GENETIC VARIATION IN THE LEUKOTRIENE PATHWAY IS ASSOCIATED WITH MYOCARDIAL INFARCTION IN THE CHINESE POPULATION

Yilan Li 1,2†, Xueming Xu 1,2†, Dandan Zhang 1,2, Wei Cheng 1, Yanan Zhang 3, Bo Yu 1,2 and Yao Zhang 1,2*

Abstract

Background: Genetic variation in the genes ALOX5 (arachidonate 5-lipoxygenase), ALOX5AP (arachidonate 5-lipoxygenase-activating protein) and LTA4H (leukotriene A4 hydrolase) has previously been shown to contribute to the risk of MI (myocardial infarction) in Caucasian and African American populations. All genes encode proteins playing a role in the synthesis of the pro-inflammatory leukotriene B mediators, possibly providing a link between MI and inflammation. The aim of the present study was to investigate whether these associations could be confirmed in the study of China MI patients. The study included 401 Han Chinese MI patients and 409 controls. Six tag single nucleotide polymorphisms (SNPs)—ALOX5 rs12762303 and rs12264801, ALOX5AP rs10507391, LTA4H rs2072512, rs2540487 and rs2540477—were selected. SNP genotyping was performed by an improved multiplex ligation detection reaction assay.

Results: The rs2540487 genotype was associated with the risk of MI in overdominant model (P = 0.008). rs12762303 and rs10507391 SNPs were significantly associated with lipid levels in MI patients (P < 0.006–0.008). Several SNPs interacted with alcohol consumption, cigarette smoking, and hypertension to modify TC, TG, LDL-C and CRE levels, and the risk of MI (P < 0.0017 for all). No association between the SNPs of LT pathway and susceptibility to MI was found (P > 0.05 for all).

Conclusions: Taken together, this study provides additional evidence that functional genetic variation of the LT pathway can mediate atherogenic processes and the risk of MI in Chinese.

Keywords: Arachidonate 5-lipoxygenase, Arachidonate 5-lipoxygenase-activating protein, Single nucleotide polymorphism, Myocardial infarction, Coronary artery disease

Background

Coronary artery disease (CAD), and its most severe complication myocardial infarction (MI), are leading causes of death and disability worldwide [1, 2]. Multiple factors, including genetic, environmental, and psychological factors, were believed to contribute to the onset of CAD [3]. A plethora of evidence has demonstrated that atherosclerosis is a major pathologic change in CAD, and inflammatory reactions and immune function disorders are implicated in the development of CAD [4, 5]. In recent years, focus has turned on the complex cascade of inflammatory processes that takes place in the vessel wall and within atherosclerotic plaques [6–8]. In this context the leukotriene pathway has received attention.

The initial enzymatic step in the leukotriene pathway is the oxidation of arachidonic acid to leukotriene A4 (LTA4) by 5-lipoxygenase (5-LO, encoded by ALOX5) [9]. A necessary cofactor in this reaction is 5-lipoxygenase-activating protein (FLAP), encoded by the arachidonate 5-lipoxygenase-activating protein (ALOX5AP) gene, is an important mediator of the activity of 5-lipoxygenase, a key enzyme in the biosynthesis of leukotrienes [10]. The LTA4H gene encodes leukotriene A4 hydrolase, a protein in the same biochemical pathway as ALOX5AP [11]. LTA4H is further hydrolyzed by leukotriene A4 hydrolase (LTA4H) to leukotriene B4 (LTB4) or conjugated to produce a series of three related...
to cysteinyl leukotrienes (LTC4, LTD4, LTE4) by the LTC4 synthase (LTC4S) enzyme [12]. LTs are thought to be potent chemotactic molecules that mediate the recruitment of neutrophils, monocytes, and other leukocytes to sites of inflammation, including the arterial wall of atherosclerotic lesions [13, 14] (Fig. 1).

Despite the accumulating evidence linking the 5-LO pathway to atherosclerosis, no Chinese genetic studies have substantiated a relationship between LT pathway polymorphisms and clinical complications of atherosclerosis including MI [10]. Several genetic linkage and associations studies as well as gene expression studies have shown an association of the ALOX5/ALOX5AP pathway to CAD. This stems from a series of biochemical, genetic, and pharmacological studies over the last few years that have provided evidence for the pro-atherogenic role of LTs [14, 15]. For example, genetic deficiency for ALOX5 in mice increases mortality after MI because of healing defects [16]. This is not mediated by a change in local blood flow, but through an altered inflammation and/or fibroblast function. Other mouse studies have reported the involvement of LT pathway genes in atherosclerosis related traits as well, including the LT receptors and ALOX5 activating protein (ALOX5AP) [17, 18].

The leukotriene pathway has been implicated in the pathogenesis of cardiovascular but the detailed mechanistic basis for their pathophysiological roles is still a matter of discussion. Moreover, none of the previous studies specifically attempted to dissect the role of LT pathway in the atherosclerosis phenotype rather than in its ‘complication’ phenotype (MI) [19]. The goal of this study was therefore to comprehensively evaluate the genetic contribution of the LT pathway in individuals with MI. Such information may have the potential to provide predictive value for assessing cardiovascular risk [12].

Results

General characteristics of the subjects

Table 1 compares the general characteristics and lipid levels between the patients and controls. The population included in the study comprises a total of 810 individuals. As illustrated, the majority of study participants are male (82.0%),

![Fig. 1](image-url)
about two-third are current smokers (60.3%), and 26.2% drinking alcohol. The mean age, gender distribution, hypercholesterolemia and triglycerides level were not different between controls and MI patients \((P > 0.05\) for all). With the exception of hypercholesterolemia and triglycerides, the risk factors generally occurred more frequently among the cases than the controls. \((p < 0.05\) for all).

Genotypic and allelic frequencies in patients and controls

The genotype and allele frequencies of six SNPs selected for study are shown in Table 2. No deviations from Hardy–Weinberg equilibrium were observed in either cases or controls. The genotype and allele frequencies of the rs12762303, rs12264801, rs10507391, rs2072512, rs2540487, rs2540477 SNPs in MI patients and controls were not significantly different (all \(P > 0.05\)).

Genotypes of the six LT pathway SNPs and the risk of MI

To explore the potential inheritance patterns, four models of inheritance including dominant, recessive, codominant and overdominant models were explored for each SNP (Table 3). The genotype of the LTA4-H rs2540487 was associated with the risk of MI after the Bonferroni correction (a value of \(P < 0.01\) was considered statistically significant) in the overdominant genetic model: \(\text{CC + TT vs. CT (OR = 1.48, 95\% CI = 1.11–1.99, } P = 0.008)\).
were observed. Similar, but weaker trends were observed for the recessive model, codominant model or overdominant model, with no significant associations of the five SNPs with MI (all \( P > 0.05 \)).

### Genotypes and lipid levels

We expected that genetic risk associated with the SNPs would be reflected by established CAD risks, including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (CRE), or fasting blood glucose (FBG). As shown in Table 4, the minor C allele of rs12762303 was associated with high FBG concentrations in MI patients compared with the control group \((P = 0.008)\) and the rs10507391 variants were associated with increased TC \((P = 0.006)\) after the Bonferroni correction of \( P \) values. None of the six SNPs were associated with TG, HDL-C, LDL-C or CRE in MI patients \((P > 0.0083)\).

### Interactions of the six SNPs and drinking, smoking, age, sex and hypertension on lipid levels and the risk of MI

The interactions of the six SNPs and drinking, smoking, age, sex and hypertension on lipid levels and the risk of MI are shown in Table 5. The SNP of rs12762303 interacted with alcohol consumption to influence TC level. Several SNPs interacted with age to influence TC (rs12264801 and rs10507391), TG (rs12264801 and rs2540477) and LDL-C (rs10507391) levels. The SNP of rs2540477 interacted with sex to modulate CRE levels. The SNP of rs2540487 interacted with hypertension to influence TG levels.

### Discussion

Recent LT pathway studies of MI have discovered multiple gene loci. However, most of the studies have focused on samples of non-Asian origin, and the identified loci altogether explain only a small fraction of the risk for MI. Moreover, the variants identified in these populations descent might not be applicable in Chinese because of underlying genetic heterogeneity. Therefore, larger scale studies in Chinese are needed to reveal new susceptibility loci and improve our understanding of LT pathway to MI. This study identified interactions between gene polymorphisms in leukotriene production enzymes and the clinical complications of atherosclerosis mainly about MI in Chinese Hans [20].

Our study showed that ALOX5 rs12762303 was associated with fasting blood glucose (FBG) levels but not with MI in Chinese population, which is consistent with the studies by Assimes et al. [10]. A recent study by Mehrhadian et al. demonstrated that Alox5−/− mice had significantly increased fat mass, plasma leptin levels and fasting glucose levels, but lower fasting insulin levels [21]. These results provide strong evidence for the recessive model, codominant model or overdominant model, with no significant associations of the five SNPs with MI (all \( P > 0.05 \)).

### Table 3 Genetic model analysis of the association of six SNPs and MI susceptibility

| SNP/group | Genotype | \( \chi^2 \) | \( P \) | OR (95% CI) |
|-----------|----------|-------------|--------|-------------|
| rs12762303 | Dominant | CC+ CT | TT | 0.10 | 0.92 | 0.98 (0.73–1.32) |
| | Recessive | CC | CT + TT | 0.16 | 0.69 | 1.14 (0.59–2.18) |
| | Codominant | TT | CT | 0.06 | 0.81 | 0.96 (0.71–1.31) |
| | Overdominant | TT + CC | CT | 0.08 | 0.76 | 1.04 (0.77–1.42) |

\*SNPs with MI (all \( P < 0.01 \) were considered statistically significant after Bonferroni correction. \* \( P \)-values < 0.01 are bold.

allele (C) with homozygous carriers of the major allele (T) revealed that the ALOX-5 rs12762303 and rs12264801 SNPs were negatively associated with MI, suggesting a dominant genetic effect. No association of ALOX-5AP rs10507391, LTA4-H rs2072512 or rs2540477 and MI were observed. Similar, but weaker trends were observed for the recessive model, codominant model or overdominant model, with no significant associations of the five SNPs with MI (all \( P > 0.05 \)).
| SNP         | Genotype (Counts) | TC mmol/L | TG mmol/L | HDL-C mmol/L | LDL-C mmol/L | CRE μmol/L | FBG mmol/L |
|-------------|-------------------|-----------|-----------|--------------|--------------|------------|------------|
| rs12762303  | case TT (270)     | 4.53 ± 1.13 | 1.66 ± 1.16 | 1.28 ± 0.47 | 2.78 ± 0.86 | 87.35 ± 31.89 | 7.36 ± 4.03 |
|             | control TT (274)  | 4.97 ± 0.85 | 1.71 ± 1.26 | 1.39 ± 0.40 | 3.28 ± 0.75 | 70.82 ± 13.74 | 6.00 ± 2.05 |
|             | rs12264801 case AA (86) | 4.65 ± 0.96 | 1.71 ± 1.22 | 1.27 ± 0.45 | 2.79 ± 0.89 | 82.11 ± 25.52 | 7.47 ± 3.22 |
|             | control AA (99)   | 4.94 ± 0.77 | 1.63 ± 1.09 | 1.31 ± 0.26 | 3.27 ± 0.76 | 70.92 ± 13.80 | 5.91 ± 1.93 |
|             | rs10507391 case TT (158) | 4.77 ± 1.03 | 1.73 ± 1.30 | 1.28 ± 0.33 | 2.85 ± 0.81 | 81.90 ± 24.50 | 7.48 ± 3.91 |
|             | control TT (167)  | 4.95 ± 0.93 | 1.58 ± 1.17 | 1.35 ± 0.31 | 3.33 ± 0.80 | 70.83 ± 12.84 | 5.87 ± 1.93 |
|             | rs2072512 case AA (74) | 4.64 ± 1.22 | 1.65 ± 1.11 | 1.23 ± 0.34 | 2.83 ± 0.93 | 92.85 ± 41.31 | 7.05 ± 4.00 |
|             | control AA (68)   | 4.88 ± 0.90 | 1.53 ± 1.11 | 1.38 ± 0.40 | 3.22 ± 0.72 | 69.85 ± 13.40 | 5.84 ± 2.08 |
|             | rs2540487 case CC (261) | 4.58 ± 1.02 | 1.70 ± 1.21 | 1.29 ± 0.45 | 2.78 ± 0.82 | 84.94 ± 30.49 | 7.17 ± 4.09 |
|             | control CC (237)  | 4.96 ± 0.86 | 1.70 ± 1.23 | 1.36 ± 0.35 | 3.30 ± 0.75 | 70.82 ± 14.12 | 6.02 ± 2.00 |

Note: Significance levels are indicated as follows: *P < 0.05; **P < 0.01
After the Bonferroni correction, a study by Guoping et al. [22], who found a consistent no association of acute coronary syndrome (ACS) with total cholesterol (TC) levels but we didn’t observe any association between rs10507391 and the risk of MI. This is in line with the findings of Guoping et al. [22], who found a consistent no association of acute coronary syndrome with the A allele of the same polymorphism. Moreover, ALOX5AP has been previously associated with atherosclerosis [15], whereas its haplotypes have been associated with myocardial infarction [11].

The most significant association detected in our discovery sample set was between a SNP of LTA4H (rs2540487) and MI. Previous human genetic study concerning the relationship of rs2540487 polymorphism with CAD have yielded inconsistent results [13]. Jaana Hartiala et al. have reported a significant association between rs2540477 and CAD with the T allele being risky. Moreover, they have demonstrated that also haplotype HapK, containing this T allele, results in an increased risk for CAD [13]. In spite of the above-reviewed positive findings, there are other studies that failed to demonstrate this type of associations for CAD or MI, including a case-control study nested within the Multi-Ethnic Study of Atherosclerosis Cohort in the US [23], a case-cohort study in Denmark [24], and a US study that recruited participants mostly from young adults [10]. The lack of consistent findings among the published studies could be due to differences in allele and haplotype frequency of underlying causal variants and extent of LD between causal and non-causal variants in different populations [25].

Several limitations should be acknowledged in the present study. First, the sample size was relatively small and the participants were limited to Chinese ethnicity. Second, there were differences in some clinical characteristics between the patients and controls. Although several confounders have been adjusted for the statistical analyses, we could not completely eliminate the potential influences of these factors on the results. Finally, the biological mechanism of genetic variants about the LT pathway were not conducted in this study. Larger studies should be followed up to assess the potential association of the SNPs with more complex, clinical-disease-related endpoints.

### Conclusion

In conclusion, the six SNPs in the leukotriene pathway were not associated with the risk of MI in this Han Chinese population, although MI patient characteristics

| SNP       | Genotype (Counts) | TC mmol/L | TG mmol/L | HDL-C mmol/L | LDL-C mmol/L | CRE μmol/L | FBG mmol/L |
|-----------|------------------|-----------|-----------|--------------|--------------|------------|------------|
| rs2540477 | case AA (79)     | 4.71 ± 1.20 | 1.71 ± 1.27 | 1.23 ± 0.32 | 2.87 ± 0.91 | 94.05 ± 38.87 | 7.04 ± 3.64 |
|           | GA (193)         | 4.54 ± 0.99 | 1.61 ± 1.14 | 1.26 ± 0.33 | 2.76 ± 0.81 | 80.42 ± 23.65 | 7.31 ± 3.40 |
|           | GG (129)         | 4.52 ± 1.02 | 1.66 ± 1.01 | 1.32 ± 0.58 | 2.71 ± 0.82 | 88.39 ± 31.50 | 6.92 ± 3.80 |
|           | P                 | 0.367 | 0.477 | 0.971 | 0.508 | 0.066 | 0.680 |
| control   | AA (70)          | 4.81 ± 0.85 | 1.51 ± 1.16 | 1.34 ± 0.38 | 3.19 ± 0.71 | 71.56 ± 13.09 | 5.91 ± 2.20 |
|           | GA (202)         | 5.03 ± 0.85 | 1.65 ± 1.21 | 1.39 ± 0.35 | 3.35 ± 0.79 | 69.74 ± 13.54 | 5.90 ± 1.89 |
|           | GG (137)         | 4.90 ± 0.84 | 1.77 ± 1.28 | 1.37 ± 0.38 | 3.23 ± 0.69 | 72.10 ± 12.99 | 6.00 ± 1.81 |
|           | P                 | 0.073 | 0.222 | 0.328 | 0.154 | 0.207 | 0.507 |

SNP single nucleotide polymorphism, TC total cholesterol, TG triglyceride, HDL-C high-density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, CRE creatinine, FBG fasting blood glucose. Results are mean ± SD. Significance (P < 0.05) was determined by the Kruskal–Wallis test. Significant difference, P < 0.0083 after the Bonferroni correction. *P-values < 0.0083 are bold
were affected by gene polymorphisms. The results of the present study showed that the rs2540487 genotype was associated with the risk of MI in overdominant model. Those with rs12762303CC genotype had higher FBG levels than those with rs12762303TT and rs12762303CT genotypes. Those with rs10507391TT genotype had higher TC levels than those with rs12762303AA and rs12762303AT genotypes. Several SNPs interacted with alcohol consumption, cigarette smoking and hypertension to modify TC, TG, LDL-C and CRE levels, and the risk of MI. However, this study was designed as a pilot study and further investigations are needed to confirm our results and to elucidate unresolved questions. The contribution of other genetic variants of these vascular-related genes to CAD and MI cannot be excluded.

### Methods

#### Sample collection

A total of 401 hospitalized MI patients were enrolled at the Second Affiliated Hospital, Harbin Medical University (China) between September 2016 and November 2017. The study protocol was approved by the local ethics review board; all participants provided written informed consent. MI was diagnosed by symptoms within 24 h of hospital admission, an electrocardiogram consistent with MI, and positive troponin-I. Patients with recent illnesses or infections were not eligible [26].

### Table 5

The P values for interactions of genotypes and age, drinking and smoking, on lipid levels and the risk of CAD

| SNP         | Factor | TC    | TG    | HDL-C | LDL-C | CRE    | FBG   |
|-------------|--------|-------|-------|-------|-------|--------|-------|
| rs12762303  | Drinking | 0.022 | 0.794 | 0.621 | 1.57E-4 | 0.883 | 0.398 |
|            | Smoking | 0.452 | 0.633 | 0.251 | 0.213 | 0.721 | 0.410 |
|            | Age     | 0.084 | 0.247 | 0.633 | 0.162 | 0.667 | 0.415 |
|            | Sex     | 0.115 | 0.560 | 0.593 | 0.200 | 0.050 | 0.090 |
|            | Hypertension | 0.863 | 0.019 | 0.050 | 0.077 | 0.641 | 0.481 |
| rs12264801  | Drinking | 0.582 | 0.549 | 0.448 | 0.503 | 0.747 | 0.693 |
|            | Smoking | 0.798 | 0.528 | 0.163 | 0.838 | 0.669 | 0.666 |
|            | Age     | 5.21E-4 | 3.47E-4 | 0.500 | 0.010 | 0.071 | 0.530 |
| rs10507391  | Drinking | 0.916 | 0.728 | 0.633 | 0.236 | 0.595 | 0.961 |
|            | Smoking | 0.899 | 0.299 | 0.082 | 0.465 | 0.631 | 0.803 |
|            | Age     | 1.92E-4 | 0.006 | 0.706 | 2.83E-4 | 0.321 | 0.924 |
| rs2072512   | Drinking | 0.361 | 0.962 | 0.629 | 0.639 | 0.436 | 0.915 |
|            | Smoking | 0.396 | 0.095 | 0.240 | 0.932 | 0.671 | 0.259 |
|            | Age     | 0.016 | 0.012 | 0.391 | 0.010 | 0.051 | 0.033 |
|            | Sex     | 0.460 | 0.402 | 0.505 | 0.784 | 0.011 | 0.019 |
|            | Hypertension | 0.114 | 0.002 | 0.157 | 0.457 | 0.930 | 0.348 |
| rs2540487   | Drinking | 0.150 | 0.359 | 0.988 | 0.020 | 0.678 | 0.941 |
|            | Smoking | 0.878 | 0.478 | 0.174 | 0.281 | 0.054 | 0.468 |
|            | Age     | 0.018 | 0.010 | 0.912 | 0.079 | 0.178 | 0.998 |
|            | Sex     | 0.808 | 0.457 | 0.900 | 0.432 | 0.517 | 0.191 |
|            | Hypertension | 0.031 | 0.001 | 0.191 | 0.546 | 0.261 | 0.150 |
| rs2540477   | Drinking | 0.787 | 0.763 | 0.898 | 0.229 | 0.717 | 0.926 |
|            | Smoking | 0.777 | 0.110 | 0.147 | 0.787 | 0.609 | 0.533 |
|            | Age     | 0.010 | 8.73E-6 | 0.320 | 0.069 | 0.095 | 0.312 |
|            | Sex     | 0.367 | 0.811 | 0.228 | 0.771 | 0.001 | 0.071 |
|            | Hypertension | 0.049 | 0.002 | 0.210 | 0.533 | 0.677 | 0.064 |

**SNP** single nucleotide polymorphism, **TC** total cholesterol, **TG** triglyceride, **HDL-C** high-density lipoprotein cholesterol, **LDL-C** low-density lipoprotein cholesterol, **FBG** fasting blood glucose, **CRE** creatinine, **MI** myocardial infarction. Significant difference, P < 0.0017 after the Bonferroni correction. *P*-values < 0.0017 are bold.
group of 409 age- (5-year bands) and sex-matched medical center patients without a history of CAD or symptoms of MI were selected as controls. Patients with cerebrovascular, neurological, or kidney disease, blood disorders, cancer, peripheral vascular disease, or autoimmune diseases were excluded from the control group. Participant age, sex, blood pressure, lipid profile, fasting glucose, medical, drug, smoking, and alcohol histories were collected.

SNP selection
Four leukotriene pathway loci were selected by a tagSNP method using Haploview version 4.2 bioinformatics software (Broad Institute, Cambridge, MA, USA; https://www.broadinstitute.org/haploview/haploview) assuming a minor allele frequency > 0.05 and a squared correlation between genotypes (\(\chi^2\)) > 0.8 for the SNPs in the Han Chinese population (CHB + CHS). The SNP information was retrieved from the 1000 Genomes Project database (http://browser.1000genomes.org) and included those associated with cardiovascular disease in recent studies.

DNA extraction and genotyping
The genomic DNA was extracted using a GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, USA) as per the product instruction. The SNP genotyping work was performed using an improved multiplex ligation detection reaction (iMLDR) technique developed by Genesky Biotechnologies Inc. (Shanghai, China). A multiplex PCR-ligase detection reaction method was used in the iMLDR. For each SNP, the alleles were distinguished by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNPs were further distinguished by different extended lengths at the 3’end. Two negative controls were set: one with double-distilled water as template and the other with DNA sample without primers while keeping all other conditions the same in one plate. Duplicate samples were designed and the results were consistent. A random sample accounting for ~ 5% (\(n = 40\)) of the total DNA samples was directly sequenced using Big Dye-terminator version 3.1 and an ABI3730XL automated sequencer (Applied Biosystems) to confirm the results of iMLDR.

Statistical analyses
All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). All tests were two-sided and \(P\)-values < 0.05 were considered significant. Between-group differences in demographic characteristics and genotype frequencies of the six SNPs were evaluated by Student’s \(t\)-test for continuous variables and \(\chi^2\) tests for categorical variables. The Hardy–Weinberg equilibrium was assessed for controls using the goodness-of-fit \(\chi^2\) test. Associations of genotypes and alleles and the risk of MI were estimated by odds ratios (ORs) and 95% confidence intervals (CIs). The association between genotypes and lipid parameters was tested by analysis of covariance (ANCOVA). Any variants associated with the lipid parameter at a value of \(P < 0.0083\) (corresponding to \(P < 0.05\) after adjusting for six independent tests by the Bonferroni correction) were considered statistically significant. Significant interactions of the six SNPs with alcohol consumption, cigarette smoking, age, sex, and hypertension with lipid levels and the risk of MI were detected by the independent-samples \(t\)-test for categorical variables and linear regression analysis for continuous variables after controlling for potential confounders, a \(P\)-value < \(< 0.0017\) after the Bonferroni correction was considered statistically significant.

Abbreviations
ALOX5: Arachidonate 5-lipoxygenase; ALOXAP: Arachidonate 5-lipoxygenase-activating protein; CAD: Coronary artery disease; LT4H: Leukotriene A4 hydrolase; MI: Myocardial infarction; SNP: Single nucleotide polymorphism

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Availability of data and materials
The genotype data of CHB and CHS are available from the 1000 Genomes Project repository at https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
YZ and BY conceived and designed the study; YL and DZ performed the experiments (DNA extraction and genotyping); YL, XX and YZ analyzed the data; and YL, WC and YZ prepared the manuscript. All authors revised and approved the final draft.

Ethics approval and consent to participate
The study protocol was approved by Ethics Committee of the 2nd Affiliated Hospital of Harbin Medical University and all experimental procedures (DNA extraction and genotyping) complied with the Declaration of Helsinki (2003). All participants gave written informed consent to take part in the present study.

Consent for publication
Not applicable.

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

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Author details
1Department of Cardiology, the 2nd Affiliated Hospital of Harbin Medical University, Harbin 150001, China. 2Key Laboratory of Myocardial Ischemia, Ministry of Education, Harbin Medical University, Harbin 150001, China.

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