Cytochrome P450 Genetic Variants and Their Metabolite Levels Associated with Plaque Stability in Patients with Ischemic Stroke

Xing-Yang Yi, Duan-Xiu Liao, Chun Wang, Wen Cheng, Xiu-Quan Fu and Biao Zhang

Department of Neurology, The People's Hospital of Deyang City, Sichuan, China

Aim: Cytochrome P450s (CYP450) enzymes regulate inflammation and atherosclerosis and can affect carotid plaque stability in patients with ischemic stroke (IS). This study aimed to investigate the association of CYP450 genetic variants with CYP plasma metabolite levels and plaque stability in patients with IS.

Methods: Eleven single nucleotide polymorphisms (SNPs) of CYP genes and their plasma metabolite [20-hydroxyeicosatetraenoic acid (HETE), total epoxyeicosatrienoic acids (EETs), and dihydroxyeicosatrienoic acids (DiHETEs)] levels were measured in 396 patients with IS who underwent high-resolution B-mode ultrasound carotid plaque detection and were stratified into the following groups: non-carotid plaque and carotid plaque groups. The carotid plaque was further classified into subgroups of echolucent plaque (ELP) and echogenic plaque (EGP).

Results: Among the 396 patients with IS, 294 cases (74%) had plaques. The frequency of rs17110453CC, rs751141 GG, and rs9333025 GG genotypes was significantly higher in patients with plaque than those without plaque. The CC, GG, GG, and GG genotypes of rs17110453, rs776746, rs751141, and rs9333025 polymorphisms were independently associated with ELP (OR, 2.62 [1.34–5.26]; OR, 1.89 [1.16–3.58]; OR, 3.12 [1.27–7.13]; and OR, 2.06 [1.34–6.33], respectively). These polymorphisms were also associated with CYP plasma metabolite levels. Patients with ELP have also shown significantly higher levels of 20-HETE and DiHETEs, but lower levels of EETs.

Conclusions: Our data demonstrates that CYP450 SNPs are associated with plasma CYP450 metabolite levels and echolucent plaques, indicating that these SNPs may be potential markers for plaque instability.

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Key words: Carotid atherosclerosis, Stable and unstable plaques, Cytochrome P450, Single nucleotide polymorphisms

Introduction

Stroke is the leading cause of mortality and disability worldwide, including China. Ischemic stroke (IS) is the most common type, accounting for up to 80% of all strokes and is usually caused by thrombosis or emboli. Brian artery disease and alterations (inflammation and atherosclerosis) could induce thrombosis. For example, carotid plaque is a distinct phenotype of subclinical atherosclerosis, which is an inflammatory lesion associated with a significantly increased risk of IS. Therefore, identifying novel risk factors for carotid plaque is important for preventing atherosclerosis and stroke. Clinically, carotid ultrasound is a well-established method for visualizing and quantifying atherosclerotic lesions and its vulnerability. Moreover, ultrasonographic assessment of carotid plaque composition has been shown to be a better predictor of carotid plaque-induced adverse events than plaque size. Indeed, echolucent, lipid-rich, histologically “soft” plaques are associated with more complications than calcified or mixed plaques. Heterogeneous plaques including a hypoechoic component were reportedly associated with frequent intra-
plaque hemorrhage, ulceration, and atherosclerosis.

Atherosclerosis is a complex inflammatory disorder and genes involved in inflammation and endothelial function were suggested in the pathogenesis of atherosclerosis. Arachidonic acid (AA) and metabolites through cyclooxygenase, lipoxygenase, and CYP pathways are the primary inflammatory mediators. For example, AA can be metabolized by CYP oxidase into 20-hydroxyeicosatetraenoic acid (20-HETE), which is a potent vasoconstrictor, and AA is also metabolized by CYP epoxygenases into four epoxyeicosatrienoic acids (EETs), which can then be metabolized by soluble epoxide hydrolase (sEH) to yield less biologically active dihydroxyepoicoic acids (DiHETEs). EETs exert vascular relaxation and protective effects on the cardiovascular system.

One study has shown that CYP450 plasma metabolite levels including 20-HETE, EETs, and DiHETEs were associated with IS. Other studies have shown that individual genetic variants of these key enzymes are associated with higher risk of IS. However, to date, only few studies have assessed the association between these genetic variants, CYP plasma metabolite levels, and carotid plaque vulnerability.

In this study, we hypothesized that there was an association between these genetic variants, CYP plasma metabolite levels, and carotid plaque vulnerability. Thus, we selected and assessed 11 SNPs from seven CYP genes and their plasma metabolite levels in 396 patients with IS; these were associated with the risk of developing particular sub-types of carotid plaque in these patients.

Materials and Methods

Study Population

We identified 396 patients with IS who received medical care in our hospitals between August 2010 and March 2013. IS was confirmed on the basis of both clinical findings and neurological examination results, which were related to atherothrombosis (AT, \( n = 270 \)) and small artery disease (SAD, \( n = 126 \)) according to the Trial of ORG 10172 in the Acute Stroke Treatment classification system. Exclusion criteria included: (i) cardiogenic cerebral embolisms or IS caused by unknown factors; (ii) calcified plaques with acoustic shadow or occluded carotid artery because reliably determining their echogenicity was technically impossible; and (iii) carotid endarterectomy or stent implantation. The study protocol was reviewed and approved by the Ethics Committee of our hospital. All patients or their family members provided written informed consent (in the Chinese language) before enrollment into this study.

Vascular risk factors including body mass index, body weight, tobacco smoking history (current and former smoking vs. never smoking), diabetes mellitus, and hypertension were collected. Fasting blood samples were analyzed for blood sugar, total plasma cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C).

Carotid Ultrasonography

Carotid ultrasonography was performed according to the standard scanning and reading protocols, using a diagnostic ultrasound device (type 512, Acuson Sequoia Apparatus, 7.5-MHz probe, Berlin, Germany). Bilateral internal and common carotid arteries, as well as bifurcations, were examined for atherosclerotic plaque presence and defined as an endoluminal protrusion of at least 1.5 mm or a focal thickening >50% of the intima-media thickness relative to the adjacent wall segment. Thereafter, patients were divided into two groups: carotid plaque and non-carotid plaque groups. Patients in the carotid plaque group were further classified into classes I to V. Class V patients were excluded from this study on the basis of the above named exclusion criteria. Then, the remaining patients in the carotid plaque group were divided into two groups: echolucent plaque (ELP; classes I and II) group and echogenic plaque (EGP; classes III and IV) group.

Carotid ultrasound was conducted by a sonographer (Dr. Wen Cheng) blinded to laboratory and questionnaire data. For a test run in this study, we assessed the reproducibility of plaque echogenicity in 33 randomly selected plaques. Plaque echogenicity analyses were performed twice by Dr. Wen Cheng and subsequently by another sonographer (Dr. Xiuquan Fu). Intraobserver and interobserver coefficients of variation for plaque echogenicity were 8.2% and 8.8%, respectively, which could be characterized as substantial. Dr. Wen Cheng assessed all 396 patients, and these data were used for our data analyses.

Selection and Genotyping of SNPs

Eleven SNPs from seven CYP genes were selected according to the following criteria: (i) SNPs with minor allele frequency >0.05; (ii) SNPs leading to amino acid changes; (iii) SNPs that have been assessed in previous studies; and (iv) tagging SNPs across different human populations. Genotyping was performed using the matrix-assisted laser desorption/ionization time of flight mass spectrometry.
method according to our previous study\(^{18}\).

**Measurement of Plasma 20-HETE, EETs, and DiHETEs Levels**

Plasma 20-HETE level was analyzed using a stable isotope dilution gas chromatography/mass spectrometer (GC/MS) as previously described\(^{17}\). Total plasma EETs and DiHETEs levels were measured using a stable isotope dilution GC/MS following base hydrolysis and separation on high performance liquid chromatography\(^{17}\).

**Statistical Analysis**

All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). For quantitative and qualitative variables of demographic and clinical data, Student's \(t\)-test and \(\chi^2\)-test were performed, respectively. Deviation of the Hardy–Weinberg equilibrium for genotype frequencies was also analyzed using the \(\chi^2\)-test. Differences in the genotype frequencies were compared using the \(\chi^2\)-test, whereas plasma 20-HETE, EETs, and DiHETEs levels were compared using the Student’s \(t\)-test. Analysis of variance (ANOVA) followed by the Student-Newman-Keuls test was employed to evaluate differences between the same groups stratified by genotypes. Multivariate logistic regression analysis was performed to adjust risk factors as the independent predictors for the association of these SNPs. Clinicopathological features with carotid plaque instability and odds ratio (OR) with 95% CI were calculated to estimate the association between the genotypes and presence of carotid plaques. \(P\) value less than 0.05 was considered statistically significant.

**Results**

**Patient Characteristics**

Among these 396 patients with IS, 294 cases (74%) had plaques, of which 106 patients had ELP.
Table 2. Genotype distribution and CYP plasma metabolite levels of patients with and without carotid plaques

|                      | Carotid plaque | No carotid plaque | P value* |
|----------------------|----------------|-------------------|----------|
|                      | (n = 294)      | (n = 102)         |          |
| rs10889160           |                |                   |          |
| AA, n (%)            | 224 (76.19)    | 77 (75.49)        |          |
| AG, n (%)            | 67 (22.79)     | 23 (22.55)        |          |
| GG, n (%)            | 3 (1.02)       | 2 (1.96)          | 0.72     |
| rs17110453           |                |                   |          |
| AA, n (%)            | 128 (43.54)    | 50 (49.02)        |          |
| AC, n (%)            | 118 (40.13)    | 46 (45.10)        |          |
| CC, n (%)            | 48 (16.33)     | 6 (5.88)          | 0.036    |
| rs1934980            |                |                   |          |
| CC, n (%)            | 44 (14.97)     | 15 (14.71)        |          |
| CT, n (%)            | 132 (44.89)    | 47 (46.08)        |          |
| TT, n (%)            | 118 (40.14)    | 40 (39.21)        | 0.89     |
| rs1799853            |                |                   |          |
| CC, n (%)            | 294 (100)      | 102 (100)         | –        |
| rs1057910            |                |                   |          |
| AA, n (%)            | 265 (90.14)    | 93 (91.18)        |          |
| AC, n (%)            | 24 (8.16)      | 8 (7.84)          |          |
| CC, n (%)            | 5 (1.70)       | 1 (0.98)          | 0.87     |
| rs776746             |                |                   |          |
| GG, n (%)            | 185 (62.93)    | 44 (43.14)        |          |
| AG, n (%)            | 99 (33.67)     | 48 (47.06)        |          |
| AA, n (%)            | 10 (3.4)       | 10 (9.80)         | <0.001   |
| rs751141             |                |                   |          |
| GG, n (%)            | 159 (54.08)    | 55 (53.92)        |          |
| AT, n (%)            | 56 (19.05)     | 19 (18.63)        |          |
| TT, n (%)            | 79 (25.87)     | 28 (27.45)        | 0.43     |
| rs2269231            |                |                   |          |
| AA, n (%)            | 6 (2.04)       | 6 (5.88)          |          |
| AG, n (%)            | 73 (24.83)     | 44 (43.14)        |          |
| GG, n (%)            | 215 (73.13)    | 52 (50.98)        | <0.001   |
| rs3093135            |                |                   |          |
| TT, n (%)            | 244 (82.99)    | 84 (82.35)        |          |
| AT, n (%)            | 44 (14.97)     | 15 (14.71)        |          |
| AA, n (%)            | 6 (2.04)       | 3 (2.94)          | 0.94     |
| rs2108622            |                |                   |          |
| GG, n (%)            | 152 (51.71)    | 51 (50.00)        |          |
| GA, n (%)            | 117 (39.79)    | 42 (41.18)        |          |
| AA, n (%)            | 25 (8.50)      | 9 (8.82)          | 0.68     |
| 20-HETE (pmol/L)     | 1726 ± 189     | 1592 ± 162        | <0.001   |
| EETs (nmol/l)        | 63.82 ± 5.62   | 67.76 ± 5.25      | <0.001   |
| DiHETEs (nmol/l)     | 82.66 ± 6.24   | 78.87 ± 5.92      | <0.001   |

CYP, cytochrome P450; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acids; DiHETEs, dihydroxyeicosatrienoic acids. *χ² test or Student’s t-test.
Association of Clinicopathological Data from Patients with Echolucent and Echogenic Plaques

Hypertension ($P<0.016$) and diabetes mellitus ($P=0.028$) were significantly more frequent in patients with ELP than in those with EGP. LDL-C level was also higher ($P=0.045$) in patients with ELP than in those with EGP. However, there was no significant difference in age, gender, tobacco smoking history, previous or ongoing drug treatments, stroke subtype, serum levels of TC, TG, HDL-C, and fasting blood-glucose, and hemoglobin A1C levels between these two groups ($P>0.05$; Table 1).

Genotype Distributions between Patients with and without Plaque

Genotype distributions of these 11 SNPs were in Hardy–Weinberg Equilibrium ($P>0.05$). Frequency of rs17110453 CC, rs776746 GG, rs751141 GG, and rs9333025 GG genotypes were significantly higher in patients with plaque than in those without plaque ($P=0.036$, $<0.001$, and $<0.001$, respectively; Table 2). There were no significant differences in the genotypes frequencies of these 11 SNPs between patients with AT and SAD.

Table 3. Association of demographic and clinicopathological data from patients with echolucent and echogenic plaques

| Characteristics                  | ELP ($n=106$) | EGP ($n=188$) | $P$-value* |
|----------------------------------|---------------|---------------|------------|
| Age (years)                      | 68.62±10.84   | 68.13±11.92   | 0.58       |
| Men (n, %)                       | 62 (58.49)    | 112 (59.57)   | 0.86       |
| Diabetes mellitus (n, %)         | 50 (47.17)    | 64 (34.04)    | 0.028      |
| Hypertension (n, %)              | 95 (89.62)    | 140 (74.47)   | 0.016      |
| Previous MI (n, %)               | 1 (0.94)      | 2 (1.06)      | 0.99       |
| Current or former smoking (n, %) | 45 (42.45)    | 75 (39.89)    | 0.87       |
| Body mass index (kg/m²)          | 24.09±2.51    | 24.02±2.59    | 0.78       |
| TC (mM)                          | 5.62±1.38     | 5.53±1.32     | 0.57       |
| LDL-C (mM)                       | 3.23±1.23     | 2.92±1.20     | 0.045      |
| HDL-C (mM)                       | 1.22±0.47     | 1.22±0.53     | 0.99       |
| TG (mM)                          | 1.92±1.09     | 1.87±1.11     | 0.96       |
| Fasting blood-glucose (mM)       | 7.08±2.14     | 6.99±2.09     | 0.28       |
| HbA1c(%)                         | 6.22±1.38     | 6.11±1.35     | 0.65       |
| Previous or ongoing drug treatments (n, %) |         |               |            |
| Antihypertensive drugs           | 32 (30.19)    | 57 (30.32)    | 0.99       |
| Hypoglycemic drugs               | 29 (27.36)    | 47 (25.00)    | 0.68       |
| Statins                          | 14 (13.21)    | 25 (13.29)    | 0.98       |
| Antiplatelet drugs               | 21 (19.81)    | 39 (20.75)    | 0.98       |
| Stroke subtype (n, %)            | 78 (73.58)    | 135 (71.81)   | 0.74       |
| AT stroke                        | 28 (26.42)    | 53 (28.19)    |            |

MI, myocardial infarction; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; HbA1c, Hemoglobin A1C; ELP, echolucent plaque; EGP, echogenic plaque; AT, atherothrombosis; SAD, small artery disease. * $\chi^2$ test or Student’s $t$-test.

Association of Clinicopathological Data from Patients with Echolucent and Echogenic Plaques

Hypertension ($P=0.016$) and diabetes mellitus ($P=0.028$) were significantly more frequent in patients with ELP than in those with EGP. LDL-C level was also higher ($P=0.045$) in patients with ELP than in those with EGP. However, there was no significant difference in age, gender, tobacco smoking history, previous or ongoing drug treatments, stroke subtype, serum levels of TC, TG, HDL-C, and fasting blood-glucose, and hemoglobin A1C levels between these two groups ($P>0.05$; Table 3).

Genotype Distribution between Patients with ELP and those with EGP

Frequency of rs17110453 CC, rs776746 GG, rs751141 GG, and rs9333025 GG were significantly higher in patients with ELP than in those with EGP ($P=0.003$, 0.022, $<0.001$, and 0.032, respectively). According to the best genetic model and after adjusting with relevant confounding variables (age, gender, hypertension, diabetes, smoking, and hypercholesterolemia) using the logistic regression analysis, we found
**Table 4.** Genotype distribution and CYP plasma metabolite levels of patients with echolucent and echogenic plaques

| Genotype   | ELP (n=106) | EGP (n=188) | P-value* | Adjusted OR† (95% CI) |
|------------|-------------|-------------|----------|-----------------------|
| rs10889160 |             |             |          |                       |
| AA, n (%)  | 81 (76.42)  | 143 (76.06) | 0.92     | 0.95 (0.55–1.64)      |
| AG, n (%)  | 24 (22.64)  | 43 (22.87)  |          |                       |
| GG, n (%)  | 1 (0.94)    | 2 (1.06)    |          |                       |
| rs17110453 |             |             |          |                       |
| AA, n (%)  | 44 (41.51)  | 84 (44.68)  |          |                       |
| AC, n (%)  | 34 (32.07)  | 84 (44.68)  |          |                       |
| CC, n (%)  | 28 (26.42)  | 20 (10.64)  | 0.003    | 2.62 (1.34–5.26)      |
| rs1934980  |             |             |          |                       |
| CC, n (%)  | 16 (15.09)  | 28 (14.89)  |          |                       |
| CT, n (%)  | 45 (42.45)  | 87 (46.28)  |          |                       |
| TT, n (%)  | 45 (42.45)  | 73 (38.83)  | 0.61     | 1.15 (0.67–1.98)      |
| rs1799853  |             |             |          |                       |
| CC, n (%)  | 106 (100)   | 188 (100)   | –        | –                     |
| rs1057910  |             |             |          |                       |
| AA, n (%)  | 93 (87.74)  | 172 (91.49) |          |                       |
| AC, n (%)  | 9 (8.49)    | 15 (7.98)   |          |                       |
| CC, n (%)  | 4 (3.77)    | 1 (0.53)    | 0.65     | 1.08 (0.54–2.14)      |
| rs776746   |             |             |          |                       |
| AA, n (%)  | 8 (7.55)    | 27 (14.36)  |          |                       |
| AG, n (%)  | 34 (32.07)  | 78 (41.49)  |          |                       |
| GG, n (%)  | 64 (60.38)  | 83 (44.15)  | 0.022    | 1.89 (1.16–3.58)      |
| rs751141   |             |             |          |                       |
| GG, n (%)  | 82 (77.36)  | 103 (54.79) |          |                       |
| AG, n (%)  | 21 (19.81)  | 78 (41.49)  |          |                       |
| AA, n (%)  | 3 (2.83)    | 7 (3.72)    | <0.001   | 3.12 (1.27–7.13)      |
| rs2269231  |             |             |          |                       |
| AA, n (%)  | 22 (20.75)  | 34 (18.09)  |          |                       |
| AT, n (%)  | 57 (53.77)  | 102 (54.26) |          |                       |
| TT, n (%)  | 27 (25.47)  | 52 (27.65)  | 0.78     | 0.94 (0.56–1.99)      |
| rs9333025  |             |             |          |                       |
| AA, n (%)  | 2 (1.89)    | 4 (2.13)    |          |                       |
| AG, n (%)  | 17 (16.03)  | 56 (29.78)  |          |                       |
| GG, n (%)  | 87 (82.08)  | 128 (68.09) | 0.032    | 2.06 (1.34–6.33)      |
| rs3093135  |             |             |          |                       |
| TT, n (%)  | 90 (84.91)  | 154 (81.91) |          |                       |
| AT, n (%)  | 14 (13.20)  | 30 (15.96)  |          |                       |
| AA, n (%)  | 2 (1.89)    | 4 (2.13)    | 0.89     | 0.96 (0.52–1.86)      |
| rs2108622  |             |             |          |                       |
| GG, n (%)  | 56 (52.83)  | 96 (51.06)  |          |                       |
| GA, n (%)  | 39 (36.79)  | 78 (41.49)  |          |                       |
| AA, n (%)  | 11 (10.38)  | 14 (7.45)   | 0.67     | 1.03 (0.51–2.16)      |

20-HETE (pmol/L) 1778 ± 183 1602 ± 168 <0.001
EETs (nmol/l) 60.32 ± 4.52 68.35 ± 4.92 <0.001
DiHETEs (nmol/l) 85.12 ± 7.53 77.82 ± 6.26 <0.001

CYP, cytochrome P450; ELP, echolucent plaque; EGP, echogenic plaque; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acids; DiHETEs, dihydroxyeicosatrienoic acids; OR, odds ratio; CI, confidence interval. *χ² test or Student’s t-test. †OR adjusted for age, gender, hypertension, diabetes, smoking, and hypercholesterolemia using logistic regression analyses.
that CC, GG, GG, and GG genotypes of rs17110453, rs776746, rs776746, and rs9333025 polymorphisms were independently associated with ELP formation (OR, 2.62 [1.34–5.26]; OR, 1.89 [1.16–3.58]; OR, 3.12 [1.27–7.13]; and OR, 2.06 [1.34–6.33]; respectively) as shown in Tables 4 and 5.

**CYP Plasma Metabolite Levels and their Association with CYP Genotype Distribution**

CYP450 plasma metabolite levels were measured in 178 patients with plaques and 40 patients without plaques. Plasma levels of 20-HETE and DiHETEs were significantly higher, whereas EETs levels were lower in patients with plaques compared with those without plaques, with ELP or with EGP (P < 0.001, Tables 2 and 4). However, there was no significant difference in 20-HETE, DiHETEs, and EETs levels between patients with AT and SAD (1712 ± 172, 83.24 ± 6.52, and 62.36 ± 5.46 vs. 1701 ± 168, 82.88 ± 6.33, and 63.02 ± 5.81, all P > 0.05). There was also no significant difference in 20-HETE, DiHETEs, and EETs levels between patients with and without statin treatment (1706 ± 69, 82.82 ± 6.74, and 61.87 ± 5.29 vs. 1698 ± 163, 82.98 ± 7.26, and 62.03 ± 5.82, respectively, all P > 0.05).

Furthermore, patients with ELP carrying the rs9333025 GG genotype had statistically higher plasma 20-HETE levels than those with EGP, whereas patients with ELP carrying the rs17110453 CC or rs776746 GG genotype had statistically lower plasma levels of EETs than those with EGP. Moreover, patients with ELP carrying the rs751141 GG genotype had statistically lower plasma levels of EETs and higher plasma levels of DiHETEs compared with those with EGP (P < 0.01, Table 6).

**Discussion**

Our current data showed that some CYP450 SNPs were associated with echolucent plaque formation in patients with IS and CYP450 plasma metabolite levels. A number of studies have explored the association of CYP450 genes with IS26. Some studies have shown that CYP450 plasma metabolite levels are associated with IS risk19, but few studies have focused on subclinical atherosclerosis. Certain carotid plaque phenotypes such as plaque instability may be important markers of vulnerable plaques susceptible to rupture, leading to stroke21. To our knowledge, our study is the first to investigate the association between the genetic variations of genes related to the CYP pathway and possible markers of vulnerable plaque or the effect of these genetic variants on CYP450 plasma metabolite levels.

The development of atherosclerosis is associated with chronic inflammatory conditions; thus, CYP4A11 and CYP4F2 genes encode CYP ω-hydroxylase to metabolize inflammation mediators such as AA and its metabolites. The genetic variant of CYP4A11 rs9333025 analyzed in our study was associated with 20-HETE levels. 20-HETE can constrict cerebral arteries, is involved in endothelial dysfunction23, and helps form oxygen radicals24. Thus, altered 20-HETE levels could contribute to atherosclerosis, plaque formation, or IS onset. In this regard, studies on CYP450 SNPs could provide a biomarker to assess IS risk. Furthermore, the CYP2 and 3 gene family encodes for the majority of epoxygenase enzymes predominantly expressed in vascular endothelial cells and heart tissues that metabolizes AA into all four EET regioisomers. Soluble epoxide hydrolase (sEH), encoded by the epoxide hydrolase-2 (EPHX2) gene, metabolizes EETs to less biologically active DiHETEs15. In the current study, CYP2C8 rs17110453 and CYP3A5 rs776746 polymorphisms were associated with lower plasma levels of EETs, whereas rs751141 GG genotype in EPHX2 locus was associated with both lower plasma levels of EETs and higher plasma levels of DiHETEs. The pharmacological inhibition or genetic deletion of sEH has been previously shown to increase EET levels and protect against stroke-induced brain injury25. In addition, certain gene polymorphisms such as human EPHX2 have been indicated to increase sEH activity; thereby, increasing IS incidence26. This specific interaction between CYP2C8 and EPHX2 genes has been recently described in an animal model as CYP2C8 overexpression coupled with EPHX2 downregulation significantly attenuated NF-κB dependent vascular inflammatory response27.

**Table 5.** Multivariate analysis of the major risk factors for echolucent plaques

| Risk factor | OR  | 95% CI     | P value* |
|-------------|-----|------------|----------|
| Cigarette smoking | 0.64 | 0.51–1.29 | 0.76     |
| AT stroke | 0.88 | 0.76–1.65 | 0.72     |
| Hypertension | 1.98 | 1.36–5.63 | 0.038    |
| Diabetes mellitus | 0.96 | 0.63–1.86 | 0.48     |
| LDL-C | 0.87 | 0.54–1.76 | 0.62     |
| rs17110453CC | 2.62 | 1.34–5.26 | 0.003    |
| rs751141 GG | 3.12 | 1.27–7.13 | <0.001   |
| rs776746 GG | 1.89 | 1.16–3.58 | 0.022    |
| rs9333025 GG | 2.06 | 1.34–6.33 | 0.032    |

OR, odds ratios; CI, confidence interval; AT, atherothrombosis; LDL-C, low-density lipoprotein cholesterol.
Thus, the study of a larger sample is necessary in future. Although this study examined the rs751141 and rs776746, statin treatment may be an impotent reason in China; showing this action of statins. The low proportion of plaque characteristics. However, our results did not may affect levels of CYP metabolites and carotid plaque; thus, anti-inflammation drugs (i.e., statins) may contribute to arterial inflammation and atherogenic pathway, resulting in the instability of carotid plaque; thus, anti-inflammation drugs (i.e., statins) may affect levels of CYP metabolites and carotid plaque characteristics. However, our results did not show this action of statins. The low proportion of statin treatment may be an impotent reason in China; thus, the study of a larger sample is necessary in future.

**Conclusion**

Our data demonstrated that CYP450 SNPs are associated with plasma CYP450 metabolite levels and echolucent plaques, indicating that these SNPs may be potential markers for plaque instability. Our results, as well as all previous observations, provide a potential explanation behind the molecular mechanism of gene actions on carotid plaque phenotypes. To our knowledge, our study is the first to investigate the association between the genetic variations of genes related to the CYP pathway and possible markers of vulnerable plaques. To our knowledge, our study is the first to investigate the association between the genetic variations of genes related to the CYP pathway and possible markers of vulnerable plaques. To our knowledge, our study is the first to investigate the association between the genetic variations of genes related to the CYP pathway and possible markers of vulnerable plaques. To our knowledge, our study is the first to investigate the association between the genetic variations of genes related to the CYP pathway and possible markers of vulnerable plaques.

**Table 6.** Association of 20-HETE, DiHETEs, and EET levels with genotype distribution in patients with echolucent and echogenic plaques

| Assay | EETs (nmol/l) | 20-HETE (pmol/L) | DiHETEs (nmol/l) |
|-------|---------------|------------------|-----------------|
|       | (n = 82)      | (n = 96)         | (n = 82)        | (n = 96) |
| rs17110453 |               |                  |                 |
| AA    | 63.95 ± 6.17  | 1527 ± 182       | 81.13 ± 6.04    |
| AC    | 62.67 ± 5.02  | 1618 ± 184       | 86.35 ± 10.72   |
| CC    | 58.72 ± 5.63  | 1638 ± 188       | 83.12 ± 10.24   |
| rs776746 |               |                  |                 |
| AA    | 62.26 ± 6.71  | 1606 ± 168       | 74.04 ± 6.74    |
| AG    | 62.87 ± 4.88  | 1628 ± 177       | 76.95 ± 6.32    |
| GG    | 57.96 ± 5.23  | 1673 ± 186       | 86.87 ± 8.92    |
| rs751141 |               |                  |                 |
| GG    | 58.24 ± 4.67  | 1706 ± 194       | 106.24 ± 11.22  |
| AG    | 61.46 ± 6.58  | 1621 ± 166       | 76.36 ± 6.15    |
| AA    | 63.68 ± 5.66  | 1633 ± 182       | 71.88 ± 4.66    |
| rs9333025 |            |                  |                 |
| AA    | 61.11 ± 5.67  | 1599 ± 167       | 82.12 ± 8.51    |
| AG    | 61.89 ± 6.17  | 1647 ± 162       | 85.89 ± 9.12    |
| GG    | 60.78 ± 5.66  | 1832 ± 188       | 83.87 ± 8.02    |

ELP, echolucent plaque; EGP, echogenic plaque; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acids; DiHETE, dihydroxyeicosatrienoic acid. * Student’s t-test.

This study does have some potential limitations. For instance, this study is just a case-control study; therefore, recruitment and survival bias cannot be excluded. Our data were obtained from patients with IS in Han Chinese populations; therefore, comorbidity may represent as a confounding factor, and generalizing our findings to other ethnicities would have unclear results. Further, this study was a single hospital study; thus, our findings need to be confirmed in multi-center studies. Although this study examined the role of several known important CYP450 genes, other known and unknown genes were not captured. Our current data indicated that levels of CYP metabolites may contribute to arterial inflammation and atherogenic pathway, resulting in the instability of carotid plaque; thus, anti-inflammation drugs (i.e., statins) may affect levels of CYP metabolites and carotid plaque characteristics. However, our results did not show this action of statins. The low proportion of statin treatment may be an impotent reason in China; thus, the study of a larger sample is necessary in future.

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**Conflict of Interest Statement**

The authors declare that there is no conflict of interest regarding this work.
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