New findings in the roles of Cyclin-dependent Kinase inhibitors 2B Antisense RNA 1 (CDKN2B-AS1) rs1333049 G/C and rs4977574 A/G variants on the risk to coronary heart disease

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ABSTRACT

The relationship between Cyclin-Dependent Kinase Inhibitors 2B Antisense RNA 1 (CDKN2B-AS1) variants rs1333049 G/C and rs4977574 A/G and the risk of coronary heart disease is unclear. We conducted an update analysis incorporating odds ratios and 95% confidence intervals to assess the correlation. Furthermore, we used in silico analysis to investigate the genes and proteins that interact with CDKN2B. Fifty case-control studies with a sample size of 35,915 cases and 48,873 controls were involved. We revealed that the rs1333049 C allele could increase the risk of coronary heart disease in the overall analysis (allele comparison, OR = 1.13, 95%CI = 1.05–1.21, P = 0.001; homozogous contrast, OR = 1.29, 95%CI = 1.11–1.49, P = 0.001; dominant comparison, OR = 1.14, 95%CI = 1.03–1.27, P = 0.011; recessive comparison, OR = 1.21, 95%CI = 1.10–1.34, P < 0.001). In subgroup analysis, positive correlations were detected in studies involving West and East Asians and in population-based control studies. The rs4977574 G allele was also a risk factor for coronary heart disease (allele comparison, P = 0.001; heterozygous comparison, P = 0.003; homozogous comparison, P < 0.001; dominant comparison, P = 0.001). These results indicate correlation of CDKN2B-AS1 rs1333049 G/C and rs4977574 A/G variants may be correlated with the risk of coronary heart disease.

Abbreviations
CDK: Cyclin Dependent Kinase; CCND: G1/S-specific cyclin-D; CDKN: Cyclin Dependent Kinase Inhibitor; GWAS: Genome-wide association study; CDKN2B-AS1: Cyclin-Dependent Kinase Inhibitors 2B Antisense RNA 1; CHD: Coronary heart disease; MAF: minor allele frequencies; HWE: Hardy-Weinberg equilibrium of controls; CI: confidence interval; COL8A2: Collagen type VIII alpha 2 chain; HB: Hospital-based; OR: odds ratios; iTGA11: Integrin subunit alpha 11; LTBP: Latent transforming factor beta binding protein; PB: Population-based; IBC: Imit Broad Care; NA: Not applicable; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; MI: Myocardial Infarction; SNP: single nucleotide polymorphism; SMAD: Mothers against decapentaplegic homolog; RT-PCR: Real-time polymerase chain reaction; UK: United Kingdom

Introduction

Coronary heart disease (CHD) is characterized by coronary artery stenosis and leading to occlusion. This disease is one of the leading causes of disability and death globally [1]. The exact pathogenesis of CHD is unclear; however, evidence indicate a crucial role of genetic factors in the development
of CHD [2]. Genome-wide association studies have provided evidence of a correlation between common variations on specific chromosome location 9p21.3 and susceptibility to cardiovascular diseases including atherosclerosis-related ischemia and coronary heart disease [3,4].

Cyclin-Dependent Kinase Inhibitors 2B Antisense RNA 1 (CDKN2B-AS1) also known as Antisense Noncoding RNA in the INK4 locus (ANRIL) is a potential CHD candidate gene located within the CDKN2A-CDKN2B gene cluster on human chromosome 9 (9p21.3). CDKN2B-AS1 can also encode a large antisense non-coding RNA, and prior studies have suggested the role of CDKN2B-AS1 gene in the progression of CHD by regulating the expression of CDKN2B and other genes in cardiac tissue [5]. Inhibition of CDKN2B-AS1 in vascular smooth muscle could affect the expression of extra-cellular matrix remodeling genes, indicating a pivotal role in vascular function [6]. Abnormal CDKN2B-AS1 expression in atherosclerotic lesions can promote atherosclerosis and thrombosis [7,8]. Therefore, it is plausible that variants in the CDKN2B-AS1 gene are associated with atherosclerosis-related diseases, including CHD.

Polymorphisms of CDKN2B-AS1 have been investigated previously and have been correlated with susceptibility to various diseases that include ischemic stroke, glaucoma, gout, and cancer [9–12]. Prior studies have assessed the potential association between CDKN2B-AS1 variants and the likelihood of CHD. The variant rs4977574 (A/G) is considered as a non-protein-coding variation located on chromosome 9p21.3 adjacent to Cyclin-Dependent Kinase Inhibitor 2B (CDKN2B). Up to now, the A to G variation can be correlated with early onset of CHD. This variation affects the expression level of CDKN2B in many tissues including coronary artery smooth muscle cells [5,13]. For rs1333049, the carrying of C allele was found to be a risk factor for CHD patients in West Siberia. The SNP (single nucleotide polymorphism) allele C, when present in the heterozygous genotype (GC) elevated CHD risk by 15–20% and when present in the homozygous SNP genotype (CC) elevated CHD risk by 30–40% [14,15]. Most of these studies are pilot researches, and their findings are far from conclusive [16,17]. In 2018, two meta-analyses explored the association between CDKN2B-AS1 polymorphisms and coronary artery disease. One analysis involved only 9 studies based on the rs1333049 variant [18] and the other included 6 studies involving the rs4977574 polymorphism [19]. Up to now, there is still no prior study to determine whether CDKN2B-AS1 rs1333049 C and rs4977574 G allele can be used as a marker for the diagnosis or prognosis of CHD. The aim of the present research was to identify all eligible case-control studies to comprehensively investigate the correlation of CDKN2B-AS1 polymorphisms and CHD [20–58]. Furthermore, we used in silico analysis to investigate the genes and proteins that interact with CDKN2B.

Materials and methods

Search strategy

A literature search of Embase, PMC, Google Scholar, and Chinese Wanfang databases for relevant published articles was performed using the search term (‘rs4977574’ OR ‘rs1333049’ OR “CDKN2B antisense RNA” OR “CDKN2B-AS” OR “9p21” OR “ANRIL”) AND (“variant” OR “variant” OR “SNP”) AND (“myocardial infarction” OR ”coronary artery disease”). The most recent search update was 1 June 2020. Besides the use of databases, eligible studies were also retrieved by searching the references cited in the published articles.

Inclusion criteria and exclusion criteria

A publication was included in the analysis only if it met the following criteria: (a) Case–control study addressing the relationship between CDKN2B-AS1 rs1333049 and rs4977574 variants and CHD; (b) Study providing available genotypic frequencies of 9p21 region polymorphisms; and (c) Full text in English or other languages. Major exclusion criteria were (a) Duplicated studies using the same data; (b) Absence of a control group; and (c) No relevant to CDKN2B-AS1 variants and CHD.

Data extraction

Information retrieved from the included studies was as follows: First author name, date of
publication, region, and ethnicity of populations used, primary outcome, source of the control samples, total sample size, gene distribution of CDKN2B-AS1 variants, evaluation of Hardy-Weinberg equilibrium (HWE), and the genotyping method. In addition, studies including Asian population were divided into East Asia and West Asia. Two investigators independently carried out data extraction and quality evaluation and differences between them were resolved by discussions until a consensus was reached.

**Statistical analyses**

Strength of the correlation between CDKN2B-AS1 rs1333049 and rs4977574 variants and CHD susceptibility was investigated using odds ratios (ORs) together with 95% confidence intervals. Five genetic models were adopted to assess the likelihood of CDKN2B-AS1 polymorphisms. For SNP rs1333049 G/C, the allele comparison represents C-allele versus (vs.) G-allele; heterozygous contrast refers to CG vs. GG; homozygous contrast represents CC vs. GG; dominant model represents CC + CG vs. GG; and recessive model refers to CC vs. CG + GG. For SNP rs4977574 A/G, the five genetic models were G-allele vs. A-allele, GA vs. AA, GG vs. AA, GG+GA vs. AA, and GG vs. GA + AA. Cochran’s Q statistic was performed to calculate the heterogeneity between ORs. If the probability (P) value < 0.05 was considered as statistically significant, indicating heterogeneity among studies. In this case, a random-effects model was adopted. Otherwise, we carried out a fixed-effects model. The HWE P value was calculated using the Fisher’s exact test, with a P value < 0.05 indicating significant bias. Stratification analyses were carried out to investigate the strength of ethnicity, control source, and type of primary outcome. Begg’s funnel plot was adopted to assess the potential publication bias. P < 0.05 represents the significance exists. Sensitivity analyses were used to test the reliability of the included studies. All statistical methods were referring to the STATA 11.0 software of StataCorp (College Station, TX).

**In silico analysis of CDKN2B**

Differentially expressed genes between the CHD and control groups in the overall population were evaluated using an online database. Moreover, we checked the minor allele frequencies (MAFs) in worldwide populations based on the online database (https://www.ncbi.nlm.nih.gov/snp). The protein–protein interactions of CDKN2B were investigated using the STRING tools (https://string-db.org/cgi/input.pl).

**Results**

**Characteristics of eligible studies**

Fifty case-control studies comprising 35,915 CHD patients and 48,873 control subjects met the inclusion criteria and were summarized in the present study (Table 1). For the rs1333049 G/C variant, 33 studies with 20,365 cases and 29,413 controls were involved. In subgroup analysis by ethnicity, the sample population of 14 studies was of Europeans, 18 studies were of Asian descendants (divided into West Asians and East Asians), and one study was on the African population. Stratification analysis based on the source of controls used revealed that 14 studies were hospital based and 17 studies were population based. In a subgroup analysis by disease type, 22 studies focused on unclassified coronary artery disease and 11 studies focused on myocardial infarction. For the rs4977574 A/G polymorphism, the sample population of 8 studies was of European descendants and 9 studies was of Asian populations (4 studies were of West Asians and 5 were of East Asians). Stratification analysis based on the source of controls revealed 7 studies as hospital based and 10 studies as population based. We also determined the MAFs in the overall and subpopulations. The MAFs for the SNP rs1333049 G/C variant were as follows: global population, 0.418; Africans, 0.213; East Asians, 0.537; European descendants, 0.472; South Asians, 0.491; and Americans, 0.455. In the current study, the MAF in case was 0.521; and in control was 0.489. The MAFs for the SNP rs4977574 were as follows: global population, 0.395; Africans,
### Table 1. Study characteristics of CDKN2B-AS1 rs1333049 G/C and rs4977574 A/G variants included in the present analysis.

| First author | Year | Origin | Type | Ethnicity | Source of control | Case | Control | Case | Control | HWE | Method |
|--------------|------|--------|------|-----------|------------------|------|---------|------|---------|------|---------|
| Suleiman     | 2019 | Iraq   | CAD  | West Asian| Hospital based   | 50   | 50      | 9    | 22      | 19  | 4       | 23   | 23  | 0.595 | Primex PCR |
| Shakhtsneider| 2019 | Russia | MI   | Caucasian | Population based | 118  | 2610    | 39   | 51      | 28  | 554     | 1330 | 726 | 0.228 | RT-PCR |
| Kalpana      | 2019 | India  | CAD  | West Asian| Population based | 91   | 436     | 30   | 38      | 23  | 102     | 222  | 112 | 0.693 | MassARRAY |
| Huang        | 2019 | China  | CAD  | East Asian| Hospital based   | 501  | 496     | 110  | 263     | 128 | 94      | 254  | 148 | 0.417 | MassARRAY |
| Kashyap      | 2018 | India  | CAD  | West Asian| Hospital based   | 512  | 272     | 117  | 316     | 79  | 46      | 176  | 50  | <0.001 | PCR-RFLP |
| Yang         | 2018 | China  | CAD  | East Asian| Hospital based   | 542  | 549     | 111  | 269     | 162 | 100     | 273  | 176 | 0.743 | MassARRAY |
| Pigkataro    | 2017 | Italy  | CAD  | Caucasian | NA               | 711  | 755     | 251  | 342     | 118 | 215     | 391  | 149 | 0.229 | NA |
| Li           | 2017 | China  | CAD  | East Asian| NA               | 555  | 480     | 198  | 239     | 118 | 129     | 223  | 128 | 0.121 | TaqMan |
| Haslacher    | 2016 | Austria| MI   | Caucasian | Population based | 493  | 431     | 118  | 236     | 139 | 97      | 222  | 112 | 0.514 | Taqman |
| Foroughmand  | 2015 | Iran   | CAD  | West Asian| Hospital based   | 170  | 100     | 31   | 111     | 28  | 25      | 67   | 8  | <0.001 | ARMS-PCR |
| Cakmak       | 2015 | Turkey | CAD  | Caucasian | Hospital based   | 220  | 240     | 54   | 120     | 46  | 85      | 115  | 40  | 0.917 | RT-PCR |
| Pinos        | 2014 | Spain  | CAD  | Caucasian | Hospital based   | 152  | 343     | 45   | 53      | 54  | 105     | 153  | 85  | 0.052 | TaqMan |
| Pinos        | 2014 | Japan  | CAD  | East Asian| Hospital based   | 742  | 920     | 158  | 373     | 211 | 193     | 485  | 242 | 0.082 | TaqMan |
| Jansen       | 2014 | Norway | CAD  | Caucasian | Population based | 818  | 2094    | 238  | 368     | 212 | 647     | 1009 | 438 | 0.224 | MassARRAY |
| Gong         | 2014 | China  | CAD  | East Asian| Hospital based   | 545  | 725     | 133  | 248     | 164 | 160     | 358  | 207 | 0.824 | MassARRAY |
| Bhanushali   | 2013 | India  | CAD  | East Asian| Hospital based   | 97   | 151     | 33   | 57      | 7   | 34      | 80   | 37  | 0.461 | Taqman |
| Bhanushali   | 2013 | India  | MI   | East Asian| Hospital based   | 120  | 151     | 38   | 60      | 22  | 34      | 80   | 37  | 0.461 | Taqman |
| Zeng         | 2013 | China  | CAD  | East Asian| Population based | 359  | 398     | 110  | 168     | 81  | 75      | 197  | 126 | 0.897 | PCR-RFLP |
| Ahmed        | 2013 | Pakistan| MI  | West Asian| Hospital based   | 294  | 290     | 63   | 166     | 65  | 23      | 180  | 87  | <0.001 | Taqman |
| Qi           | 2012 | China  | MI   | East Asian| Hospital based   | 142  | 192     | 21   | 79      | 42  | 43      | 99   | 50  | 0.651 | PCR-RFLP |
| Lin          | 2011 | Taiwan | MI   | East Asian| Hospital based   | 423  | 1361    | 105  | 218     | 100 | 311     | 655  | 395 | 0.213 | Taqman |
| Guo          | 2011 | China  | CAD  | East Asian| Population based | 670  | 1340    | 156  | 327     | 187 | 358     | 661  | 321 | 0.643 | RT-PCR |
| Xie          | 2011 | China  | CAD  | East Asian| Population based | 2305 | 1061    | 659  | 1140    | 506 | 241     | 525  | 295 | 0.810 | Taqman |
| Scheffold    | 2011 | Germany| MI   | Caucasian | Population based | 976  | 999     | 246  | 518     | 212 | 205     | 502  | 292 | 0.688 | RT-PCR |
| Mendonca     | 2011 | Portugal| CAD | Caucasian | Population based | 723  | 683     | 258  | 348     | 117 | 200     | 321  | 162 | 0.136 | Taqman |
| Ghazoouani   | 2010 | Tunisia | CAD  | African | Population based | 292  | 323     | 72   | 137     | 83  | 88      | 151  | 84  | 0.244 | Taqman |
| Saleheen     | 2010 | Pakistan| MI  | West Asian| Population based | 2387 | 2573    | 697  | 1273    | 617 | 609     | 1290 | 674 | 0.865 | IBC array |
| Peng         | 2009 | China  | MI   | East Asian| Population based | 520  | 560     | 156  | 265     | 99  | 116     | 285  | 159 | 0.572 | Taqman |
| Hiura        | 2008 | Japan  | MI   | East Asian| Population based | 586  | 2432    | 170  | 279     | 137 | 592     | 1204 | 636 | 0.638 | Taqman |
| Hinohara     | 2008 | Korea  | CAD  | East Asian| Hospital based   | 679  | 706     | 186  | 335     | 158 | 161     | 353  | 192 | 0.959 | Taqman |
| Hinohara     | 2008 | Japan  | CAD  | East Asian| Hospital based   | 604  | 1151    | 178  | 312     | 114 | 259     | 606  | 286 | 0.069 | Taqman |
| Samani       | 2007 | Germany| MI   | Caucasian | Population based | 844  | 1605    | 158  | 453     | 233 | 425     | 831  | 349 | 0.130 | GeneChip |
| Samani       | 2007 | UK     | CAD  | Caucasian | Population based | 1924 | 2936    | 586  | 960     | 378 | 676     | 1431 | 829 | 0.222 | GeneChip |

### (Continued)
The strength of the correlation between CDKN2B-ASI SNPs rs1333049 and rs4977574 is summarized in Table 2. For the rs1333049 G/C variation, when all studies pooled together, we observed that individuals carrying CC allele had a 1.29-fold higher risk of CHD than those carrying GG allele (95% CI = 1.11–1.49, \( P = 0.001 \), Figure 2(a)). In subgroup analyses, we revealed that West Asians with CC allele had a 1.73-fold increased susceptibility than those with GG allele (95% CI = 1.14–2.64, \( P = 0.011 \)). For East Asians, the ratio was 1.32 (95% CI = 1.11–1.57, \( P = 0.001 \), Figure 2(a)). Moreover, similar findings were indicated for the subgroup with population-based control (C allele vs. G allele, OR = 1.15, 95% CI = 1.04–1.27, \( P = 0.006 \); CC vs. GG, OR = 1.32, 95% CI = 1.08–1.60, \( P = 0.006 \); dominant model, OR = 1.17, 95% CI = 1.02–1.35, \( P = 0.028 \); and recessive model, OR = 1.23, 95% CI = 1.08–1.39, \( P = 0.002 \), Figure 3(a)). In stratification by phenotype of CHD, we identified that individuals with CC allele had a 1.26-fold higher risk of coronary artery disease than those with GG allele (95% CI = 1.05–1.51, \( P = 0.012 \)). For myocardial infarction groups, the ratio was 1.25 (95% CI = 1.01–1.53, \( P = 0.037 \), Figure 4(a)). For the rs4977574 A/G variant, a positive association was observed for all studies when combined. Individuals carrying GG allele had a 1.39-fold higher risk of CHD than those carrying AA allele (95% CI = 1.16–1.67, \( P < 0.001 \), Figure 2(b)). Stratification analysis revealed West Asians with GG allele had a 1.28-fold increased susceptibility than those with AA allele (95% CI = 1.12–1.46, \( P < 0.001 \), Figure 2(b)). For East Asians the ratio was 1.53 (95% CI = 1.13–2.08, \( P = 0.006 \), Figure 3(b)). In subgroup analysis by phenotype, we revealed that individuals carrying GG allele had a 1.43-fold increased susceptibility of coronary artery disease than those with AA allele (95% CI = 1.13–1.82, \( P = 0.004 \)). The ratio was 1.38 in
myocardial infarction groups (95%CI = 1.06–1.79, $P = 0.018$, Figure 4(b)).

**In silico analysis of CDKN2B**

Protein-protein crosstalk of CDKN2B was investigated by the STRING tools. Interaction of at least 20 proteins with CDKN2B was identified in Figure 5. The most relevant interactions were with the following proteins: Cyclin-Dependent Kinase (CDK) 4, CDK 6, Cyclin-Dependent Kinase Inhibitor (CDKN) 1A, CDKN 1B, CDKN 1C, Mothers against decapentaplegic homolog (SMAD) 4, G1/S-specific cyclin-D (CCND) 1, CCND 2, SMAD 3, and SMAD 2 (Figure 5(b)). The online database was also utilized to assess the differentially expressed genes between the CHD and control groups (Figure 6(a)). The most probable correlations with CDKN2B in CHD included the genes for latent transforming factor beta binding protein 2 (LTBP2, Figure 6(b)), integrin subunit alpha 11 (ITGA11, Figure 6(c)), and collagen type VIII alpha 2 chain (COL8A2, Figure 6(d)).

**Publication bias and sensitivity analysis**

We constructed the Begg’s funnel plots to detect the publication bias among the included studies. We identified no significant asymmetry of the funnel plots in any of these models when evaluating the variants of rs1333049 (Figure 7(a), $P > 0.05$) and rs4977574 (Figure 7(b), $P > 0.05$). Furthermore, we conducted sensitivity analysis by removing single studies. Single study did not have an impact on the significance of ORs for both rs1333049 G/C (Figure 7(c)) and rs4977574 A/G (Figure 7(d)) polymorphisms.

**Discussion**

CHD is still the main cause of mortality globally and imposes a huge social and economic burden [59,60]. The relationship between the CDKN2B-AS1 variants rs1333049 and rs4977574 and the risk of CHD has been previously reported; however, a comprehensive analysis of the relationship was not available. Several meta-analyses have pooled the data of various studies; however, the number of studies included was insufficient. In 2018, Xu et al evaluated six articles on CDKN2B-AS1 SNP rs4977574 indicating increased likelihood of CHD due to the variation [19]. Hu et al in 2019 evaluated the association between SNP rs1333049 and CHD using 7 studies and reported increased risk of CHD with rs1333049 in the East Asian population [61]. The present analysis, which involved a total of 50 case-control studies with 35,915 CHD patients and 48,873 control subjects, is by far the most comprehensive analysis evaluating the relationship between CDKN2B-AS1 variants rs1333049 and rs4977574 and the risk of CHD. Our analysis revealed a significant association of rs1333049 G/C and rs4977574 A/G variants.
Table 2. Stratified analysis of CDKN2B-AS1 rs1333049 and rs4977574 variants on susceptibility to coronary heart disease.

| Variables | N | Case/Control | OR(95%CI) | p-value (OR) | p-value (OR) | p-value (OR) | p-value (OR) | p-value (OR) | p-value (OR) | p-value (OR) |
|-----------|---|--------------|-----------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| rs1333049 G/C | 20365/29413 | 1.13(1.05–1.21) | 0.001 | 0.108(0.99–1.18) | 0.001 | 1.29(1.11–1.49) | 0.001 | 1.14(1.03–1.27) | 0.001 | 1.21(1.10–1.34) | 0.001 |
| Ethnicity | | | | | | | | | | | |
| West Asian | 8 | 3921/4023 | 1.25(1.07–1.45) | 0.005 | 1.10(0.98–1.23) | 0.072 | 0.91 | 1.73(1.14–2.64) | 0.001 | 1.26(0.98–1.62) | 0.018 | 1.52(1.14–2.01) | 0.002 |
| Caucasian | 14 | 6979/12696 | 1.05(0.89–1.24) | 0.575 | 1.01(0.82–1.25) | 0.001 | 0.916 | 1.10(0.79–1.53) | 0.001 | 1.04(0.81–1.33) | 0.001 | 1.10(0.89–1.35) | 0.001 |
| East Asian | 10 | 9173/12371 | 1.15(1.06–1.25) | 0.001 | 1.12(1.02–1.23) | 0.039 | 0.023 | 1.32(1.11–1.57) | 0.001 | 1.18(1.05–1.33) | 0.001 | 1.23(1.09–1.39) | 0.001 |
| African | 1 | 292/323 | 0.90(0.72–1.25) | 0.381 | 0.92(0.63–1.34) | 0.661 | 0.395 | 0.83(0.54–1.28) | 0.91 | 0.89(0.62–1.26) | 0.501 | 0.87(0.61–1.25) | 0.465 |
| Source | | | | | | | | | | | | |
| HB | 14 | 4510/5840 | 1.07(0.96–1.20) | 0.001 | 1.03(0.88–1.21) | 0.008 | 0.696 | 1.20(0.92–1.57) | 0.001 | 1.07(0.90–1.27) | 0.001 | 1.15(0.95–1.39) | 0.001 |
| PB | 17 | 14589/22338 | 1.15(1.04–1.27) | 0.006 | 1.11(0.98–1.25) | 0.001 | 0.092 | 1.32(1.08–1.60) | 0.001 | 1.17(1.02–1.35) | 0.001 | 1.23(1.08–1.39) | 0.001 |
| NA | 2 | 1266/1235 | 1.27(1.13–1.42) | 0.001 | 1.13(0.92–1.39) | 0.010 | 0.249 | 1.56(1.24–1.95) | 0.001 | 1.29(1.06–1.56) | 0.067 | 1.43(1.20–1.69) | 0.001 |
| Phenotype | | | | | | | | | | | | |
| CAD | 22 | 13262/16209 | 1.12(1.02–1.22) | 0.001 | 1.06(0.94–1.20) | 0.001 | 0.307 | 1.26(1.05–1.51) | 0.001 | 1.12(0.98–1.29) | 0.001 | 1.20(1.08–1.34) | 0.001 |
| MI | 11 | 7103/13204 | 1.15(1.01–1.30) | 0.001 | 1.11(0.98–1.27) | 0.010 | 0.102 | 1.35(1.02–1.77) | 0.001 | 1.17(1.00–1.38) | 0.001 | 1.25(1.01–1.53) | 0.001 |
| rs4977574 A/G | 15550/19460 | 1.18(1.07–1.29) | 0.001 | 1.16(1.05–1.29) | 0.001 | 0.003 | 1.39(1.16–1.67) | 0.001 | 1.24(1.09–1.40) | 0.001 | 1.26(1.10–1.44) | 0.001 |
| Ethnicity | | | | | | | | | | | | |
| West Asian | 4 | 4294/3528 | 1.13(1.06–1.21) | 0.001 | 1.03(0.92–1.16) | 0.372 | 0.607 | 1.28(1.12–1.46) | 0.057 | 1.11(0.99–1.25) | 0.007 | 1.24(1.12–1.38) | 0.045 |
| Caucasian | 8 | 7392/12030 | 1.18(1.00–1.40) | 0.005 | 1.18(0.99–1.40) | 0.001 | 0.071 | 1.33(0.99–1.94) | 0.001 | 1.25(0.99–1.56) | 0.001 | 1.23(0.97–1.56) | 0.001 |
| East Asian | 5 | 3864/3902 | 1.12(0.83–1.43) | 0.002 | 1.22(1.10–1.37) | 0.633 | 0.001 | 1.53(1.13–2.08) | 0.002 | 1.31(1.18–1.45) | 0.137 | 1.29(0.97–1.72) | 0.001 |
| Source | | | | | | | | | | | | |
| HB | 7 | 2257/1892 | 1.17(0.93–1.47) | 0.001 | 1.27(1.08–1.48) | 0.190 | 0.003 | 1.39(0.91–2.13) | 0.001 | 1.27(0.96–1.66) | 0.012 | 1.23(0.87–1.74) | 0.024 |
| PB | 10 | 13293/17568 | 1.18(1.06–1.31) | 0.001 | 1.14(1.02–1.28) | 0.001 | 0.027 | 1.38(1.12–1.70) | 0.001 | 1.22(1.05–1.40) | 0.001 | 1.27(1.09–1.47) | 0.001 |
| Phenotype | | | | | | | | | | | | |
| CAD | 9 | 5747/5660 | 1.18(1.04–1.34) | 0.001 | 1.28(1.16–1.41) | 0.199 | 0.001 | 1.43(1.13–1.82) | 0.001 | 1.28(1.09–1.51) | 0.001 | 1.28(1.04–1.57) | 0.001 |
| MI | 8 | 9803/13800 | 1.18(1.03–1.35) | 0.001 | 1.14(0.99–1.31) | 0.001 | 0.065 | 1.38(1.06–1.79) | 0.001 | 1.22(1.03–1.45) | 0.001 | 1.27(1.02–1.50) | 0.001 |

CAD: Coronary artery disease; HB: Hospital based; MI: Myocardial Infarction; NA: Not applicable; PB: Population based.

*Number of comparisons

p_{het}: P value of Q-test for heterogeneity test.
with the likelihood of CHD, when all studies were pooled together.

For the SNP rs1333049, C allele was a risk factor for both West Asians and East Asians in the subgroup analysis by race. In the stratified analysis by source of control population, there is a positive correlation between rs1333049 variant and population-based studies. In a subgroup analysis based
on disease type, we observed that individuals carrying CC allele had an increased susceptibility of coronary artery disease and myocardial infarction patients’ group. Our conclusion is not consistent with the meta-analysis performed by Xie et al, who observed no positive relationship between this variant and susceptibility of myocardial infarction groups (allele contrast, $P$ value $= 0.17$, OR $= 0.87$, 95% confidence intervals $= 0.72–1.06$; dominant comparison, $P$ value $= 0.14$, OR $= 0.83$, 95% confidence intervals $= 0.64–1.07$; recessive genetic model, $P$ value $= 0.28$, OR $= 1.25$, 95% confidence intervals $= 0.84–1.86$) [18]. A possible reason for the difference in study outcomes may be the relatively small number of studies included in their meta-analysis. For the SNP rs4977574, we detected a significant correlation between the G allele and the risk of CHD among West Asian and East Asian populations in a stratification analysis by ethnicity and the findings are consistent with the results in a previous study [62]. In stratification analysis by control population source, there was a positive correlation with population-based studies. Based on previous randomized controlled trial, CDKN2B-AS1 rs1333049 G/C and rs4977574 A/G variants were not correlated with higher risk in African patients with CHD [63].

Evidence from genome-wide association study showed that no major locus could individually reveal the high risk of coronary heart disease in African Americans [64]. Moreover, we checked the MAFs in worldwide populations based on the online database. The MAF for the CDKN2B-AS1 rs1333049 G/C variant in Africans is 0.21. It is lower than that in other populations and global average. Similar result was indicated for the rs4977574 A/G variant. A possible reason is that CDKN2B-AS1 rs1333049 G/C and rs4977574 A/G variants may be not associated with the CHD susceptibility in African population. Additionally, an online database was employed to explore differentially expressed genes between the CHD and control groups. We found that expression of LTBP2, ITGA11, and COL8A2 correlated with the expression of CDKN2B in CHD. The online database contains scant data on the specific mechanism of these genes. Future functional analyses and in vitro experiments are needed to demonstrate the correlations in detail.

The current analysis has several limitations. First, we observed significant heterogeneity in the overall analysis when evaluating the

![Figure 4](image-url)
CDKN2B-AS1 rs1333049 G/C and rs4977574 A/G variations. Although the DerSimonian and Laird method was employed [65], potential bias may influence the conclusion. Second, the
pathogenesis of CHD is very complex. Thus, a single gene polymorphism is unlikely to make a significant contribution to its development. All OR values obtained in the current study are all < 2. Therefore, further studies elucidating the gene-gene or gene-environment connections to demonstrate correlation are recommended. In addition, the analysis of the protein-protein crosstalk of CDKN2B by the STRING tool, identified interactions with more than 20 proteins (Figure 5), however, these interactions need be confirmed by in vitro and in vivo analyses. Third, the study does not include adjusted analysis for sex, lifestyle, and smoking exposure, which may have helped in better segregation and evaluation of the different groups.

**Conclusion**

Taken together, our study demonstrates that CDKN2B-AS1 rs1333049 C allele and rs4977574 G allele is correlated with the risk of CHD. These polymorphisms may serve as genetic biomarkers for CHD, especially in people of East and West Asian ancestry.
YW and LFZ conceived of the study, WZ, ZBR and LZ prepared the data, WZ and YL were involved in the data analyses, WZ and YYM drafted the original manuscript. YYM and LFZ prepared the figures. All the authors agreed to the submission of the present work.

Disclosure statement
The authors declare that they have no competing financial interests.

Data availability
All data in the present research are available from this manuscript.

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Figure 7. Publication bias and sensitivity analysis for CDKN2B-AS1 rs1333049 G/C and rs4977574 A/G polymorphisms. We revealed no evidence of publication bias according to rs1333049 G/C (a) and rs4977574 (b). No significant change of the result was detected in the sensitivity analysis for rs1333049 G/C (c) and rs4977574 (d) variants.
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