Effects of CDKN2B-AS1 polymorphisms on the susceptibility to coronary heart disease

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Abstract
Background: Coronary heart disease (CHD) is one of the most severe cardiovascular diseases. Cyclin-dependent kinase inhibitor 2B antisense RNA 1 (CDKN2B-AS1) is a significant susceptibility locus for cardiovascular disease by regulating inflammation response and cell cycle. The aim of this study was to assess whether CDKN2B-AS1 polymorphisms are associated with CHD risk in the Chinese Han population.

Methods: A total of 501 CHD patients and 496 healthy controls were recruited from Central South University Xiangya School of Medicine Affiliated Haikou Hospital, five CDKN2B-AS1 polymorphisms (rs10115049, rs75227345, rs2383205, rs10738606, and rs1333049) were analyzed by the Agena MassARRAY platform. The association of CDKN2B-AS1 polymorphisms and CHD risk was determined by odd ratios (OR) and 95% confidence intervals (CI) using logistic regression.

Results: CDKN2B-AS1 rs10738606 was significantly associated with CHD under codominant (p = .03), dominant (p = .019), recessive (p = .010), additive (p = .003), and allele (p = .003) models. Gender-based subgroup tests showed that four polymorphisms (rs75227345, rs2383205, rs10738606 and rs1333049) were associated with CHD in males (p < .05). And age-based subgroup tests indicated that rs2383205 and rs10738606 were associated with CHD among individuals, respectively (p < .05). For CHD patients, rs1333049 decreased the risk of diabetes under heterozygote (p = .014) and dominant (p = .024) models.

Conclusions: In conclusion, CDKN2B-AS1 polymorphisms were associated with CHD risk in the combined or subgroup tests, suggesting an important role of CDKN2B-AS1 in CHD susceptibility.

KEYWORDS
case–control study, CDKN2B-AS1, coronary heart disease, polymorphism, subgroups analysis

1 BACKGROUND

Coronary heart disease (CHD) is one of the most common cause of morbidity and mortality in cardiovascular diseases (CVD) worldwide, especially in the developed countries (Gaunt & Davey, 2015). CHD is characterized by the deposition of excessive cholesterol in the arterial intima (Lusk et al., 2014). The interaction of genetic and environmental factors...
can explain the majority of CHD cases (Peyser, 1997). Genetic factors play a vital role in the occurrence and development of CHD (Cunnington, Koref, Mayosi, Burn, & Keavney, 2010; Roberts, 2014). Single nucleotide polymorphisms (SNPs) are the most frequent genetic variation. Therefore, further exploration of the gene SNPs is much more significant and helpful for specific diagnosis on CHD.

Cyclin-dependent kinase inhibitor 2B antisense RNA 1 (CDKN2B-AS1), also called ANRIL, is located within the CDKN2A-CDKN2B cluster. Its product is a functional RNA molecule that interacts with polycomb repressive complex-1 (PRC1) and -2 (PRC2), leading to epigenetic silencing of other genes in this cluster (Jing et al., 2018). CDKN2B-AS1 is expressed in vascular endothelial cells and coronary smooth muscle cells (Broadbent et al., 2008). The regulation of CDKN2B-AS1 expression level affects vascular cell proliferation and senescence. Genome-wise association studies (GWAS) have reported that CDKN2B-AS1 contains multiple genetic markers for CHD (Kunnas, Piesanen, & Nikkari, 2011). However, the definite polymorphisms of CDKN2B-AS1 affect CHD risk remain unclear, especially in the subgroups of age, gender, CHD patients with hypertension, and diabetes. Previous studies have shown that CDKN2B-AS1 polymorphisms are associated with susceptibility to many diseases, including brain diseases (Sun et al., 2017), gout (Hsu et al., 2012), myocardial infarction (MI; Ivanova et al., 2017), and cancers (Gong et al., 2017). Although there have been several studies about the relationship between rs1333049 and CHD risk, the conclusions were not entirely consistent (Foroughmand, Nikkhah, Galehdari, & Jadbabaee, 2015; Lian et al., 2014; Pignataro et al., 2017; Roberts, 2014). Moreover, there were no studies regarding the association of rs10115049, rs75227345, rs2383205, rs10738606, and CHD susceptibility.

Hence, we conducted a case–control study to investigate the association of CDKN2B-AS1 polymorphisms (rs10115049, rs75227345, rs2383205, rs10738606, and rs1333049) and CHD risk in the Chinese Han population.

2  |  METHODS

2.1  |  Study subjects

This study included 501 CHD patients (320 males and 181 females) and 496 healthy controls (318 males and 178 females) enrolled from Central South University Xiangya School of Medicine Affiliated Haikou Hospital, China. Patients were diagnosed with CHD according to standardized coronary angiography. The healthy controls were healthy individuals determined by medical history and clinical examinations. The healthy controls who had congenital heart disease, family history of CVD or known disease, were excluded in this study. The demographic and clinical characteristics of the participants are recorded in Table 1. Our study protocol was approved by the Medical Ethics Committees of Central South University Xiangya School of Medicine Affiliated Haikou Hospital. And written informed consent was obtained from all study objects.

2.2  |  SNP selection and genotyping

Genomic DNA was extracted from peripheral blood stored with EDTA using blood DNA kit (GoldMag Co. Ltd.). The concentration of the DNA samples was measured with Nanodrop 2000 (Thermo Scientific). In this study, five SNPs in CDKN2B-AS1 were selected from UCSC database and each candidate SNP had larger than 5% minor allele frequency in Chinese Han population. The primers used in this

| TABLE 1 | Demographic and clinical characteristics of the study objects |
|-----------------|-----------------|-----------------|
| Characteristics | CHD patients (N = 501) | Healthy controls (N = 496) | p |
| Age, years | 61.32 ± 11.70 | 60.69 ± 6.43 | .289 |
| >61 | 250 (49.9%) | 233 (47.0%) | .947 |
| ≤61 | 251 (50.1%) | 263 (53.0%) | .585 |
| Sex | | | |
| Male | 320 (63.9%) | 318 (64.1%) | .895 |
| Female | 181 (36.1%) | 178 (35.9%) | .895 |
| HDL (mmol/L) | 1.13 ± 0.25 | 1.09 ± 0.23 | <.001 |
| LDL (mmol/L) | 1.92 ± 0.82 | 2.55 ± 0.71 | <.001 |
| PLT (10^9/L) | 169.37 ± 75.18 | 211.10 ± 55.50 | <.001 |
| PDW (%) | 14.30 ± 2.87 | 13.74 ± 2.87 | .010 |
| MPV (FL) | 13.01 ± 7.14 | 10.91 ± 1.23 | .000 |
| PCT (%) | 1.08 ± 3.15 | 0.30 ± 0.89 | .000 |
| WBC | 11.68 ± 15.45 | 5.93 ± 1.50 | <.001 |
| RBC | 14.72 ± 35.86 | 4.84 ± 0.45 | <.001 |
| HGB | 132.67 ± 31.56 | 148.48 ± 14.77 | <.001 |
| Urea | 5.81 ± 6.51 | 7.01 ± 21.29 | .241 |
| UA (μmol/L) | 292.30 ± 88.75 | 330.55 ± 82.58 | <.001 |
| TG (mmol/L) | 1.78 ± 1.48 | 1.77 ± 1.13 | .947 |
| TC (mmol/L) | 4.09 ± 1.16 | 4.82 ± 5.47 | .006 |
| Hypertension | 296 (60%) | 296 (60%) | .000 |
| Diabetes | 101 (20%) | 101 (20%) | .000 |
| Gastritis | 59 (12%) | 59 (12%) | .000 |

Note: Numbers in bold mean statistical significance. Abbreviations: CHD, coronary heart disease; HDL, high-density lipoprotein; HGB, hemoglobin; LDL, low-density lipoprotein; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; PLT, platelet; RBC, red blood cells; TC, total cholesterol; TG, triglyceride; UA, uric acid; WBC, white blood cells.
study were designed using MassARRAY Assay Design 3.0 software (Table S1), and the genotyping was performed on the MassARRAY iPLEX platform (Agena Bioscience) (Sun et al., 2017). We checked the quality of the genotype determination by the same method. We predicted functions of selected polymorphisms by HaployReg v4.1. Agena Bioscience TYPER version 4.0 software was used to perform data management and analyses.

2.3 | Statistical analysis

Differences in categorical and continuous variables between cases and controls were assessed using the chi-squared test and t test, respectively. Hardy–Weinberg equilibrium (HWE) was conducted for each SNP in controls using Fisher's exact test. Genotype and allele distributions were compared using the chi-squared test. The relationships between CDKN2B-AS1 polymorphisms and CHD risk were evaluated in multiple genetic models using PLINK software. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression analysis after adjusting with gender and age. In addition, haplotype analysis and linkage disequilibrium (LD) were conducted by PLINK software and Haploview software (version 4.2; Kaushal et al., 2007). All statistical analyses were performed using the SPSS 17.0 (IBM®). The p-values were two-sided in our study, and p < .05 was considered statistical significant.

3 | RESULTS

3.1 | Characteristics of the study objects

A total of 501 CHD cases (mean age: 61.32 ± 11.70) and 496 healthy controls (mean age: 60.69 ± 6.43) were included in this study. Demographic and clinical characteristics of the study objects that include age, sex, high-density lipoprotein (HDL), low-density lipoprotein (HDL), platelet (PLT), platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT), white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), urea, uric acid (UA), triglyceride (TG), total cholesterol (TC) are shown in Table 1. There were no significant differences in the age and sex distribution between two groups (p > .05). Among the CHD patients, 296 (60%) individuals with hypertension, 101 (20%) individuals with diabetes, and 59 (12%) individuals had gastritis.

3.2 | Associations between CDKN2B-AS1 polymorphisms and CHD risk

As shown in Table 2, the genotype distributions of all the five SNPs in controls met HWE (p > .05). Genotype and
allele frequencies of CDKN2B-AS1 polymorphisms are listed in Table 2. In Table S2, all CDKN2B-AS1 polymorphisms are located in intronic region, and these SNPs are related to the regulation of DNase, Motifs changed, Selected eQTL hits, Enhancer histone marks and NHGRI/EBI GWAS hits. In Table 3, logistic regression analyses revealed that rs10738606 conferred a decreased risk of CHD in codominant (OR = 0.54, 95% CI = 0.36–0.81, \( p = .003 \)), dominant (OR = 0.74, 95% CI = 0.57–0.95, \( p = .019 \)), recessive (OR = 0.60, 95% CI = 0.41–0.89, \( p = .010 \)) and additive (OR = 0.75, 95% CI = 0.63–0.91, \( p = .003 \)) models. A allele carriers of rs10738606 significantly decreased CHD risk (OR = 0.76, 95% CI = 0.63–0.91, \( p = .003 \)). No significant associations were observed in the other CDKN2B-AS1 polymorphisms and CHD risk (\( p > .05 \)).

### 3.3 Associations between CDKN2B-AS1 polymorphisms and CHD risk in subgroups

The relationships between CDKN2B-AS1 polymorphisms and CHD risk were further assessed in four subgroups (age, gender, hypertension, and diabetes). The significant associations were presented in Table 4. Rs75227345, rs2383205, rs10738606, and rs1333049 were associated with CHD risk in males. Rs75227345 and rs1333049 increased the risk of CHD (rs75227345: homozygote, OR = 2.62, 95% CI = 1.07–6.44, \( p = .036 \); recessive: OR = 2.49, 95% CI = 1.02–6.10, \( p = .036 \)).

| SNP       | Model   | Genotype/Allele | OR (95% CI)   | \( p \) |
|-----------|---------|-----------------|---------------|--------|
| rs10115049| Codominant | AA/GG          | 1.08 (0.73–1.59) | .690   |
|           |         | GA/GG          | 0.86 (0.66–1.12) | .257   |
|           | Dominant | AA-AH/HH       | 0.91 (0.70–1.16) | .438   |
|           | Recessive | AA/AA/HH      | 1.17 (0.81–1.68) | .396   |
|           | Additive | AA/H/G         | 0.99 (0.83–1.18) | .894   |
|           | Allele   | A/G             | 0.99 (0.82–1.19) | .905   |
| rs75227345| Codominant | TT/CC          | 1.59 (0.85–2.99) | .147   |
|           |         | TC/CC          | 1.15 (0.87–1.53) | .332   |
|           | Dominant | TT-CT/CC       | 1.20 (0.92–1.58) | .178   |
|           | Recessive | TT/CT-CC      | 1.53 (0.82–2.86) | .181   |
|           | Additive | TT/H-CC        | 1.20 (0.96–1.50) | .110   |
|           | Allele   | T/C             | 1.22 (0.96–1.53) | .097   |
| rs2383205 | Codominant | AA/GG          | 0.72 (0.31–1.66) | .435   |
|           |         | GA/GG          | 0.80 (0.60–1.07) | .130   |
|           | Dominant | AA-AG/GG       | 0.79 (0.60–1.05) | .104   |
|           | Recessive | AA/AG-GG      | 0.76 (0.33–1.75) | .515   |
|           | Additive | AA/H-GG        | 0.81 (0.64–1.04) | .103   |
|           | Allele   | A/G             | 0.82 (0.64–1.05) | .108   |
| rs10738606| Codominant | AA/TT          | 0.54 (0.36–0.81) | .003   |
|           |         | TA/TT          | 0.80 (0.61–1.05) | .108   |
|           | Dominant | AA-AT/TT       | 0.74 (0.57–0.95) | .019   |
|           | Recessive | AA/AT-TT      | 0.60 (0.41–0.89) | .010   |
|           | Additive | AA/H-T         | 0.75 (0.63–0.91) | .003   |
|           | Allele   | A/T             | 0.76 (0.63–0.91) | .003   |
| rs1333049 | Codominant | CC/GG          | 1.36 (0.95–1.96) | .095   |
|           |         | GC/GG          | 1.20 (0.90–1.61) | .218   |
|           | Dominant | CC-CG/GG       | 1.25 (0.94–1.65) | .122   |
|           | Recessive | CC/GC-GG      | 1.21 (0.89–1.65) | .231   |
|           | Additive | CC/G-C         | 1.17 (0.98–1.40) | .089   |
|           | Allele   | C/G             | 1.16 (0.97–1.38) | .103   |

**Note:** Numbers in bold mean statistical significance.

**Abbreviations:** 95% CI, 95% confidence interval; CHD, coronary heart disease; OR, odds ratio; SNP, single nucleotide polymorphism.
p = .045; additive: OR = 1.35, 95% CI = 1.01–1.81, p = .040; allele: OR = 1.38, 95% CI = 1.03–1.86, p = .033; rs1333049: homozygote, OR = 1.67, 95% CI = 1.06–2.64, p = .027; recessive: OR = 1.59, 95% CI = 1.08–2.37, p = .018; additive: OR = 1.28, 95% CI = 1.02–1.60, p = .034; allele: OR = 1.26, 95% CI = 1.01–1.58, p = .037), whereas rs2383205 and rs10738606 decreased CHD risk (rs2383205: dominant, OR = 0.67, 95% CI = 0.49–0.97, p = .035; additive, OR = 0.67, 95% CI = 0.48–0.92, p = .014; allele, OR = 0.67, 95% CI = 0.49–0.93, p = .014; rs10738606: homozygote, OR = 0.44, 95% CI = 0.27–0.73, p = .001; dominant, OR = 0.64, 95% CI = 0.47–0.88, p = .006; recessive, OR = 0.52, 95% CI = 0.33–0.84, p = .007; additive, OR = 0.68, 95% CI = 0.54–0.85, p = .001; allele, OR = 0.67, 95% CI = 0.53–0.85, p < .001). For the individuals equal or younger than 61 years old, rs2383205 had a lower risk of CHD in heterozygote (OR = 0.61, 95% CI = 0.39–0.95, p = .030) and dominant (OR = 0.63, 95% CI = 0.41–0.97, p = .035) models. Among the elderly group (age > 61), rs10738606-A allele was a protective factor of CHD (OR = 0.74, 95% CI = 0.57–0.96, p = .023). In addition, we found that rs1333049 decreased the risk of diabetes for CHD patients (heterozygote, OR = 0.53, 95% CI = 0.32–0.88, p = .014; dominant: OR = 0.58, 95% CI = 0.36–0.93, p = .024).

### 3.4 Genotypes and clinical characteristics

We evaluated the association between genotypes of CDKN2B-AS1 polymorphisms and clinical characteristics of patients, including HDL, LDL, PLT, PDW, MPV, PCT, WBC, RBC, HGB, urea, UA, TG, and TC (Table S3 and Table 5). We observed that patients carried different genotypes of CDKN2B-rs75227345 had significant differences in MPV, PCT, WBC, RBC, and HGB (p < .05).

### 3.5 Haplotype analysis of CDKN2B-AS1 polymorphisms and CHD risk

We also performed haplotype analysis of CDKN2B-AS1 polymorphisms and CHD risk. We found one block including rs10115049 and rs75227345 (Figure 1). As shown in Table S4, there was no significantly association between haplotypes of CDKN2B-AS1 polymorphisms and CHD risk (p > .05).

### 4 DISCUSSION

In this study, we genotyped five SNPs of CDKN2B-AS1 and evaluated the association between these SNPs and CHD risk in the Chinese Han population. We found that CDKN2B-AS1 polymorphisms had strong relationships with CHD risk, especially rs10738606 could protect the Chinese Han population from CHD. Age, sex, and
complications of CHD significantly influenced the association of CDKN2B-AS1 polymorphisms and CHD risk. Our results gave a clue in the prevention, diagnosis, and individual treatment of CHD.

**CDKN2B-AS1** encodes a 3.8 kb lnc RNA which consists of 19 exons, and is located at chromosome 9p21 (Holdt et al., 2011; Kong, Sharma, Nwosu, & Alonso, 2016). The 9p21.3 locus was first identified by GWAS to be strongly associated with CHD and MI (Glinsky, 2008; Ruth et al., 2007). It then reported that this locus was associated with PLT reactivity and polymorphisms at 9p21 influence inflammatory signaling and vascular cell proliferation (Harismendy et al., 2011; Musunuru et al., 2010; Visel et al., 2010). We observed significant difference in PLT between CHD patients and healthy controls, and genotypes of rs75227345 also associated with PLT, it may explain the association of polymorphism with CHD. According to several published studies, rs2383206, rs10757274, and rs10757278 may serve as genetic biomarkers of CHD in Caucasians, East Asians, and West Asian (Wang, Dong, & Yang, 2016). The expression of CDKN2B-AS1 genetic variants could influence CHD susceptibility in the Iranian patients (Bochenek et al., 2013). Among the selected SNPs, rs1333049 is the frequently studied polymorphism. Rs1333049 was found to be associated with CHD in the Turkish (Cakmak et al., 2015), Indians (Kashyap et al., 2018), Japanese (Pinós et al., 2014) population, but no studies focused on the Chinese population. As shown in our study, rs1333049 was remarkably associated with CHD susceptibility in subgroups. In addition, we found rs10738606 was a protective factor for CHD.

Age and gender disparities widely existed in the prevalence of CHD (Chen et al., 2016; Cline & Beckie, 2013). The incidence of CHD is 0.6% for the people younger than 40 years old, it will increase twofold or more with aging (Yan et al., 2013). In our study, rs2383205 was associated with a decreased CHD risk among younger people (age ≤ 61), whereas rs10738606 decreased CHD risk among elderly people (age > 61). It revealed an age-based mechanism on the genetic variations. Previous studies have indicated that gender differences could influence gene expression and then affect disease progression (Coban et al., 2014; Xu et al., 2008). We found rs75227345,
rs2383205, rs10738606, and rs1333049 had relationships with CHD risk in males, no associations were found in females. It confirmed previous results. Additionally, hypertension and diabetes are considered as traditional risk factors of CHD. In this study, we studied the relationship of CDKN2B-AS1 polymorphisms and complications (hypertension and diabetes) among CHD cases. Rs1333049 could protect CHD patients from diabetes under heterozygote and dominant models. Nevertheless, larger sample size and well-designed studies are required to validate our results.

Some limitations could not be ignored in this study. First, all samples were collected from hospital, which inevitably exist the choosing bias. Second, we did not evaluate some factor that could have effects on CHD risk, because of a lack of data from both CHD patients and healthy controls. Third, this study did not analysis the mechanisms of CDKN2B-AS1 polymorphisms influence CHD risk. Further experiments on cell or animal level are required to explain the detailed molecular mechanism.

5 | CONCLUSION

Our results indicate that CDKN2B-AS1 polymorphisms are associated with CHD risk in the Chinese Han population. These SNPs may serve as biomarkers for CHD in the Chinese Han population.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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