INTRODUCTION

Apolipoprotein E (ApoE) is a 34-kDa glycoprotein with 299 amino acids that 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.

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.

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.

Key Words

Apolipoprotein E, Polymorphisms, Brain atrophy, WML.
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. 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 were 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. 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. 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 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 2000TM 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. 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), ApoA1 (p<0.0001), TCh (p<0.0001), Lp(a) (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 white matter lesions and brain atrophy 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, Hyper tension, 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±5.54            | 71.58±8.89                                    | 64.12±10.66          | <0.0001|
| ApoA1 (g/L)              | 1.35±0.22  | 1.31±0.26             | 1.30±0.202                                    | 1.26±0.187           | 0.05   |
| 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 (1.60–2.84)                                | 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)              | 66.77 (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 allele; ε3 allele=ε3/ε3+ε2/ε3+ε3/ε4; genotype; ε4 allele=ε3/ε4+ε2/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 metabolic parameters between the different groups. Table 2 shows the association of apoE allele and genotype between disease group and control group.

| Genotype (%) | WML | Brain atrophy | Co-occurrence of WML and brain atrophy | p value |
|--------------|-----|---------------|----------------------------------------|---------|
| E2/2         | 2 (3.45) | 0 (0.00) | 2 (1.79) | 0.016  |
| E2/3         | 3 (5.17) | 13 (10.16) | 25 (22.32) | 0.016  |
| E2/4         | 0 (0.00) | 3 (2.34) | 2 (1.79) | 0.016  |
| E3/3         | 38 (65.52) | 89 (69.53) | 64 (57.14) | 0.016  |
| E3/4         | 13 (22.41) | 23 (17.97) | 17 (15.18) | 0.016  |
| E4/4         | 2 (3.45) | 0 (0.00) | 2 (1.79) | 0.016  |

**Allele (%)**

| Allele (%) | WML | Brain atrophy | Co-occurrence of WML and brain atrophy | p value |
|------------|-----|---------------|----------------------------------------|---------|
| E2         | 5 (8.62) | 13 (10.16) | 27 (24.11) | 0.016  |
| E3         | 38 (65.52) | 92 (71.88) | 66 (58.93) | 0.016  |
| E4         | 15 (25.86) | 2 (2.34) | 2 (1.79) | 0.016  |

**Combined metabolic disease (%)**

| Combined metabolic disease (%) | WML | Brain atrophy | Co-occurrence of WML and brain atrophy | p value |
|-------------------------------|-----|---------------|----------------------------------------|---------|
| 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±0.234 | 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  |

ε2 allele=ε2/ε2+ε2/ε3 genotypes; ε3 allele=ε3/ε3 genotype; ε4 allele=ε3/ε4+ε4/ε4 genotypes. WML: White matter lesions
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/E4 genotype

As shown in Table 6, 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) | 360 (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) | 0.42 |
| 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
ApoE Polymorphisms with WML and Brain Atrophy

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. 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.20,21 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.

| 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±1.50   | 1.26±1.93             | 1.33±0.173                                    | 0.095   |
| ApoB (g/L)                           | 0.93±0.220  | 0.80±0.241            | 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

|                        | WML (N=13)  | brain atrophy (N=23)  | Co-occurrence of WML and brain atrophy (N=17) | p value |
|------------------------|-------------|-----------------------|-----------------------------------------------|---------|
| 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   |

DISCUSSION

Some articles have confirmed the clinical results of the presence of WML and brain atrophy on MRI.19 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. The above data indicates that age is closely related to the progression and type of disease and genotype, Allele.
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, and E4/E4. 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, APOE isoforms are thought to play an important role in the pathogenesis of vascular disease. 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. 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 current 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 \( [3.192 \text{ (1.289-7.904)}] \), age \( [0.900 \text{ (0.860-0.942)}] \) and E2 allele \( [0.197 \text{ (0.051-0.762)}] \) are protective factors for white matter disease. In the same way, hyperhomocysteinemia \( [0.472 \text{ (0.247-0.900)}] \) and uric acid \( [0.995 \text{ (0.992-0.999})] \) are protective factors for brain atrophy.

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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. Writing—original draft: ZhiLi Niu. Writing—review & editing: ZhiLi Niu.

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