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Association of Apolipoprotein *E* Polymorphisms with White Matter Lesions and Brain Atrophy

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Objective Apolipoprotein E (ApoE) is mainly synthesized in the liver. So far, it is unknown the relationship among *APOE* gene polymorphisms and WML, brain atrophy. Therefore, the aim of the study was to assess the associations of *APOE* gene polymorphisms in patients with WML and brain atrophy.

Methods A total of 58 patients with WML, 128 patients with brain atrophy, 112 patients with co-occurrence of WML and brain atrophy and 95 healthy elderly volunteers were recruited from Renmin Hospital of WuHan University.

Results Allele *E3* was the most common allele. The alleles *E2* had significantly higher levels of ApoB and lower age in WML group. The alleles *E2* was associated with the lower level of ApoB, LDL-Ch, TCh, and sdLDL in co-occurrence group. The *E3/E3* genotype has higher level of sdLDL, but lower age and female frequency in WML. The *E3/E4* genotype had higher level of TG, but lower age in WML. Gender, Age, *E2*, Hyperhomocysteinemia and UA were also significantly associated with disease progression.

Conclusion This study found that clinical data, lipids and metabolic complications were closely related to ApoE genotypes and alleles, and also disease progression and type.
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Key Words Apolipoprotein E, Polymorphisms, Brain atrophy, WML.

INTRODUCTION

Apolipoprotein E (ApoE) is a 34-kDa glycoprotein with 299 amino acids that is mainly synthesized in the liver. The apoE gene with 3.6 kb is located on chromosome 19q13.32.[1] *APOE* has three major isoforms, *APOE2*, *E3*, and *E4*, which are encoded by three alleles (*E2*, *E3*, and *E4*), resulting in six different genotypes (*E2/E2*, *E3/E3*, *E2/E3*, *E3/E4*, *E2/E4*, and *E4/E4*).[2,3] The *E3* allele differs from the *E2* allele by change cysteine to arginine at codon 158, while the *E4* differs from *E3* by a substitution of arginine for cysteine at residue 112. ApoE protein plays an important role in lipoprotein metabolism. *E2* allele was associated with decreased plasma cholesterol level but *E4* with elevations in LDL-C. Moreover, some studies have reported that the *E4* allele is associated with the risk of coronary heart disease.[4]

Brain atrophy was mainly caused by cell apoptosis and considered as an irresistible physiological process, characterized by widening of the sulci, decreased specific grey or white matter volumes and increased ventricular volumes, which was a common finding on MRI in the elderly.[5,6] White matter disease (WMD) was a neuroimaging term, also known as leukoaraiosis (LA). The concept of LA was first proposed by Hachinski in 1987.[7] WML appeared as a speckled or patchy change in the subcorolla or periventricular white matter, showing a low-density lesion on CT, and also T1WI image with low signal or an equal signal, and the T2WI and T2 FLAIR images with high signals on the MRI. In addition to brain atrophy, WML was considered as the result of cerebral small-vessel disease and also often found on MRI in the elderly.[8,9] Brain atrophy and WML had many of the same features as followed. First, evidence has accumulated that vascular factors including hypertension, hyperlipidemia, diabetes mellitus, obesity, alcohol, and smoking play an important role in the etiology of brain atrophy and WML.[10] Second, they were associated with future cognitive decline and dementia.[11,12] Then, they were common

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accompaniment of ageing and accelerate in older age.

Several studies had demonstrated the association of *APOE* polymorphisms with cardiovascular and nervous system diseases, Alzheimer's disease, diabetic nephropathy, atherosclerosis, diabetes.[13,14] Few studies had investigated apoE polymorphism in the Chinese population with White matter lesions and/or brain atrophy. More recently, concomitant brain atrophy and WML have frequently been observed in elderly people on magnetic resonance imaging (MRI). However, it is unknown which factors may explain the co-occurrence of WML and brain atrophy. Therefore, the objective of this study was to assess the genetic associations of *APOE* allele/genotype frequencies in patients with White matter lesions and/or brain atrophy.

METHODS

Participants

Patients and controls were recruited from Renmin Hospital of WuHan University, Hubei Province in China from January 2018 to December 2018. This study included 393 participants, which consisted of 58 patients with White matter lesions, 128 patients with White matter lesions, 112 patients with White matter lesions and 95 healthy elderly volunteers who underwent a regular health examination as healthy controls. WMLs was identified on MRI scan and its severity was graded using the Fazekas method to include a score for the deep white matter and periventricular regions when patients were rechecked MRI scan.[15] The brain atrophy disease was scored on the frontal, temporal, and parietal atrophy by the cortical atrophy scale and the subject's head MRI T1 image.[16] The above assessment was performed by two neurologists who were blinded to the basic information of the patients. Written informed consent was obtained from all participants before the collection of biological samples, and the protocol was approved by the Medical Ethics Review Committee of Renmin Hospital of WuHan University (WDRY2019-K105).

Biochemical measurement

Approximately 5 mL of blood was taken from each study participant in the morning after 12-hour overnight fast and all subjects were told to consume a bland diet before blood testing, and serum samples were separated immediately by centrifugation. Serum samples were stored at −80°C until analysis. Serum high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TCh), triglyceride (TG), small dense density lipoprotein (sdLDL), uric acid (UA), creatinine (Cr) were measured by enzymatic methods with commercially available kits on SEIMENS ADVIA 2400 (Siemens Healthcare Diagnostics ltd, Frimley, Camberley, UK). Lipoprotein(a) [Lp(a)], apolipoprotein A1 (Apo-A1), and apolipoprotein B (Apo-B) levels were measured by immunoturbidimetric method, using reagent from SEIMENS ADVIA 2400 (Siemens Healthcare Diagnostics Inc, Akishima, Tokyo, Japan). Reference intervals were: ApoA1: 1.0−1.6 g/L, ApoB: 0.75−1.00 g/L, HDL-Ch: 1.00−1.55 mmol/L, LDL-Ch: 1.9−3.1 mmol/L, Lp(a): <300 mg/L, TCh: 3.1−5.2 mmol/L, TG: 0.6−1.7 mmol/L, sdLDL: 0.25−1.17 mmol/L, UA: 155−357 μmol/L, Urea: 2.6−7.5 mmol/L, Cr: 41−73 μmol/L. In addition to Biochemical Measurement, the following risk factors were also recorded for each individual: Hyperuricemia, Hypertension, Hyperhomocysteinemia, Hyperlipidemia, Type 2 diabetes.

DNA extraction and APOE genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells by using a QIAamp DNA Blood Mini Kit [Kaijie Enterprise Management (Shanghai) Co., Ltd., Xuhui, Shanghai, China] following the manufacturer's instructions, and DNA concentration was quantified by using a NanoDrop 2000™ spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). PCR was performed according to the following protocol: 50°C for two minutes, pre-denaturation at 95°C for 15 minutes, followed by 45 cycles at 94°C for 30 seconds and 65°C for 45 seconds. The amplified products were detected by using an APOE Gene typing Detection kit (gene-chip assay) (Sinochips Bioscience Co., Ltd., Zhuhai, Guangdong, China) (@ Association of APOE Gene Polymorphisms with Cerebral Infarction in the Chinese Population).

Genetic analysis

The detection of three isoforms of the apoE protein (*E2*, *E3*, and *E4*) resulting from the polymorphisms of the gene was performed as described in the literature.[17]

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 19.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. The data were reported as mean±standard deviation (SD), quarter median and frequencies. The chi-square test and ANOVA were used to analyze the association between specific APOE genotypes and clinical characteristics. Multivariate logistic regression analysis was carried out to estimate the odds ratio (OR), with 95% confidence intervals (CI), in order to assess basic information, clinical indicators and APOE genotypes and alleles risk factors for progress of the disease. The results were considered to be significant differences when the p value was less than 0.05.

RESULTS

Clinical characteristics of the patient and control groups

As showed in Table 1, comparison between disease group (White matter lesions, brain atrophy, Combined with white matter lesions and brain atrophy) and the controls indicated statistically significant differences in the clinical parameters, which included Gender (p<0.0001), Age (p<0.0001), ApoB (p<0.0001), HDL-Ch (p=0.005), Lp(a) (p=0.001), TCh (p< 0.0001), TG (p<0.0001), sdLDL (p<0.0001), UA (p<0.0001), Urea (p=0.002), Cr (p<0.0001), except for LDL-Ch and ApoA1.

The distribution and frequencies of the APOE genotypes and alleles in the patient and control groups

Table 2 showed that carriers of genotype *E3/E3* was the most common type in three groups (65.52%, 69.53%, 57.14% of White matter lesions, brain atrophy, and Combined with brain atrophy and white matter lesions groups, respectively), followed by *E3/E4* genotype (22.41%, 17.97% of White matter lesions and brain atrophy groups, respectively), but the genotype frequency of *E2/E3* was higher than *E3/4* in Combined with white matter lesions and brain atrophy group (p=0.016). Allele *E3* was the most common allele, followed by allele *E4* and allele *E2* in White matter lesions, brain atrophy, and Combined with white matter lesions and brain atrophy groups, respectively (p=0.011). The type 2 diabetes of combined metabolic disease was significantly different among the three groups (p=0.015). However, others including Hyperuricemia, Hypertension, Hyperhomocysteinemia and Hyperlipidemia were not significantly different.

Comparison of metabolic parameters among the different ApoE alleles

We further analyzed the effects of the *APOE* genotypes on the clinical and metabolic parameters in White matter lesions group. As shown in Table 3, Age and the levels of ApoB and Urea were significantly different among the *APOE* genotype groups in White matter lesions group. Specifically, the subjects with an alleles *E4* was associated with the lowest level of Urea incidence (p=0.021), however, subjects with the alleles *E2* had significantly higher levels of ApoB and lower age compared with the *E3* and *E4* group (p=0.040, p=0.026, respectively). In addition, we compared the relationship between *ApoE* alleles and Combined metabolic disease, which was not statistically significant (p>0.05).

Comparison of metabolic parameters among the different ApoE alleles

We also analyzed the effects of the *APOE* genotypes on the clinical and metabolic parameters in brain atrophy group. As shown in Table 4, there was no significantly different among clinical, metabolic parameters and *APOE* genotype groups in

Table 1. Comparison of clinical data between disease group and control group

Clinical characteristics	WML (N=58)	Brain atrophy (N=128)	Co-occurrence of WML and brain atrophy (N=112)	Control group (N=95)	p value
Gender (male, %)	29 (50.00)	92 (71.88)	73 (65.18)	38 (40.00)	<0.0001
Age (years)	62.9±10.06	70.58±9.54	71.58±8.89	64.12±10.66	<0.0001
ApoA1 (g/L)	1.35±0.222	1.31±0.206	1.30±0.202	1.26±0.187	0.050
ApoB (g/L)	0.93±0.24	0.85±0.25	0.84±0.24	0.73±0.157	<0.0001
HDL-Ch (mmol/L)	1.05 (0.83−1.25)	1.00 (0.84−1.17)	1.05 (0.88−1.21)	1.15 (0.98−1.32)	0.005
LDL-Ch (mmol/L)	2.52 (1.83−2.95)	2.30 (1.71−2.92)	2.28 (1.6 0−2.84)	2.14 (1.72−2.76)	0.240
Lp(a) (mg/L)	199.40 (97.00−477.33)	137.45 (70.40−332.25)	134.10 (71.10−429.00)	118 (55.2−183)	0.001
TCh (mmol/L)	4.53 (3.79−5.18)	4.21 (3.57−4.90)	4.08 (3.43−4.87)	3.83 (3.39−4.39)	<0.0001
TG (mmol/L)	1.41 (1.07−2.23)	1.25 (0.99 −1.78)	1.32 (0.90−1.68)	0.97 (0.75−1.18)	<0.0001
sdLDL (mmol/L)	0.92 (0.58−1.22)	0.78 (0.54−1.06)	0.71 (0.48−1.01)	0.57 (0.46−0.75)	<0.0001
UA (μmol/L)	372.50 (322.50−447.75)	362.50 (299.50−432.25)	386.00 (307.00−486.00)	259 (210−296)	<0.0001
Urea (mmol/L)	4.77 (3.92−5.61)	5.31 (4.25−6.43)	5.22 (4.30−6.44)	4.56 (3.85−5.65)	0.002
Cr (μmol/L)	66.50 (55.50−77.00)	73.00 (63.00−84.50)	72.00 (58.00−89.00)	55 (49−62)	<0.0001

ε2 allele=ε2/ε2+ε2/ε3 genotypes; ε3 allele=ε3/ε3+ε2/ε4; genotype; ε4 allele=ε3/ε4+ε4/ε4 genotypes. All values were adjusted for ethnicity, age and gender except the PWV was adjusted for age, SBP and ethnicity. Biological reference interval: ApoA1: 1−1.6 g/L; ApoB: 0.75−1.0 g/L; HDL-Ch: 1.0−1.55 mmol/L; LDL-Ch: 1.9−3.1 mmol/L; Lp(a): <300 mg/L; TCh: 3.1−5.2 mmol/L; TG: 0.56−1.70 mmol/L; sdLDL: 0.25−1.17 mmol/L; UA: 155−357 μmol/L; Urea: 2.6−7.5 mmol/L; Cr: 41−73 μmol/L. ApoA1: Apolipoprotein A, ApoB: apolipoprotein B, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, Lp(a): lipoprotein (a), TCh: total cholesterol, TG: triglyceride, sdLDL: small dense density lipoprotein, UA: uric acid, Cr: creatinine, WML: White matter lesions

brain atrophy group (p>0.05). The same conclusion appeared in the combined metabolic disease group (p>0.05).

Comparison of metabolic parameters among the different *ApoE* alleles

We finally analyzed the effects of the *APOE* alleles on the

Table 2. Association of *apoE* Allele and genotype between disease group and control group

Clinical characteristics	WML	Brain atrophy	Co-occurrence of WML and brain atrophy	p value
Genotype (%)				0.016
E2/2	2 (3.45)	0 (0.00)	2 (1.79)	
E2/3	3 (5.17)	13 (10.16)	25 (22.32)	
E2/4	0 (0.00)	3 (2.34)	2 (1.79)	
E3/3	38 (65.52)	89 (69.53)	64 (57.14)	
E3/4	13 (22.41)	23 (17.97)	17 (15.18)	
E4/4	2 (3.45)	0 (0.00)	2 (1.79)	
Allele (%)				0.011
E2	5 (8.62)	13 (10.16)	27 (24.11)	
E3	38 (65.52)	92 (71.88)	66 (58.93)	
E4	15 (25.86)	23 (17.97)	19 (16.96)	
Combined metabolic disease (%)				
Hyperuricemia	16 (27.59)	30 (23.44)	37 (33.04)	0.254
Hypertension	42 (72.41)	79 (61.72)	75 (66.96)	0.343
Hyperhomocysteinemia	12 (20.69)	46 (35.94)	28 (25.00)	0.054
Hyperlipidemia	20 (34.48)	33 (25.78)	32 (28.57)	0.477
Type 2 diabetes	11 (18.97)	43 (33.59)	21 (18.75)	0.015

ε2 allele=ε2/ε2+ε2/ε3 genotypes; ε3 allele=ε3/ε3 genotype; ε4 allele=ε3/ε4+ε4/ε4 genotypes. WML: White matter lesions

Table 3. Comparison of baseline characteristics of participants with *E2*, *E3*, and *E4* alleles in WML

Genotype	E2 (N=5)	E3 (N=38)	E4 (N=15)	p value
Gender (male%)	4 (4/5)	15 (15/38)	10 (10/15)	0.076
Age (years)	60.40±5.550	61.79±10.624	66.53±9.234	0.026
ApoA1 (g/L)	1.50±0.255	1.32±.234	1.36±0.162	0.218
ApoB (g/L)	1.05 (1.04−1.24)	0.85 (0.77−1.03)	0.89 (0.69−1.02)	0.040
HDL-Ch (mmol/L)	1.23±0.430	1.07±0.342	1.05±0.202	0.516
LDL-Ch (mmol/L)	3.02±1.085	2.49±0.821	2.38±0.816	0.342
Lp(a) (mg/L)	208.00 (92.30−1000.10)	190.30 (85.25−343.13)	222.50 (100−727)	0.717
TCh (mmol/L)	5.15±1.020	4.56±1.076	4.49±0.944	0.453
TG (mmol/L)	1.28 (1.04−2.46)	1.36 (0.97−2.23)	1.52 (1.08−2.41)	0.895
sdLDL (mmol/L)	1.08±0.254	0.94±0.381	0.87±0.423	0.569
UA (μmol/L)	331 (239−377.5)	398 (335.5−456.5)	381(297−444)	0.102
Urea (mmol/L)	5.12 (4.72−6.62)	5.13 (4.20−5.99)	3.83 (3.21−4.78)	0.021
Cr (μmol/L)	63 (47−84.5)	66.5 (56.75−73.25)	69 (51−88)	0.937
Combined metabolic disease (%)				
Hyperuricemia	1 (20.00)	10 (26.35)	5 (33.33)	0.809
Hypertension	5 (100)	24 (63.15)	13 (86.67)	0.080
Hyperhomocysteinemia	3 (60.00)	6 (15.79)	3 (20.00)	0.072
Hyperlipidemia	2 (13.33)	12 (31. 58)	6 (40.00)	0.814
Type 2 diabetes	0 (0.00)	8 (21.05)	3 (20.00)	0.525

ε2 allele=ε2/ε2+ε2/ε3 genotypes; ε3 allele=ε3/ε3+ε2/ε4, genotype; ε4 allele=ε3/ε4+ε4/ε4 genotypes. WML: White matter lesions

Table 4. Comparison of baseline characteristics of participants with *E2*, *E3* and *E4* alleles in brain atrophy

Genotype	E2 (N=13)	*E3* (N=92)	E4 (N=23)	p value
Gender (male%)	11 (11/13)	67 (67/92)	14 (14/23)	0.292
Age (years)	69 (63.5−76)	68 (6325−78.75)	74 (68−83)	0.054
ApoA1 (g/L)	1.33±0.193	1.32±0.210	1.26±0.193	0.423
ApoB (g/L)	0.88±0.327	0.86±0.236	0.80±0.241	0.599
HDL-Ch (mmol/L)	1 (0.88−1.26)	1.01 (0.84−1.13)	0.98 (0.85−1.24)	0.874
LDL-Ch (mmol/L)	2.8 (1.48−3.27)	2.3 (1.72−2.96)	2.25 (1.76−2.75)	0.839
Lp(a) (mg/L)	254 (54.95−461)	131.5 (70.4−317.75)	137 (73.7−345)	0.831
TCh (mmol/L)	4.06 (3.37−5.16)	4.28 (3.67−4.97)	3.85 (3.24−4.37)	0.376
TG (mmol/L)	1.24 (1.04−2.51)	1.27 (0.98−1.83)	1.17 (0.89−1.50)	0.518
sdLDL (mmol/L)	0.89±0.374	0.83±0.389	0.78±0.440	0.763
UA (μmol/L)	381.62±89.511	369.64±116.971	346.74±94.852	0.595
Urea (mmol/L)	4.82 (3.4−5.88)	5.34 (4.35−6.45)	5.77 (4.04−6.44)	0.277
Cr (μmol/L)	76 (66−80.5)	72.5 (63.00−85.00)	73.00 (62.00−86.00)	0.974
Combined metabolic disease (%)				
Hyperuricemia	4 (30.77)	23 (25.00)	3 (13.04)	0.387
Hypertension	9 (69.23)	59 (64.13)	11 (47.83)	0.299
Hyperhomocysteinemia	4 (30.77)	32 (34.78)	10 (43.48)	0.680
Hyperlipidemia	6 (46.15)	24 (26.09)	3 (13.04)	0.092
Type 2 diabetes	3 (23.08)	35 (38.04)	5 (21.74)	0.233

ε2 allele=ε2/ε2+ε2/ε3 genotypes; ε3 allele=ε3/ε3+ε2/ε4, genotype; ε4 allele=ε3/ε4+ε4/ε4 genotypes

clinical and metabolic parameters in Combined with white matter lesions and brain atrophy group. As shown in Table 5, the levels of ApoB, LDL-Ch, TCh, and sdLDL were significantly different among the APOE genotype groups in Combined with white matter lesions and brain atrophy group. Specifically, the subjects with an alleles E2 was associated with the lower level of ApoB, LDL-Ch, TCh, and sdLDL compared with the *E3* and *E4* group (p<0.0001, p<0.0001, p=0.005, p=0.002, respectively). In addition, we compared the relationship between ApoE alleles and combined metabolic disease, only Type 2 diabetes of combined metabolic disease was different among different *APOE* alleles (p=0.016).

Comparison of metabolic parameters among the different disease groups of the subjects with *E3/E3* genotype

As shown in Table 6, clinical information including Gender and Age and the levels of sdLDL were significantly different among the different disease groups of the subjects with E3/E3 genotype. Specifically, the subjects with White matter lesions has higher level of sdLDL but lower age and female frequency compared other disease groups (p=0.039, p<0.0001, p=0.002, respectively). In addition, we compared the relationship between disease groups of the subjects with E3/E3 genotype and combined metabolic disease, only type 2 diabetes of combined metabolic disease was significantly different in the three disease groups of the subjects with E3/E3 genotype (p=0.004).

Comparison of metabolic parameters among the different disease groups of the subjects with *E3/E4* genotype

As shown in Table 7, clinical information including age and the levels of ApoB and TG were significantly different among the different disease groups of the subjects with *E3/E4* genotype. Specifically, the subjects with White matter lesions has higher level of TG than other disease groups (p=0.013), but lower age than other disease groups (p=0.001, p=0.026, respectively). Interestingly, the subjects with brain atrophy has lower level of ApoB than other disease groups (p=0.026). In addition, we compared the relationship between disease groups of the subjects with *E3/E4* genotype and combined metabolic disease, there was no significantly different in the three disease groups of the subjects with *E3/E4* genotype (p>0.05).

The multivariate logistic regression analysis of Basic Information, Clinical indicators and *APOE* among different disease groups

Table 8 presents that Gender (p=0.012, OR=3.192, 95% CI: 1.289−7.904) and Age (p<0.0001, OR=0.900, 95% CI: 0.860−0.942) of basic information and E2 Allele(p=0.019, OR=0.197,

Table 5. Comparison of baseline characteristics of participants with *E2*, *E3*, and *E4* alleles in co-occurrence of WML and brain atrophy

Genotype	E2 (N=27)	E3 (N=66)	E4 (N=19)	p value
Gender (male%)	18 (18/27)	41 (41/66)	14 (14/19)	0.636
Age (years)	74 (67–80.25)	71 (66–78.25)	68 (64–77)	0.644
ApoA1 (g/L)	1.27±0.184	1.30±0.218	1.32±0.169	0.716
ApoB (g/L)	0.70±0.242	0.86±0.216	0.98±0.219	<0.0001
HDL-Ch (mmol/L)	1.02 (0.82–1.19)	1.07 (0.86–1.23)	1.06 (0.92–1.14)	0.571
LDL-Ch (mmol/L)	1.75±0.801	2.31±0.788	2.74±0.804	<0.0001
Lp(a) (mg/L)	131.45 (67.82–491)	140.05 (79.50–431)	108 (54.60–353.10)	0.653
TCh (mmol/L)	3.73±1.007	4.21±1.030	4.77±1.128	0.005
TG (mmol/L)	1.24 (0.87–1.68)	1.32 (0.90–1.66)	1.46 (1.10–2.35)	0.277
sdLDL (mmol/L)	0.63±.351	0.75±0.319	1.00±0.392	0.002
UA (μmol/L)	406 (294.25–493.75)	360.50 (297.25–446.25)	427 (326–468)	0.384
Urea (mmol/L)	4.79 (4.38–6.26)	5.60 (4.38–6.63)	4.60 (3.90–6.95)	0.348
Cr (μmol/L)	76 (61–85.50)	68.50 (57.00–95.00)	77.00 (57.00–93.00)	0.949
Combined metabolic disease (%)				
Hyperuricemia	11 (40.74)	20 (30.30)	6 (31.58)	0.617
Hypertension	19 (70.37)	43 (65.15)	13 (68.42)	0.879
Hyperhomocysteinemia	6 (22.22)	19 (28.79)	3 (15.79)	0.478
Hyperlipidemia	8 (29.63)	18 (27.27)	6 (46.15)	0.926
Type 2 diabetes	4 (14.81)	9 (13.64)	8 (42.11)	0.016

ε2 allele=ε2/ε2+ε2/ε3 genotypes; ε3 allele=ε3/ε3+ε2/ε4, genotype; ε4 allele=ε3/ε4+ε4/ε4 genotypes. WML: White matter lesions

Table 6. Comparison of clinical data and complications among three disease groups of APOE *E3/E3*

Genotype	WML (N=38)	Brain atrophy (N=89)	Co-occurrence of WML and brain atrophy (N=64)	p value
Gender (male, %)	15 (39.47)	65 (73.03)	40 (62.50)	0.002
Age (years)	61.79±10.624	69.61±9.822	71.78±8.682	<0.0001
ApoA1 (g/L)	1.32±0.234	1.32±0.213	1.30±0.216	0.738
ApoB (g/L)	0.85 (0.77–1.03)	0.86 (0.64–1.03)	0.87 (0.71–1.01)	0.599
HDL-Ch (mmol/L)	1.02 (0.79–1.25)	1.01 (0.84–1.13)	1.05 (0.86–1.22)	0.852
LDL-Ch (mmol/L)	2.52 (1.83–2.85)	2.30 (1.71–2.96)	2.29 (1.70–2.88)	0.678
Lp(a) (mg/L)	190.30 (85.25–343.13)	132.00 (70.15–329.50)	133.55 (78.50–423.43)	0.420
TCh (mmol/L)	4.56±1.076	4.30±1.020	4.19±0.986	0.199
TG (mmol/L)	1.36 (0.97–2.23)	1.27 (0.98–1.82)	1.32 (0.91–1.67)	0.287
sdLDL (mmol/L)	0.94±0.381	0.83±0.395	0.75±0.313	0.039
UA (μmol/L)	398.00 (335.50–456.50)	364.00 (302.50–441.00)	363.50 (300.25–468.75)	0.312
Urea (mmol/L)	5.13 (4.20–5.99)	5.36 (4.40–6.45)	5.55 (4.35–6.68)	0.483
Cr (μmol/L)	66.50 (56.75–73.25)	72.00 (63.00–84.00)	68.50 (57.00–95.00)	0.150
Combined metabolic disease (%)				
Hyperuricemia	10 (26.32)	22 (24.72)	20 (31.25)	0.663
Hypertension	24 (63.16)	57 (64.04)	41 (64.06)	0.995
Hyperhomocysteinemia	6 (15.79)	31 (34.83)	18 (28.13)	0.094
Hyperlipidemia	12 (31.58)	24 (26.97)	17 (26.56)	0.839
Type 2 diabetes	8 (21.05)	33 (37.08)	9 (14.06)	0.004

WML: White matter lesions

Table 7. Comparison of clinical data and complications among three disease groups of APOE *E3/E4*

Genotype	WML (N=13)	brain atrophy (N=23)	Co-occurrence of WML and brain atrophy (N=17)	p value
Gender (male, %)	10 (76.92)	14 (60.87)	13 (76.47)	0.462
Age (years)	63.92±6.383	74.83±8.516	71.00±8.208	0.001
ApoA1 (g/L)	1.39±.150	1.26±.193	1.33±0.173	0.095
ApoB (g/L)	0.93±.220	0.80±.241	1.01±0.215	0.026
HDL-Ch(mmol/L)	1.12 (0.86−1.23)	098 (0.85−1.24)	1.07 (0.93−1.29)	0.501
LDL-Ch(mmol/L)	2.51±0.783	2.25±0.765	2.84±0.796	0.073
Lp(a) (mg/L)	191 (89.9−679.1)	137 (73.7−345)	108 (54.8−301.05)	0.453
TCh (mmol/L)	4.64 (3.86−5.13)	3.85 (3.24−4.37)	4.76 (4.25−5.48)	0.049
TG (mmol/L)	1.53 (1.31−2.50)	1.17 (0.89−1.50)	1.46 (1.13−2.52)	0.013
sdLDL (mmol/L)	0.96±0.387	0.78±0.440	1.03±0.407	0.174
UA (μmol/L)	381.38±92.151	346.74±94.852	416.65±103.780	0.089
Urea (mmol/L)	4.24±1.208	5.43±1.435	5.16±1.690	0.071
Cr (μmol/L)	66.31±24.243	74.35±15.865	73.12±18.415	0.459
Combined metabolic disease				
Hyperuricemia	4 (30.77)	3 (13.04)	6 (35.29)	0.226
Hypertension	11 (84.62)	11 (47.83)	12 (70.59)	0.069
Hyperhomocysteinemia	3 (23.08)	10 (43.48)	3 (17.65)	0.173
Hyperlipidemia	5 (38.46)	3 (13.04)	6 (35.29)	0.151
Type 2 diabetes	2 (15.38)	5 (21.74)	7 (41.18)	0.226

WML: White matter lesions

95% CI: 0.051−0.762) in White matter lesions group were also significantly associated with disease progression when Combined with white matter lesions and brain atrophy was set as the reference group. Similarly, the results showed that Hyperhomocysteinemia of Combined metabolic disease (p=0.023, OR=0.472, 95% CI: 0.247−0.900) and UA (p=0.004, OR=0.995, 95% CI: 0.992−0.999) in brain atrophy group were also significantly associated with disease progression when Combined with white matter lesions and brain atrophy was set as the reference group.

DISCUSSION

Some articles have confirmed the clinical results of the presence of WML and brain atrophy on MRI.[10] Previous studies have found that age and gender are risk factors for brain atrophy and white matter.[18,19] However, few articles had reported the relationship between gender and alleles, genotypes in different diseases. Here we show that the prevalence of men in brain atrophy, co-occurrence of WML and brain atrophy disease group is higher than that of WML, the co-occurrence of WML and brain atrophy as a control group, the multivariate logistic analysis of clinical data showed that Gender was a risk factor and gender was a protective factor in the WML group in brain atrophy group. More importantly, *E4* allele carriers are older than E2 and E3 allele carriers in WML not in other disease groups. We also analyzed the relationship between clinical data, and different disease groups in *E3/E3* and *E3/E4* genotype group, respectively. The results showed that age, gender were significantly different in different disease groups of *E3/E3* carriers, while age was also significantly different in different disease groups of *E3/E4* carriers. Interestingly, the age of the co-occurrence group was higher than the other groups in both *E3/E3* and *E3/E4* groups. The above data indicates that age is closely related to the progression and type of disease and genotype, Allele.

According to a previous report cardiovascular disease is associated with high blood lipid levels, type 2 diabetes and other metabolic diseases, such as hyperhomocysteinemia, hyperuricemia, hypertension.[20-22] In the present study, the metabolic index of the disease group (including WML, brain atrophy, co-occurrence of WML and brain atrophy) was higher than that of the healthy group, although most of the indicators were within the normal reference range. It is worth mentioning that the uric acid concentration in the disease group is higher than the normal range. The multivariate logistic analysis of metabolic indicators showed that serum uric acid was a protective factor in brain atrophy group.

Table 8. Multivariate logistic regression analysis among different disease groups

	WML			Brain atrophy		
	OR	95% confidence interval	p value	OR	95% confidence interval	p value
Basic information						
Gender	3.192	1.289–7.904	0.012	0.707	0.353–1.412	0.326
Age	0.900	0.860–0.942	<0.0001	0.995	0.963–1.029	0.771
Combined metabolic disease						
Hyperhomocysteinemia	0.823	0.327–2.073	0.680	0.472	0.247–0.900	0.023
Hyperuricemia	1.019	0.398–2.609	0.968	0.843	0.400–1.777	0.653
Hypertension	0.705	0.302–1.646	0.419	1.291	0.705–2.364	0.408
Hyperlipidemia	1.188	0.468–3.015	0.717	1.280	0.612–2.675	0.512
Diabetes	1.223	0.480–3.114	0.673	0.521	0.266–1.024	0.059
Clinical indicators						
UA	0.999	0.995–1.003	0.587	0.995	0.992–0.999	0.004
ApoA1	0.562	0.021–15.256	0.732	1.165	0.097–14.003	0.904
ApoB	0.405	0.003–56.254	0.719	0.186	0.004–8.247	0.385
HDL-Ch	4.131	0.232–73.637	0.335	1.861	0.220–15.764	0.569
LDL-Ch	1.755	0.351–8.774	0.494	2.308	0.591–9.014	0.229
Lp(a)	1.001	0.999–1.002	0.341	1.000	0.999–1.001	0.427
TCh	0.652	0.172–2.468	0.529	0.658	0.211–2.049	0.470
TG	1.437	0.649–3.181	0.371	1.571	0.811–3.043	0.180
sdLDL	3.007	0.337–26.842	0.324	1.683	0.324–8.739	0.536
Urea	0.921	0.733–1.158	0.480	0.963	0.810–1.145	0.670
Cr	0.997	0.975–1.020	0.820	1.001	0.993–1.009	0.813
Allele						
E=2	0.197	0.051–0.762	0.019	0.514	0.191–1.381	0.187
E=3	0.508	0.199–1.296	0.157	1.305	0.617–2.762	0.486
E=4	The reference group					
Genotype						
APOE=E2/2+E2/4+E4/4	0.527	0.082–3.399	0.500	0.689	0.120–3.964	0.676
APOE=E2/3	0.210	0.043–1.026	0.054	0.468	0.166–1.316	0.150
APOE=E3/3	0.985	0.377–2.572	0.976	1.154	0.531–2.510	0.717
APOE=E3/4	The reference group					

co-occurrence of WML and brain atrophy was set as the reference group. WML: White matter lesions

In the Chinese population, the predominant genotype is *E3/E3* in the Chinese population, whereas the most common allele is E3, followed by *E2/E3*, *E3/E4*, *E2/E4*, *E4/E4,* and *E2/E2*.[23-25] Our research also confirmed that E3/E3 and E3 were dominant. However, in this research, the E3/E4 genotype follows the *E3/E3* genotype in WML group and brain atrophy group, respectively, but the *E2/E2* genotype follows the *E2/E3* genotype in the co-occurrence of WML and brain atrophy disease group. The same conclusion is given for *E2* and *E4* alleles. The difference in the above results may be that the proportion of *APOE* genotypes is related to the type of disease.

Since *APOE* gene polymorphism plays a key role in the regulation of plasma lipid levels,[26,27] APOE isoforms are thought to play an important role in the pathogenesis of vascular disease.[28,29] The results show that, First, serum ApoB and urea levels of patients with WML are different among allele groups. In addition, ApoB level of E2 allele carriers was higher than others. Second, there is no difference in clinical data and metabolic indicators between different alleles of brain atrophy patients. Then, ApoB, LDL-Ch, TCh and sdLDL levels of the metabolic indicators of co-occurrence of WML and brain atrophy patients are significantly different among different alleles. It is worth mentioning that ApoB, LDL-Ch, TCh and sdLDL levels of *E2* carriers was lower than that of *E3* and *E4*

carriers. The above conclusions can be speculated that the relationship between alleles and metabolic indicators is closely related to disease type and progression. Interestingly, genotype has a certain relationship with disease progression. Further analysis of genotypes and metabolic indicators revealed that sdLDL was significantly different in different disease groups of *E3/E3* carriers, while APOB and TG were significantly different in different disease groups of *E3/E4* carriers.

Metabolic disorder can lead to a greater potential of type 2 diabetes, lipid disorders, cardiovascular disease, hepatic steatosis, and other circulatory disorders.[30,31] We found that the incidence of type 2 diabetes has significant differences among three disease groups. In addition, type 2 diabetes also has significant differences among different allele groups of co-occurrence group, not WML and brain atrophy groups. Further comparison of the incidence of metabolic complications among different disease groups of the certain genotype, and found that type 2 diabetes has significant differences among different diseases groups of E3/E3 carriers, rather than *E3/E4* carriers.

At last, multivariate logistic regression analysis of the factors affecting the disease progression, the co-infected group as a control group, the data show that gender is a risk factor [3.192 (1.289−7.904)], age [0.900 (0.860−0.942)] and *E2* allele [0.197 (0.051-0.762)] are protective factors for white matter disease. In the same way, hyperhomocysteinemia [0.472 (0.247−0.900)] and uric acid [0.995 (0.992−0.999)] are protective factors for brain atrophy.

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None.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: ZhiLi Niu. Data curation: ZhiLi Niu. Formal analysis: PingAn Zhang. Funding acquisition: Dong Li. Investigation: ChengLiang Zhu. Methodology: LiNa Feng. Project administration: Ge Xiong. Resources: NaNa Song. Software: Pei Tang. Supervision: PingAn Zhang. Validation: Feng Liu. Visualization: ZhiLi Niu. Writing—original draft: ZhiLi Niu. Writing—review & editing: ZhiLi Niu.

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