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·Letter to the Editor·

Apolipoprotein E gene polymorphisms associated with processing speed and executive functions in healthy Han Chinese

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)[1]. But the results are not entirely consistent. For example, Scarmeas *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[5]. 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/- (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 *APOE*ε2/- (i.e., ε2/ε2 and ε2/ε3) participants spent more time completing the trail-making-A test compared to those with *APOE*ε3/ε3 (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[6]. 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[6]. 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

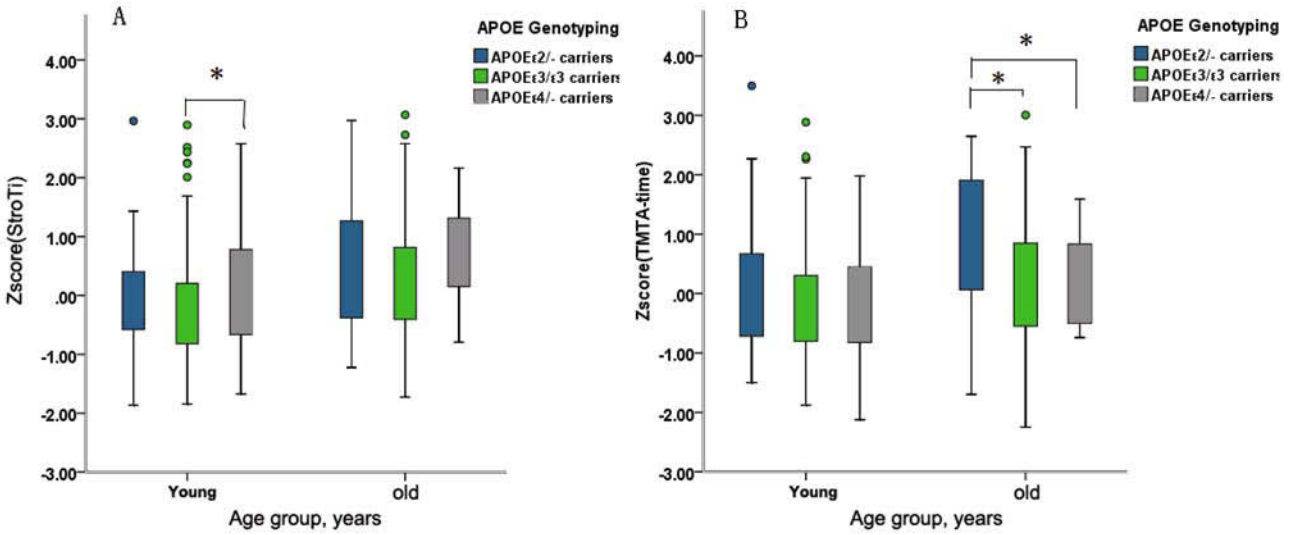

Fig. 1. Comparison of standardized residuals of Stroop color (StroTi) (A) and trail-making-A (TMT-A) (B) tests in young and old groups. APOEε2/- includes ε2/ε2 and ε2/ε3; APOEε4/- includes ε3/ε4 and ε4/ε4. StroTi, completion time of reading the color in Stroop color and color-word interference tests. TMTA-time, completion time of trail-making, part A. Young group: 16–39 years. Old group: 40–70 years. ****P* **<0.05.

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[12]. 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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Supplementary Material

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)[1] 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 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[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[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[7-10]. Stroop color and color-word interference tests reflect cognitive plasticity and executive functions[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: *APOE*ε2/-, *APOE*ε3/ε3, and *APOE*ε4/-. *APOE*ε2/- and *APOE*ε4/- included heterozygous and homozygous *APOE*ε2 (i.e., ε2/ε2 and ε2/ε3) and *APOE*ε4 (i.e., ε3/ε4 and ε4/ε4), respectively. Subjects with *APOE*ε2/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 χ^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 (*APOE*ε2/-, *APOE*ε3/ε3, and *APOE*ε4/-) 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[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

Demographic variables/ Cognitive tests	Age group									
	16-39 years					40-70 years				
	*APOE*ε2/-	*APOE*ε3/ε3	*APOE*ε4/-	χ^2/F	*P* value	*APOE*ε2/-	*APOE*ε3/ε3	*APOE*ε4/-	χ^2/F	*P* value
Subjects	35	200	47			18	95	18		
Sex (male:female)	15/20	107/93	21/26	2.17	0.338	9/9	44/51	6/12	1.24	0.539
Age (years)	28.29(6.58)	27.44(6.37)	26.87(5.94)	0.502	0.606	55.83(5.83)	49.80(7.69)	50.22(8.03)	0.152	0.859
Age range (years)	16-39	16-39	16-37			41-62	40-70	40-68		
Education(years)	10.17(3.80)	11.02(3.59)	11.30(3.75)	1.046	0.353	9.33(2.79)	8.83(3.27)	8.94(4.71)	0.162	0.851
StroTi (s)	66.00(2.31)	63.36(0.95)	69.18(1.97)	3.728	0.025	76.92(4.11)	77.21(1.79)	81.57(4.36)	0.449	0.639
StroCWTi (s)	161.99(8.55)	161.93(3.57)	171.26(7.37)	0.665	0.515	189.25(11.89)	198.15(5.32)	207.10(12.62)	0.533	0.588
StroCW2R (numbers)	71.65(3.48)	72.94(1.45)	70.98(2.86)	0.215	0.807	60.22(5.21)	62.39(2.21)	60.69(5.21)	0.103	0.903
TMTA-Ti (s)	46.742(2.36)	44.35(0.98)	43.67(2.02)	0.543	0.581	63.46(3.57)	53.86(1.55)	51.69(3.78)	3.47	0.034
TMTB-Ti (s)	59.61(2.92)	62.38(1.42)	62.49(2.50)	0.404	0.668	94.11(6.00)	81.68(2.62)	86.52(6.37)	1882	0.157

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-memoryIM(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-memoryDE(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

Age group	Genotype						Allele frequency		
	ε2/ε2 (%)	ε2/ε3(%)	ε2/ε4(%)	ε3/ε3 (%)	ε3/ε4(%)	ε4/ε4 (%)	ε2 (%)	ε3 (%)	ε4(%)
Young group	1(0.3)	34(11.7)	9(3.1)	200(68.7)	43(14.8)	4(1.4)	45(7.7)	477(82)	60(10.3)
Old group	1(0.7)	17(12.7)	3(2.2)	95(70.9)	17(12.7)	1(0.7)	22(8.2)	224(83.6)	22(8.2)
Total	2(0.5)	51(12.0)	12(2.8)	295(69.4)	60(14.1)	5(1.2)	67(7.9)	701(82.5)	82(9.0)

The SNPs of the *APOE* gene did not deviate from Hardy–Weinberg equilibrium in this population (χ^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 (χ^2 = 1.28, P = 0.94) and allele-wise (χ^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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