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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