The association between CYP1A1 genetic polymorphisms and coronary artery disease in the Uygur and Han of China

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Abstract

Background: The cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) gene is expressed in the vascular endothelium, which metabolizes arachidonic acid into 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs). 20-HETE mediates cardiovascular homeostasis and growth response in vascular smooth muscle cells (VSMCs) as well as the anti-platelet effect. EETs are potent endogenous vasodilators and inhibitors of vascular inflammation. This study assessed the association between human CYP1A1 gene polymorphisms and coronary artery disease (CAD) in the Uygur and Han in China.

Methods: Two independent case–control studies that recruited Han (389 patients with CAD and 411 controls) and Uygur participants (293 patients with CAD and 408 controls) analyzed the relationship between CYP1A1 single nucleotide polymorphisms (SNPs: rs4886605, rs12441817, rs4646422 and rs1048943) and CAD. All patients with CAD and controls were genotyped for the four SNPs of CYP1A1 using TaqMan SNP genotyping assays.

Results: In the Uygur group, the distribution of the dominant model (CC vs CT + TT) of rs4886605 for the total sample and the males was significantly different between CAD patients and control participants (P = 0.001 and P = 0.012, respectively). The difference remained significant after a multivariate adjustment (P = 0.018, P = 0.015, respectively). The rs12441817 was also associated with CAD in a dominant model for all participants (P = 0.003) and men (P = 0.012), and the difference remained significant after a multivariate adjustment (P = 0.016, P = 0.002, respectively). However, we did not observe differences in the Uygur females and Han group with regard to the allele frequency or genotypic distribution of rs4886605 and rs12441817 between patients with CAD and control participants. Patients with CAD did not significantly differ from the control participants with regard to the distributions of rs4646422 and rs1048943 genotypes, the dominant model, the recessive model, or allele frequency in the Han and Uygur groups.

Conclusion: Both rs4886605 and rs12441817 SNPs of the CYP1A1 gene are associated with CAD in the Uygur population of China.

Keywords: CYP1A1, Single nucleotide polymorphism, Coronary artery disease, Case–control study

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Introduction
Coronary artery disease (CAD) accounts for nearly 40% of all the Causes of mortality in developed countries [1,2]. CAD is a complex, multifactorial and polygenic disorder thought to result from the interaction between an individual’s genetic makeup and various environmental factors [3]. Various gene variants are associated with CAD [4,5].

Cytochrome P450 (CYP) is a super-family of cytochrome heme enzymes that mediate the oxidative metabolism of exogenous and endogenous molecules [6]. CYP enzymes play an important role in maintaining cardiovascular health because they catalyze the formation and/or metabolism of several endogenous molecules, such as cholesterol, androgens, estrogens, and several arachidonic acid metabolites, that affect several cardiovascular functions [7,8]. Mounting evidence demonstrates that CYP enzymes are involved in the pathogenesis of CAD [9,10]. Polymorphisms of CYP genes such as, CYP2C8, CYP2C9, CYP2J2 (epoxyeicosatrienoic acid[EET] synthesis) [11-13], CYP8A (prostacyclin synthesis) [14], CYP1B2 (aldosterone synthesis) [15], CYP17, and CYP19 (sex hormone synthesis) are related to CAD [16]. The human gene cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) is expressed in the vascular endothelium. In addition to the roles that CYP1A1 plays in metabolizing exogenous compounds such as poly-cyclic aromatic hydrocarbons (PAHs) and aromatic amines that increase the development of atherosclerotic lesions, it can metabolize arachidonic acid to terminal 20-hydroxyeicosatetraenoic acid (20-HETEs; 75–90%) and, to a lesser extent, epoxyeicosatrienoic acids (EETs; 5–7%) [17-19]. 20-HETE plays critical roles in the regulation of cardiovascular, renal and pulmonary homeostasis as well as the growth response in vascular smooth muscle cells (VSMCs), cardiac function and vascular tone [7]. In addition, 20-HETE has beneficial anti-platelet effects and inhibits sodium reabsorption in the renal tubules [20]. EETs generally have cardioprotective effects [1].

Recently, several reports investigated the association between CYP1A1 genetic polymorphisms and the risk of CAD associated with cigarette smoking. Wang et al. [9], Manfredi et al. [21], and Cornelis et al. [10], studied the association between the CYP1A1 MspI polymorphism, cigarette smoking, and the risk of CAD. They provided inconsistent results. According to the physiological and pathological mechanism of CYP1A1, the interaction between CYP1A1 and smoking can cause atherosclerosis. Furthermore, this gene can metabolize arachidonic acid into 20-HETE and EETs, which can directly lead to atherosclerosis [1]; However, few studies have excluded the factors related to smoking and independently assessed the relationship between CYP1A1 and coronary heart disease.

The present case–control study aimed to assess the association between the human gene CYP1A1 and CAD excluding the risk factors related to smoking in the Uygur and Han population who are two major ethnic groups in Xinjiang, China. It can be further clarified physiological and pathological mechanism that CYP1A1 can cause CAD through Arachidonic acid metabolites such as 20-HETE and EETs.

Materials and methods
Ethical approval of the study protocol
Written informed consent was obtained from all participants, who explicitly provided permission for all DNA analyses and the collection of relevant clinical data. The Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China) approved this study, which was conducted according to the standards of the Declaration of Helsinki.

Participants
The participants were recruited from the Han and Uygur population who live in the Xinjiang Uygur Autonomous Region of China. All patients and controls received differential diagnoses for chest pain at the Cardiac Catheterization Laboratory of the First Affiliated Hospital of Xinjiang Medical University between 2006 and 2013. Highly skilled physicians performed all coronary angiography procedures using the Judkins approach [22]. At least two experienced imaging specialists interpreted the coronary angiography findings, and the final CAD diagnosis was made based on the angiography report.

We randomly sampled 293 Uygur patients with CAD and 408 ethnically and geographically matched participants for the control group. In addition, we randomly sampled 389 Han patients with CAD and 411 ethnically and geographically matched participants for the control group. CAD was defined as the presence of at least one significant coronary artery stenosis of more than 50% lumenal diameter according to coronary angiography for all groups. All control participants also underwent a coronary angiogram and did not have coronary artery stenoses or show clinical or electrocardiogram evidence of myocardial infarction (MI) or CAD. Control participants were not healthy individuals; some were diagnosed with hypertension, DM or hyperlipidemia. Thus, the control group was exposed to the same risk factors of CAD; however, their coronary angiogram results were normal. Data and information regarding traditional coronary risk factors including hypertension, diabetes mellitus (DM), and smoking were collected from all study participants. The diagnosis of hypertension was established if patients were on antihypertensive medication or if the mean of 3 measurements of systolic blood pressure (SBP) was >140 mm Hg or diastolic blood pressure (DBP) >90 mm Hg, respectively.
Diabetes mellitus was diagnosed using the criteria of the World Health Organization (WHO). In addition, individuals with a fasting plasma glucose of >7.0 mmol/L, or with a history of diabetes or treatment with insulin were considered as diabetic. The smoking variable was dichotomized as smokers (including current and ex-smokers) and non-smokers. All patients with impaired renal function, malignancy, connective tissue disease, valvular disease or chronic inflammatory disease were excluded.

Blood collection and DNA extraction
Fasting blood samples drawn via venipuncture in the catheter room were taken from all participants before cardiac catheterization. The blood samples were drawn into a 5-mL ethylene diamine tetraacetic acid (EDTA) tube and centrifuged at 4000 × g for 5 min to separate the plasma content. Genomic DNA was extracted from the peripheral leukocytes using the standard phenol-chloroform method. The DNA samples were stored at −80°C until use. For use, the DNA was diluted to a concentration of 50 ng/μL.

Genotyping
CYP1A1 is located on chromosome 15q24. This gene consists of approximately 6.068 kilobase pairs (kbp) and contains seven exons that are separated by six introns (Figure 1). A total of 287 SNPs are listed for the human CYP1A1 gene in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP). Using Haploview 4.2 and the HapMap phase II database, we obtained four tag SNPs (rs4886605, rs12441817, rs4646422 and rs1048943) using minor allele frequency (MAF) of >0.1 and linkage disequilibrium patterns with r² ≥ 0.5 as a cut off. The position of SNP1, SNP2, SNP3 and SNP4 (rs4886605, rs12441817, rs4646422 and rs1048943) occurred in order of decreasing distance from the CYP1A1 gene 3‘ end (Figure 1). SNP1 (rs4886605) was located 8.037 kbp upstream from the start codon in exon 1; SNP2 (rs12441817) was located 7.863 kbp upstream from the start codon in exon 1; SNP3 (rs4646422) was observed in the exon 2 region of the gene; and SNP4 (rs1048943) was observed in the exon 7 region of the gene. Genotyping was undertaken using the TaqMan® SNP Genotyping Assay (Applied Biosystems). The primers and probes used for the TaqMan® SNP Genotyping Assays (ABI) were chosen based on information on ABI’s website (http://appliedbiosystems.com.cn/). Thermal cycling was conducted using the Applied Biosystems 7900HT Standard Real-Time PCR System. Plates were read using the Sequence Detection Systems (SDS) Automation controller software v2.4 (ABI). PCR amplification was performed using 2.5 μL of TaqMan Universal Master Mix, 0.15 μL probes and 1.85 ddH2O in a 6-μL final reaction volume containing 1 μL DNA. Thermal

![Figure 1 Structure of the human CYP1A1 gene.](image-url)

The gene consists of seven exons (boxes) separated by six introns (lines; intergenic regions). Filled boxes indicate the coding regions, while arrows indicate the locations of single nucleotide polymorphism (SNPs). kbp, kilobase pairs.
cycling conditions were as follows: 95°C for 5 min; 40 cycles of 95°C for 15 s; and 60°C for 1 min. All 96-well plates were read using Sequence Detection Systems (SDS) automation controller software v2.4 (ABI).

Biochemical analyses
Serum concentrations of total cholesterol (TC), triglyceride (TG), glucose (Glu), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were measured using standard methods at the Central Laboratory of the First Affiliated Hospital of Xinjiang Medical University.

Statistical analyses
All continuous variables (e.g., age, BMI, pulse, and cholesterol levels) are presented as the means ± standard deviation (S.D.). The differences between the CAD and control groups were analyzed using independent-samples T-test. The differences with regard to the frequencies of smoking, hypertension, diabetes mellitus, and CYP1A1 genotypes were analyzed using \( \chi^2 \) test or Fisher's exact test where appropriate. The Hardy-Weinberg equilibrium was assessed using a \( \chi^2 \) analysis. Logistic regression analyses with effect ratios (odds ratios [ORs] and 95% CIs) were used to assess the contribution of the major risk factors. All statistical analyses were performed using SPSS 16.0 for Windows (SPSS Institute, Chicago, USA). P-value is 2-tailed, and P-values of less than 0.05 were considered significant.

Results
Table 1 shows the clinical characteristics of the Han and Uygur study participants. For total, men, and women, the plasma concentrations of Glu and LDL-C as well as the rates of EH, DM, and smoking were all significantly higher for patients with CAD than control participants in the two ethnic populations, and the plasma concentrations of Glu was significantly higher only for total and men group. BMI was significantly higher among total, men, and women patients with CAD than their control counterparts in Uygur populations, and the plasma concentration of TC was significantly higher for women group.

Tables 2 and 3 show the distribution of the genotypes and alleles for the four CYP1A1 gene SNPs in the Han and Uygur populations. The genotypic distribution of each SNP matched the predicted Hardy-Weinberg equilibrium values for both ethnicities (P > 0.05 for the CAD and control groups; data not shown).

The distribution of the SNP1 (rs4886605) genotypes (P = 0.004) and the additive model (P = 0.027) significantly differed between the CAD and control participants in the entire Uygur sample. The distribution of SNP1 (rs4886605) alleles and the dominant model (CC vs CT + TT) significantly differed between CAD and control participants (alleles: P = 0.006 [total sample] and P = 0.021 [men]; dominant model: P = 0.001 [total sample] and P = 0.012 [men]). The T allele of rs4886605 was found significantly more frequently in patients with CAD than controls (total: 46.76% vs 39.09%; men: 46.43% vs 38.54%). The dominant model (CC vs CT + TT) of rs4886605 was significantly lower among patients CAD than controls (total: 25.60% vs 37.50%; men: 24.80% vs 36.10%). The distribution of SNP2 (rs12441817) genotypes (P = 0.010) and the additive model (P = 0.048) significantly differed between patients with CAD and controls for the entire sample. The distribution of SNP2 (rs12441817) alleles and the dominant model (TT vs CC + CT) significantly differed between patients CAD and controls (alleles: P = 0.004 [total sample] and P = 0.013 [men]; dominant model: P = 0.003 [total sample] and P = 0.012 [men]). The C allele of rs12441817 was found significantly more frequently in patients with CAD than control participants (total: 40.27% vs 32.84%; men: 40.48% vs 32.20%). The dominant model (TT vs CC + CT) of rs12441817 was significantly lower among patients with CAD than control participants (total: 34.10% vs 45.30%; men: 33.30% vs 45.40%). Moreover, significant differences were not observed between patients with CAD and control participants (for total participants, males or females) in the Uygur group with regard to the distributions of rs4646422 or rs1048943, the dominant model, the recessive model, or allele frequency (P > 0.05). Similarly, significant differences were not observed between patients with CAD and control participants (for total participants, males or females) within the Han group with regard to the distributions of rs4886605, rs12441817, rs4646422 or rs1048943, the dominant model, the recessive model, or allele frequency (P > 0.05).

Tables 4 and 5 show that multiple logistic regression analyses were performed with TG, TC, HDL, Glu, LDL-C, EH, DM, and smoking because these variables are the major confounding factors for CAD. Table 4: The significant difference observed with regard to rs4886605 was retained after adjustment for TG, TC, HDL, Glu, LDL-C, EH, DM, and smoking within the Uygur population (total participants: OR = 0.368, 95% confidence intervals [CI] = 0.185–0.530, P = 0.018; men: OR = 0.350, 95% CI: 0.235–0.546, P = 0.015). Table 5: The significant difference observed with regard to rs12441817 was retained after multivariate adjustment for TG, TC, HDL, Glu, LDL-C, EH, DM, and smoking within Uygur population (total participants: OR = 0.253, 95% confidence intervals [CI] = 0.231–0.530, P = 0.018; men: OR = 0.241, 95% CI = 0.132–0.478, P = 0.002).

Discussion
We found that variation in CYP1A1 gene is associated with CAD in the Uygur population of China. After multivariate adjustment, the associations between CYP1A1 gene polymorphisms with CAD were not modified. Our study is the
### Table 1: Han and Uygur population characteristics of study participants

|                | Han               | Uygur            |
|----------------|-------------------|------------------|
|                | Total  Men  Women | Total  Men  Women |
|                | CAD  Control  P value | CAD  Control  P value | CAD  Control  P value | CAD  Control  P value |
| Number (n)     | 389  294  95      | 210  83          |
| Age (years)    | 58.73 ± 7.32      | 56.97 ± 5.58     |
|                | 57.82 ± 8.53      | 55.93 ± 5.59     |
| BMI (kg/m²)    | 27.62 ± 3.71      | 30.63 ± 1.78     |
|                | 28.23 ± 2.74      | 30.17 ± 1.75     |
| Pulse (beats/min) | 73.82 ± 10.23   | 73.13 ± 10.02    |
| Glu (mmol/L)   | 6.12 ± 2.34       | 6.48 ± 3.12      |
|                | 5.28 ± 1.67       | 5.42 ± 1.59      |
| TC (mmol/L)    | 4.35 ± 1.08       | 4.24 ± 1.08      |
| HDL (mmol/L)   | 1.14 ± 0.23       | 0.98 ± 0.13      |
| LDL (mmol/L)   | 2.53 ± 0.79       | 2.86 ± 0.86      |
| EH (%)         | 59.32 ± 7.95      | 42.68 ± 8.42     |
| DM (%)         | 27.23 ± 3.15      | 26.71 ± 12.84    |
| Smoke (%)      | 58.48 ± 3.14      | 33.15 ± 17.97    |

BMI, body mass index; Glu, glucose; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; EH, essential hypertension; DM, diabetes mellitus. Continuous variables were expressed as the means ± standard deviations. The P-values of continuous variables were calculated using independent-samples T-tests. The P-values of categorical variables were calculated using X² test. *P < 0.05.
| Variants       | Genotyping | Dominant model | Recessive model | Additive model | Allele | rs4886605(SNP1) | Zou et al. Lipids in Health and Disease 2014, 13:145 | http://www.lipidworld.com/content/13/1/145 |
|---------------|------------|----------------|----------------|----------------|--------|----------------|-----------------------------------|-------------------------------------------|
|               | CAD n (%)  | Control n (%)  | P Value        | CAD n (%)  | Control n (%)  | P Value        | CAD n (%)  | Control n (%)  | P Value        | CAD n (%)  | Control n (%)  | P Value        |
| rs4886605(SNP1)|            |                |                | rs12441817(SNP2) |            |                |            | rs4646422(SNP3) |            |                  |                |
| Genotyping    |            |                |                | Genotyping    |            |                |            | Genotyping    |            |                  |                |
| CC            | 79(20.3%)  | 96(23.4%)      |                | 120(30.8%)   | 115(28.0%)   | 0.494          | 310(79.7%) | 315(76.6%)   | 0.297          | 269(69.2%) | 296(72.0%)      | 0.373          |
| CT            | 190(48.8%) | 200(48.7%)     |                | 120(30.8%)   | 115(28.0%)   | 0.494          | 190(48.8%) | 200(48.7%)   |                | 199(51.1%) | 211(51.3%)      | 0.959          |
| TT            | 120(30.8%) | 115(28.0%)     |                | 300(75.2%)   | 300(75.2%)   |                | 300(75.2%) | 300(75.2%)   |                | 430(55.3%) | 430(55.2%)      | 0.236          |
| Allele        |            |                |                | CC + CT      |            |                |            | CC + CT      |                |            |                |                |
| C             | 348(44.7%) | 392(47.7%)     |                | 269(69.2%)   | 296(72.0%)   | 0.373          | 348(44.7%) | 392(47.7%)   |                | 348(44.7%) | 392(47.7%)      |                |
| T             | 430(55.3%) | 430(55.2%)     |                | 269(69.2%)   | 296(72.0%)   | 0.373          | 430(55.3%) | 430(55.2%)   |                | 430(55.3%) | 430(55.2%)      |                |
| rs12441817(SNP2)|            |                |                | rs4646422(SNP3) |            |                |            |            |                |            |                  |                |
| Genotyping    |            |                |                | Genotyping    |            |                |            | Genotyping    |            |                  |                |
| CC            | 83(21.3%)  | 83(20.2%)      |                | 101(26.0%)   | 129(31.4%)   | 0.235          | 101(26.0%) | 129(31.4%)   | 0.235          | 83(21.3%)  | 83(20.2%)       | 0.235          |
| CT            | 205(52.7%) | 199(48.4%)     |                | 288(74.0%)   | 282(68.6%)   | 0.09           | 205(52.7%) | 199(48.4%)   |                | 205(52.7%) | 199(48.4%)      | 0.09           |
| TT            | 101(26.0%) | 129(31.4%)     |                | 101(26.0%)   | 129(31.4%)   | 0.235          | 101(26.0%) | 129(31.4%)   | 0.235          | 101(26.0%) | 129(31.4%)      | 0.235          |
| Allele        |            |                |                | CC + CT      |            |                |            | CC + CT      |                |            |                |                |
| C             | 371(47.7%) | 365(44.4%)     |                | 284(48.3%)   | 365(44.4%)   | 0.188          | 284(48.3%) | 365(44.4%)   | 0.188          | 371(47.7%) | 365(44.4%)      | 0.188          |
| T             | 407(52.3%) | 457(55.6%)     |                | 284(48.3%)   | 365(44.4%)   | 0.188          | 407(52.3%) | 457(55.6%)   | 0.188          | 407(52.3%) | 457(55.6%)      | 0.188          |
first case—control study to investigate the association be-
tween the human CYP1A1 gene and CAD in the Uygur and Han populations in western China. Several CYP enzyme families have been identified in the heart, endothelium, and smooth muscle of blood vessels. A link between the expression and activity of CYP and cardio-
vascular diseases (CVDs) such as hypertension, CAD, heart failure, stroke, cardiomyopathy, and arrhythmia has been established [7]. CYP1A1 polymorphisms can affect the me-
tabolism of arachidonic acid, thereby resulting in an altered
generating capacity of 20-HETE and EETs. 20-HETE have 5 physiological functions. First, 20-HETE increases the intracellular calcium concentration and regulates the vaso-
constrictor responses of angiotensin II, vasopressin, and norepinephrine [23-25]. Second, 20-HETE increases thymi-
dine incorporation in cells and regulates growth responses in vascular smooth muscle cells (VSMCs) in response to norepinephrine and angiotensin II [26-28]. Third, 20-HETE inhibits Na + − K + − ATPase activity and sodium transport in the proximal tubule and is a key mediator of the long-
term control of arterial pressure [29-31]. Fourth, 20-HETE significantly contributes to ischemia -reperfusion injury, and the exogenous administration of 20-HETE significantly increases infarct size [32,33]. Fifth, 20-HETE inhibits plate-
let aggregation and the formation of thromboxane A2 (TxA2) during platelet activation [34], in addition, CYP1A1 also metabolize arachidonic acid to EETs, which are potent endogenous vasodilators, inhibitors of vascular inflamma-
tion, and possessors of potent vasodilatory and antiapoptotic and anti-thrombotic properties in the cardiovascular system [35]. Several genetic polymorphisms have been reported in the CYP1A1 gene including T6235C and T5639C in the 3′-flanking region, A4889G and C4887A at exon 7 [36]. Functional studies have revealed that these polymor-
phisms are associated with increased CYP1A1 activity and/or inducibility [37]. Wang et al. [9] reported signifi-
cant association between CYP1A1 MspI polymorphism and the risk of coronary artery disease (CAD) in cigarette smoking. This increased risk was more evident among light smokers with OR of 3.44, but not observed in non-
smokers or heavy smokers. Nevertheless, Manfredi et al.

| Table 2 Genotype and allele distributions in the control subjects and patients with CAD in the Han population (Continued) |
|---------------------------------------------------------------|
| GG | 17(4.4%) | 10(2.4%) | 0.294 | 14(4.8%) | 5(2.2%) | 0.187 | 3(3.2%) | 5(2.7%) |
| AA + AG | 372(95.6%) | 401(97.6%) | 0.129 | 280(95.2%) | 219(97.8%) | 0.129 | 92(96.8%) | 182(97.3%) | 0.818 |
| Additive model | | | | | | | | |
| AG | 102(26.2%) | 115(28.0%) | 0.252 | 74(25.2%) | 67(29.9%) | 0.28 | 28(29.5%) | 48(25.7%) |
| AA + GG | 287(73.8%) | 296(72.0%) | 0.576 | 220(74.8%) | 157(70.1%) | 0.23 | 67(70.5%) | 139(74.3%) | 0.496 |
| Allele | | | | | | | | |
| A | 642(82.5%) | 687(83.6%) | 486(82.7%) | 371(82.8%) | 156(82.1%) | 316(84.5%) |
| G | 136(17.5%) | 135(16.4%) | 102(17.3%) | 77(17.2%) | 34(17.9%) | 58(15.5%) |
| rs1048943(SNP4) | | | | | | |
| Genotyping | | | | | | |
| CC | 20(5.1%) | 17(4.1%) | 15(5.1%) | 8(3.6%) | 5(5.3%) | 9(4.8%) |
| CT | 152(39.1%) | 148(36.0%) | 115(39.1%) | 80(35.7%) | 37(38.9%) | 68(36.4%) |
| TT | 217(55.8%) | 246(59.9%) | 0.47 | 164(55.8%) | 136(60.7%) | 0.45 | 53(55.8%) | 110(58.8%) | 0.888 |
| Dominant model | | | | | | |
| TT | 217(55.8%) | 246(59.9%) | 164(55.8%) | 136(60.7%) | 53(55.8%) | 110(58.8%) |
| CC + CT | 172(44.2%) | 165(40.1%) | 0.244 | 130(44.2%) | 88(39.3%) | 0.26 | 42(44.2%) | 77(41.2%) | 0.626 |
| Recessive model | | | | | | |
| CC | 20(5.1%) | 17(4.1%) | 15(5.1%) | 8(3.6%) | 5(5.3%) | 9(4.8%) |
| TT + CT | 369(94.9%) | 394(95.9%) | 0.499 | 279(94.9%) | 216(96.4%) | 0.402 | 90(94.7%) | 178(95.2%) | 0.87 |
| Additive model | | | | | | |
| CT | 152(39.1%) | 148(36.0%) | 115(39.1%) | 80(35.7%) | 37(38.9%) | 68(36.4%) |
| CC + TT | 237(60.9%) | 263(64.0%) | 0.371 | 179(60.9%) | 144(64.3%) | 0.429 | 58(61.1%) | 119(63.6%) | 0.671 |
| Allele | | | | | | |
| C | 192(24.7%) | 182(22.1%) | 145(24.7%) | 96(21.4%) | 47(24.7%) | 86(23.0%) |
| T | 586(75.3%) | 640(77.9%) | 0.231 | 443(75.3%) | 352(78.6%) | 0.223 | 143(75.3%) | 288(77.0%) | 0.645 |

CAD, coronary artery disease; SNP, single-nucleotide polymorphism.
Table 3 Genotype and allele distributions in control subjects and patients with CAD in the Uygur population

| Variants   | Total                          | Men                          | Women                         |
|------------|--------------------------------|------------------------------|-------------------------------|
|            | CAD n (%)                     | Control n (%)                | CAD n (%)                     | Control n (%) | P Value | CAD n (%)                     | Control n (%) | P Value |
| rs4886605(SNP1) |                               |                              |                              |               |         |                               |               |         |
| Genotyping |                               |                              |                              |               |         |                               |               |         |
| CC         | 75(25.60%)                    | 153(37.50%)                  | 52(24.80%)                    | 74(36.10%)    | 23(27.70%) | 79(38.90%)                    |               |         |
| CT         | 162(55.30%)                   | 191(46.80%)                  | 121(57.60%)                   | 104(50.70%)   | 41(49.40%) | 87(42.90%)                    |               |         |
| TT         | 56(19.10%)                    | 64(15.70%)                   | 37(17.60%)                    | 27(13.20%)    | 19(22.90%) | 37(18.20%)                    |               |         |
| Dominant model |                             |                              |                              |               |         |                               |               |         |
| CC         | 75(25.60%)                    | 153(37.50%)                  | 52(24.80%)                    | 74(36.10%)    | 23(27.70%) | 79(38.90%)                    |               |         |
| TT + CT    | 218(74.40%)                   | 255(62.50%)                  | 158(75.20%)                   | 131(63.90%)   | 60(72.30%) | 124(61.10%)                   |               |         |
| Recessive model |                             |                              |                              |               |         |                               |               |         |
| TT         | 56(19.10%)                    | 64(15.70%)                   | 37(17.60%)                    | 27(13.20%)    | 19(22.90%) | 37(18.20%)                    |               |         |
| CC + CT    | 237(80.90%)                   | 344(84.30%)                  | 173(82.40%)                   | 178(86.80%)   | 64(77.10%) | 166(81.80%)                   |               |         |
| Additive model |                             |                              |                              |               |         |                               |               |         |
| CT         | 162(55.30%)                   | 191(46.80%)                  | 121(57.60%)                   | 104(50.70%)   | 41(49.40%) | 87(42.90%)                    |               |         |
| CC + TT    | 131(44.70%)                   | 217(53.20%)                  | 89(42.40%)                    | 101(49.30%)   | 42(50.60%) | 116(57.10%)                   |               |         |
| Allele     |                               |                              |                                |               |         |                               |               |         |
| C          | 312(53.24%)                   | 497(60.91%)                  | 225(53.57%)                   | 252(45.46%)   | 87(52.41%) | 245(60.34%)                   |               |         |
| T          | 274(46.76%)                   | 319(39.09%)                  | 195(46.43%)                   | 158(38.54%)   | 79(47.59%) | 161(39.66%)                   |               |         |
| rs12441817(SNP2) |                             |                              |                              |               |         |                               |               |         |
| Genotyping |                               |                              |                              |               |         |                               |               |         |
| CC         | 43(14.70%)                    | 45(11.00%)                   | 30(13.40%)                    | 20(9.80%)     | 13(15.70%) | 25(12.30%)                    |               |         |
| CT         | 150(51.20%)                   | 178(43.60%)                  | 110(52.40%)                   | 92(44.90%)    | 40(48.20%) | 86(42.40%)                    |               |         |
| TT         | 100(34.10%)                   | 185(45.30%)                  | 70(33.30%)                    | 93(45.40%)    | 30(36.10%) | 92(45.30%)                    |               |         |
| Dominant model |                             |                              |                              |               |         |                               |               |         |
| TT         | 100(34.10%)                   | 185(45.30%)                  | 70(33.30%)                    | 93(45.40%)    | 30(36.10%) | 92(45.30%)                    |               |         |
| CC + CT    | 193(65.90%)                   | 223(54.60%)                  | 140(66.70%)                   | 112(54.60%)   | 53(63.90%) | 111(54.70%)                   |               |         |
| Recessive model |                             |                              |                              |               |         |                               |               |         |
| CC         | 43(14.70%)                    | 45(11.00%)                   | 30(13.40%)                    | 20(9.80%)     | 13(15.70%) | 25(12.30%)                    |               |         |
| TT + CT    | 250(85.30%)                   | 363(89.00%)                  | 180(85.70%)                   | 185(90.20%)   | 70(84.30%) | 178(87.70%)                   |               |         |
| Additive model |                             |                              |                              |               |         |                               |               |         |
| CT         | 150(51.20%)                   | 178(43.60%)                  | 110(52.40%)                   | 92(44.90%)    | 40(48.20%) | 86(42.40%)                    |               |         |
| CC + TT    | 143(48.80%)                   | 230(56.40%)                  | 100(47.60%)                   | 113(55.10%)   | 43(51.80%) | 117(57.60%)                   |               |         |
| Allele     |                               |                              |                                |               |         |                               |               |         |
| C          | 236(40.27%)                   | 268(32.84%)                  | 170(40.48%)                   | 132(32.20%)   | 66(39.78%) | 136(33.50%)                   |               |         |
| T          | 350(59.73%)                   | 548(67.16%)                  | 250(59.52%)                   | 278(67.8%)    | 100(60.22%) | 270(66.5%)                    |               |         |
| rs4646422(SNP3) |                             |                              |                              |               |         |                               |               |         |
| Genotyping |                               |                              |                              |               |         |                               |               |         |
| AA         | 251(85.7%)                    | 350(85.80%)                  | 178(84.80%)                   | 177(86.30%)   | 73(88.00%) | 173(85.20%)                   |               |         |
| AG         | 39(13.30%)                    | 53(13.00%)                   | 32(15.20%)                    | 26(12.70%)    | 7(8.40%)   | 27(13.30%)                    |               |         |
| GG         | 3(1.00%)                      | 5(1.20%)                     | 0(0.00%)                      | 2(1.00%)      | 3(3.60%)   | 3(1.50%)                      |               |         |
| Dominant model |                             |                              |                              |               |         |                               |               |         |
| AA         | 251(85.70%)                   | 350(85.80%)                  | 178(84.80%)                   | 177(86.30%)   | 73(88.00%) | 173(85.20%)                   |               |         |
| GG + AG    | 42(14.30%)                    | 58(14.20%)                   | 32(15.20%)                    | 28(13.70%)    | 10(12.00%) | 30(14.80%)                    |               |         |
| Recessive model |                             |                              |                              |               |         |                               |               |         |
found no significant association was observed between CYP1A1 MspI polymorphism and the presence of CAD, or the number of significantly diseased vessels in smokers. In agreement with this study, Cornelis et al. [10] reported no significant association between CYP1A1 polymorphism and the risk of myocardial infarction (MI) in a study from Costa Rica. Chih-Ching Yeh et al. [38] report CYP1A1*2C polymorphism, particularly in non-smokers, may be associated with the individual susceptibility to CAD. From the above example, we have considered

### Table 3 Genotype and allele distributions in control subjects and patients with CAD in the Uygur population (Continued)

| Genotype | Total | Men | Women |
|----------|-------|-----|-------|
| GG       | 1486  | 876 | 610   |
| AA + AG  | 403   | 212 | 191   |
| Additive |       |     |       |
| AG       | 379   | 198 | 181   |
| AA + GG  | 355   | 197 | 190   |
| Allele   |       |     |       |
| A        | 855   | 512 | 343   |
| G        | 631   | 378 | 253   |
| rs1048943(SNP4) Genotyping |       |     |       |
| CC       | 5     | 2   | 3     |
| CT       | 112   | 69  | 43    |
| TT       | 285   | 179 | 106   |
| Additive model |       |     |       |
| AG       | 32    | 18  | 14    |
| AA + GG  | 35    | 20  | 13    |
| Allele   |       |     |       |
| A        | 51    | 30  | 21    |
| G        | 97    | 54  | 44    |

CAD, coronary artery disease; SNPs, single-nucleotide polymorphism; *P < 0.05.

Table 4 Multiple logistic regression analysis for CAD patients and control subjects of Uygur population (rs4886605)

| Total | Men | Women |
|-------|-----|-------|
| OR    | 95% CI | P  | OR    | 95% CI | P  | OR    | 95% CI | P  |
| Dominant model (CC vs CT + TT) | 0.368 | 0.185–0.530 | 0.018 | 0.35 | 0.235–0.568 | 0.015 | 0.626 | 0.321–1.412 | 0.204 |
| Smoking | 8.09 | 5.365–12.846 | <0.001 | 10.25 | 5.713–18.375 | <0.001 | 0.232 | 0.039–1.825 | 0.262 |
| EH     | 6.65 | 3.957–10.952 | <0.001 | 9.81 | 4.94–20.513 | <0.001 | 4.103 | 1.875–9.184 | <0.001 |
| DM     | 1.52 | 0.949–2.495 | 0.284 | 1.902 | 0.926–3.854 | 0.087 | 0.95 | 0.358–2.243 | 0.757 |
| Glu    | 1.35 | 1.241–1.482 | <0.001 | 1.348 | 1.183–1.517 | <0.001 | 1.485 | 1.253–1.762 | <0.001 |
| TG     | 1.34 | 1.273–1.638 | 0.251 | 1.384 | 1.169–1.615 | 0.015 | 1.653 | 1.321–1.859 | 0.021 |
| TC     | 1.86 | 1.765–2.193 | 0.031 | 1.846 | 1.713–2.197 | 0.013 | 1.89 | 1.694–2.132 | 0.027 |
| HDL    | 0.25 | 0.086–0.541 | 0.032 | 0.231 | 0.069–0.928 | 0.021 | 0.516 | 0.285–0.930 | 0.012 |
| LDL    | 0.985 | 0.891–1.153 | 0.068 | 0.924 | 0.897–1.358 | 0.21 | 0.427 | 0.203–0.947 | 0.135 |

CAD, coronary artery disease; EH, essential hypertension; DM, diabetes mellitus; Glu, glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, Low-density lipoprotein.
Association between CYP1A1 and CAD was different on account of polymorphisms of the CYP1A1 gene. Ethnic differences, environmental factors, CYPIA1 might cause CAD in some smokers or non-smokers. Their mechanisms of CYPIA1 were different. CYPIA1 metabolizes arachidonic acid to 20-HETE, EETs, which cause CAD, in non-smoking.

In this study, we hypothesized that variability in the CYPIA1 gene might affect the risk of CAD through CYPIA1 as an arachidonic acid epoxygenase enzyme in nonsmoking. We genotyped four SNPs of this gene in the Uygur and Han of China and assessed the association between CYPIA1 polymorphisms and CAD using a case–control analysis.

A significant difference was observed in the genotypic distribution of SNP1 (rs4886605) between the patients with CAD and controls within the full Uygur sample. When men and women were analyzed separately, the T allele frequency of rs4886605 was higher among men with CAD than their control counterparts. No such differences were observed among women. Thus, the risk of CAD was significantly lower among patients with CAD than controls for all participants and men; furthermore, this difference remained significant after adjusting for Glu, LDL-C, EH, DM, and smoking (Table 5). This finding indicates that the risk of CAD was decreased with the presence of the TT genotype in rs12441817 among Uygur men.

We found associations between CAD and the CYPIA1 SNPs rs4886605 and rs12441817 among Uygur men. This result might be caused by two reasons. First, it might be because of sex hormones; for instance, estrogen protects against oxidative stress and is vasoprotective [39-41]. The vascular protective effects of estradiol are metabolized into 2-hydroxyestradiol (2HE) by CYPIA1, and 2HE is converted into 2-methoxyestradiol (2ME) by catechol-O-methyl transferase. 2ME is antimitogenic, anti-angiogenic and pro-apoptotic [42]. Second, it might be because of less female samples. However, patients with CAD and controls participants in the Han group did not significantly differ with regard to the distributions of the rs4886605 and rs12441817 genotypes, the dominant model, the recessive model, or allele frequency (P > 0.05). This genetic difference associated with coronary heart disease might differ across ethnic groups because of race, diet, or lifestyle.

Although SNP4 (rs1048943) was observed in the exon 7 region of the CYPIA1 gene and the polymorphisms caused a loss of transcription factor binding at site Sp7, the synthesis of 20-HETE and EETs excluding the 2-hydroxyestradiol (2HE) by CYPIA1, and 2HE is converted into 2-methoxyestradiol (2ME) by catechol-O-methyl transferase. 2ME is antimitogenic, anti-angiogenic and pro-apoptotic [42]. Second, it might be because of less female samples. However, patients with CAD and controls participants in the Han group did not significantly differ with regard to the distributions of the rs4886605 and rs12441817 genotypes, the dominant model, the recessive model, or allele frequency (P > 0.05). This genetic difference associated with coronary heart disease might differ across ethnic groups because of race, diet, or lifestyle.

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Differences in populations such as race, geographical and environmental factors, might explain these results between Chinese and Taiwanese population.

Few studies have focused on the association between SNP3 (rs4646422) in the exon 2 and CAD. We did not find significant differences between the patients with CAD and controls with regard to the distributions of the rs4646422 genotypes, the dominant model, the recessive model, or allele frequency among the Han and Uygur samples.

Our study has several limitations. On one hand, the present study analyzed only a small sample data, More studies will need to incorporate a large samplesize for confirming the association. On the other hand, further studies will need to be undertaken in order to clarify the underlying molecular mechanism that polymorphism of CYP1A1 gene was associated with CAD.

Conclusions
In conclusion, we found that rs4886605 and rs12441817 might be two novel polymorphisms of the CYP1A1 gene associated with CAD in the Uygur population in China. The CC genotype of rs4886605 and the TT genotype of rs12441817 in the CYP1A1 gene might be protective genetic markers of CAD, whereas the T allele of rs4886605 and the C allele of rs12441817 might be genetic risk markers of CAD in the Uygur population in China.

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

Authors' contributions
Conceived and designed the experiments: JG-Z, XX, Y-TM; Performed the experiments: JG-Z, FL, Analyzed the data: JG-Z, SP, DLR-AD, Conceived and designed the experiments: JG-Z, XX, Y-TM; Performed the experiments: JG-Z, FL, Analyzed the data: JG-Z, FL, Y-NY, SP, DLR-AD, All authors read and approved the final manuscript.

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