ORIGINAL RESEARCH

Relationship Between Genetic Polymorphism and Cognitive Impairment in Patients with Acute Ischemic Stroke (APOE)

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ABSTRACT

Objective • To investigate the relationship between apolipoprotein E (ApoE) gene polymorphism and cognitive impairment (PSCI) in patients after acute ischemic stroke (AIS).

Methods • A total of 150 AIS patients were treated in Chengde Central Hospital from December 2022 to December 2023 and were selected and divided into a disorder group (n=88) and a normal group (n=62) according to the presence or absence of PSCI. Clinical data of patients in the two groups were collected, ApoE genotype and allele distribution of patients in the disabled group and the normal group were detected, Montreal Cognitive Assessment and Mini-Mental State Examination scores of patients with different ApoE gene subtypes were compared, and the risk factors of PSCI after AIS were analyzed by unconditional Logistic regression.

Results • The proportion of patients with acute lesions (≥3.0 cm) and the degree of carotid artery stenosis (moderate, severe, complete occlusion) in the disorder group was higher than that in the normal group, and the National Institutes of Health Stroke Scale score was higher than that in the control group, with statistical significance ($P < .05$). There were significant differences in the genotype and allelic distribution of ApoE between the two groups ($P < .05$). In both groups, the highest genotype frequency of ApoE was the ε3/3 homozygous type, which was 47.73% (in the disorder group) and 72.58% (in the normal group) respectively. In contrast, there were no significant differences in the genotype frequencies of ε2/2, ε2/3, ε2/4 and ε4/4 alleles in the two groups ($P > .05$). This means that in both groups of patients, the frequency of the ApoE ε3/3 genotype was the highest, while the genotype frequencies of ε2/2, ε2/3, ε2/4 and ε4/4 alleles were not significant between the two groups. difference. The distribution differences of these genotypes and alleles may be related to aspects such as disease risk and physiological function, providing valuable information for in-depth exploration of the role of ApoE in patients. The genotype frequency of ε3/3 in the disorder group was lower than that in the normal group. The frequency of the ε3/4 genotype was higher than that of the normal group, and the difference was statistically significant ($P < .05$). In both groups, the highest allele frequency was ε3 (68.75% in the disorder group and 83.06% in the normal group), and there was no difference in the frequency of ε2 allele between the two groups ($P > .05$). The frequency of the ε3 allele in the disorder group was lower than that in the normal group, and the frequency of the ε4 allele was higher than that in the normal group, the difference was statistically significant ($P < .05$). In the patients with cognitive impairment after AIS (disorder group), the MOCA and MMSE scores of patients with different ApoE subtypes (ε2, ε3, ε4) were compared, and the differences among the three groups were statistically significant ($P < .05$). The MOCA and MMSE scores in the ε4 group were lower than those in the ε2 and ε3 groups. The difference was statistically significant ($P < .05$). Logistic regression analysis showed that the degree of carotid artery stenosis, NIHSS score, and ApoEε4 gene were independent risk factors for PSCI in patients with AIS ($P < .05$).

Conclusion • APOE gene polymorphism is associated with cognitive impairment in post-AIS patients, and carrying the ApoE Epsilon 4 gene may be associated with PSCI in post-AIS patients. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Acute ischemic stroke (AIS) may cause sudden loss of neurological function, manifested as limb dysfunction, language impairment, and cognitive dysfunction. [1] Among these consequences, poststroke cognitive impairment (PSCI) has attracted increasing attention, with significant negative impacts on long-term recovery and quality of life. [2] Apolipoprotein E (ApoE) is a lipid metabolism regulatory protein with different alleles (ε2, ε3, and ε4), which plays a key role in the recovery of brain functions such as nerve injury recovery, lipid metabolism, and inflammatory response. [3] Previous studies have confirmed that ApoE is an important genetic risk factor for Alzheimer's disease and other neurodegenerative diseases. [4,5] Given some clinical and biomarker similarities between PSCI and Alzheimer's disease, some researchers have begun to pay attention to the potential association of ApoE gene polymorphisms with PSCI. [6] However, the currently available literature remains relatively limited and under-researched. Therefore, this study aimed to investigate the relationship between ApoE gene polymorphisms and PSCI to provide new biological evidence for the future prevention and treatment of PSCI.

For application in clinical practice, our findings may contribute to individualized risk assessment and intervention

for PSCI. By gaining a deeper understanding of the relationship between ApoE gene polymorphisms and PSCI, we can more accurately identify individuals at risk for PSCI among patients with AIS. This helps doctors take more targeted measures in early diagnosis and intervention, thereby improving patients' recovery and quality of life. In addition, our study may provide a basis for the future development of treatment strategies targeting specific ApoE genotypes and promote the development of personalized medicine. This in-depth study of the relationship between ApoE and PSCI will provide clinicians with more information about patients' brain functional recovery and support the development of more effective treatment plans.

MATERIALS AND METHODS

General information

One hundred and fifty patients after acute ischaemic stroke (AIS) who were treated in Chengde Central Hospital from December 2022 to December 2023 were selected. According to whether the patients had cognitive impairment (PSCI) or not, the patients were divided into cognitively impaired group (n=88) and cognitively normal group (n=62).

Inclusion criteria: (1) acute ischemic stroke met the diagnostic criteria in the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018 [7], and the patients in the cognitive impairment group met the diagnostic criteria for cognitive dysfunction; (2) patients were conscious and clear, had normal visual and auditory functions, and were able to actively cooperate with completing the examination; (3) patients had complete clinical data; (4) approval was obtained from the Medical Ethics Committee (CDCHIL2022-401).

Exclusion criteria: (1) suffering from neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, etc.; (2) history of previous cognitive impairment; (3) history of major traumatic brain injury; (4) patients who have been treated with medications that may affect cognitive function in the past 30 days; (5) suffering from depression, schizophrenia, etc. that may interfere with the assessment of cognition; (6) surgery affecting the central nervous system within the past 6 months.

General data collection

Collect the basic data of the two groups of patients, including gender, age, BMI, whether combined with hypertension, diabetes mellitus, coronary heart disease, the degree of carotid artery stenosis, and so on.

Gene polymorphism detection

A 2 mL morning fasting blood sample was collected from the patient's vein, and EDTA2Na was added as an anticoagulant. A 2 ml morning fasting blood sample was collected from the patient's vein and EDTA2Na was added as an anticoagulant. We used a DNA extraction kit to extract genomic DNA from whole blood, following the instructions provided by the manufacturer. The name of the DNA extraction kit is PCR DNA extraction kit, the manufacturer is GeneEx, Inc., and the country of origin is the United States. On the ABI 7500 PCR instrument, each reaction mixture is placed into a PCR tube and subsequently PCR amplified. The reaction mixture in the PCR system kit includes an appropriate amount of buffer, primers, etc., and the mixing ratio is in accordance with the recommendations of GeneEx, Inc. The primer sequences used in PCR amplification are: front primer: 5'-AGT GCA ATG CGG AAC TGA AC-3'; back primer: 5'-GAA GGC ACC ACC ACG TTA C-3'. These details are intended to provide readers with a clear understanding of experimental methods and reagents and to ensure experimental reproducibility and accuracy. The amplification process started with pre-denaturation at 95°C for 5 min, followed by 45 cycles, each cycle consisting of denaturation at 95°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s. The final extension was performed for 10 min at 72°C. PCR products were subjected to 2% agarose gel electrophoresis and observed under UV light. Next, the PCR product is digested with restriction enzymes in an appropriate buffer and incubated for 1-2 hours. The size of the PCR product is (please mention the actual measured PCR product size). For restriction endonuclease digestion, we used Dicerase X (assuming Dicerase X was used). According to prediction, the PCR product will obtain two specific fragments after enzyme digestion, with sizes X1 and X2 respectively. The purpose of this step is to verify the specificity of the PCR product through restriction endonuclease digestion and ensure that the obtained product is consistent with the expected target. The digested product was further electrophoresed in 10% polyacrylamide gel and the gel was silver-stained to see the DNA bands. After the silver staining treatment, the gel was imaged using a UV gel imager, and the results obtained were analyzed. The upstream primer in the primer sequence of this study: is 5'-TCCAAGGAGCTGCAGGCGGCGCA-3'. Downstream primer 5'-ACAGAATYCGCCCCGGGCCTGGTACACTGCCA-3'.

Cognitive function assessment

The cognitive functions of the patients were assessed using the Montreal Cognitive Assessment Scale (MOCA)[8] and the Brief Mental Status Examination (MMSE).[9]

MOCA: This scale covers different domains such as attention, memory, executive functioning, language, and visuospatial ability, and based on the participant's performance in these tasks, a total score can be derived, known as the MoCA score. The total MoCA score is 30, with a score of >26 representing normal cognitive functioning, and a score of ≤26 representing cognitive dysfunction.

MMSE: This is a questionnaire that assesses cognitive functioning in several domains including time and place orientation, memory, attention and calculation, naming, repetition, comprehension, reading, writing, and designing copies. The MMSE has a total score of 30, with a score of ≥24 representing normal cognitive functioning, and a score of <24 representing cognitive dysfunction.

Table 1. Comparison of general information between the two groups [n(%), ± *s*]

Classification	Cognitively impaired group (n=88)	Cognitively normal group (n=62)	t/x^2	*P* value
Sex (cases)				
Male	48(54.55)	36(58.06)	0.183	.669
Female	40(45.45)	26(41.94)		
Age/year	65.56±10.12	64.13±9.87	0.861	.391
BMI (kg/m²) kg/m²	22.58±2.50	23.12±2.15	1.379	.170
Hypertension (cases)	52(59.09)	27(43.55)	3.525	.061
Diabetes mellitus (case)	37(42.05)	19(30.65)	2.021	.155
Coronary heart disease (case)	30(34.09)	16(25.81)	1.174	.279
Acute lesion (case)				
<1.5 cm	11(12.50)	17(27.42)	15.290	.000
1.5~<3.0 cm	35(39.77)	26(41.94)		
≥3.0 cm	42(47.73)	19(30.65)		
Degree of carotid artery stenosis (cases)				
Mild	16(18.18)	32(51.61)	24.407	.000
Moderate	40(45.45)	23(37.10)		
Severe	20(22.73)	7(11.29)		
Complete occlusion	12(13.64)	0(0.00)		
NIHSS score (points)	10.83±3.42	8.57±2.95	4.214	.000

Statistical analysis

In statistical analysis, Statistical Package for Social Science (SPSS) version 26.0 was used for data analysis. For measurement information that is approximately normally distributed or normally distributed, the mean $\bar{x} \pm s$ is used to represent the standard deviation, and the difference between the two groups is compared through the independent sample *t*-test; the count information is expressed in the form of n (%), using χ^2 The test was used for comparison between groups; and for analyzing the risk factors of PSCI in AIS patients, unconditional logistic regression was used.

RESULTS

Comparison of the general information of the two groups

There is no difference between the two groups in terms of gender, age, BIM, hypertension, diabetes mellitus, and coronary artery disease ($P > .05$), and the patients in the cognitively impaired group have a larger acute lesion size and carotid stenosis than those in the cognitively normal group, and their NIHSS scores are higher than those of the cognitively normal group, and the difference is statistically significant ($P < .05$). See Table 1.

Comparison of genotype and allele distribution of ApoE in the two groups

Comparison of genotype and allele distribution of ApoE in the two groups showed statistical differences ($P < .05$). In both groups, the highest genotype frequency of ApoE was ε3/3 (47.73% in the cognitively impaired group and 72.58% in the cognitively normal group), and there was no difference in comparing the genotype frequencies of ε2/2, ε2/3, ε2/4 and ε4/4 in the two groups ($P > .05$).

Table 3 presents the allelic distribution of ApoE in the two groups, consisting of a cognitively impaired group (n=88) and a cognitively normal group (n=62). The allelic frequencies for ε2, ε3, and ε4 are detailed in the table. Notably, the cognitively impaired group showed a significantly different distribution compared to the cognitively normal group (χ^2=13.802, $P = .001$). Specifically, the ε3 allele was more prevalent in the cognitively impaired group, while the ε4 allele showed higher frequency in this group compared to the cognitively normal group ($P < .05$). See Table 2 and Table 3, Figure 1, and Figure 2.

Table 2. Genotype distribution of ApoE in the two groups [n (%)]

Group	ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	x^2	*P* value
Cognitively impaired group (n=88)	2(2.27)	10(11.36)	4(4.55)	42(47.73)[a]	27(30.68)[a]	3(3.41)	58.67	.000
Cognitively normal group (n=62)	2(3.23)	9(14.52)	1(1.61)	45(72.58)	4(6.45)	1(1.61)		

[a]$P < .05$ compared to the cognitively normal group.

Table 3. Allelic distribution of ApoE in the two groups [n (%)]

Group	ε2	ε3	ε4	x^2	*P* value
Cognitively impaired group (n=88)	18(10.23)	121(68.75)[a]	37(21.02)[a]	13.802	.001
Cognitively normal group (n=62)	14(11.29)	103(83.06)	7(5.65)		

[a]$P < .05$ compared to the cognitively normal group.

Figure 1. Genotype distribution of ApoE in the two groups

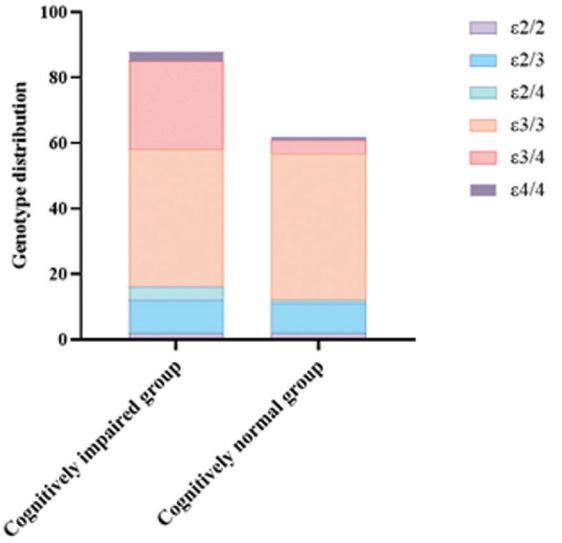


Figure 2. Allelic distribution of ApoE in the two groups

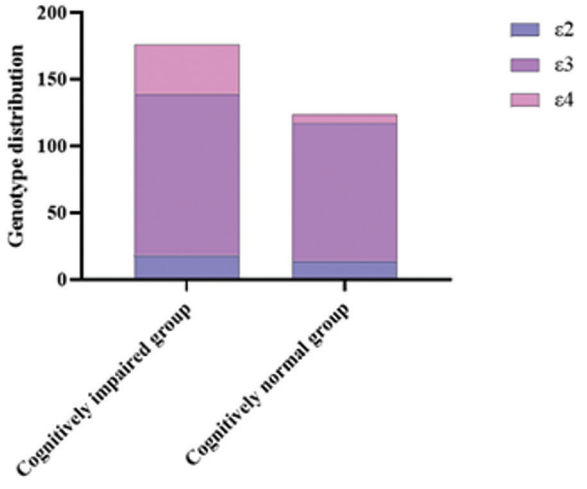


Table 4. Comparison of MoCA and MMSE scores in patients with different ApoE subtypes ± *s*)

Indicator	ε2 group (n=12)	ε3 group (n=58)	ε4 group(n=34)	*F*	*P* value
MoCA Total Score	21.55±1.89	20.26±2.63	17.58±1.78[a,b]	19.629	.000
MMSE Total Score	20.26±2.12	19.85±3.05	15.22±2.04[a,b]	35.726	.000

[a]*P* < .05 compared with ε2
[b]*P* < .05 compared with ε3

Figure 3. Comparison of MOCA and MMSE scores in patients with different ApoE subtypes

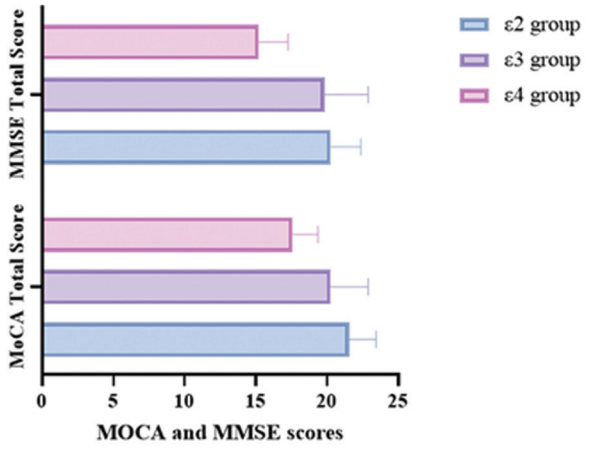
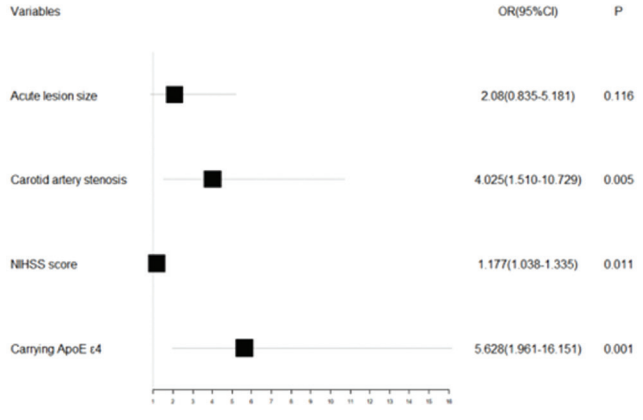


Table 5. Analysis of risk factors for the occurrence of PSCI in patients after AIS

Classification	B	S	*P* value	OR	95%CI
Acute lesion size	0.733	0.466	.116	2.080	0.835~5.181
Carotid artery stenosis	1.393	0.500	.005	4.025	1.510~10.729
NIHSS score	0.163	0.064	.011	1.177	1.038~1.335
Carrying ApoE ε4	1.728	0.538	.001	5.628	1.961~16.151

Figure 4. Analysis of risk factors for the occurrence of PSCI in patients after AIS



Comparison of MOCA and MMSE scores of patients with different ApoE subtypes in the cognitive impairment group

A comparison of MOCA and MMSE scores among patients with different ApoE subtypes is presented. The patients were categorized into ε2, ε3, and ε4 groups based on their ApoE subtype. The mean scores for MoCA and MMSE are provided for each group. Statistical analysis revealed significant differences among the ApoE subtypes for both MoCA (F=19.629, *P* = .000) and MMSE (F=35.726, *P* = .000) scores. Specifically, the ε4 group exhibited lower mean scores in both cognitive assessments compared to the ε2 and ε3 groups (*P* < .05), indicating a potential association between the ApoE subtype and cognitive performance. See Table 4, Figure 3.

Analysing the factors influencing the occurrence of PSCI in patients after AIS

Factors such as acute lesion size, degree of carotid artery stenosis, NIHSS score, and whether or not to carry the ApoEε4 gene were included in the unconditional logistic regression analysis, and the results showed that the degree of carotid artery stenosis, NIHSS score, and carrying the ApoEε4 gene were the independent risk factors for the occurrence of PSCI in patients after AIS (*P* < .05). See Table 5, Figure 4.

DISCUSSION

AIS is one of the major causes of disability and death worldwide, and with the advances in stroke treatment, the survival rate of patients has been increasing year by year, but the incidence of complications such as PSCI has also increased.[10] According to statistics, the incidence of cognitive impairment after AIS is as high as 26.05% to 63.04%.[11] PSCI not only seriously affects the quality of life of patients, but also increases the burden on the healthcare system. Despite a large number of studies exploring multiple possible mechanisms of PSCI, its exact etiology remains unclear. ApoE is part of the apolipoprotein family, which is synthesized and metabolized mainly in the liver. The main function of ApoE, as a protein with multiple morphologies, is to participate in the metabolism and transport of lipoproteins, which are particles used for storing and transporting fats in the body.[12] Different ApoE genotypes may have different effects on how lipoproteins are metabolized and transported, and how they relate to lipid levels.[12,13] These variants are found in exon 4 of the gene, where there are three different variants or alleles of amino acid codons at positions 112 and 158: ε2, ε3, and ε4. These alleles can combine in different ways to produce six different genotypes: ε2/2, ε2/3, ε2/4, ε3/3, ε3/4, and ε4/4.[13] Considering the potential association of ApoE with a wide range of diseases, especially neurodegenerative diseases, the study of its genetic polymorphisms may provide critical clues to understanding the etiology and development of these diseases.

The results of this study showed that acute lesion size, degree of carotid stenosis, and NIHSS score were significantly higher in the cognitively impaired group than in the cognitively normal group, suggesting that these factors have a strong correlation with PSCI after AIS. Meanwhile, further logistic regression analysis identified the degree of carotid artery stenosis and NIHSS score as independent risk factors for the development of cognitive impairment in patients after

AIS ($P < .05$). The reason for this analysis was that the carotid artery is the main blood vessel supplying the brain, and its stenosis leads to reduced cerebral blood flow. Sustained hypoperfusion triggers local hypoxia in the brain, prompting neuronal apoptosis and microglial activation.[14] Secondly, under sustained hypoperfusion, brain nerve cells are under metabolic stress for a long period, which is prone to induce the release of multiple inflammatory mediators, further aggravate nerve cell damage, and lead to the reduction of cognitive function.[15] In addition, carotid artery stenosis is prone to plaque formation, and these plaques may dislodge to form small blood clots, leading to the blockage of cerebral microvessels and the formation of multiple microischemic brain injuries. The cumulative effect of these multiple microischemic brain injuries may have a significant impact on cognitive function.[16] Further, the NIHSS score reflects the severity of the stroke and the extent of damage. A higher score implies more extensive damage to the brain and also implies that the function of multiple functional areas of the brain may be impaired. In particular, when this damage involves brain regions that are closely related to cognitive function, such as the prefrontal, temporal lobe, and hippocampal structures, the risk of cognitive impairment is significantly increased in patients.[17]

In this study, we also examined the distribution of ApoE genotypes and alleles in the two groups of patients. The results showed that in both groups, the most common ApoE genotype was ε3/3, but in the cognitively impaired group, the frequency of its occurrence was significantly lower than that in the cognitively normal group, whereas the frequency of the ε3/4 genotype was significantly higher, which suggests that the ε3/4 genotype may be associated with the risk of PSCI. From an allelic perspective, ε3 was the most common but appeared less frequently in the cognitively impaired group than in the cognitively normal group, whereas the frequency of ε4 was higher. This further emphasizes the association between the ε4 allele and the risk of PSCI. To further clarify the correlation between its causal phenotype and allele distribution with PSCI, the cognitive functions of the patients were assessed in this study, and the results showed that in the cognitively impaired group, patients with the ApoE ε4 genotype had significantly lower MoCA and MMSE scores than those with the ε2 and ε3 genotypes ($P < .05$). It was further clarified after logistic regression analysis that carrying the ApoE ε4 genotype was an independent risk factor for the development of cognitive impairment in patients after AIS ($P < .05$).

However, the exact reason why the ApoE ε4 genotype affects PSCI is currently unknown, there is currently no clear explanation of how the ApoE ε4 genotype affects post-stroke cognitive impairment (PSCI), some studies have provided supporting evidence suggesting that ApoE ε4 may be involved in cognitive decline and neurological disease. Experimental studies in neurobiology also try to reveal the influence mechanism of ApoE ε4. Some of these studies have found that ApoE ε4 may be involved in neurobiological mechanisms such as neuronal damage, inflammatory response, and amyloid deposition, which may be related to the development of PSCI. The analysis may be related to the following mechanisms.[18] Firstly, high activity of ApoE ε4 can lead to massive lipid uptake by cells, which increases cholesterol levels in the body. Cholesterol accumulation then leads to dysregulation of lipid metabolism, further catalyzing the progression of atherosclerosis, which is a key factor in triggering cerebral infarction.[18] Secondly, ApoE plays a central role in nerve damage repair, especially in the metabolism of myelin phospholipids. In particular, ApoE ε3 seems to be able to benefit this process, whereas ε4 does not, and therefore, neurological recovery may be impeded in ApoE ε4 carriers. Finally, ε4 is more susceptible to oxidative stress than ε3, and its protection against ischaemic nerve damage is relatively weak. This suggests that individuals carrying the ApoE ε4 gene may suffer more severe neuronal cell damage when exposed to ischaemic nerve injury. Thus, multiple mechanisms of ApoE ε4 may act together to increase the risk of cognitive impairment after cerebral infarction.

Although our study found a significant association between ApoE genotype and cognitive function, however, we recognize that how this association affects clinical practice and patient management requires further consideration. A more comprehensive assessment of the risk for cognitive impairment by the ApoE genotype may aid in the development of personalized treatment strategies. First, knowledge of ApoE genotypes may provide clinicians with an earlier assessment of risk for cognitive impairment. This means that during the initial diagnosis of a patient, doctors can implement more targeted preventive measures or monitoring plans to reduce the risk of cognitive decline. In addition, depending on the ApoE genotype, doctors can more personalized treatment plans to better meet the specific needs of patients. Second, our findings also have potential implications for the development of pharmacotherapy plans and supportive care. For patients carrying specific ApoE genotypes, more frequent cognitive function assessments and closer follow-up, as well as more aggressive lifestyle interventions, may be needed. This personalized treatment approach can maximize the effectiveness of treatment and slow the rate of cognitive decline. Taken together, our study not only reveals the relationship between ApoE genotype and cognitive function but also provides some useful insights for clinical practice and patient management. By exploring the clinical implications of these findings in greater depth, we can better guide medical professionals to use this information more effectively in practice to provide patients with more personalized and comprehensive medical services.

Although this study provides us with important insights into the complex relationship between cognitive impairment and genetic factors, we are also aware of many unanswered questions. Future studies could explore a wider range of genetic markers to more fully understand the pathogenesis of cognitive impairment. Given the rapid advances in genetics, we can focus on other potential genetic variants, such as emerging genetic markers involved in genomic association

studies (GWAS). Furthermore, the introduction of functional neuroimaging studies may be a critical step in gaining insights into neural networks in the development of cognitive impairment. By combining genetics and neuroimaging, we can more fully reveal the association between genetic variation and brain structure and function, leading to a better understanding of the mechanisms of reduced cognitive function. These suggested future research directions will help fill the gaps in current research, prompt us to gain a deeper understanding of the pathogenesis of cognitive impairment, and provide more effective strategies for future intervention and treatment.

In conclusion, there is a correlation between APOE gene polymorphisms and cognitive impairment in patients after AIS, and carrying the ApoE ε4 gene may be associated with the development of PSCI in patients after AIS.

ETHICAL COMPLIANCE

This study was approved by the ethics committee of Chengde Central Hospital. Signed written informed consent was obtained from the patients and/or guardians.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

AUTHOR CONTRIBUTIONS

CM, DW and LC designed the study and performed the experiments, ZH and XL collected the data, ZH, XL, and HZ analyzed the data, and CM, DW, and LC prepared the manuscript. All authors read and approved the final manuscript.

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REFERENCE

1. Herpich F, Rincon F. Management of Acute Ischemic Stroke. *Crit Care Med*. 2020;48(11):1654-1663. doi:10.1097/CCM.0000000000004597
2. Liu T, Wu LY, Yang XM, et al. Influence of Nutritional Status on Prognosis of Stroke Patients With Dysphagia. *Altern Ther Health Med*. 2022;28(7):26-33.
3. Martens YA, Zhao N, Liu CC, et al. ApoE Cascade Hypothesis in the pathogenesis of Alzheimer's disease and related dementias. *Neuron*. 2022;110(8):1304-1317. doi:10.1016/j.neuron.2022.03.004
4. Serrano-Pozo A, Das S, Hyman BT. APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *Lancet Neurol*. 2021;20(1):68-80. doi:10.1016/S1474-4422(20)30412-9
5. Lanfranco MF, Ng CA, Rebeck GW, Apo E. ApoE Lipidation as a Therapeutic Target in Alzheimer's Disease. *Int J Mol Sci*. 2020;21(17):6336. doi:10.3390/ijms21176336
6. Lanfranco MF, Sepulveda J, Kopetsky G, Rebeck GW. Expression and secretion of apoE isoforms in astrocytes and microglia during inflammation. *Glia*. 2021;69(6):1478-1493. doi:10.1002/glia.23974
7. Wang G, Fang B, Yu X, Li Z. [Interpretation of 2018 guidelines for the early management of patients with acute ischemic stroke]. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*. 2018;30(4):289-295. doi:10.3760/cma.j.issn.2095-4352.2018.04.001
8. Ciesielska N, Sokołowski R, Mazur E, Podhorecka M, Polak-Szabela A, Kędziora-Kornatowska K. Is the Montreal Cognitive Assessment (MoCA) test better suited than the Mini-Mental State Examination (MMSE) in mild cognitive impairment (MCI) detection among people aged over 60? Meta-analysis. *Psychiatr Pol*. 2016;50(5):1039-1052. doi:10.12740/PP/45368
9. Arevalo-Rodriguez I, Smailagic N, Roqué-Figuls M, et al. Mini-Mental State Examination (MMSE) for the early detection of dementia in people with mild cognitive impairment (MCI). *Cochrane Database Syst Rev*. 2021;7(7):CD010783. doi:10.1002/14651858.CD010783.pub3
10. Chen Z, Wang N, Na R, et al. Bioinformatic prediction of depression-related signaling pathways regulated by miR-146a in peripheral blood of patients with post-stroke depression. *Cell Mol Biol (Noisy-le-grand)*. 2022;68(6):40-47. doi:10.14715/cmb/2022.68.6.7
11. Zhong Z, Wu H, Ye M, et al. Association of APOE Gene Polymorphisms with Cerebral Infarction in the Chinese Population. *Med Sci Monit*. 2018;24:1171-1177. doi:10.12659/MSM.905979
12. Henningfield CM, Arreola MA, Soni N, Spangenberg EE, Green KN. Microglia-specific ApoE knock-out does not alter Alzheimer's disease plaque pathogenesis or gene expression. *Glia*. 2022;70(2):287-302. doi:10.1002/glia.24105
13. Lee S, Devanney NA, Golden LR, et al. APOE modulates microglial immunometabolism in response to age, amyloid pathology, and inflammatory challenge. *Cell Rep*. 2023;42(3):112196. doi:10.1016/j.celrep.2023.112196
14. Chen D, Huang X, Lu S, et al. RBP4/Lp-PLA2/Netrin-1 signaling regulation of cognitive dysfunction in diabetic nephropathy complicated with silent cerebral infarction. *Exp Clin Endocrinol Diabetes*. 2017;125(8):547-553. doi:10.1055/s-0043-109099
15. Jin X, He W, Zhang Y, et al. Association of APOE ε4 genotype and lifestyle with cognitive function among Chinese adults aged 80 years and older: A cross-sectional study. *PLoS Med*. 2021;18(6):e1003597. doi:10.1371/journal.pmed.1003597
16. Cong L, Ren Y, Wang Y, et al. Mild cognitive impairment among rural-dwelling older adults in China: A community-based study. *Alzheimers Dement*. 2023;19(1):56-66. doi:10.1002/alz.12629
17. Angelopoulou E, Paudel YN, Papageorgiou SG, Piperi C. APOE Genotype and Alzheimer's Disease: The Influence of Lifestyle and Environmental Factors. *ACS Chem Neurosci*. 2021;12(15):2749-2764. doi:10.1021/acschemneuro.1c00295
18. Lindner K, Beckenbauer K, van Ek LC, et al. Isoform- and cell-state-specific lipidation of ApoE in astrocytes. *Cell Rep*. 2022;38(9):110435. doi:10.1016/j.celrep.2022.110435