Effect of apolipoprotein C3 genetic polymorphisms on serum lipid levels and the risk of intracerebral hemorrhage

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Abstract

Background: Serum lipid levels are associated with the risk of intracerebral hemorrhage (ICH). Genetic variants in the apolipoprotein C3 (APOC3) gene were associated with plasma triglyceride (TG) and very-low-density lipoprotein (VLDL) levels. The aim of this study was to evaluate the effect of two genetic variants (1100 C/T and 3238 C/G) of APOC3 on serum lipid levels and risk of ICH.

Methods: A prospective hospital-based case–control design and logistic regression analysis were utilized. We enrolled 150 ICH patients and 150 age- and gender-matched controls. The APOC3 gene polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: ICH patients had a significantly higher frequency of APOC3 3238 GG genotype [odds ratio (OR) =2.97, 95% confidence interval (CI) = 1.20, 7.38; \( P = 0.02 \)] and APOC3 3238 G allele (OR =1.53, 95% CI = 1.03, 2.27; \( P = 0.04 \)) than controls. The APOC3 3238 G allele was significantly associated with increasing plasma TG levels and VLDL levels both in ICH cases (\( P =0.01 \)) and controls (\( P =0.02 \)). No association was found between APOC3 1100 C/T polymorphisms and ICH.

Conclusion: To the best of our knowledge, this is the first report in the literature that the APOC3 3238 GG genotype and G allele might contribute to an increased risk of ICH as a result of its effect on serum lipid levels.

Keywords: Intracerebral hemorrhage, Lipid, Apolipoprotein C3, Gene polymorphism

Introduction

Intracerebral hemorrhage (ICH) occurs at an annual incidence rate of 15 to 19 per 100,000 [1]. ICH accounts for about 15% of all strokes and is associated with 3-month mortality of approximately 25% [1, 2]. Even with state-of-the-art medical care, ICH results in death, or severe disability in more than 50% of cases [3, 4]. ICH may occur due to hypertension, diabetes mellitus, vascular malformation, menopause, cerebral amyloid angiopathy, current cigarette smoking, trauma, coagulopathy [5–9], and serum lipid levels [10–12].

Apolipoprotein C3 (APOC3) is a major component of triglyceride (TG)-rich lipoproteins, and a minor component of high-density lipoprotein (HDL) [13]. APOC3 gene, located in the chromosome 11q23, was involved in transport, and clearance of chylomicron remnants, and very-low-density lipoprotein (VLDL), and HDL from the bloodstream [14, 15]. APOC3 encodes a 79-amino-acid glycoprotein produced mainly in the liver inhibiting the action of lipoprotein lipase and interfering with receptor-mediated lipoprotein uptake [16]. Two common single nucleotide polymorphisms (SNPs) have been identified in the APOC3 gene: 1100 C/T and 3238 C/G [17, 18]. Genetic variants in the APOC3 gene were associated with plasma TG and VLDL levels [19, 20].

We hypothesized that common genetic variants in APOC3 gene influenced the risk of ICH. To test this hypothesis, we performed a prospective hospital-based case–control study to evaluate the effect of two genetic variants (1100 C/T and 3238 C/G) of APOC3 on serum lipid levels and risk of ICH.
Materials and methods

Study population
This is a prospective hospital-based case–control study between July 2011 and July 2013 in the Department of Neurosurgery, West China Hospital, Sichuan University, China. We enrolled 150 ICH patients and 150 age- and gender-matched controls. Eligibility for ICH patients required neuroimaging (CT or MRI) confirmation of hemorrhagic stroke. Exclusion criteria were defined as: presence of a vascular malformation, aneurysmal subarachnoid hemorrhage, hemorrhagic transformation of acute infarction, traumatic ICH, brain neoplasm, or any other suspected cause of secondary ICH. Controls were confirmed to have no medical history of ICH, Alzheimer’s disease, or pre-enrollment dementia by means of interview and review of medical records. In addition, similar to the cases the controls were all required to be born in China to native Chinese Han parents. To confirm the diagnosis, two physicians reviewed the hospital records and validated each case. Collected clinical data included age, sex, body mass index (BMI), smoking status, and medical history including hypertension, diabetes mellitus, hyperlipidemia, ischemic stroke, and previous ICH. Medications included the use of warfarin, antiplatelet therapy, and statins. All data points were collected through interviews with the patient or their families/surrogates. All parts of the study were approved by the Institutional Ethical Committee of the West China Hospital, Sichuan University, and informed consent according to the Declaration of Helsinki was obtained from all participants or their families/surrogates.

DNA extraction and genotyping
Genomic DNA was isolated from white blood cells by the commercially available Qiagen kit (QIAGEN Inc., Valencia, CA, USA). The APOC3 gene polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Briefly, the primers designed for APOC3 1100 C/T and 3238 C/G were 5’-AGA GGC CGA TCC ACC CCA CTC AGC C-3’ (forward) and 5’-GAG GGT CTT GGT GGC GTG CTT CAG G-3’ (reverse); 5’-CAT GGT TGC CTA CAG AGG AGT-3’ (forward) and 5’-TGA CCT TCC GCA CAA AGC TGT-3’ (reverse), respectively. The amplified PCR products were digested with SstI (3238 C/G) and SacI (1100 C/T) (New England BioLabs, Mississauga, ON). Details of PCR conditions have been described elsewhere [21]. Electrophoresis in a 2.5 % agarose gel followed by ethidium bromide staining and ultraviolet illumination allowed detection of the alleles. For quality control, two independent observers, read all genotypes without knowing about the case or control status. When replicate quality control samples were evaluated, genotypes showed 100 % concordance.

Table 1 General characteristics of ICH patients and controls

| Variables                     | ICH   | Controls | P     |
|-------------------------------|-------|----------|-------|
| Number of subjects            | 150   | 150      |       |
| Age (years), (mean ± SD)      | 65.3 ± 11.2 | 64.8 ± 10.9 | 0.69  |
| Sex (Male/Female)             | 89/61 | 92/58    | 0.72  |
| BMI (Kg/m²)                   | 25.7 ± 3.3 | 25.3 ± 3.2 | 0.29  |
| Smoking status (Ever/Never)   | 31/119 | 17/133   | 0.03  |
| Hypertension (Positive/Negative)| 91/59 | 43/107   | <0.001|
| Diabetes (Positive/Negative)  | 35/115 | 14/136   | 0.001 |
| Hyperlipidemia (Positive/Negative)| 43/107 | 22/128   | 0.004 |
| TG (mg/dL)                    | 176.3 ± 87.5 | 149.8 ± 75.6 | 0.005 |
| TC (mg/dL)                    | 196.8 ± 59.5 | 178.4 ± 51.4 | 0.004 |
| HDL (mg/dL)                   | 34.5 ± 15.6 | 38.9 ± 16.7 | 0.02  |
| LDL (mg/dL)                   | 127.0 ± 46.5 | 109.5 ± 43.7 | 0.001 |
| VLDL (mg/dL)                  | 35.3 ± 17.5 | 30.0 ± 15.1 | 0.005 |
| ApoA1 (mg/dL)                 | 124.1 ± 21.5 | 122.7 ± 21.8 | 0.58  |
| ApoB (mg/dL)                  | 89.3 ± 19.4 | 88.5 ± 19.2 | 0.72  |

ICH intracerebral hemorrhage, BMI body mass index, TG triglyceride, TC total cholesterol, HDL high-density lipoprotein, LDL low-density lipoprotein, VLDL very-low-density lipoprotein, Apo apolipoprotein
total cholesterol, HDL-C, and LDL-C. We also have adjusted for medicine use by which lipid levels are affected in this analysis. The plasma lipid levels were compared among different genotypes by ANCOVA, adjusted for gender, age, and BMI. Statistical significance was taken at nominal \( P \)-value < 0.05 for all comparisons.

### Results

#### Characteristics of participants

General characteristics of ICH patients and controls were presented in Table 1. There were no significant differences between the ICH patients and controls in age, sex, BMI, smoking status, history of hypertension, or diabetes, total cholesterol, HDL-C, LDL-C, and medicine use by which lipid levels are affected. Statistical significance was taken at nominal \( P \)-value < 0.05 for all comparisons.

#### APOC3 3238 C/G polymorphisms, serum lipid levels, and ICH

ICH patients had a significantly higher frequency of APOC3 3238 GG genotype (OR =2.97, 95% CI = 1.20, 7.38; \( P = 0.02 \)) and APOC3 3238 G allele (OR =1.53, 95% CI = 1.03, 2.27; \( P = 0.04 \)) than controls (Table 2). The APOC3 3238 G allele was significantly associated with increasing plasma TG levels and VLDL levels both in ICH cases (\( P = 0.01 \)) and controls (\( P = 0.02 \)) (Table 3).

#### APOC3 1100 C/T polymorphisms, serum lipid levels, and ICH

No association was found between APOC3 1100 C/T polymorphisms and ICH (Table 2).

### Discussion

In this study, we evaluated the effect of two genetic variants (1100 C/T and 3238 C/G) of APOC3 on serum lipid levels and risk of ICH in a Chinese population. This prospective hospital-based case–control study revealed that the APOC3 3238 GG genotype and G allele might contribute to an increased risk of ICH as a result of its effect on serum lipid levels. No association was found between APOC3 1100 C/T polymorphisms and ICH. To the best of our knowledge, this is the first report in the literature that evaluated the effect of two genetic variants (1100 C/T and 3238 C/G) of APOC3 on serum lipid levels and risk of ICH.

The APOC3 gene polymorphisms were also associated with many other diseases. A nested case–control study demonstrated a diet-gene interaction between APOC3 rs5128 polymorphism and the western dietary patterns in relation to metabolic syndrome risk [22]. Two other case–control study also suggested that two genetic variants (−482 C/T and −455 T/C) of APOC3 were associated with the metabolic syndrome [23, 24]. The GENOCOR study identified −482 C > T of APOC3 as an additive biomarker for ischemic heart disease in an Italian cohort of ischemic patients [25]. A systematic review of 20 studies

### Table 2 Genotype and allele frequencies of APOC3 gene polymorphisms among ICH cases and healthy controls

| Genotypes | ICH (%) | Controls (%) | OR (95%CI) | \( P \) |
|-----------|---------|--------------|------------|--------|
| 1100 CC   | 58(38.7)| 63(42.0)     | 1.00(Reference) |       |
| 1100 CT   | 65(43.3)| 53(35.3)     | 1.33(0.80,2.22) | 0.27  |
| 1100 TT   | 27(18.0)| 34(22.7)     | 0.86(0.47,1.60) | 0.64  |
| 1100 C allele frequency | 181(60.3)| 179(59.7)     | 1.00(Reference) |       |
| 1100 T allele frequency | 119(39.7)| 121(40.3)     | 0.97(0.70,1.35) | 0.87  |
| 3238 CC   | 95(63.3)| 104(69.3)    | 1.00(Reference) |       |
| 3238 CG   | 36(24.0)| 39(26.0)     | 1.01(0.59,1.72) | 0.97  |
| 3238 GG   | 19(12.7)| 7(4.7)       | 2.97(1.20,7.38) | 0.02  |
| 3238 C allele frequency | 226(75.3)| 247(82.3)    | 1.00(Reference) |       |
| 3238 G allele frequency | 74(24.7)| 53(17.7)     | 1.53(1.03,2.27) | 0.04  |

Adjustment for conventional risk factors, including age, sex, BMI, smoking status, history of hypertension, or diabetes, total cholesterol, HDL-C, LDL-C, and medicine use by which lipid levels are affected.

**ICH** intracerebral hemorrhage.

### Table 3 Lipid profiles of ICH cases and controls according to APOC3 3238 C/G polymorphisms

|            | ICH                  | \( P \) value | Controls              | \( P \) value |
|------------|----------------------|--------------|-----------------------|--------------|
| TG (mg/dL) | CC 159.7 ± 85.3      | 0.01         | CC 131.5 ± 68.7       | 0.02         |
|            | CG 196.3 ± 92.1      |              | CG 173.4 ± 79.8       |              |
|            | GG 236.7 ± 103.4     |              | GG 219.5 ± 89.6       |              |
| TC (mg/dL) | CC 192.1 ± 59.1      | 0.58         | CC 174.6 ± 51.0       | 0.83         |
|            | CG 208.5 ± 60.8      |              | CG 191.5 ± 58.7       |              |
|            | GG 198.8 ± 59.8      |              | GG 161.8 ± 44.9       |              |
| HDL (mg/dL)| CC 33.7 ± 17.6       | 0.74         | CC 35.8 ± 15.1        | 0.54         |
|            | CG 38.2 ± 18.3       |              | CG 42.4 ± 19.6        |              |
|            | GG 31.5 ± 9.8        |              | GG 35.4 ± 14.8        |              |
| LDL (mg/dL)| CC 126.5 ± 45.7      | 0.63         | CC 110.5 ± 38.1       | 0.02         |
|            | CG 131.0 ± 48.2      |              | CG 114.4 ± 38.5       |              |
|            | GG 120.1 ± 41.6      |              | GG 82.5 ± 33.8        |              |
| VLDL (mg/dL)| CC 31.9 ± 17.1      | 0.01         | CC 26.3 ± 13.7        | 0.02         |
|            | CG 39.3 ± 18.4       |              | CG 34.7 ± 16.0        |              |
|            | GG 47.3 ± 20.7       |              | GG 43.9 ± 17.9        |              |
| ApoA1 (mg/dL)| CC 122.5 ± 22.9    | 0.26         | CC 119.3 ± 20.6       | 0.37         |
|            | CG 127.3 ± 24.5      |              | CG 132.1 ± 26.7       |              |
|            | GG 126.1 ± 23.4      |              | GG 120.6 ± 21.5       |              |
| ApoB (mg/dL)| CC 87.2 ± 18.7      | 0.46         | CC 85.1 ± 17.7        | 0.51         |
|            | CG 96.2 ± 21.6       |              | CG 98.0 ± 22.4        |              |
|            | GG 86.7 ± 18.1       |              | GG 86.1 ± 18.2        |              |

\( P \) values were calculated by ANCOVA, adjusted for age, sex, and BMI.
comprising 15,591 participants found that APOC3 Sst I and T-455C polymorphisms might be associated with coronary heart disease risk [17]. A case–control study suggested that the APOC3 3238 G allele might contribute to an increased risk of coronary artery disease as a result of its effect on TG and VLDL-C metabolism [26]. A case–control study found that the minor alleles of APOC3 −455 T/C polymorphisms were closely associated with acute coronary syndrome [27]. A prospective case–control study suggested that APOC3 (−455 T > C) genetic variation was involved in the susceptibility to developing nonalcoholic fatty liver disease, insulin resistance, hypertension, hypertriglyceridemia, and low HDL in the Southern Chinese Han population [28]. Another case–control study found that the polymorphisms −482 C/T and −455 T/C in APOC3 were associated with nonalcoholic fatty liver disease and insulin resistance [29].

Although our study suggested that the APOC3 GG genotype and G allele might contribute to an increased risk of ICH as a result of its effect on serum lipid levels, the clear mechanism of this association is unclear. The Bogalusa Heart Study found that APOC3 3238 C/G polymorphisms were associated with higher serum triglyceride levels [30]. A recent prospective case–control study also found that the APOC3 3238 G allele might contribute to an increased risk of coronary artery disease as a result of its effect on TG and VLDL-C metabolism [26]. Serum lipid levels may be associated with the risk of ICH [10–12]. We also observed that the proportions of smoking status, hypertension, diabetes, and hyperlipidemia were significantly higher, and levels of TG, TC, LDL, and VLDL were significantly higher, and levels of HDL were significantly lower in ICH patients than in controls.

Some shortcomings of this study should be mentioned. First of all, this study is limited by its size and lack of replication. Further large scale research on the role of APOC3 in ICH and replication of our results is necessary. Second, although we have adjusted for medicine use by which lipid levels are affected in this analysis, no information could be received on the baseline lipid levels of these patients. Third, this study only considers a Chinese population that may limit the application of these findings to other ethnic populations. Fourth, ICH is induced by multiple genes and environmental factors, which were not explored in the present study. Finally, potential selection bias might have been present, because this is a hospital based case control study and the subjects may not be representative of the general population.

Conclusion
To the best of our knowledge, this is the first report in the literature that the APOC3 3238 GG genotype and G allele might contribute to an increased risk of ICH as a result of its effect on serum lipid levels. We found that ICH patients had a significantly higher frequency of APOC3 3238 GG genotype and APOC3 3238 G allele than controls. The APOC3 3238 G allele was significantly associated with increasing plasma TG levels and VLDL levels both in ICH cases and controls. No association was found between APOC3 1100 C/T polymorphisms and ICH. Additional large scale studies are needed to confirm this finding.

Competing interest
The authors declare that they have no competing interests.

Authors’ contributions
YJ and CY carried out the molecular genetic studies and drafted the manuscript. JM carried out the genotyping. HL and YL participated in the design of the study and performed the statistical analysis. YJ, JM, HL, YL, and CY conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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