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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-wide association studies (GWAS) have reported that *CDKN2B-AS1* contains multiple genetic markers for CHD (Kunnas, Piesanen, & Nikkari, 2018). Genetic variants of *CDKN2B-AS1* are related to CVD by mediating the response to inflammatory signalling (Harismendy et al., 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; Pinós et al., 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.

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%)	
≤61	251 (50.1%)	263 (53.0%)	
Sex			
Male	320 (63.9%)	318 (64.1%)	.895
Female	181 (36.1%)	178 (35.9%)	
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	**<.001**
PCT (%)	1.08 ± 3.151	0.30 ± 0.89	**<.001**
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%)		
Diabetes	101 (20%)		
Gastritis	59 (12%)		

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.

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

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

TABLE 2 Comparison of genotype and allele frequencies between cases and controls

SNP	Location: Position	Groups	Genotype (counts)			*p*	Allele (counts)		*p*	MAF	HWE *p*
rs10115049	Chr9: 22032120		AA	GA	GG		A	G			
		Cases	73 (14.6%)	208 (41.5%)	220 (43.9%)		354 (35.3%)	648 (64.7%)		0.353	
		Controls	63 (12.7%)	227 (45.8%)	206 (41.5%)	.370	353 (35.6%)	639 (64.4%)	.905	0.356	1.000
rs75227345	Chr9: 22042298		TT	TC	CC		T	C			
		Cases	26 (5.2%)	139 (27.7%)	336 (67.1%)		191 (19.1%)	811 (80.9%)		0.191	
		Controls	17 (3.4%)	127 (25.6%)	352 (71.0%)	.257	161 (16.2%)	831 (83.8%)	.097	0.162	0.188
rs2383205	Chr9: 22060936		AA	GA	GG		A	G			
		Cases	10 (2.0%)	115 (23.0%)	376 (75.0%)		135 (13.5%)	867 (86.5%)		0.134	
		Controls	13 (2.6%)	133 (26.8%)	350 (50.4%)	.265	159 (16.0%)	833 (84.0%)	.108	0.160	0.869
rs10738606	Chr9: 22088091		AA	AT	TT		A	T			
		Cases	48 (9.6%)	224 (45.0%)	226 (45.4%)		320 (32.1%)	606 (67.9%)		0.321	
		Controls	74 (15.0%)	230 (46.7%)	188 (38.3%)	**.011**	378 (38.4%)	676 (61.6%)	**.003**	0.384	0.776
rs1333049	Chr9: 22125504		CC	CG	GG		C	G			
		Cases	110 (22.0%)	263 (52.5%)	128 (25.5%)		483 (48.2%)	519 (51.8%)		0.482	
		Controls	94 (19.0%)	254 (51.2%)	148 (29.8%)	.241	442 (44.6%)	550 (55.4%)	.103	0.446	0.467

Note: Numbers in bold mean statistical significance.

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

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,

TABLE 3 The association between *CDKN28-AS1* polymorphisms and CHD risk

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-AG/GG	0.91 (0.70–1.16)	.438
	Recessive	AA/AG-GG	1.17 (0.81–1.68)	.396
	Additive		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		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		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		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		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.

TABLE 4 The association between *CDKN28-AS1* polymorphisms and CHD risk in the subgroups

SNP	Subgroup	Homozygote		Heterozygote		Dominant		Recessive		Additive		Allele	
		OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
rs75227345	Male	2.62 (1.07–6.44)	**.036**	1.21 (0.84–1.73)	.309	1.32 (0.94–1.86)	.109	2.49 (1.02–6.10)	**.045**	1.35 (1.01–1.81)	**.040**	1.38 (1.03–1.86)	**.033**
rs2383205	Age ≤ 61	0.89 (0.23–3.45)	.864	0.61 (0.39–0.95)	**.030**	0.63 (0.41–0.97)	**.035**	1.01 (0.26–3.91)	.987	1.01 (0.26–3.91)	.987	0.72 (0.51–1.03)	.071
	Male	0.30 (0.08–1.12)	0.072	0.71 (0.49–1.02)	.065	0.67 (0.47–0.96)	**.028**	0.32 (0.09–1.21)	.094	0.67 (0.48–0.92)	**.014**	0.67 (0.49–0.93)	**.014**
rs10738606	Age > 61	0.60 (0.33–1.08)	.089	0.86 (0.58–1.28)	.453	0.79 (0.54–1.15)	.217	0.64 (0.37–1.13)	.124	0.80 (0.61–1.04)	.100	0.74 (0.57–0.96)	**.023**
	Male	0.44 (0.27–0.73)	**.001**	0.72 (0.51–1.00)	.051	0.64 (0.47–0.88)	**.006**	0.52 (0.33–0.84)	**.007**	0.68 (0.54–0.85)	**.001**	0.67 (0.53–0.85)	**<.001**
rs1333049	Male	1.67 (1.06–2.64)	**.027**	1.08 (0.74–1.56)	.698	1.22 (0.86–1.73)	.264	1.59 (1.08–2.34)	**.018**	1.28 (1.02–1.60)	**.034**	1.26 (1.01–1.58)	**.037**
	Diabetes	0.72 (0.39–1.32)	.284	0.53 (0.32–0.88)	**.014**	0.58 (0.36–0.93)	**.024**	1.07 (0.63–1.82)	.791	0.81 (0.59–1.12)	.199	0.81 (0.60–1.11)	.187

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 = 0.47–0.97, $p = .035$; 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

TABLE 5 Clinical characteristics of rs75227345 on CHD patients

Characteristics	*CDKN2B*-rs75227345			
	TT	CT	CC	*p*
HDL (mmol/L)	1.10 ± 0.29	1.13 ± 0.24	1.14 ± 0.26	.786
LDL (mmol/L)	1.84 ± 0.60	1.96 ± 0.82	1.91 ± 0.84	.753
PLT (10^9/L)	170.77 ± 37.87	157.71 ± 80.58	174.15 ± 74.70	.097
PDW (%)	14.08 ± 2.61	14.41 ± 3.13	14.28 ± 2.80	.846
MPV (FL)	11.08 ± 0.93	14.18 ± 8.67	12.67 ± 6.64	**.044**
PCT (%)	0.19 ± 0.04	1.60 ± 3.72	0.93 ± 2.98	**.038**
WBC	7.03 ± 2.65	14.83 ± 20.20	10.72 ± 13.42	**.009**
RBC	4.82 ± 0.75	22.02 ± 46.70	12.44 ± 31.35	**.011**
HGB	138.00 ± 20.16	125.37 ± 36.47	135.23 ± 29.61	**.006**
Urea	5.43 ± 2.53	5.75 ± 2.11	5.86 ± 7.80	.942
UA (μmol/L)	309.72 ± 81.47	297.77 ± 82.16	288.74 ± 91.79	.372
TG (mmol/L)	1.69 ± 1.10	1.88 ± 1.67	1.75 ± 1.43	.644
TC (mmol/L)	3.93 ± 1.01	4.27 ± 1.31	4.03 ± 1.09	.110

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.

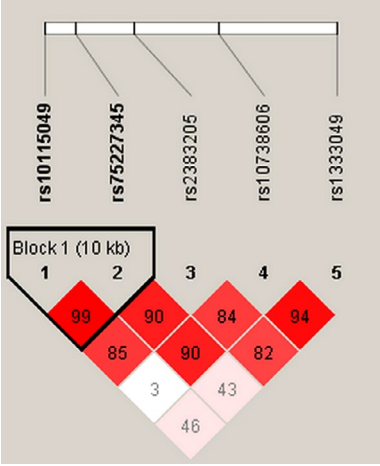

FIGURE 1 Linkage disequilibrium plots containing five polymorphisms from *CDKN2B-AS1*. Block 1 includes rs10115049 and rs75227345. The numbers inside the diamonds indicate the D′ for pairwise analyses

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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