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ORIGINAL ARTICLE

Correlations Between Single Nucleotide Polymorphisms, Cognitive Dysfunction, and Postmortem Brain Pathology in Alzheimer's Disease Among Han Chinese

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Abstract In this study, the distribution of five Alzheimer's disease (AD)-related single nucleotide polymorphisms (SNPs) in the Han population was examined in combination with the evaluation of clinical cognition and brain pathological analysis. The associations among SNPs, clinical daily cognitive states, and postmortem neuropathological changes were analyzed in 110 human brains from the Chinese Academy of Medical Sciences/Peking Union Medical College (CAMS/PUMC) Human Brain Bank. *APOE ε4* (OR = 4.482, $P = 0.004$), the *RS2305421* GG genotype (adjusted OR = 4.397, $P = 0.015$), and the *RS10498633* GT genotype (adjusted OR = 2.375, $P = 0.028$) were associated with a higher score on the ABC (Aβ plaque score, Braak NFT stage, and CERAD neuritic plaque score) dementia scale. These results advance our understanding of the pathogenesis of AD, the relationship between pathological diagnosis and clinical diagnosis, and the SNPs in the Han population for future research.

Keywords Human brain bank · Alzheimer's disease · *APOE ε4* · *ADAM10* · *SLC24A4*

Background

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss and cognitive dysfunction. The increasing number of AD patients has imposed huge burdens on individuals, families, and societies [1, 2] and scientists' exploration of its pathogenesis continues [3]. Since Dr. Alzheimer discovered AD and described its pathological features in 1906, Aβ and tau protein have been the major foci in the exploration of AD mechanisms. Over the years, studies of Aβ have dominated basic research, biomarker development [4], molecular imaging, and drug development. However, with the frequent frustrations of Aβ-targeting drugs [5], it is currently considered that tau protein-related pathological changes are more related than Aβ to clinical symptoms in the dissemination process in the brain and better reflect the severity of AD [6].

Integrating a series of single-nucleotide polymorphisms (SNPs) can help to predict the risk of dementia [7]. *APOE ε4* is well-known as the most important AD susceptibility gene. Allen Roses found that the risk of early-onset and late-onset AD in *APOE ε4* carriers is significantly higher; carrying one copy of the gene increases the risk by four

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times, while carrying two copies increases the risk by 12 times [8]. *APOE ε4* promotes the accumulation of Aβ in the brain and also interacts closely with tau protein [9].

RS2075650 is located on chromosome 19q13, close to *APOE ε4*. Since the first AD genome-wide association study (GWAS), it has been found that genes located near the *APOE ε4* gene on chromosome 19 also have a close relationship with AD [10]. After further exploration of *APOE* coding, a small-sample analysis showed that different evolutionary branches can be distinguished by a poly-T morphology repeated in the *TOMM40* (translocase of outer mitochondrial membrane 40) intron. Long poly-T is associated with an increased risk of AD and a lower age at onset [11].

The *RS2305421* locus is located on human chromosome 15q21.3-q23, and is one of the most common variants in the *ADAM10* gene (disintegrin and metalloprotease 10). *ADAM10* is an anti-amyloidogenic protease that can significantly mitigate Aβ formation [12]. Kim *et al*. found that the *RS2305421* has a genetic association with AD [13].This correlation has also been confirmed in a GWAS [14].

RS10498633, located on chromosome 14q32.12, encodes the SLC24A4 protein (24 solute carrier family member 4), which is a member of the K^+-dependent Na^+/Ca^{2+} exchanger protein family. These exchanger proteins are widely expressed in many tissues of the human body, especially neurons, suggesting that SLC24A4 may play an important role in the nervous system. The SLC24A4 protein is involved in the repair and the development of normal neurons [15], and inhibits the expression of inflammatory mediators in neurons.

Thus, because of their potential relationships with AD, we chose these five SNPs to fulfil the aim of the study which was to determine the distribution of AD-related SNPs in the Han population and analyze the correlation between clinical cognition and brain pathology based on cases from the Chinese Academy of Medical Sciences/Peking Union Medical College (CAMS/PUMC) Human Brain Bank.

Methods

One hundred and ten cases from the CAMS/PUMC Human Brain Bank in Beijing, China were included in this research. The protocols were approved by the Institutional Review Board of the Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China (Approval Number: 009-2014;031-2017). All ante-mortem written informed consent forms were received from both the potential donor and his/her next-of-kin to guarantee that the donation was completely voluntary and ethically-approved use of the brain tissues in future scientific research was permitted. The brain tissues were collected from 2012 to 2018, all following the international standard human brain banking procedure [16]. The clinical cognitive status was determined using the ECog Insider Questionnaire [17]. For neuropathological analysis, we used the "ABC" dementia score for each case according to the National Institute on Aging and Alzheimer's Association (NIA-AA) guidelines [18]. Six cases were excluded as they met the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria for vascular dementia [19].

Cognitive Function Assessment

ECog scales including 39 questions aim at assessing ante-mortem everyday function of the brain donor by comparing the participant's current ability with that of 10 years earlier. There are 6 sub-items in this questionnaire: memory, language, visuospatial functions, planning, organization, and divided attention. The subdivided areas are also associated with different patterns of progression in AD [20].

Ratings were based on a four-point scale: 1, no significant change or better; 2, questionable problems; 3, consistently worse to a small extent; 4, consistently worse to a large extent. An average was calculated for both the global ECog score and the separate score for each ECog domain. The relative items were marked "unknown" if informant could not recall or respond. If more than half of the items were marked "unknown", the average score was not calculated.

Cognitively normal was defined as ECog $\leq$ 1.0; mild cognitive impairment as ECog 1.0–2.0; and dementia as ECog $>$ 2.0. ECog 2.0 was set as the dividing point for MCI (mild cognitive impairment) and dementia [21], indicating that at least two cognitive domains were impaired. ECog scales have been demonstrated to be sensitive to early functional damage in both MCI [22] and dementia [18].

Neuropathological Classification

Brain Tissue Preparation and Fixation

Brains were cut into 2 halves. One half was frozen for molecular analysis and the other was fixed in 10% formalin for neuropathological experiments. After two weeks of formalin fixation, the meninges, severity of basilar and carotid artery sclerosis, and cerebral atrophy and infarction were assessed. By cutting along the superior colliculus and mammillary bodies, the midbrain and brainstem together

with the cerebellum were separated from the cerebrum. The brainstem and cerebellum were sliced at 0.5 cm along the sagittal axis, and the coronal cerebral sections were cut at 0.3 cm. Based on the NIA-AA guidelines, the following brain regions were sampled for conventional paraffin embedding: superior frontal cortex, primary motor cortex, inferior temporal cortex, hippocampus, anterior cingulate cortex, amygdala, supramarginal cortex, caudate/putamen, midbrain, pons, medulla oblongata, and cerebellar dentate nucleus.

Histopathological Score

In accord with the 2012 NIA-AA guidelines [18], the ABC score was used to incorporate amyloid-β deposits (A), neurofibrillary tangles (NFTs) (B), and neuritic plaques (C). The A score reflects the order of amyloid-β appearance in the brain in a tiered manner. For A and B scoring, the thickness of paraffin-embedded tissue was 5 μm, while for C scoring it was 10 μm.

A and B scores were both based on immunohistochemistry against Aβ (DAKO M0872, mouse monoclonal antibody, diluted 1:200) and p-tau (Thermo MN1020, mouse monoclonal antibody, 1:800). The above primary antibodies were incubated separately overnight at 4 °C, then processed for 30 min with the secondary antibody. The results were visualized with Polink-2 plus (ZSGB-BIO PV-9001, Beijing, China) and 3,3-diaminobenzidine (Boster AR1022, Beijing, China). The C score was determined by modified Bielschowsky stain for neuritic process in senile plaques [23]. The general ABC score was categorized to 4 gradations: none, low, intermediate, and high.

The severity of cerebrovascular changes, such as cerebral amyloid angiopathy and arteriosclerosis, was scored as mild, moderate, or severe according to previous research [24, 25].

SNP Genotyping

Genomic DNA was extracted from frozen prefrontal cortex using tissue DNA extraction kit (GeneOn BioTech GO-BTCD-400, Beijing, China) and detected by PCR-restriction fragment length polymorphism. The genotyping using second-generation sequencing technology was performed by Beijing Zixi Bio Tech Co., Ltd.

Statistical Analysis

We used Spearman analysis to evaluate the correlations among clinical cognitive status, pathological changes, SNPs, and demographic variables (education, gender, age, and post-mortem delay). Age was found to influence both cognition and pathological changes. Therefore, age was set as a control factor in the subsequent analysis of ABC and ECog scores. Since cognitive status (normal, MCI, or dementia) and ABC score (none, low, intermediate, or high) were ordinal variables, OLR (ordinal logistic regression) models were used [26, 27]. First, age, *APOE ε4*, and three SNPs (*RS2075650*, *RS2305421*, and *RS10498633*) were separately analyzed using a univariate OLR model. Then multivariate OLR analysis was conducted. In multivariate model 1, age and one of the three SNPs were included. In multivariate model 2, age, *APOE ε4*, and one of the three SNPs were included. In each regression, the estimated parameter of each SNP was divided by the estimated parameter of age to evaluate the influence of the SNP on the progression of cognitive impairment or AD pathology. $P < 0.05$ was regarded as statistically significant.

Results

Effect of Demographic Variables on Cognition and Pathological Changes

The age at death ranged from 31 years to 102 years, and the average was 78 years. Among the elderly (> 80 years old), 38.5% showed cognitive impairment (Global ECog Score > 2), while this occurred in 10.4% of the younger cases (≤ 80 years old). The ECog score correlated with age in an almost continuous fashion (ANOVA, $P = 0.007$) (Fig. 1A).

There was no significant difference in education (Kruskal-Wallis H, $P = 0.737$), gender (Kruskal-Wallis H, $P = 0.267$), and post-mortem delay (Kruskal-Wallis H, $P = 0.751$) between the different general ABC groups (Tables S1 and S2).

Spearman Correlations Among Everyday Cognition Scores, ABC Scores, and Demographic Variables

Spearman correlation analysis was used to further confirm the relationships between ABC scores, ECog scores, and demographic variables. Older participants tended to present with more evident pathological changes and worse cognitive conditions. Neither education nor gender was significantly related to the above concurrent pathologies (Table S3).

The average Global scores for the Intermediate and High groups were 2.0 and 2.1, while those for the None and Low groups were 1.0 and 1.0. As the pathological conditions worsened, cognitive function was more severely impaired (Fig. 1B–H).

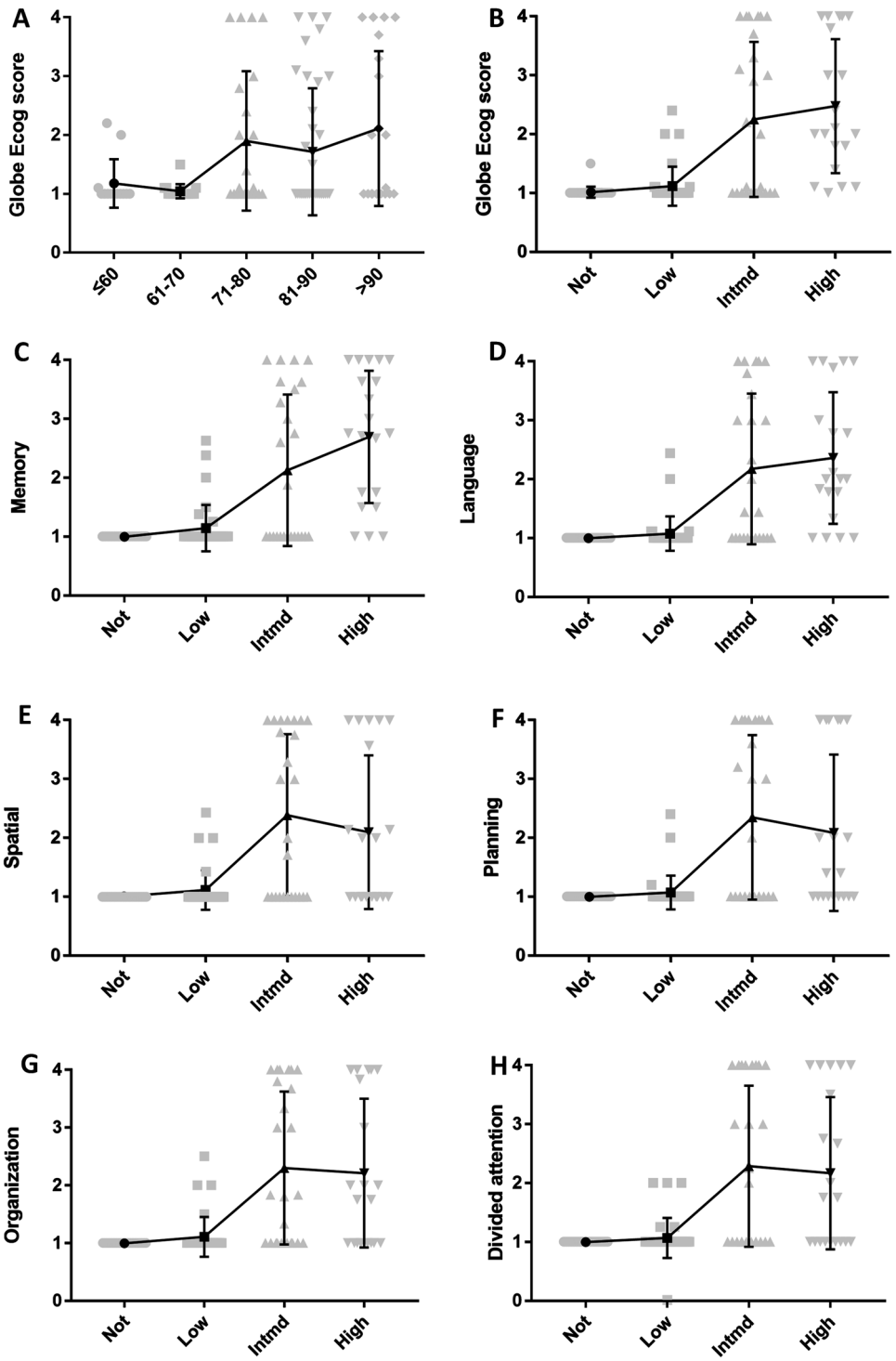

Fig. 1 Correlations between ECog scores and age or progression of AD pathology. **A** Correlations between age and Global ECog scores. **B** Correlations between ECog and ABC scores. **C** Correlations between memory and ABC scores. **D** Correlations between language and ABC scores. **E** Correlations between spatial and ABC scores. **F** Correlations between planning and ABC scores. **G** Correlations between organization and ABC scores. **H** Correlations between divided attention and ABC scores. Intmd, Intermediate.

Partial correlation analysis was applied to the ECog domains along with A, B, and C. There were extensive connections between them and the B score was more strongly correlated with the ECog score than A and C, as analysis of the B score showed $R > 0.4$ and $P < 0.001$ in all domains (Table 1).

SNPs

Univariate OLR showed that age and *APOE ε4* significantly influenced the cognitive state. Greater age (OR = 1.051, $P = 0.004$) and *APOE ε4* (OR = 5.607, $P = 0.002$) were associated with the outcome of a worse cognitive

Table 1 Partial correlations between ABC scores, concurrent pathologies and cognitive assessment scales of ECog scores

	Global ECog	Memory	Language	Spatial	Planning	Organization	Divided Attention
General ABC score	0.490 (< 0.001)***	0.534 (< 0.001)***	0.484 (< 0.001)***	0.393 (< 0.001)***	0.379 (< 0.001)***	0.417 (< 0.001)***	0.417 (< 0.001)***
A score	0.397 (< 0.001)***	0.424 (0.067)	0.406 (0.001)**	0.329 (0.001)**	0.299 (0.002)**	0.372 (< 0.001)***	0.347 (< 0.001)***
B score	0.465 (< 0.001)***	0.465 (< 0.001)***	0.465 (< 0.001)***	0.410 (< 0.001)***	0.410 (< 0.001)***	0.418 (< 0.001)***	0.405 (< 0.001)***
C score	0.446 (< 0.001)***	0.444 (< 0.001)***	0.454 (< 0.001)***	0.397 (< 0.001)***	0.373 (< 0.001)***	0.435 (< 0.001)***	0.363 (< 0.001)***
Cerebral amyloid angiopathy	−0.065 (0.516)	−0.045 (0.653)	−0.042 (0.677)	−0.077 (0.442)	−0.084 (0.402)	−0.095 (0.341)	−0.042 (0.674)
Arteriosclerosis	0.04 (0.693)	0.004 (0.971)	−0.022 (0.831)	0.020 (0.844)	0.021 (0.836)	−0.017 (0.866)	0.011 (0.913)

The correlation was adjusted by age. The results are presented as correlation coefficients (*P* value). **P* < 0.05 (2-tailed), ***P* < 0.01 (2-tailed), ****P* < 0.001 (2-tailed)

state. Thus, age and *APOE ε4* were included in the multivariate models as confounding factors.

The unadjusted OR for a worse cognitive state in *RS2075650* AG carriers was 6.417 ($P < 0.001$) relative to AA carriers. After adjusting for age, the OR was 6.508 ($P < 0.001$). The effect of *RS2075650* remained significant (OR = 5.697, $P = 0.021$) after adjusting for age and *APOE ε4* (Table 2). According to the regression results, the progression of cognitive impairment began 31.6 years earlier in *RS2075650* AG carriers than in AA carriers (Table 3, Fig. 2A). Taking the situation at 80 years of age as an example, there was a probability of 51.4% to have dementia and 21.3% to have MCI for *RS2075650* AG carriers with *APOE ε4*, but for *RS2075650* AA carriers without *APOE ε4*, the probability of having dementia was 13.1% and MCI 14.4%.

The unadjusted OR of *RS2305421* GG carriers was 4.267 ($P = 0.027$) and the OR of AG carriers was 2.869 ($P = 0.028$) relative to AA carriers. After adjusting for age, the OR of GG carriers was 4.027 ($P = 0.037$) and the OR of AG carriers was 2.971 ($P = 0.026$). After adjusting for both age and *APOE ε4*, the OR of GG carriers was 5.254 ($P = 0.018$) and the OR of AG carriers was 3.869 ($P = 0.010$) (Table 2). The progression of cognitive impairment began 30.1 years earlier in *RS2305421* AG carriers and 36.9 years earlier in GG carriers than in AA carriers (Table 3, Fig. 2B). Taking the situation at 80 years as an example, there was a probability of 68.4% to have dementia and 17.0% to have MCI for *RS2305421* GG carriers with *APOE ε4*, but for *RS2305421* AA carriers without *APOE ε4*, the probability of having dementia was 5.8% and MCI 8.6%.

Besides, *RS10498633* showed no evidence of influencing cognitive state before or after adjusting for age and *APOE ε4* (Table 2).

Univariate OLR showed that age and *APOE ε4* significantly influenced the ABC score. Greater age (OR = 1.070, $P < 0.001$) and *APOE ε4* (OR = 4.482, $P = 0.004$) were associated with the outcome of a higher ABC score. Thus, age and *APOE ε4* were included in the multivariate models as confounding factors.

The unadjusted OR of *RS2075650* AG carriers was 3.615 ($P = 0.007$) relative to AA carriers. After adjusting for age, the OR was 3.340 ($P = 0.012$). However, the effect of *RS2075650* was not significant after adjusting for *APOE ε4* (Table 4).

The unadjusted OR of *RS2305421* GG carriers was 4.187 ($P = 0.016$) relative to AA carriers. After adjusting for age, the OR was 3.896 ($P = 0.025$). After adjusting for both age and *APOE ε4*, the OR of GG carriers was 4.397 ($P = 0.015$) and the OR of AG carriers was 2.321 ($P = 0.040$) (Table 4). The progression of AD pathology began 12.8 years earlier in *RS2305421* AG carriers and

Table 2 Results of ordinal logistic regression for *RS2075650*, *RS2305421*, *RS10498633*, and cognitive state.

Variables	Cognitive state			Univariate model		Multivariate model 1*		Multivariate model 2*	
	Normal	MCI	Dementia	Unadjusted OR	*P*	Adjusted OR	*P*	Adjusted OR	*P*
RS2075650									
AA	60	12	11	Reference		Reference		Reference	
AG	6	3	10	6.417 (2.370, 17.357)	< 0.001	6.508 (2.340, 18.120)	< 0.001	5.697 (1.303, 24.903)	0.021
RS2305421									
AA	31	4	4	Reference		Reference		Reference	
AG	30	9	13	2.869 (1.122, 7.338)	0.028	2.971 (1.139, 7.752)	0.026	3.869 (1.381, 10.827)	0.010
GG	6	3	4	4.267 (1.181, 15.425)	0.027	4.027 (1.090, 14.895)	0.037	5.254 (1.326, 20.822)	0.018
RS10498633									
GG	30	4	7	Reference		Reference		Reference	
GT	33	12	14	1.988 (0.859, 4.604)	0.109	1.728 (0.731, 4.084)	0.212	1.826 (0.736, 4.527)	0.194

*Age and one of the three SNPs were included in multivariate model 1; age, *APOE ε4*, and one of the three SNPs were included in multivariate model 2.

Table 3 Parameter estimates of cognitive state for *RS2075650* and *RS2305421* in model 2.

Variables	Age		SNP		Change in progression of cognitive impairment (years)
	Estimate	*P*	Estimate	*P*	
RS2075650	0.055	0.006			
AA			Reference		
AG			1.740	0.021	31.6
RS2305421	0.045	0.012			
AA			Reference		
AG			1.353	0.010	30.1
GG			1.659	0.018	36.9

22.4 years earlier in GG carriers than in AA carriers (Table 5, Fig. 3A). Taking the situation at 80 years as an example, the probability to have an ABC score of none, low, intermediate, or high was 3.0%, 14.0%, 35.5%, or 47.5% respectively for *RS2305421* GG carriers with *APOE ε4*, but was 34.9%, 43.1%, 17.0%, or 5.0% for *RS2305421* AA carriers without *APOE ε4*.

The unadjusted OR of *RS10498633* GT carriers was 2.686 ($P = 0.009$) relative to GG carriers. After adjusting for age, the OR was 2.226 ($P = 0.040$). After adjusting for both age and *APOE ε4*, the OR of GT carriers was 2.375 ($P = 0.028$) (Table 4). The progression of AD pathology began 14.4 years earlier in *RS10498633* GT carriers than GG carriers (Table 5, Fig. 3B). Taking the situation at 80 years as an example, the probability to have an ABC score of none, low, intermediate, or high was 3.0%, 14.4%, 34.6%, or 48.0% respectively for *RS10498633* GT carriers with *APOE ε4*, but was 32.0%, 43.9%, 18.2%, or 5.8% for *RS10498633* GG carriers without *APOE ε4*.

Discussion

NFT Stage Widely Correlated with Cognitive Performance

Our results showed that the higher the NFT score, the worse the general cognitive impairment. Both CERAD (Consortium to Establish a Registry for Alzheimer's Disease) and Thal scoring can be applied as indicators of

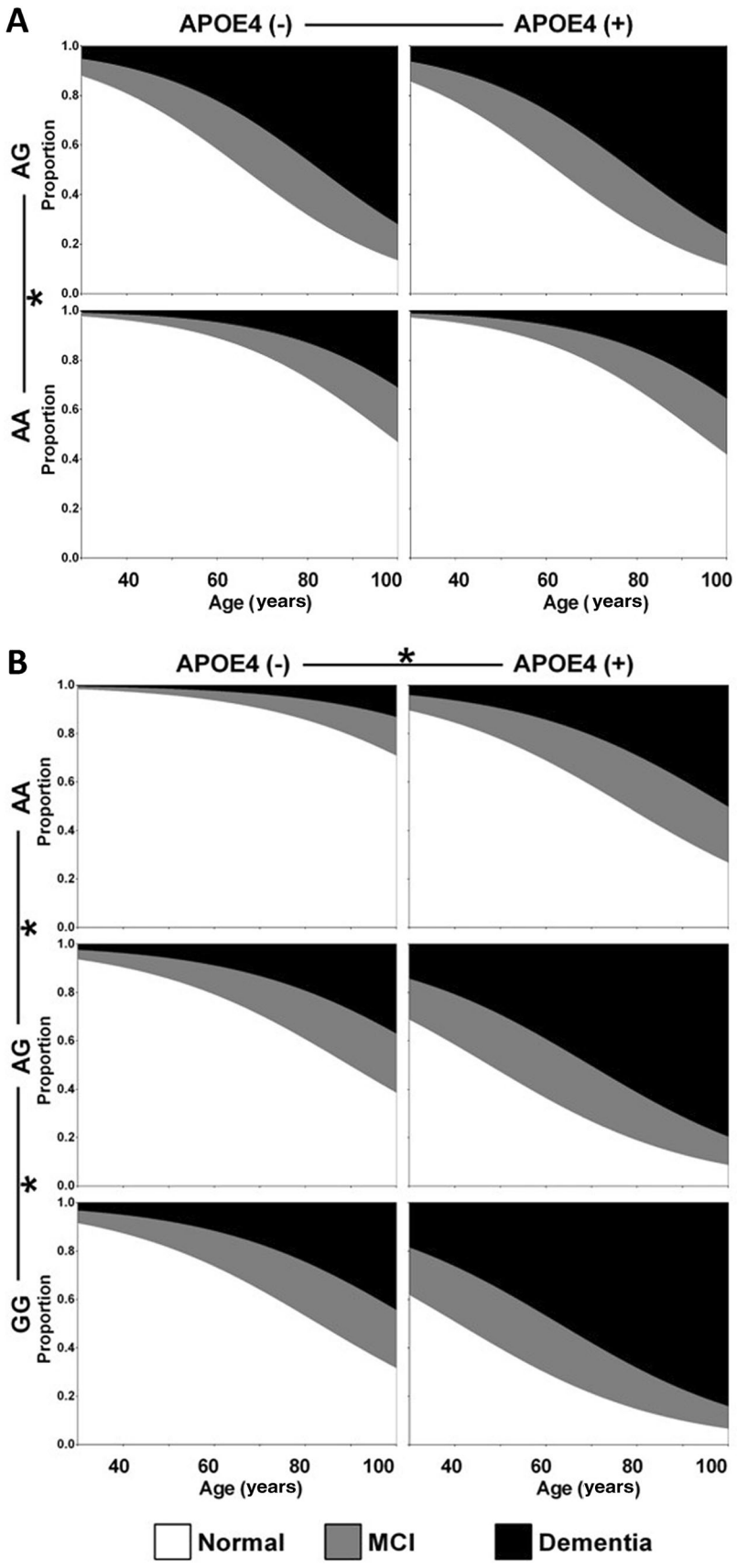

Fig. 2 Influence of *RS2075650* and *RS2305421* on the progression of cognitive impairment. **A** Influence of *RS2075650* on the progression of cognitive impairment fitted by ordinal logistic regression in model 2. **B** Influence of *RS2305421* on the progression of cognitive impairment fitted by ordinal logistic regression in model 2.

Table 4 Results of ordinal logistic regression for *RS2075650*, *RS2305421*, *RS10498633*, and ABC score.

Variables	ABC score[#]				Univariate model		Multivariate model 1*		Multivariate model 2*	
	N	L	I	H	Unadjusted OR	*P*	Adjusted OR	*P*	Adjusted OR	*P*
RS2075650										
AA	27	27	21	8	Reference		Reference		Reference	
AG	2	6	4	7	3.615 (1.428, 9.152)	0.007	3.340 (1.306, 8.542)	0.012	2.430 (1.562, 9.217)	0.192
RS2305421										
AA	13	18	5	3	Reference		Reference		Reference	
AG	15	13	15	9	2.014 (0.938, 4.323)	0.073	2.136 (0.969, 4.711)	0.060	2.321 (1.041, 5.176)	0.040
GG	1	4	5	3	4.187 (1.311, 13.383)	0.016	3.896 (1.190, 12.743)	0.025	4.397 (1.326, 14.600)	0.015
RS10498633										
GG	16	14	8	3	Reference		Reference		Reference	
GT	11	20	16	12	2.686 (1.275, 5.658)	0.009	2.226 (1.038, 4.773)	0.040	2.375 (1.095, 5.150)	0.028

[#]ABC score was divided into four grades: N (none), L (low), I (intermediate), H (high).

*Age and one of the three SNPs were included in multivariate model 1; age, APOE ε4 and one of the three SNPs were included in multivariate model 2.

Table 5 Parameter estimates of ABC score for *RS2305421* and *RS10498633* in model 2.

Variables	Age		SNP		Change in progression of AD pathology (years)
	Estimate	*P*	Estimate	*P*	
RS2305421	0.066	< 0.001			
AA			Reference		
AG			0.842	0.040	12.8
GG			1.481	0.015	22.4
RS10498633	0.06	< 0.001			
GG			Reference		
GT			0.865	0.028	14.4

β-amyloid deposition according to the 2012 NIA-AA guidelines [4]. The A score reveals the distribution of Aβ deposits in separate brain regions, and the C score reflects the density of cortical neuritic plaques [28].

NFTs result from intracellular tau protein accumulation and fibrilization, originally appearing in the entorhinal cortex, then diffusing to the hippocampus and isocortex [29]. Because of hyperphosphorylation, tau loses its normal physiological role in constructing microtubules and maintaining cellular stability [30, 31]. A recent study has also shown that phosphorylation of tau protein is necessary for β-amyloid-induced synapse loss [32]. In recent decades, it has gradually become known that neurofibrillary tau pathology correlates more closely than Aβ with the clinical symptoms of dementia and related diseases such as AD [33, 34, 35].

A meta-analysis has shown that Braak NFT staging is better correlated with cognitive status than the staging of neuritic plaques and diffuse Aβs [36]. In our study, the correlation between Aβ and cognition was not as evident as that for p-tau. A possible reason may be that Aβ is more related to aging than cognitive impairment. A previous study has demonstrated that, along with aging, reduced antioxidant content, mitochondrial dysfunction, and synaptic aging all contribute to the vulnerability to Aβ toxicity [37].

APOE ε4 and AD

We demonstrated that *APOE ε4* had a significant negative effect on both cognition and AD pathology, consistent with previous studies [8, 9]. The *APOE* classification depends on the specific SNPs at the *RS429358* and *RS7412* loci, which produce low-density lipoproteins, ligands for very-low-density lipoproteins, and chylomicron receptors. As early as 30 years ago, *APOE ε4* was shown to be related to AD [38]. The three protein phenotypes of human *APOE* are *APOE ε2*, *APOE ε3*, and *APOE ε4*. About 15% of the population carry the *APOE ε4* gene while 65% of AD patients carry it. Among them, the *APOE ε2* and *APOE ε3*

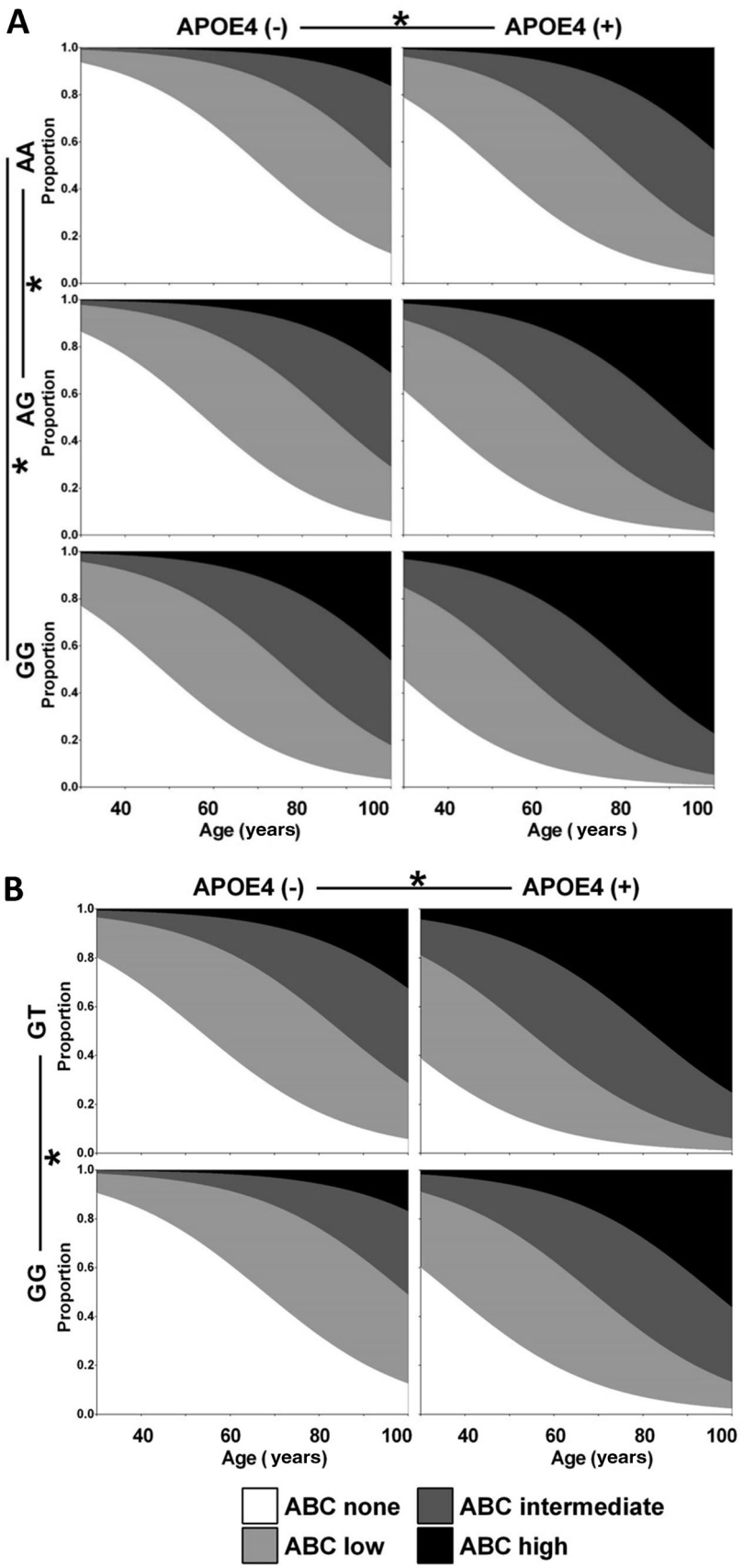

Fig. 3 Influence of *RS2305421* and *RS10498633* on the progression of AD pathology. **A** Influence of *RS2305421* on the progression of AD pathology fitted by ordinal logistic regression in model 2. **B** Influence of *RS10498633* on the progression of AD pathology fitted by ordinal logistic regression in model 2.

gene products process lipids better in glial cells, while *APOE ε4* is almost incapable of this task, resulting in abnormal accumulation of lipid droplets in glial cells and ineffective isolation of lipid peroxides. With aging, these deficiencies are magnified, and the fundamental functional differences among *APOE* subtypes make certain individuals more vulnerable to oxidative stress [39]. It is the presence of *APOE ε4* that leads to neuronal dysfunction [40].

APOE and tauopathy are closely interlinked. Experiments have found that tau levels are significantly increased in *APOE ε4* transgenic mice, and tau proteins have a greater degree of cell body-dendritic redistribution [3]. Epidemiological statistics show that *APOE ε4* carriers have elevated blood cholesterol levels, which accelerate the progression of coronary atherosclerosis. There is increasing evidence that these changes damage the blood-brain barrier and promote the deposition of tau protein in the brain [41]. Another experiment showed that higher cerebrospinal fluid (CSF) tau levels are closely associated with more rapid disease progression in AD patients with the *APOE ε4* genotype. And when cells are incubated in culture media rich in tau protein or in CSF from AD patients with the *APOE ε4* genotype, the apoptotic activity is higher. If, and only if, linked to *APOE ε4* is CSF detrimental to astrocyte survival, cortical plasticity, and disease progression [42].

RS2075650 and AD

TOMM40 is the most important of the TOMMs; its abnormal expression directly affects mitochondrial protein import by blocking the nuclear transport of encoded proteins to the mitochondria, so they cannot enter the mitochondria to function properly, eventually leading to mitochondrial dysfunction. Followed by increased production of oxygen free radicals, this genetic abnormality starts a vicious cycle, affecting the normal function of the brain [43, 44].

Ma *et al*. investigated TOMM40 polymorphism and AD in a Northern Han Chinese population and found that three SNP loci (*RS157580*, *RS2075650*, and *RS157581*) play a role in pathogenesis [45]. However, Bagnoli *et al*. investigated the polymorphisms of TOMM40 in AD and frontotemporal dementia patients in Italy. By investigating 3 SNPs (*RS157580*, *RS2075650*, and *RS157581*), they found that TOMM40 polymorphism was associated with *APOE*, and there was no evidence that TOMM40 worked independently from *APOE ε4* and became an AD risk factor itself [46]. Bernardi *et al*. found that in a familial AD population, the age at onset of AD was slightly different under the influence of the TOMM40 (*RS10524523*) gene, but it was also particularly difficult to exclude the effects of other genetic variants around this SNP [47]. In our study, the effect of *RS2075650* on ECog was independent of *APOE ε4*, but its effect on ABC scores was not significant after adjusting for *APOE ε4*. Further research is needed to clarify the influence of *RS2075650* on the pathogenesis of AD.

RS2305421 and AD

Our data demonstrated that *RS2305421* is related to the cognitive impairment and pathogenesis of AD. *RS2305421* is considered to be the main component of amyloid precursor protein (APP) α-secretase [48, 49]. Aβ, which is produced by the cleavage of APP by β-secretase and γ-secretase, plays a critical role in AD. α-secretase, on the other hand, converts APP to soluble APPs-α, which hinders the production of Aβ. This phenomenon reported in a northern Asian Han population is consistent with our findings [50]. In order to explore its role in the pathogenesis of AD, further studies on a larger scale involving more ethnic groups are needed.

ABC Score Correlation with *RS10498633*

In our study, *RS10498633* influenced the pathology of AD but not the degree of cognitive impairment. *RS10498633* is a mutation in SLC24A4, which is associated with enamel growth, hair color, and skin pigmentation [51–55]. A study of 25,580 AD patients and 48,466 controls confirmed that *RS10498633* is significantly associated with AD risk in Caucasians [56]. However, another study showed that *RS10498633* may not play a major role in the AD susceptibility of Chinese [57]. In specific case and control groups, allele and gene frequencies in different ethnic groups may vary [58]. Our research provides new evidence for this, but it still needs further investigation to determine whether this mutation is a risk factor or a predictive factor of AD.

Limitations

Our study was cross-sectional, so neither the sequence of exposure and the timing of outcome, nor the causal relationship between exposure and outcome was taken into account. Our statistical data volume was 110, and the number of cases used to study the AD relationship was 104 (6 cases were excluded). Although the amount of data has been greatly extended compared to our previous report [59], it is still relatively small, resulting in some conclusions that are not sufficiently accurate or persuasive. Thus, it should be pointed out that non-significant results do not necessarily mean that an SNP is not related to AD. Since AD is a multifactorial disease, other variables such as marital, mental, and nutritional status, should be added to the collection of clinical data. In addition, long post-mortem intervals in sampling may affect the tissue quality and have an impact on correlation analysis.

Conclusion

The B score, a measure of NFTs, had the strongest correlation with cognitive dysfunction and AD-related pathological changes. The A score, a measure of Aβ plaques, and the C score, a measure of neuritic plaques, also had specific influences. *APOE4* was the most important susceptibility gene for AD in our data. *RS2305421* and *RS10498633* may have correlations with the ABC score, but further evidence is required to confirm or deny this correlation. These results promote the understanding of the pathogenesis of AD, the relationship between the pathological diagnosis and the clinical diagnosis, and the SNPs in a northern Han Chinese population, and provide directions for future research.

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