Interactions Among CYP2C8, EPHX2, and CYP4A11 Variants and CYP Plasma Metabolite Levels in Ischemic Stroke

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Aim: To better understand the relationship between the interactions among rs17110453, rs751141, and rs9333025 variants and plasma levels of cytochrome P450 (CYP) metabolites, i.e., 20-hydroxyeicosatetraenoic acid (20-HETE), epoxyeicosatrienoic acids (EETs), and dihydroxyeicosatetraenoic acids (DiHETEs) in ischemia stroke (IS).

Methods: We measured plasma CYP metabolite levels in 218 acute IS cases and 126 controls, and a subset of samples were assessed to further understand the association between relevant variants and IS risk in our previous study. We assessed the associations between variant interactions and levels of 20-HETE, EETs, and DiHETEs as well as the associations between levels of 20-HETE, EETs, and DiHETEs and IS risk after adjusting for other potential confounders. Furthermore, the association between variant interactions and IS risk after adjusting for other covariates, including CYP metabolite levels, was evaluated.

Results: The interactions among variants rs17110453, rs751141, and rs9333025 were significantly associated with high 20-HETE, high DiHETEs, and low EETs after adjusting for the status of diabetes mellitus and hypertension. High 20-HETE, high DiHETEs, and low EETs were independent risk factors for IS after adjusting for hypertension, diabetes mellitus, and the interactions among rs17110453, rs751141, and rs9333025. Furthermore, the interactions among rs17110453, rs751141, and rs9333025 were significantly associated with a higher risk of IS after adjusting for CYP metabolites (OR = 2.02, 95% CI: 1.28–5.27, P = 0.007).

Conclusion: The association between the interactions among rs17110453, rs751141, and rs9333025 and IS risk in Chinese population may be partly but not exclusively mediated by plasma levels of 20-HETE, EETs, and DHETEs. Further well-designed studies are warranted to replicate this finding.

Key words: Ischemia Stroke, Cytochrome P450, CYP genes, Polymorphism, CYP metabolites

Introduction

Ischemic stroke (IS) is a severe complex disease, which causes a huge public health burden globally. It is critical to better understand its etiology for the better management with regard to prevention and treatment of this disease. Besides the risk factors of hypertension, smoking, diabetes mellitus, and other chronic and inflammatory diseases, genetic factors have also been suggested to explain a proportion of the etiology of IS.

Arachidonic acid, a membrane fatty acid, can be metabolized to a variety of bioactive compounds by the cytochrome P450 (CYP) enzymes, including epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE). EETs can be further metabolized to dihydroxyeicosatetraenoic acids (DiHETEs). EETs can relax vessels and play protective roles in the cardiovascular system. 20-HETE has also been detected in the cerebral vasculature of the stroke-prone sponta-
necessarily hypertensive rat and contributes to stroke severity\textsuperscript{7}. Pharmacological inhibition of 20-HETE synthesis also reduces infarct size in rats following transient occlusion of the middle cerebral artery\textsuperscript{8,9}. Despite these preclinical links between CYP metabolites and IS, little is known about the role of 20-HETE, EETs, and DiHETEs in the development of human IS. Although research has shown that plasma CYP metabolite levels are potentially associated with IS\textsuperscript{10}, the exact relationship, particularly whether variants of CYP pathway genes play roles in the relationship, remains insufficiently understood.

Several studies have shown that individual genetic variants of CYP pathway may be associated with a risk of IS\textsuperscript{11-14}. In a previous study, we demonstrated that the gene–gene interactions among variants rs17110453, rs751141, and rs9333025 conferred a higher risk of IS\textsuperscript{15}. However, the underlying mechanism for such an association is not well understood.

Considering that the assessed variants are predicted to be functional, and the proteins encoded by the corresponding genes are potentially important in the development of stroke\textsuperscript{10}, we hypothesize that the potential effect of the identified variant interactions on IS risk is at least partially mediated by the influence on plasma levels of relevant CYP metabolites (20-HETE, EETs, and DiHETEs). The aim of the current study is to test this hypothesis to better understand the relationship between interactions among rs17110453, rs751141, and rs9333025, relevant CYP metabolite levels, and IS risk.

Materials and Methods

Ethics Statement

This study was approved by the Ethics Committee of The People’s Hospital of Deyang City. All patients provided written informed consent before their enrollment into this study.

Study Populations

The detailed procedures for the recruitment of acute IS cases and controls were described in a previous study\textsuperscript{15}. Briefly, 396 acute IS patients who suffered their first IS related to atherothrombosis (n = 270) or small artery disease (n = 126) according to the Trial of ORG 10172 in the Acute Stroke Treatment classification system\textsuperscript{16} and who were admitted to The People’s Hospital of Deyang City within 72 hours of their index stroke were consecutively recruited between August 2010 and March 2013. The exclusion criteria were as follows: (1) any clinically relevant arrhythmia (including atrial fibrillation), cerebral embolism, or other determined or undetermined etiological syn-

omes of IS; (2) a family history of apoplexy or a previous history of stroke; and (3) cerebral hemorrhage. A total of 378 controls were selected from outpatients with no history of stroke, as confirmed by medical history combined with physical and laboratory examinations at the hospital. Controls had no family history of stroke and were genetically unrelated to the included IS patients. Relevant characteristics and clinical variables of involved subjects, including data regarding age, sex, blood pressure/hypertension, body mass index (BMI), diabetes mellitus, cigarette smoking, alcohol intake, total plasma cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), were collected. The genotypes of 10 variants, including CYP2J2 rs10889160, CYP2C8 rs17110453, CYP2C8 rs1934980, CYP2C9 rs1799853, CYP2C9 rs1057910, CYP3A5 rs776746, epoxide hydrase 2 (EPHX2) rs751141, CYP4A11 rs2269231, CYP4A11 rs9333025, and CYP4F2 rs3093135, were examined using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method. According to our previous analyses\textsuperscript{15}, IS patients have a higher prevalence of history of hypertension and diabetes as well as an older age. The frequency of the GG genotype for rs9333025 was significantly higher in the IS patients than in the controls (P \textless 0.001). There was a significant influence of gene–gene interactions among rs17110453, rs751141, and rs9333025 on IS risk. Individuals with a combination of rs17110453CC, rs751141GG, and rs9333025GG had a significantly higher risk of IS than those with a combination of rs17110453AA, rs751141AA, and rs9333025AA (OR = 2.86, 95% CI: 1.24–7.26, P = 0.004).

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

Among the original 396 IS patients and 378 controls, we measured the plasma CYP metabolite levels in a randomly selected subset of 218 cases and 126 controls. Based on previous studies, including one conducted by us\textsuperscript{10,17}, such a sample size should be sufficient to support our research. Thus, we used such a sample size in the current study and will expand the study size in future. A blood sample (4 ml) was collected into EDTA/butylated hydroxytoluene (BHT)/glutathione at the second day after admission. Plasma was isolated following centrifugation, and samples were stored at \textasciitilde 80°C until analysis. Specifically, plasma 20-HETE level was measured using a stable isotope dilution gas chromatography/mass spectrometer (GC/MS). Total plasma EETs and DiHETEs levels were measured using a stable isotope dilution GC/MS following base hydrolysis and separation on high per-
Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A P value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL).

### Results

#### Clinical Characteristics

Continuous variables, including plasma CYP metabolite levels, age, BMI, TC, TG, and LDL-C, were all normally distributed as suggested by the Shapiro–Wilk test (all P > 0.05). The main characteristics of the 218 IS cases and 126 controls in whom the CYP metabolite levels were measured are shown in Table 1. Generally, there were no significant differences between cases and controls regarding age, gender, myocardial infarction history, cigarette smoking, alcohol intake, BMI, previous drug treatment as well as TC, LDL-C, and TG concentrations (all P > 0.05). Furthermore, these characteristics did not differ significantly between subgroups of cases according to subtypes of stroke (atherothrombotic stroke or small artery disease stroke) (all P > 0.05). On the other hand, the proportions of cases with diabetes mellitus or hypertension are higher than those of controls (P = 0.042 and P < 0.001, respectively).

#### Plasma CYP Metabolite Levels

Plasma levels of 20-HETE and DiHETEs were significantly higher in IS cases than in controls, and EETs levels were significantly lower in cases (all P < 0.001, Table 2). Adjustment for diabetes mellitus or
hypothesis did not alter results for plasma 20-HETE, DiHETEs, and EETs.

**Association between Plasma CYP Metabolite Levels and CYP Genetic Variants**

We did not detect associations between CYP metabolite levels and 10 assessed variants of CYP pathway genes (all \( P > 0.05 \), Supplementary Table). However, stratified analyses based on different genotype combinations of the three relevant variants revealed differences in CYP metabolite levels between subgroups of variant genotype combinations. Specifically, IS patients carrying the genotype combination of rs17110453CC, rs751141GG, and rs9333025GG tended to have higher 20-HETE and DiHETEs levels and lower EETs level than controls who carried the same genotype combination (all \( P < 0.001 \), Table 3). When focusing on IS patients, individuals with the genotype combination of rs17110453CC, rs751141GG, and rs9333025GG tended to have higher 20-HETE and DiHETEs levels and lower EETs level than individuals with other genotype combinations of these variants (all \( P < 0.001 \), Table 3).

**Logistic Regression Analyses for Assessing the Relationship between Variant Interactions, CYP Metabolite Levels, and IS Risk**

Our previous analysis suggested that the interactions among rs17110453, rs751141, and rs9333025 were significantly associated with an increased risk of IS (OR = 2.36, 95% CI 1.23–5.30, \( P = 0.005 \))\(^{[5]}\). In the current study, we found that the genotype combination of rs17110453CC, rs751141GG, and rs9333025GG was significantly associated with 20-HETE (OR = 2.01, 95% CI 1.32–5.62, \( P = 0.004 \)), DiHETEs (OR = 1.93, 95% CI 1.22–4.28, \( P = 0.006 \)), and EETs (OR = 1.88, 95% CI 1.17–3.34, \( P = 0.018 \)) after adjusting for status of diabetes mellitus and hypertension. The 20-HETEs and DiHETEs as well as EETs were significantly associated with a risk of IS after adjusting for hypertension, diabetes mellitus, and the genotype combination of rs17110453CC, rs751141GG, and rs9333025GG (Table 4). Furthermore, the interactions among rs17110453CC, rs751141GG, and rs9333025GG were identified to be independently associated with a higher risk of IS after adjusting for covariates, including CYP metabolites (OR = 2.11, 95% CI: 1.27–5.53, \( P = 0.006 \)).

**Discussion**

In the present study, we detected that the interactions among variants rs17110453, rs751141, and rs9333025 were significantly associated with CYP metabolite levels; these levels were significantly associated with IS risk, suggesting that our previous finding of the association between the interactions among rs17110453, rs751141, and rs9333025 and IS risk may be mediated by CYP metabolite levels. On the other hand, it was detected that the interactions among rs17110453CC, rs751141GG, and rs9333025GG was independently associated with a higher risk of IS after adjusting for other covariates, including CYP metabolites, suggesting that such an association was not exclusively mediated by CYP metabolite levels. Our findings generate new knowledge for the relationship between rs17110453, rs751141, and rs9333025 interactions, CYP metabolite levels, and stroke risk.

In our study, we found that the plasma levels of 20-HETE and DiHETEs were significantly higher in IS cases than in controls and that EETs levels were significantly lower in these cases. The adjustment for diabetes mellitus or hypertension did not alter results for plasma 20-HETE, DiHETEs, and EETs.

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In our study, we found that the plasma levels of 20-HETE and DiHETEs were significantly higher in IS cases than in controls and that EETs levels were significantly lower in these cases. The adjustment for diabetes mellitus or hypertension did not alter the results. Ward et al.\(^ {10} \) and Lee et al.\(^ {20} \) also observed elevated levels of total plasma and urinary DiHETEs in IS patients compared with controls, which is in accordance with our findings. 20-HETE is a potent vasoconstrictor\(^ {19} \) and is involved in endothelial dysfunction\(^ {21} \); it also helps in the formation of oxygen radicals\(^ {22} \). The inhibition of 20-HETE synthesis reduces both cerebral 20-HETE levels and total infarction volume following transient occlusion of the middle cerebral artery\(^ {4, 9} \). In contrast to the effect of 20-HETE, which is a vasoconstrictor, is that of EETs, which are vasodilators\(^ {5} \). The vascular actions of EETs are modulated by their metabolism to the inactive DiHETEs\(^ {3} \).
In the current study, we detected that DiHETEs are significantly higher and EETs are significantly lower in IS cases than in controls, suggesting that there may be increased soluble epoxide hydrolase (sEH) activity in IS patients. In a mouse model of stroke, inhibiting sEH results in reduced ischemic damage and elevated cortical blood flow during vascular occlusion. Our study and those conducted by others indicate that CYP metabolites might play a role in the pathophysiology of acute IS.

CYP genes encode for enzymes responsible for arachidonic acid metabolism. Numerous studies have indicated that genetic variants of CYP pathway genes may be associated with a risk of IS. We have previously demonstrated that the gene–gene interaction of the variants rs17110453 (CYP2C8), rs751141 (EPHX2), and rs9333025 (CYP4A11) predispose patients to IS risk. CYP2C8 encodes for a major epoxidehydrogenase enzyme, and its functional variants may decrease circulating EET metabolite level. EPHX2 encodes for sEH, which can metabolize EETs to less biologically active DHET. The dysregulation of sEH has been implicated in IS development. CYP4A11 encodes for CYP ω-hydroxylase, which can metabolize AA into 20-HETE. Functional variants within this gene may be associated with IS by regulating 20-HETE levels. In the current study, it was suggested that the interaction effect of the variants rs17110453, rs751141, and rs9333025 on IS risk was partially but not exclusively mediated by CYP450 metabolite levels. We speculate that the interactions among rs17110453CC, rs751141GG, and rs9333025GG could potentially confer individuals with this specific genotype combination to have lower circulating EET levels and higher 20-HETE levels than those with other genotype combinations, thereby increasing the risk of IS. Our findings warrant further investigations to clarify the underlying mechanism.

Our study has several strengths. We explored the research question using a subset of subjects evaluated in our previous study, ensuring assessing the relationship of genotype information, metabolite levels, and disease status in the same group of subjects. Our study helps to better understand the relationship between the identified variant interactions, CYP metabolites levels, and IS risk. The findings of the current study will lead further research to better understand the genetic basis for the complex pathogenesis of IS.

Potential limitations of our study need to be acknowledged. First, we detected significant differences between the evaluated cases and controls with regard to diabetes mellitus and hypertension status. One previous study demonstrated that 20-HETE excretion might be associated with hypertension. However, our regression analyses for the research question adjusted for these potential covariates and provided adjusted estimates. In our study sample there were no significant differences between IS cases and controls with regard to age and cigarette smoking, which were suggested to be IS risk factors in previous research. Selection bias might exist in the current study, and our findings need to be replicated in further studies. Second, subjects in the current study are only a subset of individuals involved in the previous analysis, precluding a more comprehensive evaluation of the research question with all previously involved subjects. However, such a subset of individuals was randomly selected, and there were no significant differences of the main characteristics (age, gender, myocardial infarction history, cigarette smoking, alcohol intake, BMI, diabetes mellitus, and hypertension as well as TC, LDL-C, and TG concentrations) between the subset of individuals included in the current study and those that were not included. Third, CYP450 metabolite levels may be affected by acute cerebral ischemia itself. In the present study, plasma CYP450 metabolite levels were measured in acute phase, and we did not eliminate potential effect of acute cerebral ischemia on CYP450 metabolite levels. Further studies are needed to evaluate CYP450 metabolite levels in

| Table 3. 20-HETE, DiHETE and EETs levels according to different genotype combinations in patients and controls |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
|                                  | Stroke patients                  | Stroke patients                  | Controls                          |
|                                  | combination of rs17110453CC,     | other genotype                   | combination of rs17110453CC,      |
|                                  | rs751141GG, and rs9333025GG      | combination (n=187)              | rs751141GG, and rs9333025GG      |
|                                  | (n=36)                           |                                  | (n=16)                            |
| 20-HETE (pmol/L)                 | 1756 ± 172††††                   | 1452 ± 161                       | 1521 ± 126                       | 1396 ± 111                 |
| DiHETEs (nmol/l)                 | 84.23 ± 5.21††††††              | 71.72 ± 4.56                     | 72.62 ± 4.73                     | 68.98 ± 4.54               |
| EETs (nmol/l)                    | 60.65 ± 4.27†††††               | 73.72 ± 5.13                     | 70.87 ± 4.56                     | 75.55 ± 5.01               |

HETE, hydroxyeicosatetraenoic acid; DiHETEs, dihydroxyeicosatrienoic acids; EET, epoxyeicosatrienoic acids. †, P < 0.001, compared with controls with the genotype combination of rs17110453CC, rs751141GG, and rs9333025GG. ††, P < 0.001, compared with stroke patients with other genotype combination.
Table 4. Multiple regression analysis of the major risk factors for ischemia stroke

| Risk factor                                                                 | Odds ratio | 95% confidence intervals | P value |
|-----------------------------------------------------------------------------|------------|--------------------------|---------|
| Hypertension                                                                | 3.23       | 2.23–10.86               | <0.001  |
| Diabetes mellitus                                                           | 1.76       | 1.04–3.75                | 0.037   |
| 20-hydroxyeicosatetraenoic acid (pmol/L)                                    | 2.16       | 1.24–5.43                | 0.006   |
| Dihydroxyeicosatrienoic acids (nmol/l)                                      | 1.99       | 1.18–5.23                | 0.011   |
| Epoxyeicosatrienoic acids (nmol/l)                                          | 1.84       | 1.16–4.59                | 0.042   |
| Combination of rs17110453CC, rs7511141GG, and rs9333025GG                   | 2.11       | 1.27–5.53                | 0.006   |
| CYP4A11 rs9333025                                                           | 0.84       | 0.75–1.38                | 0.353   |
| Age (>68 yr)                                                                | 0.68       | 0.82–1.67                | 0.212   |
| Cigarette smoking                                                          | 0.89       | 0.84–1.38                | 0.371   |
| Low density lipoprotein-cholesterol (mM)                                   | 1.01       | 0.91–2.26                | 0.091   |
| Total cholesterol (mM)                                                      | 0.85       | 0.77–1.54                | 0.264   |

Other input variables including: body mass index, alcohol intake, CYP2J2 rs10889160, CYP2C8 rs17110453, CYP2C8 rs1934980, CYP2C9 rs1799853, CYP2C9 rs1057910, CYP3A5 rs776746, EPHX2 rs751141, CYP4A11 rs2269231, and CYP4F2 rs3093135.

OR for continuous variables means per 1-unit increase.

chronic phase and clarify the effect of acute cerebral ischemia on CYP450 metabolite levels. Fourth, due to the nature of the limited sample size and single-center study, findings of the current study need to be validated in larger, multi-center studies. Studies focusing on populations beyond Chinese are warranted to determine whether our findings can be generalized to other populations. It is also meaningful to extend our study to include follow-up data to better understand the research question of interest. We are in the process of collecting follow-up information to conduct more comprehensive analyses.

In conclusion, our study suggests that the association between the identified interactions among rs17110453, rs751141, and rs9333025 and IS risk in Chinese population is partially but not exclusively mediated by CYP450 metabolite levels. Further studies are warranted to better understand the relationship.

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Conflict of Interest

The authors declare no conflicts of interest.

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**Supplementary Table.** Association of 20-HETE, DiHETEs and EET levels with genotype distribution ischemia stroke patients and controls

| Assay | EETs (nmol/l) | 20-HETE (pmol/L) | DiHETEs (nmol/l) |
|-------|---------------|-----------------|-----------------|
|       | Stroke patients | Controls | Stroke patients | Control | Stroke patients | Control |
|       | (n=218)       | (n=126)       | (n=218)         | (n=126) | (n=218)          | (n=126) |
| rs17110453 |               |              |                 |         |                  |         |
| AA    | 61.76±6.25    | 73.11±5.64   | 1712±182        | 1539±173 | 83.13±6.04      | 69.18±7.14 |
| AC    | 60.62±5.02    | 74.23±6.36   | 1694±184        | 1511±182 | 83.35±10.72     | 70.68±7.22 |
| CC    | 62.72±5.63    | 72.45±6.64   | 1687±188        | 1436±191 | 82.98±10.24     | 71.87±6.13 |
| rs776746 |               |              |                 |         |                  |         |
| AA    | 61.26±6.71    | 73.89±6.76   | 1666±168        | 1489±175 | 84.04±6.74      | 70.96±6.25 |
| AG    | 60.87±4.88    | 74.02±6.52   | 1728±177        | 1423±164 | 83.95±6.32      | 70.24±5.46 |
| GG    | 61.96±5.23    | 73.24±6.97   | 1706±186        | 1498±185 | 82.87±8.92      | 69.66±7.33 |
| rs751141 |               |              |                 |         |                  |         |
| AA    | 61.75±6.64    | 74.26±6.72   | 1746±194        | 1479±182 | 84.24±7.22      | 68.24±6.37 |
| AG    | 61.64±6.58    | 72.42±6.35   | 1708±166        | 1536±172 | 82.86±6.15      | 69.16±6.13 |
| GG    | 61.68±5.66    | 74.77±6.44   | 1657±182        | 1542±175 | 83.01±4.66      | 70.78±5.22 |
| rs10889160 |             |            |                |          |                  |         |
| AA    | 61.18±5.19    | 72.27±6.24   | 1686±167        | 1523±179 | 82.12±8.51      | 69.36±9.28 |
| AG    | 61.76±6.34    | 73.15±6.76   | 1747±187        | 1489±164 | 85.89±9.12      | 69.86±8.76 |
| GG    | 61.37±5.75    | 72.98±6.81   | 1695±189        | 1452±161 | 83.87±8.02      | 68.98±7.01 |
| rs 9333025 |            |             |                |          |                  |         |
| AA    | 61.11±5.67    | 72.96±6.82   | 1756±197        | 1479±182 | 83.72±6.36      | 69.36±6.28 |
| AG    | 61.56±6.12    | 73.25±6.26   | 1717±184        | 1436±176 | 84.23±7.16      | 70.62±7.76 |
| GG    | 61.78±5.87    | 73.25±6.97   | 1686±188        | 1486±176 | 83.87±6.02      | 71.01±7.22 |
| rs1934980 |            |             |                |          |                  |         |
| CC    | 61.76±6.45    | 74.01±7.82   | 1699±168        | 1523±189 | 84.12±8.42      | 70.36±6.31 |
| CT    | 61.89±6.47    | 73.85±6.63   | 1756±178        | 1493±181 | 83.89±7.12      | 69.35±7.46 |
| TT    | 60.99±4.66    | 72.98±5.98   | 1701±198        | 1445±164 | 82.87±8.02      | 71.02±8.23 |
| rs1799853 |            |             |                |          |                  |         |
| CC    | 61.76±4.52    | 73.68±4.88   | 1711±162        | 1465±123 | 83.16±5.24      | 69.73±4.22 |
| rs 1057910 |           |             |                |          |                  |         |
| AA    | 61.59±8.61    | 74.18±6.36   | 1699±162        | 1467±182 | 82.68±7.52      | 69.27±5.28 |
| AC    | 60.87±6.15    | 73.14±6.86   | 1747±162        | 1429±173 | 83.89±6.12      | 69.56±8.27 |
| CC    | 61.78±7.66    | 72.21±6.89   | 1702±188        | 1494±162 | 82.88±7.44      | 70.24±6.68 |
| rs2269231 |           |             |                |          |                  |         |
| AA    | 61.86±6.23    | 74.15±7.21   | 1659±167        | 1467±179 | 83.56±7.35      | 69.97±8.55 |
| AT    | 61.06±7.12    | 73.14±6.74   | 1707±165        | 1419±174 | 82.45±8.81      | 69.23±7.34 |
| TT    | 61.96±6.66    | 73.36±6.87   | 1732±184        | 1485±166 | 83.11±7.53      | 69.58±6.47 |
| rs3093135 |           |             |                |          |                  |         |
| TT    | 61.78±6.34    | 73.32±6.57   | 1706±172        | 1567±156 | 83.25±7.96      | 69.36±8.28 |
| AT    | 61.56±6.05    | 73.36±6.38   | 1747±171        | 1619±149 | 82.89±6.68      | 70.07±8.35 |
| AA    | 60.98±7.66    | 74.51±7.27   | 1732±176        | 1645±161 | 83.07±8.11      | 69.82±7.14 |

HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acids; DiHETE, dihydroxyeicosatrienoic acid.