Polymorphisms of rs2483205 and rs562556 in the PCSK9 gene are associated with coronary artery disease and cardiovascular risk factors

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PCSK9 plays a crucial role in lipid metabolism. This case–control study explored the associations of novel single nucleotide polymorphisms (SNPs) of the PCSK9 gene with coronary artery disease (CAD) (≥ 1 coronary artery stenosis ≥ 50%) and its risk factors in the Han population in Xinjiang, China. Four tag SNPs (rs11583680, rs2483205, rs2495477 and rs562556) of the PCSK9 gene were genotyped in 950 CAD patients and 1082 healthy controls. The distributions of genotypes in rs2483205 and rs562556 were significantly different between the groups (all \(p < 0.05\)). The TT genotype of rs2483205, GG genotype of rs562556, and their H4 (T-G) haplotype were associated with CAD [odds ratio (OR) 0.65, confidence interval (CI) 0.45–0.95, \(p = 0.024\); 0.63, 0.45–0.90, \(p = 0.011\); 0.50, 0.35–0.70, \(p < 0.001\), respectively]. Additionally, the model (TT + CT vs. CC) of rs2483205 was associated with increased risk of obesity, and the G allele of rs562556 was associated with lower low-density lipoprotein cholesterol (LDL-C), blood glucose, body mass index (BMI), and mean platelet volume (MPV) (all \(p < 0.05\)). rs2483205, rs562556, and their H4 haplotype of the PCSK9 gene were associated with CAD. Additionally, rs2483205 is associated with obesity, and rs562556 is associated with LDL-C, blood glucose, BMI, and MPV.

Coronary artery disease (CAD) is the main cause of death worldwide. Genes are one of the main factors that affect the development of CAD, accounting for 40–60% of cases1. Currently, genes such as PCSK9 have been proven to significantly affect lipid metabolism and are associated with the development of CAD2.

The human PCSK9 gene, located on chromosome 1p32.3, is mainly expressed as an amino acid glycoprotein. As studies have reported, PCSK9 has effects on lipid metabolism mainly through the degradation of low-density lipoprotein (LDL) receptors4. The specific mechanisms are as follows: intracellularly, PCSK9 binds to the LDL receptor to promote lysosomal degradation of the receptor4,5, while extracellularly, PCSK9 conjoins the EGF-A domain of LDL receptors to prevent LDL receptors from recycling to the cell surface5. Dozens of studies have indicated that PCSK9 genetic mutations are associated with CAD, and more than 50 functional PCSK9 genetic mutations affect cholesterol levels in plasma6,7. Individuals with a loss-of-function (LOF) mutation in PCSK9 are likely to present with life-long low total cholesterol (TC) and LDL-C levels and a notably reduced incidence of CAD risk. In contrast, individuals with a gain-of-function (GOF) mutation probably manifest hypercholesterolemia and susceptibility to CAD8,9. According to studies, 2.6% of nonsense mutations in PCSK9 are responsible for a 28% decrease in LDL-C levels and an 88% decrease in the risk of CAD9. Some missense mutations of the PCSK9 gene also have a notable effect on plasma LDL-C levels and usually cause mild hypcholesterolaemia to protect against CAD9.

Previously, most studies on the PCSK9 gene were focused on several polymorphisms, such as E670G or R46L, and the findings suggested that the PCSK9 gene was significantly associated with LDL-C levels and CAD10–12.
Recently, an increasing number of studies have implied that PCSK9 might affect CAD beyond its effects on lipids and that it also interacts with cardiovascular risk factors, such as insulin resistance, inflammation, platelet activation, and thrombosis. However, these multieffects of PCSK9 are still unclear, and whether there are novel genetic targets of PCSK9 that could be applied to therapy for atherosclerotic cardiovascular disease (ASCVD) with safer, more efficient, and pleiotropic effects requires further exploration.

Polymorphic sites of the PCSK9 gene vary in different regions and races. The polymorphisms of the PCSK9 gene in the Han population living in the northwestern part of China have not ever been reported. Moreover, finding novel polymorphisms and potential effects of the PCSK9 gene would contribute to finding potential therapeutic targets, determining its potential mechanisms, and expanding its applications in the clinic for CAD. Therefore, this study investigated whether there are novel polymorphisms of the PCSK9 gene associated with CAD and its risk factors in the Han population in Xinjiang, China.

### Results

**Clinical characteristics of participants.** There were 950 CAD subjects (mean age of 58.68 ± 7.30 and 47.14% men) and 1082 controls (mean age of 58.68 ± 7.29 and 46.43% men) who were involved in the analysis. As Table 1 shows, comparing the groups, the baseline clinical characteristics of CAD patients were characterized by lower high-density lipoprotein (HDL) and higher body mass index (BMI), glucose, blood pressure, uric acid, triglycerides (TGs), and prevalences of smoking, hypertension, alcohol intake, diabetes, and statin therapy (all p < 0.05). However, the parameters of age, sex, LDL-C, and TC did not show any differences between the groups.

### Distribution of genotypes of the polymorphisms of the PCSK9 gene between the CAD and control groups.

Four SNPs of PCSK9 (rs11583680 C>T, rs2483205 C>T, rs2495477 A>G and rs562556 G>A) were genotyped in both the CAD and control groups. As Table 2 shows, the distribution of each genotype, genetic model, and allele of the four SNPs was separately examined in CAD and controls. Except for rs2495477 in the CAD group, all the genotype frequencies in both groups were in Hardy–Weinberg equilibrium (HWE) (p > 0.05). For rs2483205, the distributions of the CC, CT, and TT genotypes and of its recessive model (TT vs. CC + CT) were different between the two groups (p = 0.025 and p = 0.008, respectively). For rs562556, the distributions of the AA, AG, and GG genotypes; A and G alleles; and its dominant model (AA vs. GG + AG) were also significantly different between the two groups (p = 0.020, p = 0.005 and p = 0.006, respectively). However, comparing the control group, the distributions of genotypes, models, or alleles of rs11583680 and rs2495477 did not show any significant differences between the CAD and control groups (p = 0.294 and p = 0.342, respectively).

### Independent risk factors for CAD.

To determine whether the polymorphisms of the PCSK9 gene were independent risk factors for CAD, we adjusted for confounding risk factors, including TGs, TC, HDL-C, LDL-C, and the prevalence of diabetes. No collinearity was existed in the regression models. We found that the recessive model (CC vs. TT + CT) of rs2483205 and the dominant model (AA vs. GG + AG) of rs562556 still showed a significant association with CAD. The TT genotype of rs2483205 indicated protective effects against CAD (OR = 0.65, 95% CI = 0.45–0.95, p = 0.024) (Table 3), and the GG genotype of rs562556 also exhibited a beneficial effect (OR = 0.63, 95% CI = 0.45–0.90, p = 0.011) (Table 4).
### Table 2. Genotype and allele distributions in control subjects and CAD patients.

| Variants | Model              | Control, n (%) (n = 1082) | CAD, n (%) (n = 950) | CAD p-value (H-W) | Control p-value (H-W) | P-value |
|----------|--------------------|---------------------------|----------------------|-------------------|----------------------|---------|
| rs11583680 (SNP1) | Genotypes         | CC                         | 882 (81.52)          | 750 (78.95)       |                      |         |
|          |                    | CT                         | 187 (17.28)          | 184 (19.37)       |                      |         |
|          |                    | TT                         | 13 (1.20)            | 16 (1.68)         | 0.294                |         |
|          | Dominant model     | CC                         | 882 (81.52)          | 750 (78.95)       |                      |         |
|          |                    | TT + CT                    | 200 (18.48)          | 200 (21.05)       | 0.231                | 0.389   | 0.146   |
|          | Recessive model    | TT                         | 13 (1.20)            | 16 (1.68)         |                      |         |
|          |                    | CC + CT                    | 1069 (98.80)         | 934 (98.32)       | 0.360                |         |
| Alleles  |                    | C allele                  | 1951 (90.16)         | 1684 (88.63)      |                      |         |
|          |                    | T allele                  | 213 (9.84)           | 216 (11.37)       | 0.114                |         |
| rs2483205 (SNP2) | Genotypes         | CC                         | 523 (48.34)          | 488 (51.37)       |                      |         |
|          |                    | CT                         | 456 (42.14)          | 402 (42.32)       |                      |         |
|          |                    | TT                         | 103 (9.25)           | 60 (6.32)         | 0.231                | 0.389   | 0.146   |
|          | Dominant model     | CC                         | 523 (48.34)          | 488 (51.37)       | 0.057                | 0.803   | 0.173   |
|          |                    | TT + CT                    | 559 (51.66)          | 462 (46.63)       |                      |         |
|          | Recessive model    | TT                         | 103 (9.52)           | 60 (6.32)         |                      |         |
|          |                    | CC + CT                    | 979 (90.48)          | 890 (93.68)       | 0.008                |         |
| Alleles  |                    | C allele                  | 1502 (69.41)         | 1298 (71.32)      |                      |         |
|          |                    | T allele                  | 662 (30.69)          | 522 (28.78)       | 0.360                |         |
| rs2495477 (SNP3) | Genotypes         | AA                         | 502 (46.40)          | 463 (48.74)       |                      |         |
|          |                    | AG                         | 486 (44.92)          | 419 (44.11)       |                      |         |
|          |                    | GG                         | 94 (8.69)            | 68 (7.16)         | 0.025                |         |
|          | Dominant model     | AA                         | 502 (46.40)          | 463 (48.74)       | 0.041                | 0.120   | 0.292   |
|          |                    | GG + AG                    | 580 (53.60)          | 487 (51.26)       |                      |         |
|          | Recessive model    | GG                         | 94 (8.69)            | 68 (7.16)         | 0.204                |         |
|          |                    | AA + AG                    | 988 (91.31)          | 882 (92.84)       | 0.342                |         |
| Alleles  |                    | A allele                  | 1490 (68.9)          | 1345 (70.79)      |                      |         |
|          |                    | G allele                  | 674 (31.1)           | 555 (29.21)       | 0.180                |         |
| rs562556 (SNP4)  | Genotypes         | AA                         | 962 (88.91)          | 879 (92.53)       |                      |         |
|          |                    | AG                         | 118 (10.91)          | 70 (7.37)         |                      |         |
|          |                    | GG                         | 2 (0.18)             | 1 (0.11)          | 0.020                |         |
|          | Dominant model     | AA                         | 962 (88.91)          | 879 (92.53)       | 0.746                | 0.411   | 0.005   |
|          |                    | GG + AG                    | 120 (11.09)          | 71 (7.47)         |                      |         |
|          | Recessive model    | GG                         | 2 (0.18)             | 1 (0.11)          |                      |         |
|          |                    | AA + AG                    | 1080 (99.82)         | 949 (99.89)       | 0.641                |         |
| Alleles  |                    | A allele                  | 2042 (94.36)         | 1828 (96.21)      |                      |         |
|          |                    | G allele                  | 122 (5.54)           | 72 (3.79)         | 0.006                |         |

Table 3. Multiple logistic regression analysis for CAD patients and control subjects. Adjust: smoking, diabetes, TG, HDL-C, and LDL-C. TG triglyceride, LDL-C low density lipoprotein-cholesterol, HDL-C high density lipoprotein-cholesterol. *p-values < 0.05.
**Linkage disequilibrium (LD) analysis.** LD analysis of the PCSK9 gene was performed (Supplementary Table S1). We identified that these four SNPs are located in the same haplotype block. Except for rs2483205 (SNP2) and rs2495477 (SNP3), all the $r^2$ values of SNPs were below 0.5, which means that we could not construct haplotypes of SNP2 and SNP3 simultaneously. In addition, because the minor allele frequency (MAF) of SNP2 is larger than that of SNP3, we used rs11583680 (SNP1), SNP2, and rs562556 (SNP4) to construct the haplotypes. Furthermore, $|D'|$ for SNP1-SNP2, SNP1–SNP3, and SNP1–SNP4 was < 0.5. Therefore, we did not use the SNP1 to construct haplotypes. Finally, SNP2 and SNP4 were used to construct haplotypes.

**Relationship between the haplotypes of the PCSK9 gene and CAD.** As Table 5 shows, we established the haplotypes by combining SNP2 and SNP4. The distribution of haplotypes constructed by SNP2–SNP4 between the CAD and control groups was analysed. The haplotype distributions of C–G (H2) and T-G (H4) were significantly different between the two groups ($p < 0.05$). The frequencies of the H2 haplotype were significantly higher in the CAD group than in the control group (OR = 1.97, 95% CI: 1.013–3.842, $p = 0.042$). However, compared with the healthy control subjects, the frequencies of the H4 haplotype were significantly lower in patients with CAD (OR = 0.50, 95% CI: 0.35–0.70, $p < 0.001$).

**The relationship between the genotypes of polymorphisms of the PCSK9 gene and cardiovascular risk factors.** To determine the effects of polymorphisms of the PCSK9 gene on CAD disease, we screened out the values of cardiovascular factors that exhibited differences among various genotypes of rs2483205 and rs562556 and explored their relationship. The results revealed that individuals with the TT genotype in rs2483205 showed decreased TC levels 0.43 mmol/L (9.91%) and increased incidence of obesity (4.28%). In the G allele group for rs562556, 0.30 mmol/L (11.07%) mean LDL-C levels and 0.52 mmol/L (9.06%) mean blood glucose levels were reduced, and 0.66 kg/m² (2.49%) mean BMI values were increased than in the A allele group. No difference was observed in the values of HDL or TGs among genotypes in either rs2483205 or rs562556 (Table 6). According to the medians of TC (4.15 mmol/L), LDL-C (2.58 mmol/L), blood glucose (5.07 mmol/L), and BMI (25.78 kg/m²), all subjects were divided into high-value and low-value groups for each parameter. The results of univariate logistic regression analysis showed that genotypes with a T allele of rs2483205 were notably associated with a 30 percent increase in the incidence of obesity (Table 7), and the G allele of rs562556 associated with a 71 percent reduction in the incidence of high levels of LDL-C (≥ 2.58 mmol/L), 42 percent reduction in the incidence of high blood glucose(≥ 5.07 mmol/L), and 37 percent increase in the risk of high BMI (≥ 25.78 kg/m²) (Table 8).

**The relationship between PSCK9 genotypes and hemocyte parameters in subjects with CAD.** To determine whether the polymorphisms of the PCSK9 gene have an effect on CAD-related hemocyte parameters in subjects with CAD, the values of hemocyte parameters were evaluated. The results showed that the red blood cell distribution width (RDW) was different among rs2483205 genotypes, and RDW, MPV, and activated partial thromboplastin time (APTT) exhibited a difference according to rs562556 alleles, and there was no difference in white blood cells (WBCs), neutrophils (NEs), lymphocytes (LYs), monocytes (MOs), platelet count, or platelet distribution width (PDW) (Supplementary Table S2). Additionally, univariate logistic regression analysis showed that genotypes of rs2483205 had no relationship with hemocyte parameters ($p > 0.05$);

| Risk factors | OR    | 95%CI   | wals | $p$   |
|--------------|-------|---------|------|------|
| rs562556(GG + AG vs. AA) | 0.63  | (0.45–0.90) | 6.42 | 0.011* |
| Smoking      | 1.76  | (1.43–2.15) | 29.40 | < 0.001* |
| Diabetes, n (%) | 2.40  | (1.56–3.68) | 16.07 | < 0.001* |
| TG, mmol/L   | 1.22  | (1.12–1.32) | 22.71 | < 0.001* |
| HDL-C, mmol/L | 0.22  | (0.16–0.29) | 104.30 | < 0.001* |
| LDL, mmol/L  | 1.11  | (0.95–1.28) | 1.79  | 0.180 |

**Table 4.** Multiple logistic regression analysis for CAD patients and control subjects. Adjust: smoking, diabetes, TG, HDL-C, and LDL-C. TG triglyceride, LDL-C low density lipoprotein-cholesterol, HDL-C high density lipoprotein-cholesterol. *$p$-values < 0.05.

| Haplotype | SNP2 | SNP4 | Control, n (%) (n=1082) | CAD, n (%) (n=950) OR [95%CI] | $p$ value |
|-----------|------|------|--------------------------|-------------------------------|-----------|
| H1        | C    | A    | 1488.21 (0.69)           | 1354.26 (0.71)               | 1.13 [0.99–1.29] | 0.082 |
| H2        | C    | G    | 13.79 (0.01)             | 23.74 (0.01)                 | 1.97 [1.01–3.84] | 0.042* |
| H3        | T    | A    | 553.79 (0.26)            | 473.74 (0.25)                | 0.97 [0.84–1.11] | 0.630 |
| H4        | T    | G    | 108.21 (0.05)            | 48.26 (0.03)                 | 0.49 [0.35–0.70] | < 0.001* |

**Table 5.** Haplotype analysis in patients with CAD and control subjects. SNP2: rs2483205; SNP4: rs562556. *: $p < 0.05$.

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however, the G allele of rs562556 was associated with two folds increase in the risk of high RDW (≥ 13.3%), and 43 percent reduction in the incidence of high MPV (≥ 10.2 fL) (Supplementary Table S3 and S4).

### Discussion

In this study, we found that two polymorphisms of rs562556, rs2483205, and their H4 haplotype of the PCSK9 gene were associated with CAD. Furthermore, the TT genotype of rs2483205 showed a 30 percent increase in the risk of obesity, and rs562556 polymorphisms were associated with high LDL-C, blood glucose, BMI, MPV, and RDW, which can increase the risk of CAD. These findings provide potential intervention targets and show the pleiotropic effects of PCSK9 in CAD.

A high LDL-C concentration is a pivotal risk factor for cardiovascular disease (CVD). Lower LDL-C levels are usually connected with a consistent and graded reduction in cardiovascular risk18–22. As a cornerstone and routine medicine in hyperlipidaemia therapy, statins could effectively lower LDL-C most of the time. However, due to potential side effects, some individuals’ poor response, and misinformation of statin therapy, many patients are reluctant to take statins or adhere to treatment22,23. In recent years, PCSK9 has been regarded as a promising therapeutic target to regulate cholesterol metabolism. Studies have indicated that even if patients are on maximum-dose statin treatment, PCSK9 inhibitors can still reduce plasma LDL-C levels by approximately 60%23. Recent studies revealed that PCSK9 inhibitors prevent CVD through mechanisms other than only reducing LDL-C levels. Several new effects of PCSK9 on diseases, such as insulin resistance and thrombus, have been revealed13,17.

A study observed no association between the rs562556 polymorphism and myocardial infarction but demonstrated an obvious relationship with LDL-C levels24. Two published studies presented significant associations between the rs562556 polymorphism and high levels of lipids in patients with hypercholesterolemia and polycystic ovary syndrome16,26. Recently, a meta-analysis study summarized that the G carriers of the rs562556 polymorphism had lower TC and LDL-C levels and relative risk than the noncarriers27. These findings are in agreement with our results that G carriers of the rs562556 polymorphism tended to have lower LDL-C levels. However, few studies have explored the relationship between the rs562556 polymorphism and CAD. Recently, a

### Table 6. The comparison of CAD associated risk factors between different genotypes of rs2483205 and rs562556 polymorphisms. LDL-C low density lipoprotein-cholesterol, TG triglyceride, TC total cholesterol, HDL high density lipoprotein-cholesterol, BMI body mass index. *p-values < 0.05.

| Parameters | rs2483205 | rs562556 |
|------------|-----------|----------|
|             | CC genotype (n = 1010) | CT genotype (n = 858) | TT genotype (n = 163) | t / χ² | p value | A allele (n = 1814) | G allele (n = 188) | t / χ² | p value |
| LDL-C, mmol/L | 2.70 ± 0.80 | 2.65 ± 0.88 | 2.61 ± 0.81 | 0.83 | 0.438 | 2.71 ± 0.99 | 2.41 ± 0.97 | 4.28 | 0.048* |
| TG, mmol/L    | 2.05 ± 2.77 | 2.06 ± 2.94 | 1.83 ± 1.33 | 0.45 | 0.636 | 1.61 ± 1.27 | 1.36 ± 0.80 | 4.25 | 0.400 |
| TC, mmol/L    | 4.34 ± 1.77 | 4.62 ± 2.62 | 3.91 ± 1.20 | 3.34 | 0.036* | 4.29 ± 0.94 | 4.17 ± 0.88 | 1.08 | 0.300 |
| HDL, mmol/L   | 1.18 ± 0.67 | 1.15 ± 0.72 | 1.13 ± 0.42 | 0.49 | 0.615 | 1.16 ± 0.66 | 1.10 ± 0.41 | 0.14 | 0.230 |
| Glucose, mmol/L | 5.78 ± 2.27 | 5.63 ± 2.14 | 5.43 ± 2.03 | 2.15 | 0.116 | 5.74 ± 2.24 | 5.22 ± 1.75 | 8.86 | 0.003* |
| Diabetes, n (%) | 65 (6.43) | 62 (7.23) | 9 (5.52) | 0.86 | 0.650 | 128 (6.95) | 8 (4.19) | 2.12 | 0.171 |
| BMI, kg/m²    | 25.76 ± 3.78 | 26.09 ± 3.83 | 26.34 ± 3.59 | 2.72 | 0.066 | 25.88 ± 3.77 | 26.54 ± 3.92 | 5.19 | 0.023* |
| Obesity, n (%) | 242 (23.94) | 250 (29.14) | 46(28.22) | 0.035* | 477 (25.91) | 94 (4.06) | 2.48 | 0.089 |

### Table 7. Univariate logistic regression analysis of rs2483205 genotypes for cardiovascular risk factors. OR odds ratio, CI confidence interval, TC total cholesterol. *p-values < 0.05.

| Parameter | rs2483205 | CT+TT(n = 1021) | CC(n = 1010) | OR 95% CI | Wals | p     |
|-----------|-----------|----------------|-------------|-----------|-------|-------|
| TC(≥ 4.15 mmol/L) | 400 (53.05%) | 376 (48.76%) | 0.84 | 0.69–1.03 | 2.80 | 0.095 |
| Obesity(BMI ≥ 28 kg/m²) | 296 (29.0%) | 242 (23.9%) | 1.30 | 1.06–1.58 | 6.60 | 0.010* |

### Table 8. Univariate logistic regression analysis of rs562556 genotypes for cardiovascular risk factors. OR odds ratio, CI confidence interval, LDL-C low density lipoprotein-cholesterol, BMI body mass index. *p-values < 0.05.

| Parameter | rs562556 | A allele(n = 1828) | G allele(194) | OR 95% CI | Wals | p     |
|-----------|---------|----------------|-------------|-----------|-------|-------|
| LDL-C(≥ 2.58 mmol/L) | 1198 (65.57%) | 63 (32.98%) | 0.26 | 0.19–0.36 | 25.68 | < 0.000* |
| Blood glucose(≥ 5.07 mmol/L) | 1013 (55.02%) | 79 (41.36%) | 0.58 | 0.43–0.78 | 12.75 | < 0.001* |
| BMI(≥ 25.78 kg/m²) | 915 (49.70%) | 110 (57.59%) | 1.37 | 1.02–1.85 | 4.28 | 0.039* |
In conclusion, rs2483205, rs562556, and their H4 haplotype of the PCSK9 gene were associated with CAD. Additionally, rs2483205 is associated with obesity, and rs562556 is associated with high LDL-C, blood glucose, BMI, MPV, and RDW. The polymorphisms of rs562556 and rs2483205 in the PCSK9 gene would be a potential therapeutic target for the treatment of CAD and its risk factors.
Methods
Ethical approval of the study. All subjects gave us permission to proceed with DNA analysis and collecting relevant clinical data and signed informed consent forms. The Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University reviewed the operating protocol and approved this study. All procedures were performed in accordance with the requirements of the Declaration of Helsinki.

Subjects. In this study, we included 950 cases and 1082 healthy controls in the Han population who lived in Xinjiang, China. This study recruited patients who underwent angiography examination and were diagnosed with CAD at the First Affiliated Hospital of Xinjiang Medical University from 2008 to 2015. The control groups were randomly selected with age-matched participants from the cardiovascular risk survey (CRS) study, which has been reported previously26. In brief, this was a cross-sectional study that collected blood samples, demographic information, lifestyle data, and cardiovascular-related clinical characteristics from seven representative regions and multiethnic populations to explore the potential cardiovascular-related risk factors in the Xinjiang population of China. Individuals were excluded if they had a history of CAD. All the DNA samples of participants were extracted from the blood samples collected in EDTA-containing tubes.

The inclusion criteria were as follows: all the patients experienced symptoms of typical chest pain, and they underwent coronary angiography examination according to the guidelines35. CAD was defined as coronary angiography examination results demonstrating at least one coronary artery stenosis > 50%. The exclusion criteria were as follows: patients with valvular heart disease, non-ischaemic cardiomyopathy, heart failure, or congenital heart disease.

Definition of cardiovascular risk factors. The standard of hypertension diagnosis was that, according to medical history and examination, individuals were diagnosed with hypertension before or at least 2 separate examination results showed systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg in a resting state36. The diagnostic standard for diabetes was a history of diabetes, a glucose value > 11.1 mmol/L (200 mg/dl) at any time, or a glucose level > 7.0 mmol/L (126 mg/dl) in fasting plasma on 2 separate occasions. Smoking was defined as declaring regular tobacco use in the last 6 months. Subjects with a BMI ≥ 28 kg/m² were defined as obesity.

Routine blood test. Using freshly collected fasting peripheral blood samples, we performed routine biochemical variable testing. Lipid parameters were tested by the Dimension AR/AVL Clinical Chemistry System (DADE Bhring, Newark, NJ). Platelet parameters were examined by the CL TOP coagulation analyzer (Instrumentation Laboratory, USA). Hemocyte parameters were detected by the XN–2000 hematology analyzer (SYSTEMEX, Japan).

Polymorphism selection and genotyping. We selected four tag SNPs by screening the 1000 Genomes Browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) and Haploview 4.2 software. Finally, rs11583680, rs2483205, rs2495477, and rs562556 were selected for the Chinese Han population. The cut-off of minor allele frequency (MAF) was set as > 0.05, and linkage disequilibrium (LD) patterns with r² were set as > 0.8. rs11583680, rs2483205, rs2495477, and rs562556 belonged to the upstream transcript variant, intron variant, intron variant, and coding variant, respectively. The SNPs were genotyped using an improved multiplex ligation detection reaction (iMLDR) technique (Genesky Biotechnologies Inc., Shanghai, China). A blinded fashion was applied in genotyping in the absence of information on the patients’ clinical data. Approximately 10% of the samples were genotyped twice to test the quality of genotyping results.

Statistical analysis. According to data categories, we used different presentation and analysis methods. Continuous variables with a normal distribution are presented as the mean ± standard deviation (SD) and as the median in cases of a nonnormal distribution. The difference between two groups was examined by the independent Student’s t-test for variables with normal distribution, and variables were analysed by the Mann–Whitney U test when variables were nonnormally distributed. The chi-square test was applied to explore the differences in categorical variables. The independent association between polymorphisms and CAD was assessed by multiple logistic regression analysis. The collinearity of variables in regression models was analyzed by collinearity diagnostics. Hardy–Weinberg equilibrium (HWE) was analysed by the chi-square test by separately calculating the frequencies of genotypes in CAD and control subjects. Haplotype construction and LD tests were conducted by the SHEsis software platform37. A p-value < 0.05 represents statistical significance (2-tailed). SPSS version 22.0 software (SPSS, Inc., Chicago, IL) was used to perform all statistical analyses.

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References
1. Roberts, R. & Stewart, A. F. Genes and coronary artery disease: Where are we? J. Am. Coll. Cardiol. 60, 1715–1721. https://doi.org/10.1016/j.jacc.2012.07.062 (2012).
2. Luo, J., Yang, H. & Song, B. L. Mechanisms and regulation of cholesterol homeostasis. Nat. Rev. Mol. Cell Biol. 21, 225–245. https://doi.org/10.1038/s41580-019-0190-7 (2020).
8. Cohen, J.

10. Adi, D.

5. Da-Wei, Z., Rita, G., Zhenze, Z. & Meghan, M. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. J. Biol. Chem. 282(25), 18602–18612. https://doi.org/10.1074/jbc.M702027200 (2007).

Abifadel, M. et al. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease. Hum. Mutat. 30, 520–529. https://doi.org/10.1002/humu.20882 (2009).

13. Davignon, J., Dubuc, G. & Seidah, N. G. The influence of PCSK9 polymorphisms on serum low-density lipoprotein cholesterol and risk of atherosclerosis. Curr. Atheroscler. Rep. 12, 308–315 (2010).

Cohen, J. et al. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat. Genet. 37, 161–165. https://doi.org/10.1038/ng1509 (2009).

Cohen, J. C., Boerwinkle, E., Mosley, T. H. Jr. & Hobbis, H. H. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N. Engl. J. Med. 354, 1264–1272. https://doi.org/10.1056/NEJMoa054013 (2006).

14. Adi, D. et al. Relationships between genetic polymorphisms of E670G in PCSK9 gene and coronary artery disease: A meta-analysis. Int. J. Clin. Exp. Med. 8(8), 13251–13258 (2015).

Cai, G., Ganwe, A., Weijin, W., Chunyan, M. & Yanbin, S. The associations between proprotein convertase subtilisin/kexin type 9 E670G polymorphism and the risk of coronary artery disease and serum lipid levels: A meta-analysis. Lipids Health Dis. 14, 149. https://doi.org/10.1186/s12944-015-0154-7 (2015).

Benn, M., Nordengaard, B. G., Grande, P., Schnoor, P. & Tybjaerg-Hansen, A. PCSK9 R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. J. Am. Coll. Cardiol. 55, 2833–2842. https://doi.org/10.1016/j.jacc.2010.02.044 (2010).

16. Cammisotto, V.

20. Brugts, J. J.

23. Sabatine, M. S. PCSK9 inhibitors: Clinical evidence and implementation. Nat. Rev. Cardiol. 16, 155–165. https://doi.org/10.1038/s41569-018-0107-8 (2019).

Shioji, K. et al. Genetic variants in PCSK9 affect the cholesterol level in Japanese. Hum. Genet. 49, 109–114. https://doi.org/10.1007/s00438-003-0114-3 (2004).

Anderson, J. M. et al. Influence of PCSK9 polymorphisms on plasma lipids and response to atorvastatin treatment in Brazilian subjects. J. Clin. Lipidol. 8, 256–264. https://doi.org/10.1016/j.jacl.2014.02.008 (2014).

Xavier, L. B.

32. Paciullo, F. et al. The association between the plasma PCSK9 levels and platelet indices in patients with stable coronary artery disease. J. Atheroscler. Thromb. 22, 76–84. https://doi.org/10.5551/jat.25841 (2015).

33. Eicher, J. D., Lettre, G. & Johnson, A. D. The genetics of platelet count and volume in humans. Platelets 29, 125–130. https://doi.org/10.1080/09537109809437599 (2010).

Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. 17, 405–424. https://doi.org/10.1038/gim.2015.30 (2015).

35. Tosi, I., Toledo-Leiva, P., Neuwirth, C., Naoumova, R. P. & Sougar, A. K. Genetic defects causing familial hypercholesterolaemia: Identification of deletions and duplications in the LDL-receptor gene and summary of all mutations found in patients attending the Hammersmith Hospital Lipid Clinic. Atherosclerosis 194, 102–111. https://doi.org/10.1016/j.atherosclerosis.2006.10.003 (2007).

Abifadel, M. et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolaemia. Nat. Genet. 34, 154–156. https://doi.org/10.1038/ng1161 (2003).

37. Schulz, R., Schlüter, K. D. & Laufs, U. Molecular and cellular function of the proprotein convertase subtilisin/kexin type 9 (PCSK9). Basic Res. Cardiol. 110, 4. https://doi.org/10.1007/s00395-015-0463-z (2015).

38. Macchi, C.

43. Chernogubova, E. et al. Common and low-frequency genetic variants in the PCSK9 locus influence circulating PCSK9 levels. Arterioscler. Thromb. Vasc. Biol. 32, 1526–1534. https://doi.org/10.1161/ATvbaha.111.240549 (2012).
40. Humphries, S. E. et al. Healthy individuals carrying the PCSK9 p.R46L variant and familial hypercholesterolemia patients carrying PCSK9 p.L137V exhibit lower plasma concentrations of PCSK9. Clin. Chem. 55, 2153–2161. https://doi.org/10.1373/clinchem.2009.129759 (2009).
41. Cao, Y. X. et al. Circulating PCSK9 and cardiovascular events in FH patients with standard lipid-lowering therapy. J. Transl. Med. 17, 367. https://doi.org/10.1186/s12967-019-2123-9 (2019).
42. Vlachopoulos, C. et al. Prediction of cardiovascular events with levels of proprotein convertase subtilisin/kexin type 9: A systematic review and meta-analysis. Atherosclerosis 252, 50–60. https://doi.org/10.1016/j.atherosclerosis.2016.07.022 (2016).
43. Zeller, M. et al. PCSK9 levels do not predict severity and recurrence of cardiovascular events in patients with acute myocardial infarction. Nutr. Metab. Cardiovasc. Dis. 31, 880–885. https://doi.org/10.1016/j.numecd.2020.11.010 (2021).
44. Ridker, P. M., Rifai, N., Bradwin, G. & Rose, L. Plasma proprotein convertase subtilisin/kexin type 9 levels and the risk of first cardiovascular events. Eur. Heart J. 37, 554–560. https://doi.org/10.1093/eurheartj/ehv568 (2016).
45. Laugum, L. E. et al. Circulating PCSK9 and risk of myocardial infarction: The HUNT study in Norway. JACC Basic Transl. Sci. 1, 568–575. https://doi.org/10.1016/j.jacbts.2016.06.007 (2016).
46. Cameron, J. et al. Serum levels of proprotein convertase subtilisin/kexin type 9 in subjects with familial hypercholesterolemia indicate that proprotein convertase subtilisin/kexin type 9 is cleared from plasma by low-density lipoprotein receptor-independent pathways. Transl. Res. 160, 125–130. https://doi.org/10.1016/j.trsl.2012.01.010 (2012).
47. Levenson, A. E. et al. Obesity and type 2 diabetes are associated with elevated PCSK9 levels in young women. Pediatr. Diabetes 18, 755–760. https://doi.org/10.1111/pedi.12490 (2017).
48. Li, S. et al. Association of plasma PCSK9 levels with white blood cell count and its subsets in patients with stable coronary artery disease. Atherosclerosis 234, 441–445. https://doi.org/10.1016/j.atherosclerosis.2014.04.001 (2014).
49. Walley, K. R. et al. PCSK9 is a critical regulator of the innate immune response and septic shock outcome. Sci. Transl. Med. 6, 258. https://doi.org/10.1126/scitranslmed.3008782 (2014).
50. Pan, S. et al. Appropriate body mass index and waist circumference cutoffs for categorization of overweight and central adiposity among Uighur adults in Xinjiang. PLoS ONE 8, e80185. https://doi.org/10.1371/journal.pone.0080185 (2013).
51. Luo, J. Y. et al. Prevalence, awareness, treatment and control of dyslipidemia among adults in northwestern China: The cardiovascular risk survey. Lipids Health Dis. 13, 4. https://doi.org/10.1186/1476-511x-13-4 (2014).
52. Gibbons, R. J. et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina—summary article: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). J. Am. Coll. Cardiol. 41, 159–168. https://doi.org/10.1016/s0735-1097(02)02848-6 (2003).
53. Whelton, P. K. et al. 2017 ACC/AHA/ACP/AAPA/ABC/ACPM/AGS/APha/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. Hypertension. 71, 1269–1324. https://doi.org/10.1161/HYPERTENSIONAHA.117.12690. (2018).
54. Li, Z. Q. et al. A partition-igation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res. 19, 519–523. https://doi.org/10.1038/cr.2009.33 (2009).

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Author contributions
Y.T.M. and B.D.C. conceived the present study; M.T.G. and D.A. designed the experiments and wrote the draft of the manuscript; Y.N.Y., G.X.M., Y.T.M., and B.D.C gave the revise suggestion for this manuscript; X.C.C., X.M., and Z.Y.F. collected data; F.L., Y.N.Y., and X.X. performed statistical analyses; M.T.G., D.A., C.X.C., X.X., and X.M. collected samples and undertook laboratory experiments; Y.T.M. and B.D.C. supervised this study. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

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