>‡</sup>						
APOE ε4 non-carriers	-0.78(2.7)	-0.63(2.8)	-0.78(2.7)	-0.77(2.4)	-0.64(2.6)	-0.81(2.7)
APOE ε4 carriers	-0.78(2.8)	-0.58(2.8)	-0.79(3.2)	-1.81(2.5)	0.33(2.5)	-0.84(2.7)

\*Including cardiac, thoracic, laparotomy, central and peripheral vascular and fracture surgeries. <sup>†</sup>Median (IQR). <sup>‡</sup>Mean (SD)

**Table 2** Mean differences in cross-sectional cognitive composite score between participants having major, minor, knee and hip replacement, or other surgeries and the group not exposed to surgery, by APOEε4 status

Surgical procedure groups	n	Differences in cognitive score (95% CI)*			
		APOEε4 non-carriers	N	APOEε4 carriers	
No surgery	673	Reference	305	Reference	
Any surgery	1340	-0.04(-0.45 to 0.38)	618	0.04(-0.60 to 0.69)	p=0.76
Major <sup>†</sup>	370	-0.04(-0.45 to 0.38)	179	0.04(-0.60 to 0.69)	p=1.00
Minor	412	0.01(-0.41 to 0.40)	193	-0.32(-0.88 to 0.24)	p=0.54
Knee/hip replacement	32	-0.33(-1.47 to 0.81)	14	0.56(-0.82 to 1.93)	p=0.67
Other	526	0.12(-0.36 to 0.39)	232	-0.07 (-0.64 to 0.50)	p=0.86

\* Multiple linear regression analyses, adjusted for sex and age at intake examination. <sup>†</sup>Including cardiac, thoracic, laparotomy, central and peripheral vascular and fracture surgeries. P-values are for interaction between APOE and surgery.

APOE = apolipoprotein

**Table 3** Mean longitudinal 6-10 years differences (slope) in cognitive composite score between participants having major, minor, knee and hip replacement, or other surgeries and the group not exposed to surgery, by APOEε4 status

Surgical procedure groups	n	Differences in cognitive score (95% CI)*				
		APOEε4 non-carriers		N	APOEε4 carriers	
No surgery	736	Reference		316	Reference	
Any surgery	422	0.08	(-0.27 to 0.42)	197	0.08	(-0.45 to 0.61) p=0.78
Major <sup>†</sup>	78	0.22	(-0.44 to 0.89)	34	0.11	(-0.90 to 1.11) p=0.89
Minor	243	0.01	(-0.41 to 0.42)	125	-0.19	(-0.66 to 0.62) p=0.99
Knee/hip replacement	27	0.89	(-0.85 to 1.02)	12	-1.21	(-2.64 to 0.22) p=0.26
Other	74	0.14	(-0.49 to 0.78)	26	1.08	(0.09 to 2.08) p=0.08

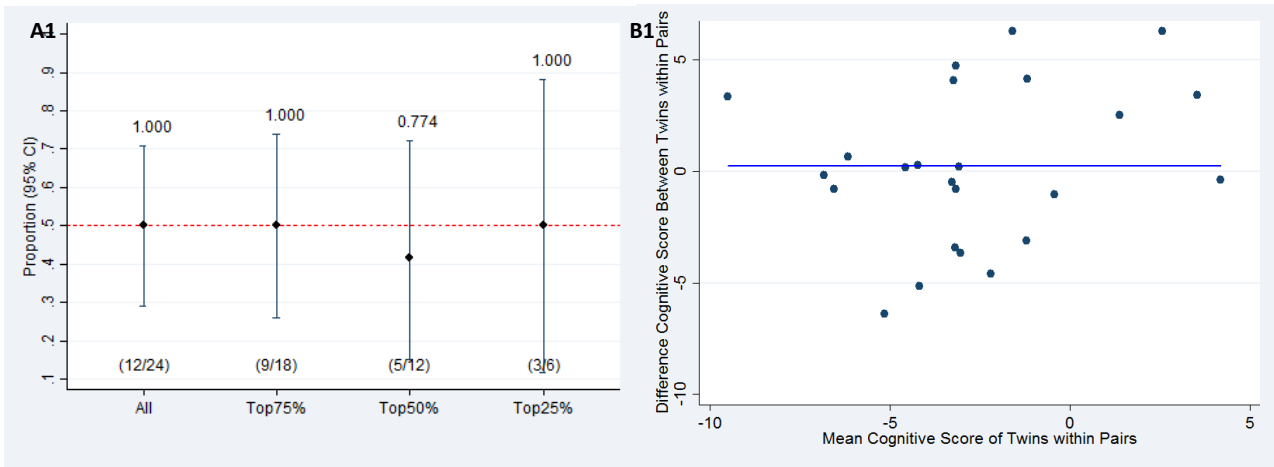
\* Multiple linear regression analyses, adjusted for sex and age at intake examination. <sup>†</sup>Including cardiac, thoracic, laparotomy, central and peripheral vascular and fracture surgeries. P-values are for interaction between APOE and surgery  
 APOE = apolipoprotein

**Figure legends:**

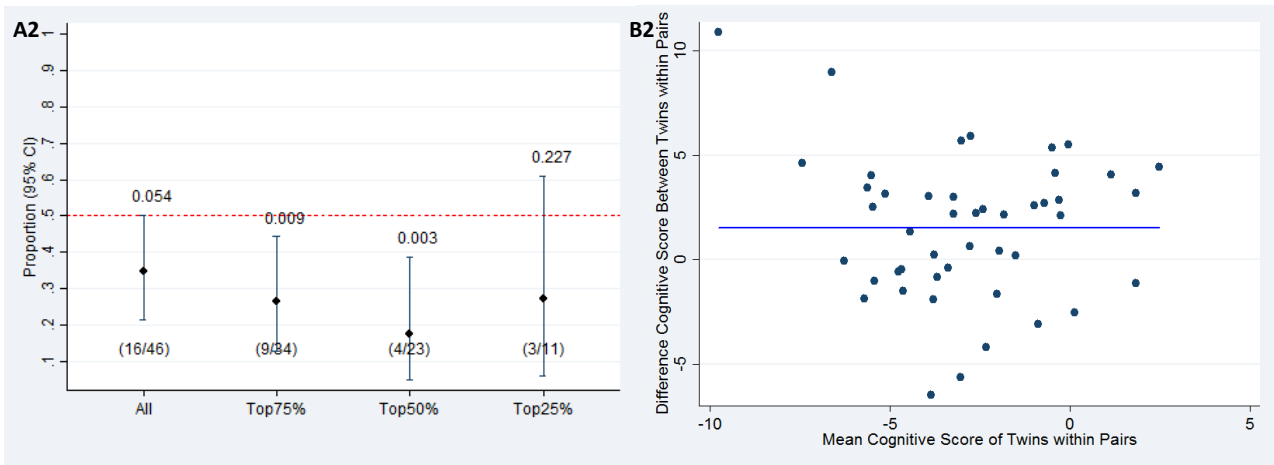
1  
2 Figure 1 (A) Proportion of twin pairs in which the twin exposed to major surgery had a lower cognitive score  
3 for all twin pairs and stratified according to the magnitude of intra-pair difference in cognitive score,  
4 separately for APOE  $\epsilon$ 4 carriers (A1) and non-carriers (A2). Above each line is the p-value from the binomial  
5 test, which tests whether the proportion equals to 0.5. (B) Bland Altman plot of difference in cognitive  
6 score between the twins (twin exposed to major surgery – twin not exposed to major surgery), separately  
7 for APOE  $\epsilon$ 4 carriers (B1) and non-carriers (B2). The solid blue lines represent the mean difference in  
8 cognitive score between twins exposed to major surgery and twins without surgery (mean 0.10 (B1) and  
9 0.42 (B2)).  
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Figure 1:

APOE ε4 carriers



APOE ε4 non-carriers



**Supplementary table 1: Frequency count (%) and test for Hardy–Weinberg equilibrium of ε2 and ε4 of the middle-age and elderly twins APOE genotypes**

APOE genotypes									
Study	ε2ε2	ε2ε3	ε3ε3	ε2 ε4	ε3ε4	ε4ε4	Total number	ε2 p-value*	ε4 p-value*
<b>Total</b>	15 (0.5 %)	388 (13.2 %)	1610 (54.8 %)	83 (2.8 %)	752 (25.6 %)	88 (3.0 %)	2936	0.16	0.90

\* Chi-square test for Hardy-Weinberg equilibrium  
 APOE = apolipoprotein E