Dear Editor,

A few studies have focused on exploring APOE gene-related effects on cognitive functions and brain activities in healthy populations. Bondi et al. found that ε4 carriers perform significantly worse on the California Verbal Learning Test than non-carriers in non-demented old subjects (mean age, 72 years)[4]. But the results are not entirely consistent. For example, Scarmascia et al. found no effect of the ε4 allele on neuropsychological performance[2] in young adults, and Jochemsen et al. found that the ε4 allele is associated with age-related cognitive decline[3]. Furthermore, protective and negative effects of the ε2 allele on cognition are inconsistent[4, 5]. APOE ε2 is thought to be a protective allele for AD in the elderly population due to its role in the superior cognitive performance of ε2 carriers compared to ε3 or ε4 carriers[6]. However, the ε2 allele has also been found to have a negative effect on AD pathology[4].

In order to test whether the ε4 and ε2 alleles of APOE affect processing speed and executive function in a healthy population, and whether there are age-specific effects, we selected 425 healthy Han Chinese aged 16 to 70 years and gave them several cognitive tests: the Stroop color and color-word interference, trail-making (A and B), logical memory, and visual reproduction tests. All participants were genotyped for two single-nucleotide polymorphisms (SNPs; rs429358 and rs7412) contributing to the APOE ε2, ε3, and ε4 alleles. We analyzed the association between APOE genotypes and attention, memory, and executive functions using analysis of covariance (ANCOVA) in participants with and without age-stratification.

We found that APOE genotypic status significantly affected the completion time to read the color of words (StroTi) in a Stroop color test ($F = 3.45$, $df = 2$, $P = 0.033$) in the total samples. Post-hoc ANCOVA revealed that participants with APOE ε4/ε4 (i.e., ε4/ε4 and ε3/ε4) showed inferior performance in the StroTi test compared to those with APOE ε3/ε3 ($P = 0.009$). Furthermore, we performed analysis in two age groups (16–39 years and 40–70 years) and found a significant difference in the young group only (ANCOVA: $F = 3.728$, $P = 0.025$; post-hoc ANCOVA: $P = 0.008$) (Fig. 1A, Supplementary Material Tables S1 and S2). Also, participants aged 40 to 70 years showed significant APOE genotypic effects on completion time in the trail-making-A test ($F = 3.47$, $P = 0.034$; Tables S1 and S2). Post-hoc ANCOVA tests revealed that APOEε2/- (i.e., ε2/ε2 and ε2ε3) participants spent more time completing the trail-making-A test compared to those with APOEε3ε3 ($P = 0.015$) or ε4/- ($P = 0.025$) (Fig. 1B). However, no difference remained significant after Bonferroni correction.

These findings suggested that the APOE ε4 allele affects executive functions and the ε2 allele affects attention in different age groups, although the effect sizes are small. This is partly consistent with previous findings that performance in neuropsychological tests, particularly those involving processing speed, executive function, and memory, is impaired in AD patients and/or normal ε4 allele carriers[8]. We found that the ε4 allele was associated with impaired performance in the StroTi test, a complex task assessing cognitive processes including cognitive plasticity, attention, and executive functions. This effect was stronger in the younger group, aged 16 to 39 years old, in particular. Although previous studies have demonstrated that the APOE ε4 allele has negative effects in elderly people with regard to cognition and neuronal activity[7], actually increasing numbers of studies have found that this effect occurs even when the participants are <40 years old[9].

The mechanism by which APOE variants impair executive functions is probably due to the effect of Aβ[6]. Ohm et al. suggested that histopathological features may precede the onset of AD by up to 50 years[8]. Han et al.[9] suggested that ε4 allele has an effect of antagonistic pleiotropy. Another view is that ε4 may have a negative impact on cognition or neuronal activity in young or middle-aged population[6, 10]. For example, Ghebremedhin et al. showed that
cognitive deficits caused by neurofibrillary tangles are more frequently seen in ε4 carriers than in non-ε4 control group between 22 and 46 years[10].

We found that APOE ε4 did not have a similar effect on executive functioning in the older group, and this does not support the findings from previous studies. One possible reason is that APOE ε4 has selective effects at different ages. A meta-analysis showed that increasing age is associated with smaller group differences between the APOE ε4 and non-APOE ε4 groups[11]. The second reason may be that the elderly were still in the early post-amyloid stage, and their declining cognitive functions were concealed by compensatory increases in brain activation, albeit followed by ultimate decline. In addition, the lack of significant differences may be due to the insufficient sample size for the 40–70 year-old group.

Furthermore, we found that the APOE ε2 allele was associated with impaired performance on the trail-making-A test, a complex problem-solving task mainly reflecting processing speed and mental flexibility in the elderly only. Previous studies have provided evidence that APOE ε2 carriers perform better than APOE ε3 or ε4 carriers, probably because the APOE ε2 allele binds more efficiently to the microtubule-associated protein tau than the ε4 allele. However, studies found that the APOE ε2 allele is a risk factor for AD[4], which suggests that APOE ε2 has negative effects on cognition similar to ε4 in the elderly. A possible mechanism may be the increased plaque pathology in individuals with APOE ε2[4] or APOE ε2 protein in disequilibrium, accelerating the pathological process of AD, initiating synaptic dysfunction and leading to cognitive decline[10]. We assume that elderly APOE ε2/- individuals are likely to show worse attention and processing speed than younger individuals due to neuropathology[4] or brain activity dysfunction[13].

Several issues should be addressed to understand the current findings. In order to capture important age-related information exhaustively in the preliminary study[14], our findings and discussion are mainly based on the results without multiple comparison correction. The multiple comparison correction is important as it reduces the probability of false-positives (type one errors) although it may increase the probability of false-negatives if the variables are not independent[15]. In conclusion, the current findings provide further evidence to support the hypothesis that the APOE gene affects processing speed and executive function in the normal population, with age-specific effects.
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METHODS

Participants
We recruited 425 healthy individuals through poster advertisements from the local area of Chengdu City, Sichuan Province, China. The study was approved by the Ethics Committee of West China Hospital, Sichuan University. Written informed consent was obtained from all participants. All subjects were screened using the non-patient version of the Structured Clinical Interview for DSM-IV (SCID-NP)\textsuperscript{11} to confirm a lifetime absence of mental disorders (especially Alzheimer’s disease, schizophrenia, bipolar disorder, major depression, and drug or alcohol abuse). Subjects with histories of brain injury, pregnancy, and physical illnesses such as cardiovascular disease or neurological disorders, as assessed by interview and medical records review, were also excluded. Also, subjects were interviewed to exclude individuals with known histories of Alzheimer’s dementia in first-degree relatives. Subjects were divided into two groups, between 16-39 years old (Young) and 40-70 years old (Old), since 40 years old is the most referential age considering the APOE gene’s age-specific role in the majority of previous studies\textsuperscript{2-5}. We regarded 40 years old as the cutoff point due to the following two reasons: first, previous studies set the 40 years old as the cutoff point; second, 40 years old was regarded as the peak stage of neurodevelopment and cognitive functions\textsuperscript{6}

Neuropsychological Testing
All participants were assessed by a trained psychiatrist using neurocognitive tests including Stroop color and color-word interference tests, Trail making tests, part A and B-M, and logical memory and visual reproduction tests\textsuperscript{7-10}. Stroop color and color-word interference tests reflect cognitive plasticity and executive functions\textsuperscript{11, 12}. In this study, three measures were recorded, including reading color completion time (StroTi; seconds), reading words completion time (StroCWTi; seconds), and the correct number of
words read within 120 s (StroCW2R). Trail making A and BM test completion times, recorded respectfully as TMTA-time and TMTBM-time (seconds), were included. The Trail making test assesses attention, processing speed and mental flexibility functioning\[^{[13, 14]}\]. The logical memory and visual reproduction tests assess individual memory and learning functions\[^{[10]}\]. We recorded and analyzed the raw scores of immediate and delayed logical memory (Log-memory IM, Log-memory DE; scores) and visual reproduction (Visu-memory IM, Visu-memory DE; scores) in this study. The detailed procedures for each test were described in other studies\[^{[8-10]}\].

**APOE Genotyping**

DNA was obtained from whole blood using the standard phenol-chloroform isolation method\[^{[15]}\]. Two single-nucleotide polymorphisms (SNPs; rs429358 and rs7412) were genotyped to identify APOE genotypes comprised of APOE ε2, ε3, and ε4 alleles using a SNaPshot assay\[^{[16]}\]. The SNaPshot assay consisted of a multiplex, PCR of all SNPs followed by a single-base extension process, and was performed following a detailed, step-by-step procedure similar to that reported by Wang et al.\[^{[17]}\]. GeneMarker software was used to read the genotyping result\[^{[18]}\]. According to previous studies\[^{[5, 19, 20]}\], individuals were divided into three subgroups according to the following genotyping: APOEε2/-, APOEε3/ε3, and APOEε4/- . APOEε2/- and APOEε4/- included heterozygous and homozygous APOEε2 (i.e., ε2/ε2 and ε2/ε3) and APOEε4 (i.e., ε3/ε4 and ε4/ε4), respectively. Subjects with APOEε2/ε4 were not included in the current study in order to clarify the genetic effects of APOE ε2 and APOE ε4 \[^{[2]}\].

**Data Analysis**

The Pearson’s $\chi^2$ test was used to compare categorical data differences. Student’s $t$ test and analysis of variance were used to analyze continuous data as appropriate. Hardy–Weinberg equilibrium was calculated using the HWE.rar package or PLINK program (http://pngu.mgh.harvard.edu/~purcell/plink/summary.shtml#hardy). An analysis of covariance (ANCOVA) was used to assess the main effect of APOE genotypic status (APOEε2/-, APOEε3/ε3, and APOEε4/-) on cognitive function performance in the total samples
and in each age group (Young and Old), using sex, years of education, and age as covariance\textsuperscript{[20-22]}.

Post-hoc ANCOVA tests were then used to assess the individual genotypic effect on cognition functions for each age group. The $P$-value threshold was set at 0.05. All analyses were performed using SPSS version 13.0 for Windows (SPSS Inc., USA).

### Table S1. Demographic variables and comparison of cognitive test results among carriers of different APOE genotypes in two age groups

<table>
<thead>
<tr>
<th>Demographic variables/ Cognitive tests</th>
<th>16-39 years</th>
<th>Age group</th>
<th>40-70 years</th>
<th>$\chi^2$/$F$</th>
<th>$P$ value</th>
<th>16-39 years</th>
<th>Age group</th>
<th>40-70 years</th>
<th>$\chi^2$/$F$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>35</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>18</td>
<td></td>
<td></td>
<td>200</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>15/20</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>9/9</td>
<td>2.17</td>
<td>0.338</td>
<td>107/93</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>44/51</td>
<td>1.24</td>
<td>0.539</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.29(6.58)</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>55.83(5.83)</td>
<td>0.502</td>
<td>0.606</td>
<td>27.44(6.37)</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>49.80(7.69)</td>
<td>0.152</td>
<td>0.859</td>
</tr>
<tr>
<td>Education(years)</td>
<td>16-39</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>41-62</td>
<td></td>
<td></td>
<td>16-39</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>40-70</td>
<td>40-68</td>
<td></td>
</tr>
<tr>
<td>StroTi (s)</td>
<td>66.00(2.31)</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>9.33(2.79)</td>
<td>3.728</td>
<td>0.025</td>
<td>63.36(0.95)</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>8.83(3.27)</td>
<td>0.449</td>
<td>0.639</td>
</tr>
<tr>
<td>StroCWTi (s)</td>
<td>161.99(8.55)</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>189.25(11.89)</td>
<td>0.665</td>
<td>0.515</td>
<td>161.93(3.57)</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>198.15(5.32)</td>
<td>0.533</td>
<td>0.588</td>
</tr>
<tr>
<td>StroCW2R (numbers)</td>
<td>71.65(3.48)</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>60.22(5.21)</td>
<td>0.215</td>
<td>0.807</td>
<td>72.94(1.45)</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>62.39(2.21)</td>
<td>0.103</td>
<td>0.903</td>
</tr>
<tr>
<td>TMTA-Ti (s)</td>
<td>46.742(2.36)</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>63.46(3.57)</td>
<td>0.543</td>
<td>0.581</td>
<td>44.35(0.98)</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>53.86(1.55)</td>
<td>3.47</td>
<td>0.034</td>
</tr>
<tr>
<td>TMTB-Ti (s)</td>
<td>59.61(2.92)</td>
<td>APOE\textsuperscript{ε}2/-</td>
<td>94.11(6.00)</td>
<td>0.404</td>
<td>0.668</td>
<td>62.38(1.42)</td>
<td>APOE\textsuperscript{ε}3/\textsuperscript{ε}3</td>
<td>81.68(2.62)</td>
<td>1882</td>
<td>0.157</td>
</tr>
</tbody>
</table>
Log-memory IM(scores) 12.89(0.65) 12.26(0.27) 12.69(0.56) 0.579 0.561 8.57(1.00) 9.40(0.44) 10.21(1.06) 0.641 0.528
Log-memory DE(scores) 10.74(0.70) 10.25(0.29) 10.27(0.60) 0.21 0.81 6.57(1.03) 7.44(0.45) 7.86(1.09) 0.414 0.662
Visu-memory IM(scores) 9.58(0.61) 10.09(0.25) 9.23(0.52) 1.235 0.292 6.45(0.81) 6.92(0.35) 8.07(0.86) 1.028 0.361
Visu-memory DE(scores) 9.13(0.59) 9.76(0.24) 9.06(0.51) 1.09 0.336 6.45(0.78) 6.47(0.34) 6.81(0.82) 0.079 0.942

Notes: Mean (s.d.). APOE ε2/- include ε2/ε2 and ε2/ε3; APOE ε4/- include ε3/ε4 and ε4/ε4. StroTi, completion time of reading the color in Stroop color and color-word interference tests; StroCWTi, completion time of reading the words; StroCW2R, the correct number of words read within 120 s; TMTA-time, the completion time of Trail making, part A; TMTB-time, the completion time of Trail making, part B; Log-memory IM, scores of immediate memory; Log-memory DE, scores of delayed logical memory; Visu-memory IM, scores of immediate visual reproduction; Visu-memory DE, scores of delayed visual reproduction.

Table S2. Genotypes and allelic distributions of the APOE gene variation in 425 healthy subjects

<table>
<thead>
<tr>
<th>Age group</th>
<th>Genotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ε2/ε2 (%)</td>
<td>ε2/ε3 (%)</td>
</tr>
<tr>
<td>Young group</td>
<td>1(0.3)</td>
<td>34(11.7)</td>
</tr>
<tr>
<td>Old group</td>
<td>1(0.7)</td>
<td>17(12.7)</td>
</tr>
<tr>
<td>Total</td>
<td>2(0.5)</td>
<td>51(12.0)</td>
</tr>
</tbody>
</table>

The SNPs of the APOE gene did not deviate from Hardy–Weinberg equilibrium in this population ($\chi^2 = 6.48, P = 0.09$). Additional tests were performed
to ensure that genotypic frequencies for rs429358 and rs7412 did not statistically deviate from Hardy–Weinberg equilibrium ($P = 0.57$ and $1$, respectively). No significant difference was found in the polymorphism frequencies, both genotype-wise ($\chi^2 = 1.28, P = 0.94$) and allele-wise ($\chi^2 = 0.95, P = 0.62$), between Young and Old groups. Young group: 16-39 years old. Old group: 40-70 years old.

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