Apolipoprotein E Gene Polymorphisms Are Associated with Primary Hyperuricemia in a Chinese Population

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Abstract

Objective: Primary hyperuricemia, an excess of uric acid in the blood, is a major public health problem. In addition to the morbidity that is attributable to gout, hyperuricemia is also associated with metabolic syndrome, hypertension, and cardiovascular disease. This study aims to assess the genetic associations between Apolipoprotein E (APOE) polymorphisms and hyperuricemia in a Chinese population.

Methods: A total of 770 subjects (356 hyperuricemic cases and 414 normouricemic controls) were recruited from the Ningxia Hui Autonomous Region, China. A physical examination was performed and fasting blood was collected for biochemical tests, including determination of the levels of serum lipid, creatinine, and uric acid. Multi-ARMS PCR was applied to determine the APOE genotypes, followed by an investigation of the distribution of APOE genotypes and alleles frequencies in the controls and cases.

Results: The frequencies of the APOE-ε2ε3 genotype (17.70% vs. 10.39%, P = 0.003) and the APOE-ε2 allele (10.53% vs. 5.80%, P = 0.001) were significantly higher in the hyperuricemic group than in the normouricemic group. Furthermore, male cases were more likely to have the APOE-ε2ε3 genotype and APOE-ε2 allele, compared with male controls. In both Han and Hui subjects, cases were more likely to have the APOE-ε2ε3 genotype and the APOE-ε2 allele compared with controls. Furthermore, multivariate logistic regression showed that carriers of the APOE-ε2ε3 genotype (P = 0.001, OR = 2.194) and the ε2 allele (P = 0.001, OR = 2.099) were significantly more likely to experience hyperuricemia than carriers of the ε3/ε3 genotype and the ε3 allele after adjustment for sex, body mass index (BMI), diastolic blood pressure (DBP), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), creatinine (Cr) and fasting blood glucose (FBG).

Conclusions: The APOE-ε2ε3 genotype and the APOE-ε2 allele are associated with serum uric acid levels in Chinese subjects, indicating that individuals carrying the APOE-ε2 allele have a higher risk of hyperuricemia than non-carriers.
APOE polymorphisms within exon 4, at codons 112 and 158[12]. APOE-ε2 (Arg158→Cys) has been shown to be associated with decreased levels of total and low density lipoprotein (LDL) cholesterol, whereas APOE-ε4 (Cys112→Arg) is associated with increased cholesterol levels [13,14].

In addition to the impact on the serum lipid profile, blood pressure levels [15], the occurrence and development of type 2 diabetes mellitus [16] and neurodegenerative disorders [17], polymorphisms of the APOE gene are also associated with the risk of cardiovascular diseases [18]. An association between APOE polymorphisms and SUA was also demonstrated in several studies. However, other studies have reported inconsistent results. Some studies suggest that the ε2 allele is independently associated with increased SUA levels [19,20] and that ε4 is associated with decreased SUA levels [21,22], but others show that the ε4 allele is associated with a higher risk of hyperuricemia [23]. Possible causes include racial and ethnic differences. At present, the correlation between APOE polymorphisms and hyperuricemia remains elusive in China, especially among individuals in an area inhabited by ethnic minorities.

Therefore, the objective of this study was to assess the genetic associations of APOE polymorphisms and hyperuricemia in a Chinese population.

Table 1. Demographic information and blood chemistry parameters of study subjects.

| Male(n = 461) | Hyperuricemic group | Normouricemic group | Female(n = 309) | Hyperuricemic group | Normouricemic group |
|--------------|---------------------|---------------------|-----------------|---------------------|---------------------|
| N(%)         | 218(47.3%)          | 243(52.7%)          | 196(63.4%)      | 113(36.6%)          | 131(43.4%)          |
| Age (years)  | 24.3(13.9–48.6)     | 24.0(15.8–45.5)     | 17.3(15.2–39.1) | 18.0(13.2–57.2)     | 17.0(13.2–57.2)     |
| Ethnicity (%)|                     |                     |                 |                     |                     |
| Han          | 103(47.2%)          | 143(58.8%)          | 108(55.1%)      | 63(55.8%)           |                    |
| Hui          | 112(51.4%)          | 97(39.9)            | 88(44.9%)       | 48(42.5%)           |                    |
| BMI (kg/m²)  | 23.0±4.8            | 24.6±4.5*           | 21.6±3.5        | 23.5±5.1*           |                    |
| WC (cm)      | 76.8±14.2           | 80.1±12.5*          | 71.3±10.1       | 74.3±12.8*          |                    |
| SBP (mmHg)   | 126.7±21.0          | 133.3±18.8*         | 121.3±17.8      | 127.6±24.2*         |                    |
| DBP (mmHg)   | 77.4±13.9           | 82.5±14.7*          | 76.2±11.6       | 79.3±14.3           |                    |
| TC (mmol/L)  | 3.95±0.86           | 4.11±0.99           | 3.95±0.84       | 4.35±1.17*          |                    |
| TG (mmol/L)  | 1.08(0.65–1.72)     | 1.33(0.76–2.39)*    | 0.94(0.58–1.30) | 1.20(0.81–1.96)*    |                    |
| HDL-C (mmol/L)| 1.13±0.25          | 1.08±0.28*          | 1.27±0.27       | 1.20±0.27*          |                    |
| LDL-C (mmol/L)| 2.20(1.76–2.69)    | 2.34(1.84–2.92)     | 2.18(1.73–2.61) | 2.51(2.03–3.06)*    |                    |
| FBG (mmol/L) | 4.96(4.68–5.33)     | 5.13(4.90–5.51)*    | 4.87(4.61–5.18) | 5.09(4.87–5.52)*    |                    |
| HbA1C (%)    | 5.10(4.90–5.40)     | 5.20(5.00–5.50)*    | 5.10(4.90–5.30) | 5.20(5.00–5.60)*    |                    |
| UA (μmol/L)  | 297.6±63.0          | 460.4±45.4*         | 239.3±55.6      | 398.4±40.0*         |                    |
| Cr (mmol/L)  | 80.37±15.4          | 88.99±18.6*         | 66.93±8.8       | 73.03±13.4*         |                    |
| BUN (μmol/L) | 4.57±1.29           | 4.92±1.47*          | 3.96±1.16       | 4.47±1.27*          |                    |

Abbreviations: BMI, Body mass index; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; FBG, Fasting blood glucose; HbA1C, Glycated hemoglobin; UA, Urine acid; Cr, Creatinine; BUN, Blood urea nitrogen.

Data are presented as n (%), mean ± standard deviation (SD); Age, TG, LDL-C, FBG and HbA1C were reported as the medians (interquartile range).

*P<0.05 for Hyperuricemic group vs. Normouricemic group within the same sex.
study and obtained their written informed consent on behalf of the minors/children enrolled in our study.

**Clinical Laboratory Tests**

All procedures were performed following a 9–12 hour overnight fast and all subjects were told to consume a bland diet before blood testing. Blood pressure (BP) was measured using an OMRON HEM-7000 electronic sphygmomanometer (OMRON Healthcare, Kyoto, Japan) after the participant had rested for $10 min.

Blood was drawn from the antecubital vein of the arm. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured with a Beckman AU Series Automatic Biochemical Analyzer (Japan), using Sekisui Medical (Japan) reagents. Fasting blood glucose (FBG), uric acid (UA), creatine (Cr), and blood urea nitrogen (BUN) were measured with the same instrument, using Beckman AU reagents. HbA1c was examined by high-performance liquid chromatography (HPLC) on a Bio-Rad Diamat automated glycosylated hemoglobin analyzer (USA).

**APOE Genotyping**

Genomic DNA for APOE genotyping was extracted from peripheral blood mononuclear cells with the Genomic DNA Purification System (Promega, Madison, WI, USA). The DNA samples were then analyzed using the amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) assay.

### Table 2. Genotype and allele frequencies of APOE polymorphisms among the study subjects.

| Genotypes | Normouricemic group (%) | Hyperuricemic group (%) | P value |
|-----------|-------------------------|-------------------------|---------|
| e2e2      | 1(0.24%)                | 3(0.84%)                |         |
| e2e3      | 43(10.39%)              | 63(17.70%)              | 0.003   |
| e2e4      | 30(7.2%)                | 61(16.9%)               |         |
| e3e3      | 304(73.43%)             | 230(64.61%)             | 0.008   |
| e3e4      | 60(14.49%)              | 52(14.61%)              | 0.522   |
| e4e4      | 3(0.72%)                | 2(0.56%)                |         |

| Alleles   | Normouricemic group (%) | Hyperuricemic group (%) | P value |
|-----------|-------------------------|-------------------------|---------|
| e2        | 48(5.80%)               | 75(10.53%)              | 0.001   |
| e3        | 711(85.87%)             | 575(80.76%)             | 0.007   |
| e4        | 69(8.33%)               | 62(8.71%)               | 0.431   |

The allelic and genotypic frequencies are indicated in absolute values and percentage. P value for the overall comparison between the hyperuricemic and normouricemic groups by the chi-square test.

The extremely rare genotypes - e2e2, e2e4 and e4e4 subjects were described but excluded from the statistical analysis.

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### Table 3. The APOE genotype and allele frequencies among the study subjects stratified by gender.

| Gender | Normouricemic group | Hyperuricemic group | P-valuea | P-valueb |
|--------|---------------------|---------------------|----------|----------|
| Male   | Normouricemic group | Hyperuricemic group | P-valuea | P-valueb |
| e2e2   | 1(0.5%)             | 2(0.8%)             |          |          |
| e2e3   | 26(11.9%)           | 46(18.9%)           |          |          |
| e2e4   | 1(0.5%)             | 5(2.1%)             |          |          |
| e3e3   | 160(73.4%)          | 154(63.4%)          |          |          |
| e3e4   | 28(12.8%)           | 34(14.0%)           |          |          |
| e4e4   | 2(0.9%)             | 2(0.8%)             |          |          |
| Total  | 218                 | 243                 |          |          |

| Female  | Normouricemic group | Hyperuricemic group | P-valuea | P-valueb |
|---------|---------------------|---------------------|----------|----------|
| e2      | 29(6.7%)            | 55(11.3%)           |          |          |
| e3      | 374(85.8%)          | 388(79.8%)          |          |          |
| e4      | 33(7.6%)            | 43(8.8%)            |          |          |
| Total   | 436                 | 486                 |          |          |

Subjects with the extremely rare genotypes - e2e2, e2e4 and e4e4 were described but excluded from the statistical analysis.

aHyperuricemic group vs. Normouricemic group in males.

bHyperuricemic group vs. Normouricemic group in females.

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### Table 4. The APOE genotype and allele frequencies among the study subjects stratified by ethnic group.

| Genotypes  | Han Normouricemic group | Han Hyperuricemic group | Hui Normouricemic group | Hui Hyperuricemic group | P-value<sup>a</sup> | P-value<sup>b</sup> |
|------------|-------------------------|-------------------------|-------------------------|-------------------------|---------------------|---------------------|
| ε2/ε2     | 1(0.5%)                 | 1(0.5%)                 | 0(0%)                   | 2(1.4%)                 |                     |                     |
| ε2/ε3     | 20(9.5%)                | 36(17.5%)               | 23(11.5%)               | 27(18.6%)               | 0.017               | 0.064               |
| ε2/ε4     | 3(1.4%)                 | 3(1.5%)                 | 0(0%)                   | 3(2.1%)                 |                     |                     |
| ε3/ε3     | 158(74.9%)              | 131(63.6%)              | 143(71.5%)              | 95(65.5%)               | 0.012               | 0.236               |
| ε3/ε4     | 28(13.3%)               | 34(16.5%)               | 32(16%)                 | 17(11.7%)               | 0.353               | 0.261               |
| ε4/ε4     | 1(0.5%)                 | 1(0.5%)                 | 2(1%)                   | 1(0.7%)                 |                     |                     |
| Total     | 211                     | 206                     | 200                     | 145                     |                     |                     |
| Alleles   |                         |                         |                         |                         |                     |                     |
| ε2        | 25(5.9%)                | 41(10.0%)               | 23(5.8%)                | 34(11.7%)               | 0.021               | 0.005               |
| ε3        | 364(86.3%)              | 332(80.6%)              | 341(85.3%)              | 234(80.7%)              | 0.027               | 0.113               |
| ε4        | 33(7.8%)                | 39(9.5%)                | 36(9%)                  | 22(7.6%)                | 0.397               | 0.509               |
| Total     | 422                     | 412                     | 400                     | 290                     |                     |                     |

Subjects with the extremely rare genotypes ε2ε2, ε2ε4 and ε4ε4 were described but excluded from the statistical analysis.

<sup>a</sup>Hyperuricemic group vs. Normouricemic group in Han.

<sup>b</sup>Hyperuricemic group vs. Normouricemic group in Hui.

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[27]. Briefly, the PCR was conducted with the following primers: Cys158/Arg158 (5’-ATGCCGATGACCTGCAGAATT-3’)/ (5’-ATGCCGATGACCTGCAGAATC-3’), Cys112/Arg112(5’- CGCGGACATGGAGGACGTTT-3’)/(5’-CGCGGACATGGAAGACGTTT-3’), and a common reverse primer (5’- GTTCAGTGATTGTCGCTGGGCA-3’). The common primer was paired with Cys158/Arg158 or Cys112/Arg112 and produced an amplicon of 451 and 588 base pairs (bp), respectively. A 218-bp fragment of the LDLR gene was co-amplified to function as an internal positive control, and the PCR primer sequences were 5’- ATGCCGATGACCTGCAGAATC-3’ and 5’- AGTG- CGCGGACATGGAGGACGTT-3’. The PCR conditions were denaturation at 95°C for 30 sec, and a final extension at 72°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 63°C for 30 sec and 72°C for 30 sec, and a final extension at 72°C for 5 min. At the end of the PCR cycles, the products were resolved by electrophoresis on 2% agarose gels to validate the amplification of the specific PCR product expected.

#### Statistical Analysis

Continuous variables with a normal distribution are given as the mean ± standard deviation (SD) and were analyzed by t-test. Variables with a non-normal distribution are given as the median (interquartile range) and were compared by the Wilcoxon rank sum test. Categorical variables (such as ethnicity, genotype distribution and allele frequency) were analyzed as percentages using the Chi-squared test. In each model, the homozygous ε2/ε2 genotype and the 2nd most frequent allele in the hyperuricemic group were significantly higher than those in the normouricemic group.

#### Results

Clinical characteristics of the normouricemic and hyperuricemic groups stratified by gender

As shown in Table 1, there was no significant difference in mean age between the normouricemic group and the hyperuricemic group for either men or women. Regardless of gender, the hyperuricemic group had significantly higher values for body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), total cholesterol (TG), fasting blood glucose (FBG), Glycated hemoglobin (HbA1C), uric acid (UA), creatine (Cr) and blood urea nitrogen (BUN), but they had lower high-density lipoprotein cholesterol (HDL-C) levels compared with the normouricemic group (P<0.05). The levels of Total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were significantly higher among women in the hyperuricemic group than in the normouricemic group, while the diastolic blood pressure (DBP) was significantly higher for men in the hyperuricemic group than in the normouricemic group.

Distribution of APOE genotypes and alleles among the study subjects

A total of 356 hyperuricemic cases and 414 normouricemic subjects were evaluated, and the frequencies of the APOE genotypes and alleles are shown in Table 2. The allele frequencies were within Hardy-Weinberg equilibrium (P>0.05). The frequencies of the ε2ε3 genotype and the ε2 allele in the hyperuricemic group were significantly higher than those in the normouricemic group.
Table 5. The effects of APOE genotypes on clinical and metabolic parameters.

|                         | Normouricemic group |                          | Hyperuricemic group |                          | P-value* | P-value# |
|-------------------------|---------------------|--------------------------|---------------------|--------------------------|----------|----------|
|                         | ε2/3 (n = 43)       | ε3/3 (n = 304)           | ε3/4 (n = 60)       | ε2/3 (n = 63)           | ε3/3 (n = 230) | ε3/4 (n = 52) |       |
| Age (years)             | 22.6 (15.0–44.2)    | 22.1 (14.9–45.4)         | 19.7 (13.0–43.7)    | 19.0 (14.0–47.1)        | 21.0 (15.7–51.4) | 19.1 (13.2–43.4) | 0.916 0.225 |
| BMI (kg/m²)             | 22.4 ± 3.86         | 22.3 ± 4.31              | 22.0 ± 4.21         | 24.5 ± 5.12             | 24.1 ± 4.68    | 24.2 ± 4.44    | 0.879 0.898 |
| WC (cm)                 | 74.6 ± 11.3         | 74.2 ± 12.7              | 73.1 ± 13.6         | 79.1 ± 13.1             | 77.8 ± 12.9    | 78.7 ± 11.3    | 0.808 0.749 |
| SBP (mmHg)              | 122.9 ± 20.1        | 124.3 ± 19.3             | 124.3 ± 22.0        | 133.8 ± 21.3            | 132.2 ± 21.3   | 135.9 ± 17.4   | 0.920 0.801 |
| DBP (mmHg)              | 75.1 ± 12.9         | 76.9 ± 12.9              | 77.3 ± 12.6         | 83.2 ± 15.4             | 80.9 ± 14.9    | 81.8 ± 13.3    | 0.666 0.547 |
| TC (mmol/L)             | 3.68 ± 0.75a        | 3.98 ± 0.87              | 4.01 ± 0.80         | 3.81 ± 1.09b            | 4.27 ± 1.04    | 4.35 ± 1.00    | 0.049 0.005 |
| TG (mmol/L)             | 0.84 (0.53–1.28)    | 0.99 (0.62–1.51)         | 1.05 (0.62–1.52)    | 1.35 (0.88–2.68)        | 1.21 (0.73–2.13) | 1.53 (0.97–2.98)c | 0.327 0.054 |
| HDL-C (mmol/L)          | 1.27 ± 0.28         | 1.20 ± 0.27              | 1.19 ± 0.27         | 1.11 ± 0.27             | 1.13 ± 0.29    | 1.05 ± 0.20    | 0.219 0.105 |
| LDL-C (mmol/L)          | 1.89 (1.45–2.38)ab  | 2.21 (1.76–2.71)         | 2.18 (1.87–2.57)    | 1.83 (1.53–2.35)ab      | 2.45 (2.01–3.06) | 2.68 (2.22–3.16) | 0.005 <0.001 |
| FBG (mmol/L)            | 4.85 (4.59–5.22)    | 4.93 (4.65–5.25)         | 4.92 (4.60–5.22)    | 5.14 (4.97–5.58)        | 5.12 (4.89–5.49) | 5.13 (4.67–5.59) | 0.536 0.349 |
| HbA1C (%)               | 5.10 (5.00–5.30)    | 5.10 (4.90–5.30)         | 5.10 (4.80–5.40)    | 5.20 (5.10–5.50)        | 5.20 (5.00–5.50) | 5.20 (5.00–5.50) | 0.932 0.922 |
| UA (μmol/L)             | 283.7 ± 57.3a       | 264.9 ± 57.4             | 273.2 ± 52.8        | 448.4 ± 54.4a           | 432.4 ± 52.1   | 437.2 ± 52.4   | 0.039 0.024 |
| Cr (mmol/L)             | 7.51 ± 13.6         | 74.2 ± 14.9              | 72.2 ± 12.4         | 83.2 ± 17.6             | 84.6 ± 18.9    | 81.0 ± 18.5    | 0.540 0.430 |
| BUN (mmol/L)            | 4.25 ± 1.31         | 43.2 ± 1.22              | 41.8 ± 1.31         | 4.52 ± 1.44             | 4.87 ± 1.44    | 4.61 ± 1.36    | 0.741 0.168 |

Abbreviations: BMI, Body mass index; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; FBG, Fasting blood glucose; HbA1C, Glycated hemoglobin; UA, Uric acid; Cr, Creatinine; BUN, Blood urea nitrogen.

LSD and SNK test were applied to analyze the differences between each pair of groups.

*ε2/3 vs. ε3/3, p < 0.05;  
#ε2/3 vs. ε3/4, p < 0.05;  
*ε3/4 vs. ε3/3, p < 0.05.  
*Comparison of genotypes in Normouricemic group.  
#Comparison of genotypes in Hyperuricemic group.  
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group ($P=0.003$ and $P=0.001$, respectively). However, the distributions of the $\varepsilon 3\varepsilon 3$ genotype and the $\varepsilon 3$ allele were significantly lower in the hyperuricemic individuals ($P=0.008$ and $P=0.007$, respectively).

### Distribution of APOE genotypes and alleles based on gender and ethnicity

Table 3 and 4 present the APOE genotype and allele frequencies distributed by gender and ethnic group, respectively. The $\varepsilon 2\varepsilon 3$ genotype frequency in the hyperuricemic group was significantly higher than that in the normouricemic group for males ($P=0.039$). Furthermore, the APOE $\varepsilon 2$ allele frequency was significantly higher in the hyperuricemic group compared with the normouricemic group both in males ($P=0.014$) and females ($P=0.049$). In addition, the frequencies of the APOE-$\varepsilon 2\varepsilon 3$ genotype and the $\varepsilon 3$ allele in the hyperuricemic group were significantly lower than those in the normouricemic group in males but not in females. Meanwhile, we observed that the prevalence of the APOE $\varepsilon 2$ allele in males was higher than that in females in both the hyperuricemic (11.3% vs. 8.8%) and the normouricemic (6.7% vs. 4.8%) groups, although the difference was not statistically significant. Similar results were obtained from different ethnic groups (Table 4). The frequencies of the $\varepsilon 2\varepsilon 3$ genotype and the $\varepsilon 2$ allele were significantly higher, but those of the $\varepsilon 3\varepsilon 3$ genotype and the $\varepsilon 3$ allele were significantly lower in the hyperuricemic group compared with the normouricemic group in the Han population. Moreover, in the Hui population, a significant difference in the distribution of the $\varepsilon 2$ allele between the control and hyperuricemic groups was identified.

### Effect of APOE gene polymorphism on metabolic parameters and serum uric acid concentrations

We further analyzed the effects of the APOE genotypes on the clinical and metabolic parameters and serum uric acid levels in both the normouricemic group and hyperuricemic group. As shown in Table 5, the levels of TC, LCL-C and UA were significantly different among the APOE genotype groups in both the normouricemic group and hyperuricemic group. The $\varepsilon 2/\varepsilon 3$ group had significantly higher levels of serum uric acid compared with the $\varepsilon 3/\varepsilon 3$ group ($P<0.05$).

### Table 6. Univariate analysis of the association between APOE polymorphism, metabolic parameters and hyperuricemia.

| Genotypes | OR (95%CI)   | $P$ value |
|-----------|--------------|-----------|
| $\varepsilon 3\varepsilon 3$ | 1 (Reference) |           |
| $\varepsilon 2\varepsilon 3$ | 1.937 (1.267–2.959) | 0.002     |
| $\varepsilon 3\varepsilon 4$ | 1.146 (0.761–1.724) | 0.515     |

| Alleles | OR (95%CI)   | $P$ value |
|---------|--------------|-----------|
| $\varepsilon 3$ | 1 (Reference) |           |
| $\varepsilon 2$ | 1.932 (1.323–2.821) | 0.001     |
| $\varepsilon 4$ | 1.111 (0.775–1.593) | 0.567     |

| Sex    | OR (95%CI)   | $P$ value |
|--------|--------------|-----------|
| Female | 1 (Reference) |           |
| Male   | 1.933 (1.439–2.597) | <0.001    |

| Age(years) | OR (95%CI)   | $P$ value |
|------------|--------------|-----------|
| 1.006 (0.998–1.013) | 0.129 |

| BMI(kg/m²) | OR (95%CI)   | $P$ value |
|------------|--------------|-----------|
| 1.097 (1.061–1.133) | <0.001 |

| WC(cm) | OR (95%CI)   | $P$ value |
|--------|--------------|-----------|
| 1.025 (1.014–1.037) | <0.001 |

| SBP | OR (95%CI)   | $P$ value |
|-----|--------------|-----------|
| 1.018 (1.010–1.025) | <0.001 |

| DBP | OR (95%CI)   | $P$ value |
|-----|--------------|-----------|
| 1.025 (1.014–1.036) | <0.001 |

| TC(mmol/L) | OR (95%CI)   | $P$ value |
|------------|--------------|-----------|
| 1.299 (1.116–1.512) | 0.001 |

| TG(mmol/L) | OR (95%CI)   | $P$ value |
|------------|--------------|-----------|
| 1.534 (1.325–1.776) | <0.001 |

| HDL-C(mmol/L) | OR (95%CI)   | $P$ value |
|---------------|--------------|-----------|
| 0.342 (0.200–0.585) | <0.001 |

| LDL-C(mmol/L) | OR (95%CI)   | $P$ value |
|---------------|--------------|-----------|
| 1.419 (1.167–1.726) | <0.001 |

| FBG(mmol/L) | OR (95%CI)   | $P$ value |
|-------------|--------------|-----------|
| 1.648 (1.303–2.084) | <0.001 |

| HbA1c (%) | OR (95%CI)   | $P$ value |
|-----------|--------------|-----------|
| 2.052 (1.447–2.910) | <0.001 |

| Cr(mmol/L) | OR (95%CI)   | $P$ value |
|------------|--------------|-----------|
| 1.040 (1.029–1.050) | <0.001 |

| BUN(mmol/L) | OR (95%CI)   | $P$ value |
|-------------|--------------|-----------|
| 1.316 (1.179–1.469) | <0.001 |

### Abbreviations: BMI, Body mass index; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; FBG, Fasting blood glucose; HbA1c, Glycated hemoglobin; Cr, Creatinine; BUN, Blood urea nitrogen.

$P$-values and their ORs for the genotypes and clinical parameters were calculated by univariate logistic regression analysis.
between some variables (for example, SBP and DBP), in the next multivariate logistic regression model, only key factors sex, BMI, DBP, TG, LDL-C, Cr and FBG were included and adjusted as potential confounding variables. As shown in Table 7, the DBP, TG, LDL-C, Cr and FBG were included and adjusted as multivariate logistic regression model, only key factors sex, BMI, DBP, TG, LDL-C, Cr and FBG.

### Table 7. Multivariate analysis of the independent association between APOE polymorphism and hyperuricemia.

| Genotypes | OR (95%CI) | P value |
|-----------|------------|---------|
| ε3c3      | 1(Reference)|         |
| ε2c3      | 2.194(1.362–3.537) | 0.001   |
| ε3c4      | 1.175(0.755–1.828)  | 0.475   |

| Alleles | OR (95%CI) | P value |
|---------|------------|---------|
| ε3      | 1(Reference)|         |
| ε2      | 2.099(1.379–3.195) | 0.001   |
| ε4      | 1.076(0.730–1.588)  | 0.710   |

| Sex | OR (95%CI) | P value |
|-----|------------|---------|
| Male| 1.093(0.757–1.579) | 0.635   |
| Female| 0.988(0.940–1.037)  | 0.621   |
| DBP | 1.000(0.986–1.014)  | 0.957   |
| TG(mmol/L) | 1.227(1.034–1.455)  | 0.019   |
| LDL-C(mmol/L) | 1.149(0.891–1.481)  | 0.285   |
| FBG(mmol/L) | 1.378(1.060–1.792)  | 0.017   |
| Cr(mmol/L) | 1.033(1.018–1.047) <0.001 |       |

### Abbreviations: BMI, Body mass index; DBP, Diastolic blood pressure; TG, Triglyceride; LDL-C, Low density lipoprotein cholesterol; FBG, Fasting blood glucose; Cr, Creatinine.

P -values and adjusted ORs for the multivariate logistic regression analyses were adjusted for sex, BMI, DBP, TG, LDL-C, FBG and Cr.

In the present study we observed that APOE gene polymorphism is associated with hyperuricemia in Chinese subjects. The frequencies of both the APOE-ε2ε3 genotype and ε2 allele were higher in the hyperuricemic group than in the normouricemic group (p<0.01), and individuals with the ε2ε3 genotype or ε2 allele had significantly higher SUA levels than ε3ε3 genotype or ε3 allele carriers. To the best of our knowledge, this is the first study to examine the possible associations between APOE polymorphism and hyperuricemia in China.

APOE gene polymorphism is known to be closely related to cardiovascular disease, hypertension, dyslipidemia, diabetes, and metabolic syndrome [28]. Hyperuricemia is an independent risk factor for coronary artery disease (CAD), hypertension, and obesity-related metabolic syndrome [29]. Therefore, it is reasonable to explore the connections between APOE gene polymorphism and hyperuricemia. Cardona et al. reported that the prevalence of the ε2 allele was greater in the patients with gout than in the healthy subjects and proposed that the reduced renal excretion of uric acid in patients with gout is mediated by the high prevalence of the ε2 allele of the APOE gene [19]. Another study in Caucasians also suggested that the APOE-ε2 allele is independently associated with increased SUA levels in healthy individuals [20]. In addition, according to various studies, the association between the ε4 allele and serum uric acid levels is controversial. Different studies have shown that APOE-ε4 allele carriers have lower [21,22] or higher [23] SUA levels compared with APOE-ε3 allele carriers.

In this Chinese population study, the frequencies of the ε2ε3 genotype and the ε2 allele in hyperuricemic individuals were significantly higher than those in normouricemic subjects, but no difference in the frequency of the ε4 allele was found between the two groups. We further analyzed the associations of gender and ethnicity with APOE polymorphism, and we found that the APOE ε2 allele frequency was also significantly higher in the hyperuricemic group compared with the normouricemic group, not only in males and females but also in the Han and Hui populations (Table 3 and Table 4). Interestingly, we observed that the prevalence of the APOE ε2 allele in males was higher than that in females in both the hyperuricemic (11.3% vs. 8.8%) and normouricemic (6.7% vs. 4.8%) groups (Table 3), which may be another reason besides differences in sex hormones for the reduced renal excretion of uric acid and the high incidence of hyperuricemia in males. Denzer et al. reported a significantly positive association between plasma testosterone and uric acid concentrations in obese children and adolescents [30]. In addition, animal studies have demonstrated gender differences in uric acid transporters and showed that male mice have a higher reabsorption of uric acid than female mice [31]. The higher APOE ε2 allele frequency in men and its possible impact requires further confirmation and clarification. The frequencies of the APOE alleles are highly variable among different ethnic populations; for example, the ε4 allele frequency is significantly higher in African-American than in Asians [32]. Therefore, in this study, we explored the difference in the frequencies of APOE gene polymorphisms between Han and Hui populations, but we did not find any significant difference between the two groups.
A logistic regression model demonstrated that the e2e3 genotype and the e2 allele were significantly positively associated with hyperuricemia, and the association remained significant after adjustment for gender, BMI, DBP, TG, LDL-C, FBG and Cr. The e2e3 genotype or e2 allele could be considered as independent risk factors of hyperuricemia. The underlying mechanisms for the increased risk of hyperuricemia in individuals with the e2e3 genotype or the e2 allele are largely unknown, but they are most likely related to increased purine metabolism and uric acid production or decreased renal clearance of uric acid. The presence of the APOE-e2 allele in patients with gout is associated with reduced renal excretion of urates [19]. In fact, in this study, the positive association between the e2 allele and hyperuricemia persists after excluding the impact of serum creatinine (SCr), suggesting that the main reason is not reduced renal clearance of uric acid, but more likely increased production of uric acid. The presence of APOE gene polymorphism has been demonstrated to influence serum levels of C-reactive protein (CRP) [33]. Moreover, epidemiological studies have confirmed that children with high concentrations of CRP are more susceptible to hyperuricemia, and the serum uric acid concentration increased with rising CRP levels [34]. Whether the APOE-e2 allele could stimulate uric acid production and the underlying mechanisms require further experimental exploration. In this study, we did not find that BMI and DBP were independently associated with hyperuricemia (Table 7), probably because of the special nature of the study population. Patients included in this study are patients with primary hyperuricemia, among whom hypertension, diabetes and other diseases have been ruled out. In addition, teenagers are the main body of the study population. Therefore, in this population, it is possible that ApoE genotype, but not BMI and DBP, is independently associated with hyperuricemia.

Dietary habits, the intensity of physical activity, drinking and smoking may also be associated with the serum uric acid level. However, we did not include these factors in the multivariable analysis, which is a limitation of our study. In addition, due to the small sample size for subjects with the e2e2, e2e4 and e4e4 genotypes, these genotypes were not included in the logistic regression analysis.

In conclusion, this study is the first to report that the APOE e2 allele is positively associated with SUA and could be an independent risk factor for hyperuricemia in a Chinese population. Larger studies in the future will further clarify the correlation between APOE polymorphisms and uric acid metabolism.

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Author Contributions

Conceived and designed the experiments: JW LQ GJZ.Performed the experiments: JW NZG LZ PCL QW LN. Analyzed the data: JW TX QC QD. Contributed reagents/materials/analysis tools: JW LQ XZG TX.

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References

1. Neogi T (2011) Clinical practice. Gout. N Engl J Med 364: 443–452.
2. Lin KC, Lin HY, Chou P (2000) The interaction between uric acid level and other risk factors on the development of gout among asymptomatic hyperuricemic men in a prospective study. J Rheumatol 27: 1501–1505.
3. Maso D, Kawauchi H, Miki H, Ohgara T, Tuck ME (2003) Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. Hypertension 42: 474–480.
4. Sundstrom J, Sullivan L, D’Agostino RB, Levy D, Kannel WB, et al. (2003) Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. Hypertension 41: 39–43.
5. Borges RL, Ribeiro AB, Zanella MT, Batista MC (2010) Uric acid as a factor in hypertension. Clin Exp Pharmacol Physiol 37: 476–482.
6. Yoo TW, Sung KC, Shin HS, Kim BJ, Kim BS, et al. (2005) Relationship between serum uric acid concentration and insulin resistance and metabolic syndrome. Circ J 69: 928–933.
7. Gaglardi AC, Manne MH, Santos RD (2009) Uric acid: A marker of increased cardiovascular risk. Atherosclerosis 202: 11–17.
8. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, et al. (2003) Is there a pathogenic role for uric acid in hypertension and cardiovascular and renal disease? Hypertension 41: 1183–1190.
9. Edwards NL (2009) The role of hyperuricemia in vascular disorders. Curr Opin Rheumatol 21: 132–137.
10. Qiu L, Cheng XQ, Wu J, Lin JT, Xu T, et al. (2011) Prevalence of hyperuricemia and its related risk factors in healthy adults from Northern and Northeastern Chinese provinces. BMC Public Health 13: 664.
11. B L, T W, Hn Z, Ww Y, Hp Y, et al. (2011) The prevalence of hyperuricemia in healthy Chinese adults. J Rheumatol 27: 132–137.
12. Rall SC, Jr., Weisgraber KH, Mahley RW (1982) Human apolipoprotein E. The complete amino acid sequence. J Biol Chem 257: 4171–4178.
13. Borges RL, Ribeiro AB, Zanella MT, Batista MC (2010) Uric acid as a factor in hypertension. Ann Rheum Dis 44: 300–308.
14. Mahley RW, Rall SC, Jr. (2000) Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 1: 507–537.
15. Liberopoulos EN, Miltiadou GA, Athyros VG, Ganotakis M, Caridou M, et al. (2005) Effect of apolipoprotein E polymorphism on serum uric acid levels in healthy subjects. J Investig Med 53: 116–122.
16. Fernandez MA, Prowen MT, Nogueira MJ, Grauzina MM, Oliveira LM, et al. (1999) Influence of apolipoprotein E genotype on blood redox status of Alzheimer’s disease patients. Int J Mol Med 4: 179–186.
17. Bazgar M, Karimi M (2012) Is the apolipoprotein E4 allele always hazardous? Serum uric acid level as a conflict. Genet Test Mol Biomarkers 16: 920–925.
18. Alvim RO, Freitas SR, Ferreira NE, Santos PC, Cunha RS, et al. (2010) APOE polymorphism is associated with lipid profile, but not with arterial stiffness in the general population. Lipids Health Dis 9: 128.
19. Becker MA, Schmacher HR, Jr., Wörtmann RL, MacDonald PA, Eastace D, et al. (2005) Febuxostat compared with allopurinol in patients with hyperuricemia and gout. N Engl J Med 353: 2450–2461.
20. Nagahama K, Inoue T, Iseki K, Toma T, Kinjo K, et al. (2004) Hyperuricemia as a predictor of hypertension in a screened cohort in Okinawa, Japan. Hypertens Res 27: 835–841.
21. Zhang W, Doherty M, Bardlin T, Pascual E, Barskova V, et al. (2006) EULAR evidence based recommendations for gout. Part II: Management. Report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis 65: 1312–1324.
22. Donohoe GG, Salomaki A, Lehtimaki T, Pulkki K, Kairisto V (2003) Rapid identification of apolipoprotein E genotypes by multiplex amplification refractory mutation system PCR and capillary gel electrophoresis. Clin Chem 49: 143–148.
23. Tang HS, Doherty M, Bardlin T, Pascual E, Barskova V, et al. (2006) EULAR evidence based recommendations for gout. Part II: Management. Report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis 65: 1312–1324.
24. Aug LS, Cruz RP, Hendel A, Granville DJ (2008) Apolipoprotein E, an important player in longevity and age-related diseases. Exp Gerontol 43: 615–622.
25. Solhaim Z, Rasheed K, Kapusta DR, Reisn E (2013) Potential role of uric acid in metabolic syndrome, hypertension, kidney injury, and cardiovascular diseases: is it time for reappraisal? Curr Hypertens Rep 15: 175–181.
26. Reddy DH, Devaraj S, Rietzschel ER, Vasan RS, Rader DJ, et al. (2008) Uric acid and risk of coronary heart disease: Meta-analysis of prospective studies. Ann Intern Med 139: 116–125.
27. Devaraj S, Rietzschel ER, Vasan RS, Rader DJ, et al. (2008) Uric acid and risk of coronary heart disease: Meta-analysis of prospective studies. Ann Intern Med 139: 116–125.
28. Devaraj S, Rietzschel ER, Vasan RS, Rader DJ, et al. (2008) Uric acid and risk of coronary heart disease: Meta-analysis of prospective studies. Ann Intern Med 139: 116–125.
29. Devaraj S, Rietzschel ER, Vasan RS, Rader DJ, et al. (2008) Uric acid and risk of coronary heart disease: Meta-analysis of prospective studies. Ann Intern Med 139: 116–125.
30. Devaraj S, Rietzschel ER, Vasan RS, Rader DJ, et al. (2008) Uric acid and risk of coronary heart disease: Meta-analysis of prospective studies. Ann Intern Med 139: 116–125.
32. Liang S, Pan M, Geng HH, Chen H, Gu LQ, et al. (2009) Apolipoprotein E polymorphism in normal Han Chinese population: frequency and effect on lipid parameters. Mol Biol Rep 36: 1251–1256.

33. Austin MA, Zhang C, Humphries SE, Chandler WL, Talmud PJ, et al. (2004) Heritability of C-reactive protein and association with apolipoprotein E genotypes in Japanese Americans. Ann Hum Genet 68: 179–188.

34. Yoshida T, Kaneshi T, Shimabukuro T, Sunagawa M, Obta T (2006) Serum C-reactive protein and its relation to cardiovascular risk factors and adipocytokines in Japanese children. J Clin Endocrinol Metab 91: 2133–2137.
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