NEURAL REGENERATION RESEARCH
March 2014, Volume 9, Issue 6
www.nrronline.org

APOE and APOC1 gene polymorphisms are associated with cognitive impairment progression in Chinese patients with late-onset Alzheimer's disease

Qin Zhou[1, 2], Dantao Peng[1], Xinrui Yuan[1], Zeping Lv[2], Shenghang Pang[2], Wenyu Jiang[2], Chuyu Yang[2], Xiaohong Shi[1], Guofang Pang[2], Yige Yang[1], Haiqun Xie[2], Wandong Zhang[3], Caiyou Hu[2], Ze Yang[1]

1 Key Laboratory of Geriatrics, Beijing Hospital & Beijing Institute of Geriatrics, Ministry of Health, Beijing, China
2 Department of Neurology, Jiangbin Hospital, Nanning, Guangxi Zhuang Autonomous Region, China
3 Human Health Therapeutics, National Research Council of Canada, Ottawa, Canada

Qin Zhou, Dantao Peng, and Xinrui Yuan contributed equally to this work.

Corresponding author:
Caiyou Hu, Department of Neurology, Jiangbin Hospital, Nanning 530021, Guangxi Zhuang Autonomous Region, China. cyhu.hua@163.com; Wandong Zhang, Human Health Therapeutics, National Research Council of Canada, Ottawa, Canada. wandong.zhang@nrc-cnrc.gc.ca; Ze Yang, Key Laboratory of Geriatrics, Beijing Hospital & Beijing Institute of Geriatrics, Ministry of Health, Beijing 100730, China. yixueshengzhouqin@163.com.

doi:*10.4103/1673-5374.130117*
http:*//www.nrronline.org/*

Accepted: *2014-02-13*

Abstract

Current evidence shows that apolipoprotein E (APOE), apolipoprotein CI (APOC1) and low density lipoprotein receptor-related protein (LRP) variations are related to late-onset Alzheimer's disease. However, it remains unclear if genetic polymorphisms in these genes are associated with cognitive decline in late-onset Alzheimer's disease patients. We performed a 30-month longitudinal cohort study to investigate the relationship between Alzheimer's disease and APOE, APOC1, and LRP. In this study, 78 Chinese Han patients with late-onset Alzheimer's disease were recruited form Guangxi Zhuang Autonomous Region in China. APOE, APOC1, and LRP genotyping was performed using polymerase chain reaction-restriction fragment length polymorphisms. The Mini-Mental State Examination and Clinical Dementia Rating Scale were used to assess patients' cognitive function. After a 30-month follow-up period, we found a significant reduction in Mini-Mental State Examination total score, a higher proportion of patients fulfilling cognitive impairment progression criteria, and a higher proportion of APOC1 H2 carriers in APOE ε4 carriers compared with non-carriers. In addition, the APOE ε4 allele frequency was significantly higher in the cognitive impairment progression group compared with the non-cognitive impairment progression group. In conclusion, APOE ε4 plays an important role in augmenting cognitive decline, and APOC1 H2 may act synergistically with APOE ε4 in increasing the risk of cognitive decline in Chinese patients with late-onset Alzheimer's disease.

Key Words: *nerve degeneration; cognitive disorders; dementia; Alzheimer's disease; polymorphism; apolipoprotein E; apolipoprotein CI; low density lipoprotein receptor-related protein; NSFC grant; neural regeneration*

Funding: *This study was supported by the National Natural Science Foundation of China, No. 81370445, 81061120527, 81241082; Major Funding from Beijing Hospital, No. BJ-2010-30; Key Project of Clinical Disciplines at the Subordinate Hospital, Ministry of Health, No. 10120101; National Department Public Benefit Research Foundation by the Ministry of Health, No. 201302008; 12th 5-year National Program from Ministry of Scientific Technology, No. 2012BAI10B01; Science and Technology Development Foundation of Guangxi Zhuang Autonomous Region, No. 1355005-6-2; and Canadian Institute of Health Research (CIHR), No. 109606.*

Zhou Q, Peng DT, Yuan XR, Lv ZP, Pang SH, Jiang WY, Yang CY, Shi XH, Pang GF, Yang YG, Xie HQ, Zhang WD, Hu CY, Yang Z. APOE and APOC1 gene polymorphisms are associated with cognitive impairment progression in Chinese patients with late-onset Alzheimer's disease. Neural Regen Res. 2014;9(6):653-660.

Introduction

Alzheimer's disease is a neurodegenerative disorder (Jin et al., 2012), and the most common cause of dementia in the aging population, accounting for an estimated 60–80% of cases (Barnes and Yaffe, 2011). The typical clinical characteristic of Alzheimer's disease is progressive loss of cognitive function, especially memory dysfunction, lasting for 2–20 years (Jonsson et al., 2013). Late stage symptoms include disorientation, confusion, impaired judgment, behavioral changes, and difficulties in speaking, swallowing, and walking. Ultimately, patients gradually lose the ability to communicate and care for themselves. There is no doubt that Alzheimer's disease is a health-threatening disease for the elderly.

According to research for the World Alzheimer Report 2012, there are an estimated 36 million people worldwide with dementia. However, there is a lack of effective treatment and prevention for Alzheimer's disease, with drugs only temporarily relieving symptoms at the early stage. Alzheimer's disease is a multi-factorial disease involving interactions between genetic and environmental factors (Meng and D'Arcy, 2012; Popp et al., 2013), and with a heritability of 60–80% (Devan et al., 2013). Further understanding the

genetic components of these devastating diseases will provide useful information for managing them. Associations between genetic polymorphisms and Alzheimer's disease have always been a hot international research topic. Pathogenic mutations in amyloid-beta (Aβ) precursor protein and presenilin 1 and 2, have been linked to early onset forms of familial Alzheimer's disease (Cruchaga et al., 2012). While there has been considerable success in identification of genes contributing to early-onset Alzheimer's disease, late-onset Alzheimer's disease, one of the most common forms, is not yet well understood.

The apolipoprotein E (APOE) genotype is the only widely accepted genetic risk factor for Alzheimer's disease (Bangen et al., 2013; Liu et al., 2013). In 1993, Strittmatter and colleagues reported that the APOE ε4 allele frequency was dramatically increased in patients with late-onset familial Alzheimer's disease (Strittmatter et al., 1993). They also found ApoE protein, encoded by APOE, binds with high avidity to Aβ. The percentage of Alzheimer's disease patients carrying an APOE ε4 allele (46.2%) is significantly higher than controls (13.2%) (Strittmatter et al., 1993). Corder and colleagues subsequently found that Alzheimer's disease risk and mean age at clinical onset were 91% and 68 years in ε4 homozygotes, 47% and 76 years in heterozygotes, and 20% and 84 years in non-carriers, indicating that APOE ε4 increases the risk of developing Alzheimer's disease at an earlier age of onset, in a gene dose dependent manner (Corder et al., 1993). Recent studies have shown genetic polymorphisms in dyslipidemia are also involved in late-onset Alzheimer's disease, *e.g.*, apolipoprotein CI (APOC1) and low density lipoprotein receptor-related protein (LRP) (Basak et al., 2012; Li et al., 2008). While inheritance of certain genotypes increases the risk of developing Alzheimer's disease, it is still unknown if the genetic polymorphisms are associated with cognitive decline in patients with late-onset Alzheimer's disease. Research on the relationship between the APOE, APOC1, and LRP genes and cognitive decline has rarely been reported.

We used a 30-month longitudinal cohort study of patients with late-onset Alzheimer's disease to determine the association between APOE, APOC1, and LRP gene polymorphisms, and cognitive decline in late-onset Alzheimer's disease. We further examined gene-gene interactions between APOE and APOC1, and APOE and LRP. These genes may have a negative impact on neural regeneration in Alzheimer's disease patients.

Subjects and Methods

Subjects

Patients with late-onset Alzheimer's disease were recruited from in- and out-patient sections of the Department of Neurology, Jiangbin Hospital in Guangxi Zhuang Autonomous Region, China from January 2009 to September 2010. The follow-up period was 30 months. After 30 months, patients were interviewed face to face.

Inclusion criteria

Patients were included if they met all the following criteria: (1) diagnosis of probable Alzheimer's disease according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (McKhann et al., 1984); (2) diagnosis of mild or moderate Alzheimer's disease according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American, 1994); (3) onset age of 65 years or older; (4) Hachinski Ischemia Scale score < 4 (Hachinski et al., 1975).

Exclusion criteria

Patients were excluded if they met any of the following criteria: (1) current or past diagnosis of other nervous system diseases (such as stroke, Parkinson's disease, traumatic brain injury); (2) current or past diagnosis of psychiatric diseases (such as schizophrenia, delirium, major depressive disorder, illusion, alcohol or substance dependence and/or misuse); (3) current or past diagnosis of metabolic disorders (such as diabetes, hypertension); (4) use of medications that affect cognitive function (such as donepezil) within 2 weeks of the baseline visit or during the study; (5) no reliable caregiver who can provide reliable information for neuropsychological and behavioral assessment.

The study adhered to the principles of the *Declaration of Helsinki*. Approval for the study was obtained from the Jiangbin Hospital Ethical Committee. Written informed consent was obtained from all patients or their guardians before study participation.

DNA extraction

At baseline visits, upper limb venous blood (5 mL) was collected from each patient and placed into ethylenediaminetetraacetic acid anticoagulated tubes. Genomic DNA was extracted from whole blood samples using standard DNA isolation methods (Loparev et al., 1991). (1) A whole blood (2.7 mL) sample was transferred to 5 mL vacutainer-ethylenediaminetetraacetic acid K3 tubes, mixed by inverting, centrifuged at 805 × *g* for 5 minutes and then 150 μL of white blood cells (middle layer) transferred to a new tube. (2) 150 μL of ddH_2O was added and mixed by inverting. (3) 300 μL of NaI (6 mol/L) was added and mixed by vortexing for 20 seconds. (4) 600 μL of chloroform and isoamyl alcohol mixture (24:1) was added to the tube and mixed by vortexing for 20 seconds. (5) The tube was centrifuged at 12,879 × *g* for 10 minutes and 450 μL of supernatant transferred to a new tube. (6) 0.6 volumes of isopropanol was added, mixed by inverting and then stood for 5 minutes. (7) The tube was centrifuged at 12,879 × *g* for 10 minutes and the supernatant removed. (8) The pellet was washed twice with 200 μL 37% isopropanol and air-dried. (9) The pellet was redissolved in 50–100 μL TE buffer and stored at −20°C.

Extracted human genomic DNA was detected using ultraviolet spectrophotometry (Pharmacia Biotech, Uppsala, Sweden). The A_{260nm}/A_{280nm} ratio (absorbance at 260 and 280 nm) was required to be 1.7–1.8, to ensure the genomic DNA was in accordance with laboratory qualification requirements.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technology

APOE, APOC1, and LRP genotyping was performed using PCR-RFLP methods (Hollenbach et al., 1998; Nillesen et al., 1990; Emi et al., 1988).

Primer sequences are shown as follows:

Genes	Sequence (5′–3′)	Product size (bp)
APOE	Forward: ACA GAA TTC GCC CCG GCC TGG TAC AC Reverse: TAA GCT TGG CAC GGC TGT CCA AGG A	244
APOC1	Forward: TTT GAG CTC GGC TCT TGA GAC AGG AA Reverse: GGT CCC GGG CAC TTC CCT TAG CCC CA	226
LRP	Forward: GGG GTC CAG GAC TGC ATG TA Reverse: CCA GGA CAG TAC TCG GAA GGT	121

PCR mixtures (total volume 20 μL) for detecting APOE, APOC1, and LRP contained 800 μmol/L dNTPs (Pharmacia, New Jersey, NJ, USA; each dNTP at 200 μmol/L), forward and reverse primers (each 5 pmol), 2.0 μL 10 × buffer, 50 ng template DNA, 1 U Taq DNA polymerase (Beijing Xin Jing Ke Biotechnology Co., Ltd., Beijing, China), and sterile deionized water. In addition, for the APOE gene, 10% DMSO was added.

The thermal cycling profile for the APOE gene: initial denaturation at 95°C for 10 minutes, 34 cycles of 95°C for 1 minute, 64°C for 1 minute, and 72°C for 1 minute, followed by 72°C for 5 minutes. The thermal cycling profile for the APOC1 and LRP genes: initial denaturation at 94°C for 5 minutes, 35 cycles of 94°C for 35 seconds, 61°C for 35 seconds, and 72°C for 35 seconds, followed by 72°C for 5 minutes.

PCR products were separated by 8% non-denaturing polyacrylamide gel electrophoresis (Bellco Biotechnology, Vineland, NJ, USA). After identification by electrophoresis, PCR products were subsequently digested in total volumes of 20 μL with restriction endonucleases at 37°C for 4 hours. Digestion products were visualized (Bio-Rad, Hercules, CA, USA) after separation by non-denaturing polyacrylamide gel electrophoresis. All products were confirmed by sequencing.

Main outcome measures

The Mini-Mental State Examination (MMSE; Cummings, 1993) and Clinical Dementia Rating Scale (CDR; Hughes et al., 1982) were used to assess patients' cognitive function. Possible total scores on the MMSE range from 0 (most severe) to 30 (normal). MMSE total score should take a participants' education into account (considered as dementia if MMSE ≤ 17 for illiterates, ≤ 20 for primary school graduates, and ≤ 24 for middle school graduates or higher education) (Zhuang et al., 2012). Higher MMSE scores suggest Alzheimer's disease patients have better cognitive function. Changes in MMSE total scores from baseline to end point were used to determine the degree of cognitive decline in patients.

The CDR rating is a five-point scale with CDR-0 indicating no cognitive impairment, and the remaining four points various stages of dementia: CDR-0.5, very mild dementia; CDR-1, mild dementia; CDR-2, moderate dementia; CDR-3, severe dementia. Increases in CDR scores from baseline to end point were used to determine cognitive impairment progress in patients. Cognitive impairment progress was defined as an increase of more than one point in patients' total CDR score. MMSE and CDR assessments were performed at baseline and 30 month follow-up visits. Two trained neurologists completed MMSE and CDR assessments together by interviewing patients or their reliable caregivers.

Statistical analysis

All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL, USA). Measurement data (*e.g.*, age and MMSE score) were expressed as mean ± SD. Q-Q plots were used to test if measurement data were normally distributed. Normally distributed data were analyzed using independent samples *t*-tests. Enumeration data (*e.g.*, gender distributions, education, genotype, alleles between groups, and cognitive impairment progress number) were expressed as percentages and analyzed using Pearson's chi-square tests. All tests were two-sided and significance levels set at $P < 0.05$.

Results

Quantitative analysis and baseline information of participants

A total of 78 patients met the inclusion criteria. Of these 78 patients, 11 were absent from the follow-up owing to migration ($n = 5$), death ($n = 3$), or unable to continue their participation ($n = 3$). Therefore, during the 30-month study period, 67 patients completed both cognitive function assessments and were included in the analysis.

All 67 patients were Han Chinese, approximately half were female ($n = 35$), the age was 73.9 ± 3.3 years (68–79 years), and years with Alzheimer's disease ranged from 2.6 to 6.0. Sixteen patients were illiterate, 13 primary school graduates, and 38 middle school or higher education graduates. The mean baseline MMSE total score was 14.71 ± 2.62 scores (10–21 scores). According to CDR ratings, 36 patients were classified with mild dementia (CDR-1) and 31 with moderate dementia (CDR-2).

Genotyping results

In the APOE gene, fragment lengths of digested products were determined by the number of restriction enzyme sites (Kontula et al., 1990). The main fragment lengths of digested products for ε2 were 91 and 83 bp, ε3 were 91, 48, and 35 bp, and ε4 were 72, 48, and 35 bp. Therefore, APOE genotypes can be determined by digesting APOE PCR products. For APOE, three alleles and five genotypes (ε2/3, ε2/ε4, ε3/3, ε3/4, and ε4/4) were identified (Figure 1).

For the APOC1 gene, the *Hpa* I restriction site is a CGTT insertion located 317 bp upstream of the APOC1 gene promoter (transcription initiation site). Thus, there are two

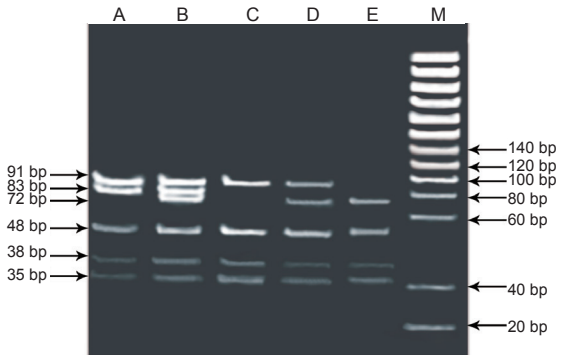

Figure 1 Gel electrophoresis of APOE genotypes following *Hha* I restriction digestion.
(A) The APOE ε2/3 genotype was digested by *Hha* I restriction endonuclease to 91, 83, 48, and 35 bp fragments. (B) The APOE ε2/4 genotype was digested by *Hha* I restriction endonuclease to 91, 83, 72, 48, and 35 bp fragments. (C) The APOE ε3/3 genotype was digested by *Hha* I restriction endonuclease to 91, 48, and 35 bp fragments. (D) The APOE ε3/4 genotype was digested by *Hha* I restriction endonuclease to 91, 72, 48, and 35 bp fragments. (E) The APOE ε4/4 genotype was digested by *Hha* I restriction endonuclease to 72, 48, and 35 bp fragments. M: Marker. The 38-bp fragment is a non-specific amplification product produced by primer-dimer formation. APOE: Apolipoprotein E.

Table 3 Comparison of APOC1 H2 and LRP T carriers in APOE ε4 carriers and non-carriers

Group	Number [*n*(%)]	APOC1		LRP	
		H_2	Non-H_2	T	Non-T
APOE ε4 carriers (*n* = 22)	22(33)	12(54)[a]	10(45)	2(9)	20(91)
APOE ε4 non-carriers (*n* = 45)	45(67)	8(18)	37(82)	5(11)	40(89)

[a]$P < 0.01$, *vs.* APOE ε4 non-carriers group. Using Pearson chi-square tests. Results are presented as *n*(%). APOE: Apolipoprotein E; APOC1: apolipoprotein CI; LRP: low-density lipoprotein receptor-related protein.

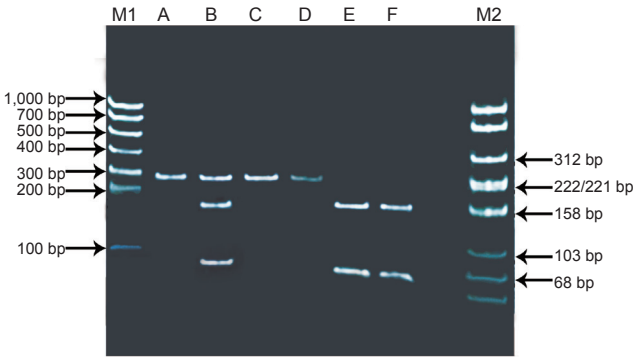

Figure 2 Gel electrophoresis of APOC1 genotypes following *Hha* I restriction digestion.
(A, C, D) APOC1 H1H1 genotypes were not digested by *Hha* I restriction endonuclease and only include a 222 bp fragment. (B) APOC1 H1H2 genotypes were digested by *Hha* I restriction endonuclease to 222, 160, and 66 bp fragments. (E, F) APOC1 H2H2 genotypes were digested by *Hha* I restriction endonuclease to 160 and 66 bp fragments. M1: pBR322/*Msp* I DNA marker; M2: SD011 DNA marker. APOC1: Apolipoprotein CI.

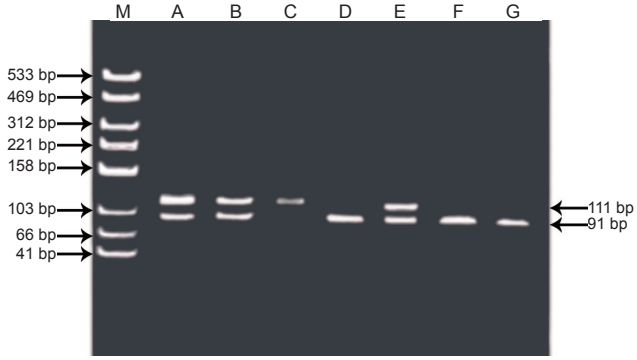

Figure 3 Gel electrophoresis of LRP genotypes following *Rsa* I restriction digestion.
(A, B, E) CT genotypes were digested by *Rsa* I restriction endonuclease to 111 and 91 bp fragments. (C) TT genotypes were digested by *Rsa* I restriction endonuclease to 111 bp fragments. (D, F, G) CC genotypes were digested by *Rsa* I restriction endonuclease to 91 bp fragments. M: Marker; LRP: low-density lipoprotein receptor-related protein.

APOC1 gene alleles. H1 has no *Hpa* I site and is the deletion allele, while H2 has the *Hpa* I restriction site and is the insertion allele. Therefore, H1 is not digested by *Hpa* I and has a length of 222 bp. The main H2 digested product fragments are 160 and 66 bp. Thus, both APOC1 alleles and three genotypes (H1H1, H1H2, and H2H2) were identified (Figure 2).

For the LRP gene, the polymorphism (C766T) in exon 3 is not a restriction endonuclease site. Therefore, a *Rsa* I restriction site (GTA) was introduced into the 3′ end of the forward primer. To ensure that the enzyme digested each PCR product, another *Rsa* I restriction endonuclease site (GTAC) was introduced into the reverse primer. T and C alleles were identified by 111 and 91 bp fragment bands, respectively. Both alleles and three genotypes (CT, TT, and CC) were identified (Figure 3).

Decline in MMSE total score after 30 months

APOE, APOC1, and LRP gene polymorphism distributions were examined in the 67 Alzheimer's disease patients. APOE ε4 carriers were defined as patients carrying at least one ε4 allele, while APOE ε4 non-carriers had no ε4 alleles. In total, 22 patients were APOE ε4 carriers and 45 were non-carriers. There were no significant differences in baseline demographic characteristics between APOE groups (Table 1). However, with cognitive decline, the change in MMSE total score from baseline to end, was significantly greater in APOE ε4 carriers than non-carriers ($t = 4.58$, $df = 65$, $P < 0.01$). No significant differences were found for change in MMSE total score in APOE ε2 and ε3, APOC1 H1 and H2, and LRP C and T carriers ($P > 0.05$).

Cognitive impairment progress after 30 months

The proportion of patients who met the criteria for cognitive impairment progress was significantly higher in APOE ε4 carriers compared with non-carriers (68% *vs.* 38%, $\chi^2 = 5.48$, $P = 0.02$, $OR = 3.53$, 95%$CI = 1.20$–10.40). No statistically significant differences were observed in APOE ε2 and ε3, APOC1 H1 and H2, and LRP C and T carriers ($P > 0.05$).

Table 1 Cognitive assessment of APOE ε4 carriers and non-carriers

	Mini-Mental State Examination (mean±SD, score)			
Group	Baseline	End-point	Reduction at end-point	Number of CIP [*n*(%)]
APOE ε4 carriers (*n* = 22)	15.82±2.23	8.21±2.82	7.62±2.51[b]	15 (68)[a]
APOE ε4 non-carriers (*n* = 45)	14.13±2.54	9.42±2.83	4.72±2.44	17 (38)

[a]$P < 0.05$, [b]$P < 0.01$, *vs.* APOE ε4 non-carriers group, using independent samples *t*-test and Pearson chi-square test. A greater reduction in Mini-Mental State Examination total score suggests more pronounced cognitive decline in Alzheimer's disease patients. Cognitive impairment progress (CIP) was defined as an increase by more than one point in Clinical Dementia Rating Scale total score. Fifteen patients met the definition of CIP in APOE ε4 carriers, with 17 in APOE ε4 non-carriers. APOE: Apolipoprotein E.

Table 2 Distribution of APOE, APOC1, and LRP alleles between CIP and non-CIP patient groups

	APOE			APOC1		LRP	
Group	ε2	ε3	ε4	H1	H2	C	T
CIP (*n* = 32)	5(8)	43(67)	16(25)[a]	50(78)	14(22)	33(51)	31(48)
Non-CIP (*n* = 35)	6(8)	57(81)	7(10)	62(88)	8(11)	37(53)	33(47)

[a]$P < 0.05$, *vs.* non-CIP group, using Pearson chi-square tests. Results are presented as *n*(%). APOE: Apolipoprotein E; APOC1: apolipoprotein CI; LRP: low density lipoprotein receptor-related protein; CIP: cognitive impairment progress.

Association between APOE, APOC1, LRP and cognitive impairment progress

The APOE ε4 allele frequency was significantly higher in the group with cognitive impairment progress, compared with the non-cognitive impairment progress group (25% *vs.* 10%, $\chi^2 = 5.29$, $P = 0.02$, $OR = 3.00$, 95%$CI = 1.14–7.87$). Allele frequency distributions for APOE ε2 and ε3, APOC1 H1 and H2, and LRP C and T polymorphisms did not differ significantly between either group ($P > 0.05$; Table 2).

Identification of gene-gene interactions

APOE ε4 and APOC1 H2

An APOC1 H2 carrier was defined as carrying at least one H2 allele. To investigate gene-gene interactions between APOE ε4 and APOC1 H2, we compared the proportion of APOC1 H2 carriers in APOE ε4 carriers *versus* non-carriers (Table 3). The proportion of patients carrying APOC1 H2 was significantly higher in APOE ε4 carriers than non-carriers (54.5% *vs.* 17.8%, $\chi^2 = 9.54$, $P = 0.002$, $OR = 5.55$, 95%$CI = 1.78–17.27$).

APOE ε4 and LRP T

An LRP T carrier was defined as carrying at least one T allele. To explore gene-gene interactions between APOE ε4 and LRP T, we compared the proportion of LRP T carriers in APOE ε4 carriers *versus* non-carriers (Table 3). The proportion of patients carrying LRP T showed no significant difference in APOE ε4 carriers compared with non-carriers (9.1% *vs.* 11.1%, $\chi^2 = 0.06$, $P = 0.80$, $OR = 0.80$, 95%$CI = 0.14–4.49$).

Discussion

Alzheimer's disease is a chronic neurodegenerative disease. Neural regeneration and degeneration involve different mechanisms. To promote neural regeneration, it is critical to understand the mechanism of neural degeneration, such as the pathogenic mechanism of Alzheimer's disease. It is known that many genetic variations/polymorphisms have a role in disease development of neurodegenerative pathologies. In this study, we focused on the association between cognitive decline in patients with late-onset Alzheimer's disease and the APOE, APOC1, and LRP genes. We also examined gene-gene interactions (APOE and APOC1, and APOE and LRP) in cognitive decline.

Association between APOE ε4 and cognitive decline in late-onset Alzheimer's disease patients

Accumulating evidence indicates that the APOE gene is associated with cognitive decline. Cognitively normal, healthy APOE ε4 homozygotes had accelerated memory decline compared with ε4 heterozygotes or non-carriers around the same age (60 years), before diagnosis of mild cognitive impairment and Alzheimer's disease (Caselli et al., 2004, 2007). In individuals diagnosed with mild cognitive impairment, ε4 carriers are associated with greater memory impairment compared with non-carriers (Smith et al., 1998). Moreover, evidence shows that APOE ε4 carriers diagnosed with mild cognitive impairment, show a significantly more rapid decline in performance on nearly all cognitive and functional domains (Whitehair et al., 2010). A meta-analysis found that patients with APOE ε4 alleles have a higher risk for progression from mild cognitive impairment to Alzheimer's disease (Elias-Sonnenschein et al., 2011). It appears that APOE ε4 is associated with cognitive decline in healthy elderly individuals and patients diagnosed with mild cognitive impairment. Nevertheless, there is still no evidence showing that the APOE gene can affect progression of cognitive impairment in patients with late-onset Alzheimer's disease. Therefore, we performed a 30-month study to determine if APOE ε4 has an effect on cognitive decline in late-onset Alzheimer's disease patients. Comparing MMSE change scores from baseline to end point, our results show they are significantly reduced in APOE ε4 carriers compared with non-carriers. Moreover,

the proportion of patients fulfilling the criteria for cognitive impairment progress is significantly higher in APOE ε4 carriers than non-carriers. The frequency of the APOE ε4 allele is also significantly higher in the cognitive impairment progress group, compared with the non-cognitive impairment progress group. Overall, our results indicate that cognitive impairment in late-onset Alzheimer's disease progresses at a faster pace in APOE ε4 carriers compared with non-carriers. Thus, APOE ε4 may play a role in progression of cognitive impairment in patients with late-onset Alzheimer's disease.

A Japanese case-control study found that APOE ε4 has a significant effect on reduction of MMSE score (reflecting memory or attention function) in Alzheimer's disease patients in their 80s (Nagata et al., 2013). Frequencies of the APOE ε2, ε3, and ε4 alleles are 2.7, 74.1, and 23.2%, respectively, in Chinese (Hong et al., 1996), and 2.0, 70, and 28.0%, respectively, in Japanese (Alzgene, 2001) populations. Therefore, allelic distribution of APOE is similar between Chinese and Japanese populations. However, we found differences between our study and Nagata's, on association between APOE ε4 and cognitive function in Japan (Nagata et al., 2013). First, our study is a 30-month longitudinal cohort study, while the Japanese study was cross-sectional. Second, the Alzheimer's disease patients in our study did not differ in baseline MMSE total score between APOE ε4 carriers and non-carriers. However, after the 30-month follow-up, Alzheimer's disease patients that were also APOE ε4 carriers, had significantly lower MMSE total scores than APOE ε4 non-carriers. In contrast, the Japanese study reported significantly lower MMSE total scores in APOE ε4 carriers than non-carriers at the beginning of their study. Third, the Alzheimer's disease patients in our study were younger, with a mean age of 73.9 years (range 68–79 years). Therefore, the results of our study indicate that APOE ε4 affects cognitive decline at a younger age than the Japanese study. The Japanese study investigated APOE ε4 influence on cognitive decline in Alzheimer's disease patients in their 80 years.

Prevailing evidence supports Aβ-dependent mechanisms link APOE ε4 status with Alzheimer's disease. Senile plaques, a major hallmark of Alzheimer's disease pathology, are extracellular deposits of beta amyloid in the gray matter of the brain. A neuropathological study of patients in their 50 years, indicated that 40.7% of APOE ε4 carriers had senile plaques compared with 8.2% in non-carriers (Kok et al., 2009). Therefore, compared with individuals with no ε4 alleles, the increased risk for senile plaque formation is eight-fold in people with ε4 alleles. Another neuropathological study also reported that Alzheimer's disease patients carrying two ε4 alleles had significantly more senile plaques in all neocortical regions than those with either one or no ε4 alleles (Tiraboschi et al., 2004). Amyloid positron emission tomography tracers, an important biomarker of Alzheimer's disease, have been developed for *in vivo* detection of brain fibrillar amyloid deposition. APOE ε4 carriers diagnosed with mild cognitive impairment had higher Pittsburgh compound-B retention than APOE ε4 non-carriers (Nordberg et al., 2013), indicating that patients diagnosed with mild cognitive impairment and being APOE ε4 carriers, have significantly more fibrillar amyloid deposition than ε4 non-carriers. Apolipoprotein E (ApoE) is a protein with 299 amino acids, encoded by the APOE gene. The three common polymorphisms in the APOE gene (ε2, ε3, and ε4) encode different ApoE proteins with single amino changes (Verghese et al., 2011). The single amino acid differences are critical because they alter the charge and structural properties of the protein, and ultimately affect functional properties of the ApoE isoforms. Differential effects of ApoE isoforms on Aβ aggregation and clearance are probably the major mechanisms in AD pathogenesis. ApoE isoform-mediated differential effects in Aβ metabolism result in different brain Aβ aggregation (ε4 > ε3 > ε2) (Holtzman et al., 2012), an upstream event known to trigger AD onset (Sperling et al., 2011). Some histopathological studies have demonstrated positive correlations between amyloid plaque density and APOE ε4 allele dose (Drzezga et al., 2009). Other potential mechanisms, such as differential modulation of tau phosphorylation and neurotoxicity by ApoE isoforms, and a role in synaptic plasticity and neuroinflammation, may also contribute mechanistically to increased AD risk and accelerated cognitive decline with the APOE gene (Kim et al., 2009).

Synergistic effect

APOE ε4 and APOC1 H2

Further analysis of APOC1 and LRP gene polymorphism distributions in APOE ε4 carriers and non-carriers showed that the proportion of patients with APOC1 H2 was significantly higher in APOE ε4 carriers than non-carriers. This suggests that APOE ε4 and APOC1 H2 may act synergistically in cognitive impairment progression in late-onset Alzheimer's disease patients. APOC1, encoded by APOC1, plays an important role in regulation of lipid metabolism and levels (Abildayeva et al., 2008). A number of studies have shown that APOC1 H2 is also an important risk factor for Alzheimer's disease (Woods et al., 2012). ApoC1 may be responsible for abnormal lipoprotein metabolism-related diseases (including Alzheimer's disease) by inhibiting clearance of triglyceride-rich lipoproteins (Berbée et al., 2006). The APOC1 gene is located in a cluster on the long arm of chromosome 19, nearly 5 kb downstream from the APOE gene (Woods et al., 2012). Interaction between APOE and APOC1 genes may play a role in onset and progress in Alzheimer's disease.

APOE ε4 and LRP

The LRP gene is located on the long arm of chromosome 12, and encodes a protein that is involved in lipid homeostasis and Aβ clearance. In human models, LRP is responsible for Aβ clearance from the central nervous system, *via* transport across the blood-brain barrier (Cuzzo et al., 2011). However, the relationship between LRP and Alzheimer's disease is controversial between studies. Studies have shown LRP as a risk factor for Alzheimer's disease (Kang et al., 1997; Kolsch et al., 2003), but others have failed to confirm this correlation (Fallin et al., 1997; Lendon et al., 1997). Studies have also found that LRP variations may decrease Alzheimer's disease risk (Kamboh et al., 1998; Zhou et al., 2008). Different

ethnic groups and small sample sizes may account for these conflicting results. Our study did not find an association between LRP and cognitive decline in Alzheimer's disease. In addition, our study showed that the proportion of patients with the LRP T allele was not significantly different between APOE ε4 carriers and non-carriers. Overall, our results do not show a gene interaction between APOE ε4 and LRP T.

Limitations

There are limitations to our study. First, the sample size is relatively small and the results, statistically different or not, may not be truly correct owing to type I or II errors. The statistical power of our study is not enough to compare cognitive decline among the genotypic groups (ε4 homozygotes, ε4 heterozygotes, and ε4 non-carriers). The MMSE subtests include different domains, such as orientation, memory, attention, calculation, language, and visual-spatial abilities. However, because of limited sample size, our study lacks the statistical power to compare different domains of cognitive function between ε4 carriers and non-carriers. Second, our results show that a synergistic effect between APOE ε4 and APOC1 H2 may contribute to cognitive impairment progression in Chinese patients with late-onset Alzheimer's disease. However, we do not know the functional connections between these two genes. Their functional roles and synergistic effect in pathogenesis of late-onset Alzheimer's disease remains to be further investigated.

In conclusion, despite these limitations, our study is the first to show that APOE ε4 plays an important role in cognitive impairment progression in Chinese patients with late-onset Alzheimer's disease. APOC1 H2 may act synergistically with APOE ε4 to increase the risk of cognitive impairment progression. Our results suggest that these alleles may have a role in promoting Alzheimer's neurodegenerative pathology leading to cognitive impairment progression and thereby, may have a negative impact on neural regeneration.

Author contributions: *Zhang WD, Hu CY and Yang Z were responsible for the study concept and design. Zhou Q, Peng DT and Yuan XR were in charge of data analysis and wrote the manuscript. Lv ZP, Pang SH, Jiang WY, Yang CY, Shi XH, Pang GF, Yang YG and Xie HQ performed the study. All authors approved the final version of the paper.*

Conflicts of interest: *None declared.*

References

Abildayeva K, Berbee JF, Blokland A, Jansen PJ, Hoek FJ, Meijer O, Lutjohann D, Gautier T, Pillot T, De Vente J, Havekes LM, Ramaekers FC, Kuipers F, Rensen PC, Mulder M (2008) Human apolipoprotein C-I expression in mice impairs learning and memory functions. J Lipid Res 49:856-869.

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders. Arlington, USA.

Bangen KJ, Beiser A, Delano-Wood L, Nation DA, Lamar M, Libon DJ, Bondi MW, Seshadri S, Wolf PA, Au R (2013) APOE genotype modifies the relationship between midlife vascular risk factors and later cognitive decline. J Stroke Cerebrovasc Dis 22:1361-1369.

Barnes DE, Yaffe K (2011) The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol 10:819-828.

Basak JM, Verghese PB, Yoon H, Kim J, Holtzman DM (2012) Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of Abeta uptake and degradation by astrocytes. J Biol Chem 287:13959-13971.

Berbée JF, van der Hoogt CC, Kleemann R, Schippers EF, Kitchens RL, van Dissel JT, Bakker-Woudenberg IA, Havekes LM, Rensen PC (2006) Apolipoprotein CI stimulates the response to lipopolysaccharide and reduces mortality in gram-negative sepsis. FASEB J 20:2162-2164.

Caselli RJ, Reiman EM, Locke DE, Hutton ML, Hentz JG, Hoffman-Snyder C, Woodruff BK, Alexander GE, Osborne D (2007) Cognitive domain decline in healthy apolipoprotein E epsilon4 homozygotes before the diagnosis of mild cognitive impairment. Arch Neurol 64:1306-1311.

Caselli RJ, Reiman EM, Osborne D, Hentz JG, Baxter LC, Hernandez JL, Alexander GG (2004) Longitudinal changes in cognition and behavior in asymptomatic carriers of the APOE e4 allele. Neurology 62:1990-1995.

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921-923.

Cruchaga C, Haller G, Chakraverty S, Mayo K, Vallania FL, Mitra RD, Faber K, Williamson J, Bird T, Diaz-Arrastia R, Foroud TM, Boeve BF, Graff-Radford NR, St JP, Lawson M, Ehm MG, Mayeux R, Goate AM (2012) Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PLoS One 7:e31039.

Cummings JL (1993) Mini-Mental State Examination. Norms, normals, and numbers. JAMA 269:2420-2421.

Cuzzo LM, Ross-Cisneros FN, Yee KM, Wang MY, Sadun AA (2011) Low-density lipoprotein receptor-related protein is decreased in optic neuropathy of Alzheimer disease. J Neuroophthalmol 31:139-146.

Devan WJ, Falcone GJ, Anderson CD, Jagiella JM, Schmidt H, Hansen BM, Jimenez-Conde J, Giralt-Steinhauer E, Cuadrado-Godia E, Soriano C, Ayres AM, Schwab K, Kassis SB, Valant V, Pera J, Urbanik A, Viswanathan A, Rost NS, Goldstein JN, Freudenberger P, et al. (2013) Heritability estimates identify a substantial genetic contribution to risk and outcome of intracerebral hemorrhage. Stroke 44:1578-1583.

Drzezga A, Grimmer T, Henriksen G, Mühlau M, Perneczky R, Miederer I, Praus C, Sorg C, Wohlschläger A, Riemenschneider M (2009) Effect of APOE genotype on amyloid plaque load and gray matter volume in Alzheimer disease. Neurology 72:1487-1494.

Elias-Sonnenschein LS, Viechtbauer W, Ramakers IH, Verhey FR, Visser PJ (2011) Predictive value of APOE-epsilon4 allele for progression from MCI to AD-type dementia: a meta-analysis. J Neurol Neurosurg Psychiatry 82:1149-1156.

Emi M, Wu LL, Robertson MA, Myers RL, Hegele RA, Williams RR, White R, Lalouel JM (1988) Genotyping and sequence analysis of apolipoprotein E isoforms. Genomics 3:373-379.

Fallin D, Kundtz A, Town T, Gauntlett AC, Duara R, Barker W, Crawford F, Mullan M (1997) No association between the low density lipoprotein receptor-related protein (LRP) gene and late-onset Alzheimer's disease in a community-based sample. Neurosci Lett 233:145-147.

Hachinski VC, Iliff LD, Zilhka E, Du Boulay GH, McAllister VL, Marshall J, Russell RW, Symon L (1975) Cerebral blood flow in dementia. Arch Neurol 32:632-637.

Hollenbach E, Ackermann S, Hyman BT, Rebeck GW (1998) Confirmation of an association between a polymorphism in exon 3 of the low-density lipoprotein receptor-related protein gene and Alzheimer's disease. Neurology 50:1905-1907.

Holtzman DM, Herz J, Bu G (2012) Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb Perspect Med 2:a6312.

Hong CJ, Liu TY, Liu HC, Wang SJ, Fuh JL, Chi CW, Lee KY, Sim CB (1996) Epsilon 4 allele of apolipoprotein E increases risk of Alzheimer's disease in a Chinese population. Neurology 46:1749-1751.

Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL (1982) A new clinical scale for the staging of dementia. Br J Psychiatry 140:566-572.

Jin C, Li W, Yuan J, Xu W, Cheng Z (2012) Association of the CR1 polymorphism with ate-onset Alzheimer's disease in Chinese Han populations: a meta-analysis. Neurosci Lett 527:46-49.

Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher J, Levey AI, Lah JJ, Rujescu D, Hampel H, Giegling I, Andreassen OA, Engedal K, Ulstein I, Djurovic S, Ibrahim-Verbaas C, Hofman A, Ikram MA, et al. (2013) Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 368:107-116.

Kamboh MI, Ferrell RE, DeKosky ST (1998) Genetic association studies between Alzheimer's disease and two polymorphisms in the low density lipoprotein receptor-related protein gene. Neurosci Lett 244:65-68.

Kang DE, Saitoh T, Chen X, Xia Y, Masliah E, Hansen LA, Thomas RG, Thal LJ, Katzman R (1997) Genetic association of the low-density lipoprotein receptor-related protein gene (LRP), and apolipoprotein E receptor, with late-onset Alzheimer's disease. Neurology 49:56-61.

Kim J, Basak JM, Holtzman DM (2009) The role of apolipoprotein E in Alzheimer's disease. Neuron 63:287-303.

Kok E, Haikonen S, Luoto T, Huhtala H, Goebeler S, Haapasalo H, Karhunen PJ (2009) Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. Ann Neurol 65:650-657.

Kolsch H, Ptok U, Mohamed I, Schmitz S, Rao ML, Maier W, Heun R (2003) Association of the C766T polymorphism of the low-density lipoprotein receptor-related protein gene with Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet 121:128-130.

Kontula K, Aalto-Setala K, Kuusi T, Hamalainen L, Syvanen AC (1990) Apolipoprotein E polymorphism determined by restriction enzyme analysis of DNA amplified by polymerase chain reaction: convenient alternative to phenotyping by isoelectric focusing. Clin Chem 36:2087-2092.

Lendon CL, J Talbot C, Craddock NJ, Woo Han S, Wragg M, Morris JC, Goate AM (1997) Genetic association studies between dementia of the Alzheimer's type and three receptors for apolipoprotein E in a Caucasian population. Neurosci Lett 222:187-190.

Li H, Wetten S, Li L, St JP, Upmanyu R, Surh L, Hosford D, Barnes MR, Briley JD, Borrie M, Coletta N, Delisle R, Dhalla D, Ehm MG, Feldman HH, Fornazzari L, Gauthier S, Goodgame N, Guzman D, Hammond S, et al. (2008) Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease. Arch Neurol 65:45-53.

Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 9:106-118.

Loparev VN, Cartas MA, Monken CE, Velpandi A, Srinivasan A (1991) An efficient and simple method of DNA extraction from whole blood and cell lines to identify infectious agents. J Virol Methods 34:105-112.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939-944.

Meng X, D'Arcy C (2012) Apolipoprotein E gene, environmental risk factors, and their interactions in dementia among seniors. Int J Geriatr Psychiatry 28:1005-1014.

Nagata T, Shinagawa S, Kuerban B, Shibata N, Ohnuma T, Arai H, Nakayama K, Yamada H (2013) Age-Related Association between Apolipoprotein E epsilon4 and Cognitive Function in Japanese Patients with Alzheimer's Disease. Dement Geriatr Cogn Dis Extra 3:66-73.

Nillesen WM, Smeets HJ, van Oost BA (1990) Human ApoCI HpaI restriction site polymorphism revealed by the polymerase chain reaction. Nucleic Acids Res 18:3428.

Nordberg A, Carter SF, Rinne J, Drzezga A, Brooks DJ, Vandenberghe R, Perani D, Forsberg A, Langstrom B, Scheinin N, Karrasch M, Nagren K, Grimmer T, Miederer I, Edison P, Okello A, Van Laere K, Nelissen N, Vandenbulcke M, Garibotto V, et al. (2013) A European multicentre PET study of fibrillar amyloid in Alzheimer's disease. Eur J Nucl Med Mol Imaging 40:104-114.

Popp J, Meichsner S, Kolsch H, Lewczuk P, Maier W, Kornhuber J, Jessen F, Lutjohann D (2013) Cerebral and extracerebral cholesterol metabolism and CSF markers of Alzheimer's disease. Biochem Pharmacol 86:37-42.

Smith GE, Bohac DL, Waring SC, Kokmen E, Tangalos EG, Ivnik RJ, Petersen RC (1998) Apolipoprotein E genotype influences cognitive 'phenotype' in patients with Alzheimer's disease but not in healthy control subjects. Neurology 50:355-362.

Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CJ, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, et al. (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:280-292.

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A 90:1977-1981.

Tiraboschi P, Hansen LA, Masliah E, Alford M, Thal LJ, Corey-Bloom J (2004) Impact of APOE genotype on neuropathologic and neurochemical markers of Alzheimer disease. Neurology 62:1977-1983.

Verghese PB, Castellano JM, Holtzman DM (2011) Apolipoprotein E in Alzheimer's disease and other neurological disorders. Lancet Neurol 10:241-252.

Whitehair DC, Sherzai A, Emond J, Raman R, Aisen PS, Petersen RC, Fleisher AS (2010) Influence of apolipoprotein E varepsilon4 on rates of cognitive and functional decline in mild cognitive impairment. Alzheimers Dement 6:412-419.

Woods AG, Sokolowska I, Taurines R, Gerlach M, Dudley E, Thome J, Darie CC (2012) Potential biomarkers in psychiatry: focus on the cholesterol system. J Cell Mol Med 16:1184-1195.

Zhou YT, Zhang ZX, Chan P, He XM, Tang MN, Wu CB, Hong Z (2008) Genetic association between low-density lipoprotein receptor-related protein gene polymorphisms and Alzheimer's disease in Chinese Han population. Neurosci Lett 444:109-111.

Zhuang JP, Wang G, Cheng Q, Wang LL, Fang R, Liu LH, Xiong R, Zhang Y, Gui YX, Kang WY, Tang HD, Chen SD (2012) Cognitive impairment and the associated risk factors among the elderly in the Shanghai urban area: a pilot study from China. Transl Neurodegener 1:22.

Copyedited by Robens J, James R, Wang J, Yang Y, Li CH, Song LP, Zhao M