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*Review Article*

# Association between CDKN2B-AS gene rs4977574 polymorphism and susceptibility to coronary heart disease: evidence from 124,752 subjects

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**Abstract:** Objectives: To investigate the association between the CDKN2B-AS rs4977574 polymorphism and coronary heart disease. Methods: We performed a computerized comprehensive search on PubMed, Embase, OVID, Web of Science, CNKI, and Wan Fang databases up to December of 2016. Two of the authors individually extracted study data and assessed the study quality using NOS scale. Odds ratios (ORs) and 95% confidence intervals (CIs) were combined for evaluation using a random effect model or fixed effect model according to study heterogeneity. Results: There were totally twenty case-control studies of 51,522 patients and 73,230 healthy controls included. Significant associations were found between CDKN2B-AS rs4977574 polymorphism and CHD in overall population (OR = 1.26, 95% CI = 1.21-1.32, p<0.001, $I^2$ = 79.2%). In subgroup analysis by ethnicity, we found significant association between the CDKN2B-AS rs4977574 polymorphism and CHD risk in both Asian (OR = 1.28, 95% CI = 1.19-1.38, $I^2$ = 68.7%) and Caucasian (OR = 1.25, 95% CI = 1.18-1.32, $I^2$ = 85.9%). Sensitivity analysis did not indicate any meaningful alternation in the odds ratios. The Begg's funnel plot was used to assess the publication bias, showing no evidence of publication bias visually. Conclusion: The rs4977574 polymorphism in the CDKN2B-AS gene has susceptibility to CHD in Asians as well as Caucasians, indicating that the rs4977574 polymorphism of CDKN2B-AS gene may contribute to the pathogenesis of CHD.

**Keywords:** Single nucleotide polymorphism, CDKN2B-AS, CHD, meta-analysis

## Introduction

Coronary heart disease (CHD), occupies the most important cause of mortality and morbidity worldwide [1]. It presents as a complex traits disease that includes asymptomatic myocardial ischemia, angina, myocardial infarction, ischemic cardiomyopathy and sudden cardiac death. CHD has been proved to be associated with hypertension, diabetes mellitus, hyperlipidemia and smoking. Actually, CHD is a polygenic disease resulting from complicated interactions between multiple genes and environmental exposures [2, 3].

It has been estimated that in the susceptibility to coronary heart disease, heritable factors account for 30%~60% in the inter-individual variation [4]. Despite extensive exploration of various genes, strong evidence of a molecular genetic association with angina pectoris or myocardial infarction remains to be obtained. Thus, in order to provide better measures for CHD. risk assessment as well as prevention and treatment, we urge to identify the underlying genetic architecture of CHD.

More than 400 genes have been revealed to be associated with the incidence, pathogenesis, and progression of CHD [5]. Recently nine single nucleotide polymorphisms were indicated by GWAS to be associated with CHD in Caucasians [6, 7]. These SNPs were rs4977574 of the CDKN2A/CDKN2B gene, rs10953541 of the BCAP-29 gene, rs1122608 of the LDLR gene, rs12190287 of the TCF21 gene, rs1241-3409 of the CNNM-2 gene, rs1412444 of the LIPA gene, rs1746048 of the CXCL-12 gene, rs3798220 of the LPA gene, and rs579459 of the ABO gene.

CDKN2B-AS gene is located in 9p21.3 region which is also called the "9p21.3 CHD risk interval". Just as its name implies, this 58-kb region containing multiple genetic variants can cause linkage disequilibrium, which is considered to be the most important locus in the pathogenesis of coronary heart disease [8]. CDKN2B-AS is also named antisense non-coding RNA in the INK4 locus (ANRIL) that locates within the CDKN2A-CDKN2B gene cluster [9-11]. Through immunologic technology, we can detect differential expression of CDKN2B-AS gene in a variety of tissues, especially in coronary smooth muscle cells (SMCs) and vascular endothelial cells [12-15]. As a result, the proliferation of coronary SMCs and vascular endothelial cells are influenced by alternation of CDKN2B-AS expression [16]. CDKN2B-AS may be a promising biomarker for assessing the risk of atherosclerosis and coronary heart diseases [17].

To date, there has been no GWAS studying the association between CDKN2B-AS rs4977574 polymorphism and the risk of CHD in Asians. For the past three years, many case-control designed studies [18-26] have investigated the association between the CDKN2B-AS polymorphism and CHD susceptibility in Asians and Caucasians but because of the low statistical power and small sample size of individual studies, the consolidated result is still controversial. So we conducted the present meta-analysis to: validate the association between rs4977574 and CHD in Caucasians and evaluate whether the association exists in Asians.

## Methods

We reported our meta-analysis according to MOOSE proposal [27].

### *Search strategy*

We conducted a systematic computerized search of literature to identify relevant English articles in PubMed, Embase, OVID, Web of Science, CNKI, and Wan Fang databases up to December of 2016, combined with a manual search of reference lists from identified articles. For the literature search, the following combination of medical subject headings or suitable key words was used: coronary heart disease, ischemic heart disease, myocardial infarction, coronary artery disease or angina pectoris and CDKN2B-AS or rs4977574 and polymorphism. We have also searched individually to discover possible eligible studies containing in the references of review articles and of all the obtained case-control studies.

### *Selection and exclusion criteria*

To elaborate the selection for studies obtained in this meta-analysis, the inclusion criteria were pre-established: 1) case-control designed studies; 2) studies evaluated the association of the CDKN2B-AS rs4977574 polymorphism and susceptibility to CHD; 3) studies that provided sufficient data to calculate odds ratios and 95% confidential intervals for extraction. The criteria for exclusion were: 1) studies that gave too limited data for extraction; 2) abstracts-only articles, reviews, meta-analysis and unpublished studies; 3) inclusion of data duplicated in other studies.

### *Data extraction*

Two of the authors (Y.F. Jiang M.D. and M. Chen M.D.) individually extracted all useful data of each study involving in this meta-analysis. Conflicts were discussed with a third investigator (Y.F. Zhou PhD.). Extraction of study data includes: first author; publication year; country of the work established, number of patients and control individuals; ethnicities, genotyping method, population-based, odds ratios(OR) and 95% confidential intervals (CI). We made attempts to contact the original authors for detailed information if the data were incomplete or missing in the publication. Study quality was evaluated according to the 9-point Newcastle-Ottawa Scale (NOS) [28].

### *Statistical analysis*

We evaluate the strength of the associations between CDKN2B-AS rs4977574 polymorphism and susceptibility to CHD by combining odds ratios (OR) and 95% confidence intervals (95% CI) under a fixed or randomized effect model according to the quantification of the heterogeneity calculated with the $I^2$ test. $I^2$ ranges between 0 to 100% and represents the extent of inter-study heterogeneity. A random effect model for pooled analysis should be adopted when $I^2 > 50\%$ indicating heterogeneity among studies. Otherwise the fixed effect model should be used. Sensitivity analysis was

**Table 1.** Characteristics of the studies included for meta-analysis

| Author | Year | Country | Ethnicity | Source of control | Genotyping method | Cases | Controls | Patients | Risk allele | Study design | NOS | HWE (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cao [18] | 2016 | China | Asian | Hospital-based | PCR-RFLP | 565 | 541 | CHD | G | Case-control | 8 | Y |
| AbdulAzeez [19] | 2016 | Saudi Arabia | Asian | Hospital-based | TaqMan | 250 | 252 | CHD | G | Case-control | 7 | Y |
| Zheng [20] | 2016 | US | Caucasian | Population-based | TaqMan | 1560 | 1751 | MI | G | Case-control | 8 | Y |
| Tang [21] | 2016 | China | Asian | Hospital-based | TaqMan | 289 | 338 | CHD | G | Case-control | 8 | Y |
| Matsuoka [22] | 2015 | Japan | Asian | Hospital-based | RT-PCR | 1822 | 2284 | MI | G | Case-control | 8 | Y |
| Hindy [23] | 2014 | Sweden | Caucasian | Population-based | TaqMan | 3164 | 20785 | CHD | G | Case-control | 8 | Y |
| Wang [24] | 2014 | China | Asian | Population-based | TaqMan | 2365 | 2678 | MI | G | Case-control | 8 | Y |
| Shanker [25] | 2014 | India | Asian | Population-based | TaqMan | 1034 | 1034 | CHD | G | Case-control | 8 | Y |
| Huang [26] | 2014 | China | Asian | Hospital-based | RT-PCR | 590 | 482 | CHD | G | Case-control | 8 | Y |
| Lee [29] | 2013 | Korea | Asian | Population-based | Affymetrix | 2293 | 4302 | CHD | G | Case-control | 8 | Y |
| Tragante [30] | 2013 | Netherlands | Caucasian | Population-based | TaqMan | 3788 | 2015 | CHD | G | Case-control | 8 | Y |
| Lin [31] | 2012 | China | Asian | Hospital-based | PCR-RFLP | 142 | 192 | MI | G | Case-control | 7 | Y |
| Qi [32] | 2011 | US | Caucasian | Hospital-based | TaqMan | 1989 | 2096 | MI | G | Case-control | 8 | Y |
| Peden1 [33] | 2011 | UK | Caucasian | Hospital-based | Illumina | 8424 | 7268 | CHD | G | Case-control | 8 | Y |
| Peden2 [33] | 2011 | UK | Asian | Hospital-based | Illumina | 6996 | 7794 | CHD | G | Case-control | 8 | Y |
| Erdmann [34] | 2011 | Germany | Caucasian | Population-based | RT-PCR | 1157 | 1748 | CHD | G | Case-control | 8 | Y |
| Saade [35] | 2011 | Lebanon | Asian | Hospital-based | Illumina | 1524 | 425 | CHD | G | Case-control | 8 | Y |
| Davies [36] | 2010 | Canada | Caucasian | Hospital-based | Affymetrix | 3323 | 2319 | CHD | G | Case-control | 8 | Y |
| Kathiresan [37] | 2009 | US | Caucasian | Hospital-based | Affymetrix | 2967 | 3075 | MI | G | Case-control | 8 | Y |
| Helgadottir [38] | 2007 | Iceland | Caucasian | Population-based | Illumina | 4479 | 7269 | MI | G | Case-control | 8 | Y |
| Samani [39] | 2007 | UK | Caucasian | Population-based | Affymetrix | 2801 | 4582 | CHD | G | Case-control | 8 | Y |

Case-control design was used in all the included studies. year = publication year; NOS = Newcastle-Ottawa scale; HWE = Hardy-Weinberg equilibrium, CHD = coronary heart disease, MI = myocardial infarction.

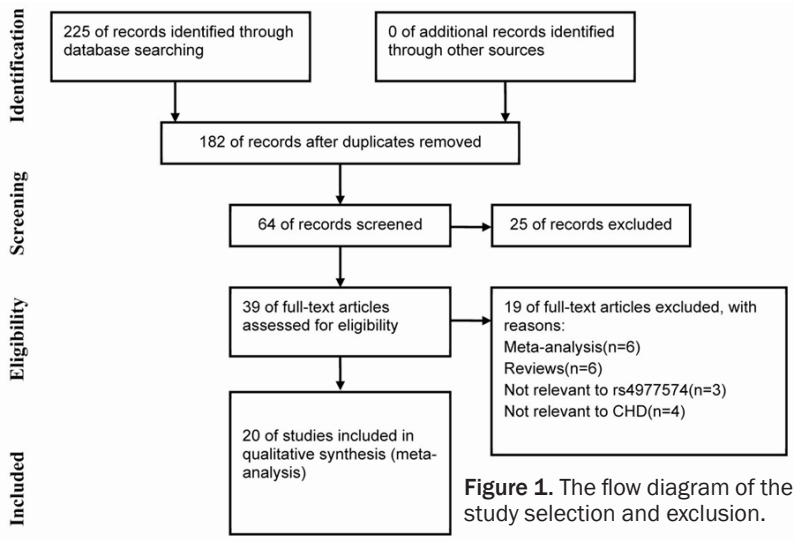


**Figure 1.** The flow diagram of the study selection and exclusion.

performed by combining ORs repeatedly with omission of each study to identify potential alternation of the overall meta result. We have also investigated publication bias vias calculating Begg's and Egger's test and drawing Begg's funnel plot. P > 0.05 was considered that there was no statistically significant bias of publication. Meta-analysis was performed using Stata version 14.0 (Stata Corporation, USA).

## Results

### *Study characteristics*

The search of the six databases identified 225 records in total. After removing duplicated studies, there were 182 studies left for screening and 25 of records were excluded. 39 studies were read by full-text, and 19 of full-text articles were excluded, including 6 meta-analyses, 6 reviews, 3 articles not relevant to rs4977574 and 4 articles not relevant to CHD. There were eventually twenty studies [18-26, 29-39] of 51,522 cases and 73,230 controls eligible for this meta-analysis on the relationship between CDKN2B-AS gene rs4977574 polymorphism and CHD. All of these articles were published in English. The sample sizes ranged from 334 to 23,949 of all eligible studies. The races of the included studies were Asian (n = 11) and Caucasian (n = 10). (Peden's study included different stages of Asian and

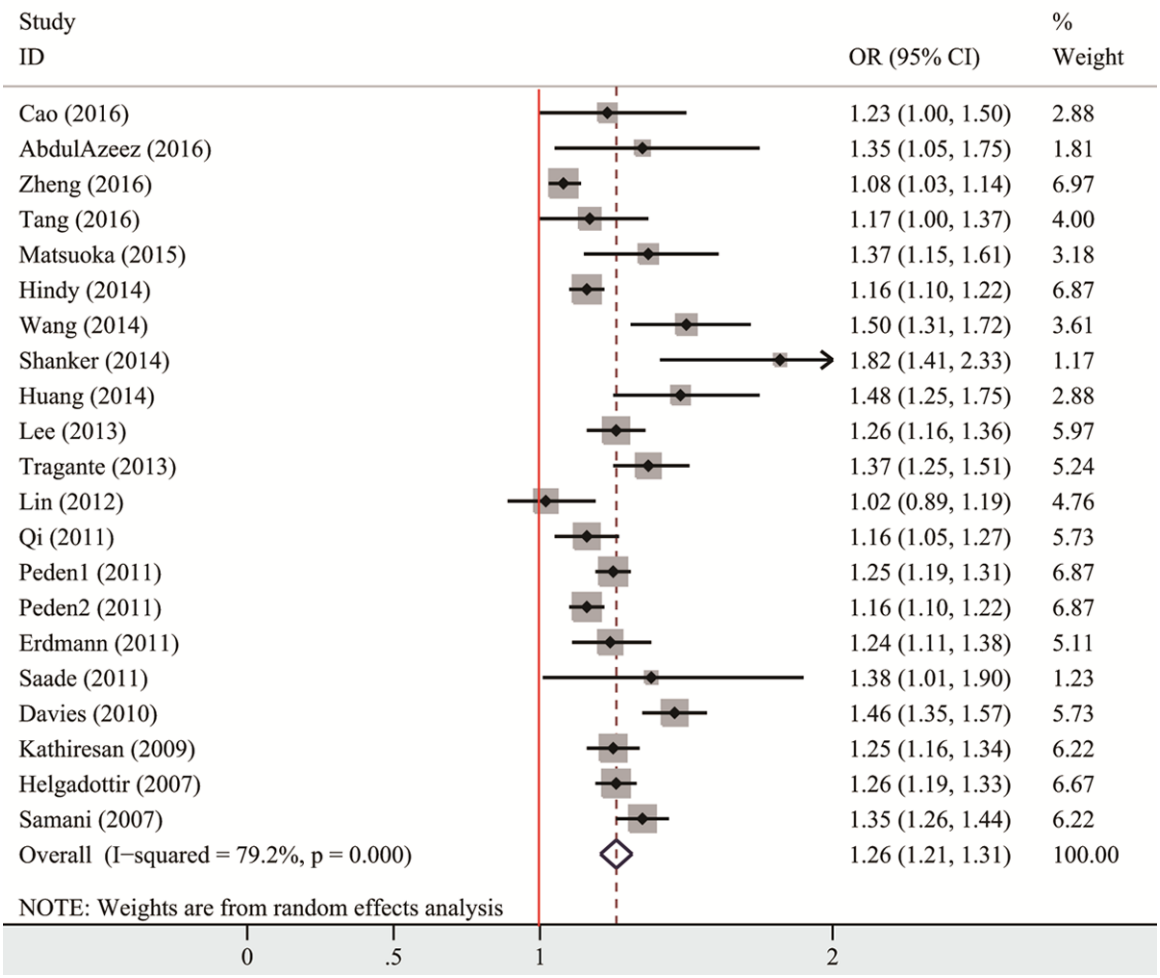


**Figure 2.** Forest plot from the meta-analysis on the association of CDKN2B-AS rs4977574 polymorphism and CHD risk in allele model (G vs A allele distribution frequency of CDKN2B-AS gene rs4977574 polymorphism). CHD = coronary heart disease, CI = confidence interval, OR = odds ratio.

Caucasian, so we divided it into two parts for meta-analysis and subgroup analysis). All the included studies fitted in with the HWE test. The NOS scores of all eligible studies in our meta-analysis were higher than 6 points, representing a good study quality. Characteristics of the studies included for meta-analysis are shown in **Table 1**. **Figure 1** shows the complete procedure of the study selection and exclusion.

*Quantitative synthesis*

Combining the 20 included studies on the association between the CDKN2B-AS gene rs4977574 polymorphism and susceptibility to CHD provided 51,522 patients and 73,230 controls for this meta-analysis. To analyze the whole datasets, a randomized effect model was used to perform the pooled analysis regarding to $I^2$ > 50%. A significant increased risk of CHD was found to be related to the CDKN2B-AS gene rs4977574 polymorphism in overall population (OR = 1.26; 95% CI = 1.21 to 1.32; p<0.001; $I^2$ = 79.2%). The forest plot is shown in **Figure 2**.

The analyses above found noticeable heterogeneity according to $I^2$ > 50%. Therefore, we conducted stratified analyses to explore the source of heterogeneity. In subgroup analysis by ethnicity, we found significant association between the CDKN2B-AS rs4977574 polymorphism and CHD risk in both Asian (OR = 1.28, 95% CI = 1.19-1.38) and Caucasian (OR = 1.25, 95% CI = 1.18-1.32). A randomized effect model was used to perform this pooled analysis regarding to $I^2$ > 50%. **Figure 3** shows the forest plot of subgroup meta-analysis result of ethnicity. We

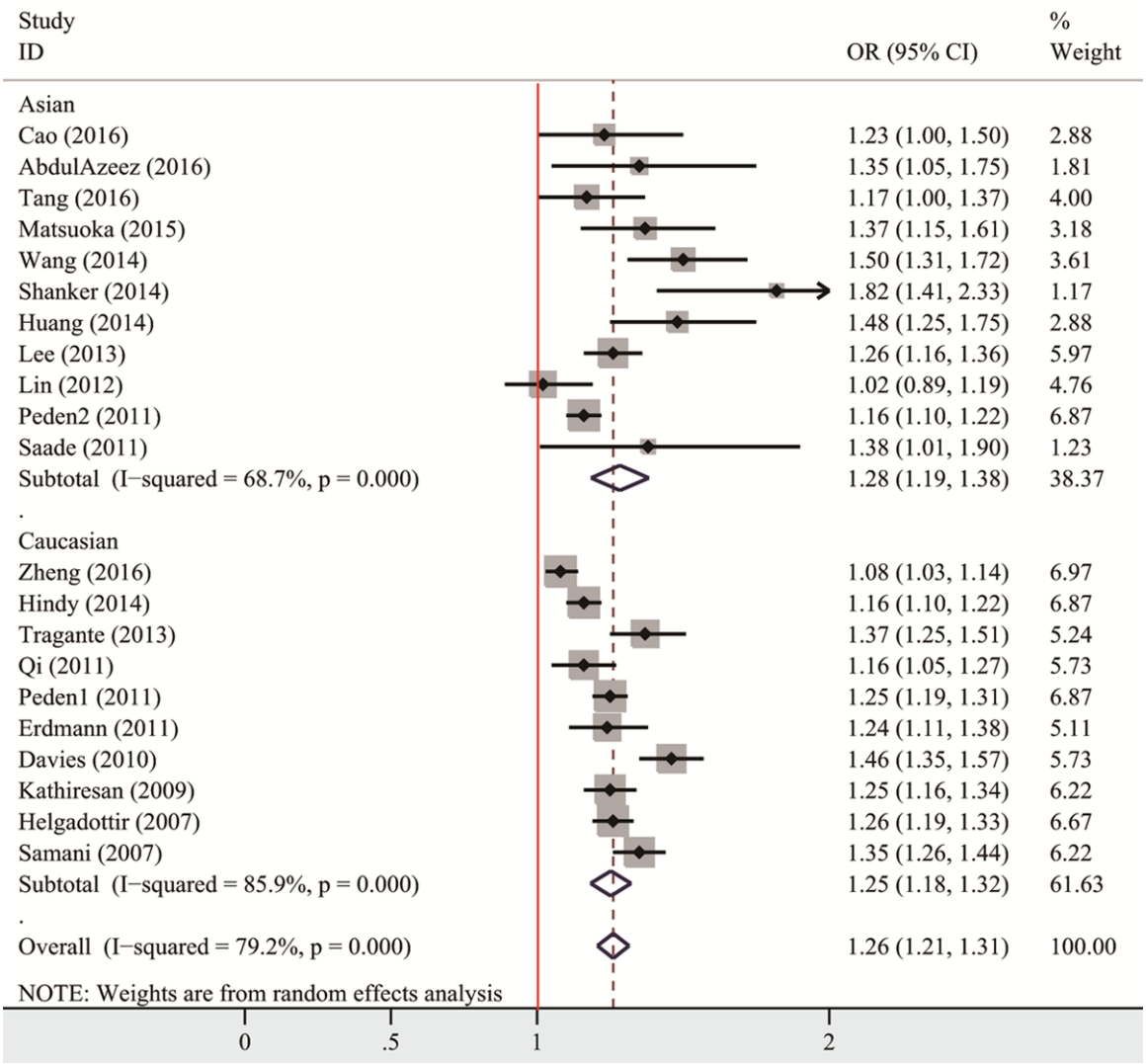


**Figure 3.** Subgroup meta-analysis by ethnicity of the relationship between CDKN2B-AS rs4977574 polymorphism and CHD risk in allele model. CHD = coronary heart disease, CI = confidence interval, OR = odds ratio.

have also performed subgroup analysis according to source of control, sample size and genotyping method. The detailed information is presented in **Table 2**. When processing the subgroup analysis by sample size, no significant association was found between CDKN2B-AS rs4977574 polymorphism and CHD risk in sample size <1000 group. There were only 3 studies [19, 21, 31] included in this subgroup analysis and the overall and other subgroup analysis result consists with each other. Based on this, we concluded that the result of subgroup analysis of sample size <1000 was due to small sample effect. Moreover, after eliminating these three studies with small sample size, the $I^2$ value for studies with sample size ≥1000 decreased to 44.3%, which confirmed that small sample size was the main source of heterogeneity.

*Sensitivity analysis*

We conducted the sensitivity analysis to discover whether the omission of each study will alter the pooled odds ratios quantitatively. As is shown in **Figure 4**, no altered results showed after the individual study was omitted, which provided reliable evidence to prove the increased risk of the CDKN2B-AS rs4977574 polymorphism to CHD susceptibility (**Figure 4**).

*Publication bias*

When performing a meta-analysis, publication bias is no doubtfully a common problem to be

**Table 2.** Subgroup analysis of association between rs4977574 polymorphism and CHD

| Subgroup | | Number | Odds ratio | 95% confidential interval | *P* value | $I^2$ (%) |
|---|---|---|---|---|---|---|
| Total | | 21 | 1.26 | (1.21, 1.32) | <0.001 | 79.2 |
| Ethnicity | Asian | 11 | 1.28 | (1.19, 1.38) | <0.001 | 68.7 |
| | Caucasian | 10 | 1.25 | (1.18, 1.32) | <0.001 | 85.9 |
| Source of control | HB | 12 | 1.25 | (1.18, 1.32) | <0.001 | 71.3 |
| | PB | 9 | 1.28 | (1.19, 1.37) | <0.001 | 85.9 |
| Sample size | ≥1000 | 18 | 1.28 | (1.22, 1.33) | <0.001 | 44.3 |
| | <1000 | 3 | 1.13 | (0.97, 1.29) | 0.17 | 80.8 |
| Genotyping method | Taqman | 13 | 1.27 | (1.21, 1.33) | <0.001 | 70.9 |
| | Non-Taqman | 8 | 1.25 | (1.15, 1.35) | <0.001 | 81.5 |

HB represents for hospital-based; and PB, population-based.

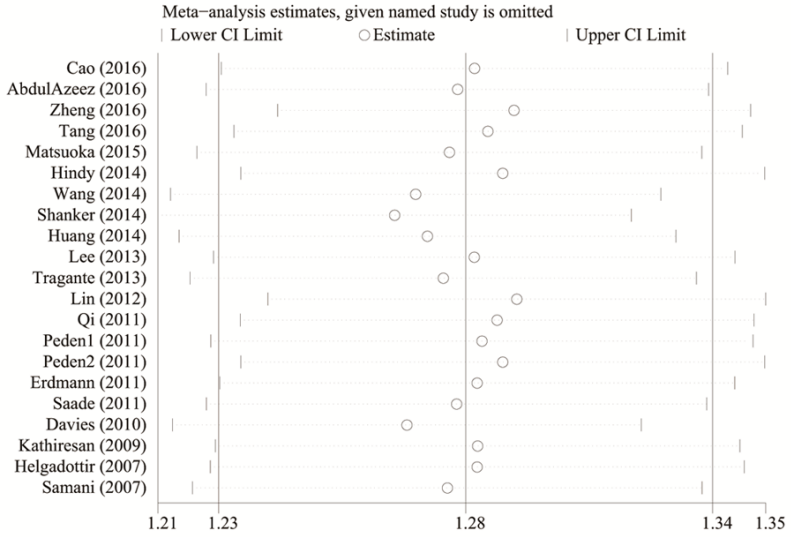


**Figure 4.** Sensitivity analysis of the pooled OR coefficients on the relationship between CDKN2B-AS rs4977574 polymorphism and CHD risk. CHD = coronary heart disease, CI = confidence interval, OR = odds ratio.

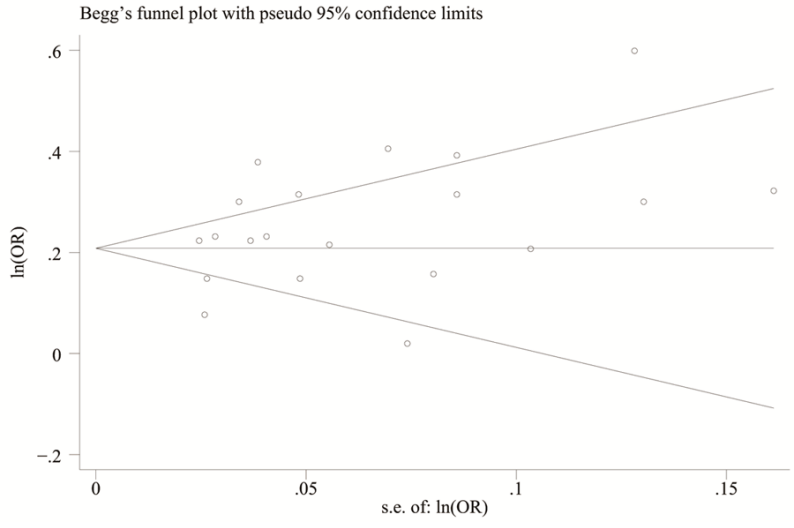


**Figure 5.** Begg's funnel plot with pseudo 95% confidence limits in allele model.

addressed. In our meta-analysis, we calculated Begg's test as well as Egger's test and drew the Begg's funnel plot to assess the publication bias. Visually from the Begg's funnel plot (**Figure 5**), we could see all the twenty studies were symmetrically distributed on the two sides which indicated that the publication bias was acceptable in our meta-analysis (Begg, p = 0.12; Egger, p = 0.06, all values of $p > 0.05$).

## Discussion

Coronary heart disease is playing a progressively vital role in people's mortality and mobility. The drug and interventional treatment of CHD is still the hot spot. However, the genetic therapy of CHD remains largely unknown. The CDKN2B-AS gene, located nearby the CDKN2B-CDKN2A gene cluster of chromosome 9p21, can result in the epigenetic silencing of other genes of this cluster by producing a functional RNA molecule. The mechanism is that the RNA molecule will interact with polycomb repressive complex-1 and -2. As CDKN2B-AS gene is

widely expressed in coronary SMCs and vascular endothelial cells, we have strong reasons to doubt that there may be a significant genetic susceptibility locus in this region and is associated with high risk of cardiovascular disease.

In previous large-scale analysis of GWAS in Caucasians, a CHD-associated locus at chromosome 9p21, a region containing the antisense non-coding RNA in the INK4 locus (ANRIL, also called CDKN2B-AS) was identified [34, 36, 37, 39]. However, there has been no large GWAS studying the association between CDKN2B-AS rs4977574 polymorphism and the risk of CHD in Asians. Recently, many case-control designed studies have investigated the association between the CDKN2B-AS polymorphism and CHD susceptibility in Asians and Caucasians but because of the low statistical power and small sample size of individual studies, the consolidated result is still controversial. So we conducted the present meta-analysis to: (1) verify the association between rs4977574 and CHD in Caucasians; (2) evaluate whether the association exists in Asians.

The present meta-analysis included 20 studies with a sum of 51,522 cases and 73,230 healthy controls. Significant associations were found between CDKN2B-AS rs4977574 polymorphism and CHD in overall population (OR = 1.26; 95% CI = 1.21 to 1.32; $p<0.001$; $I^2$ = 79.2%). However, we found significant heterogeneity among ORs in the pooled analysis. Possible factors underlying this high heterogeneity may include ethnicity, age, gender, source of control, sample size and genotyping method. In subgroup analysis by ethnicity, we found significant association between the CDKN2B-AS rs4977574 polymorphism and CHD risk in both Asian (OR = 1.28, 95% CI = 1.19-1.38) and Caucasian (OR = 1.25, 95% CI = 1.18-1.32). No differences were detected after source of control, genotyping method subgroup analyses. The only difference of subgroup analysis of sample size <1000 may not be meaningful due to small sample size effect. Gender and age differences were also considered; however, due to the lack of reported data, we could not perform this analysis.

For publication bias analysis, Begg's test, Egger's test and a Begg's funnel plot was used for assessment. Visually from the Begg's funnel plot (**Figure 5**), we could see all the twenty studies were symmetrically distributed on the two sides which indicated that the publication bias was acceptable in our meta-analysis (Begg, p = 0.12; Egger, p = 0.06; all values of $p > 0.05$). Based on these, our conclusion of meta-analysis is reliable.

Yet the underlying mechanism of CDKN2B-AS rs4977574 polymorphism to increase susceptibility to CHD is unclear. More animal trials should be carried out to study the specified pathway or gene expression of CDKN2B-AS rs4977574 polymorphism to CHD. As an antisense non-coding RNA in the INK4 locus, it could be a promising loci for genetic therapy in the clinical management of CHD in the future.

Compared to another meta-analysis [26] of eleven studies (nine studies on Caucasian, two studies on Asian), we included eight more studies on Asian. So our meta-analysis could provide other researchers with a comprehensive and improved perspective on the association between the CDKN2B-AS gene rs4977574 polymorphism and CHD. The conformation to HWE and relatively large sample size has strengthened our meta-analysis, but some limitations do exist. First, all of the 20 studies included in this meta-analysis were written in English, so studies in other languages and possible unpublished articles did not attend this meta-analysis, which may cause selection bias. Second, there were no studies including Africans. Third, the genetic susceptibility may also depend on the coincidence of several gene polymorphisms acting together, which may influence the results.

## Conclusion

By performing this meta-analysis, we finally concluded that the rs4977574 polymorphism in the CDKN2B-AS gene significantly increases the risk of CHD, which indicates that the rs4977574 polymorphism of CDKN2B-AS gene may contribute to the pathogenesis of CHD. As an antisense non-coding RNA in the INK4 locus, it could be a promising loci for genetic therapy in the clinical management of CHD in the future and more case-control studies need to be carried out to further validate the conclusion of this meta-analysis.

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## Disclosure of conflict of interest

None.

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## References

[1] Zhou L, Cai J, Liu G, Wei Y and Tang H. Associations between interleukin-1 gene polymorphisms and coronary heart disease risk: a meta-analysis. PLoS One 2012; 7: e45641.

[2] Arnett DK, Baird AE, Barkley RA, Basson CT, Boerwinkle E, Ganesh SK, Herrington DM, Hong Y, Jaquish C and Mcdermott DA. Relevance of genetics and genomics for prevention and treatment of cardiovascular disease A scientific statement from the American heart association council on epidemiology and prevention, the stroke council, and the functional genomics and translational B. Circulation 2007; 27: 1682-1686.

[3] Roberts R, Wells GA, Stewart AF, Dandona S and Li C. The genome-wide association study-a new era for common polygenic disorders. J Cardiovasc Transl Res 2010; 3: 173-182.

[4] Marenberg ME, Risch N, Berkman LF, Floderus B and De FU. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 1994; 330: 1041.

[5] Roberts R. Genetics of coronary artery disease. Circ Res 2014; 114: 1890-1903.

[6] Schunkert H, König IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, Preuss M, Stewart AF, Barbalic M and Gieger C. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011; 45: 25-33.

[7] Peden JF, Hopewell JC, Saleheen D, Chambers JC, Hager J, Soranzo N, Collins R, Danesh J, Elliott P and Farrall M. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet 2011; 43: 339-344.

[8] Samani NJ and Schunkert H. Chromosome 9p21 and cardiovascular disease: the story unfolds. Circ Cardiovasc Genet 2008; 1: 81-84.

[9] Mcpherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybjaerghansen A and Folsom AR. A common allele on chromosome 9 associated with coronary heart disease. Science 2007; 316: 1488-1491.

[10] Wan G, Mathur R, Hu X, Liu Y, Zhang X, Peng G and Lu X. Long non-coding RNA ANRIL (CDKN2B-AS) is induced by the ATM-E2F1 signaling pathway. Cell Signal 2013; 25: 1086.

[11] Anna S, Witold P, Ewa W, Dominika M, Małgorzata S, Anna NJ, Robert M, Sławomir D, Jerzy MW and Adam KK. Polymorphism of 9p21.3 locus Is associated with 5-year survival in high-risk patients with myocardial infarction. PLoS One 2013; 8: e72333.

[12] Jarinova O and Stewart AR. Functional analysis of the chromosome 9p21.3 coronary artery disease risk locus. Arteriosclerosis Thrombosis & Vascular Biology 2009; 29: 1671-1677.

[13] Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, Mohlke KL, Ibrahim JG, Thomas NE, Sharpless NE. INK4/ARF transcript expression is associated with chromosome 9p21 variants linked to atherosclerosis. PLoS One 2009; 4: e5027.

[14] Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, Clarke R, Collins R, Franzosi MG and Tognoni G. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet 2008; 17: 806.

[15] Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, Ibrahim JG, Thomas NE and Sharpless NE. Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of human aging. Aging Cell 2009; 8: 439.

[16] Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, Uitterlinden AG, Harris TB, Witteman JCM and Boerwinkle E. Cohorts for heart and aging research in genomic epidemiology (CHARGE) consortium: design of prospective meta-analyses of genome-wide association studies from five cohorts. Circ Cardiovasc Genet 2009; 2: 73.

[17] Zhang W, Chen Y, Liu P, Chen J, Song L, Tang Y, Wang Y, Liu J, Hu FB and Hui R. Variants on chromosome 9p21.3 correlated with ANRIL expression contribute to stroke risk and recurrence in a large prospective stroke population. Stroke 2012; 43: 14-21.

[18] Cao XL, Yin RX, Feng H, Wu JZ and Chen WX. Chromosome 9p21 and ABCA1 genetic variants and their interactions on coronary heart disease and ischemic stroke in a Chinese Han population. Int J Mol Sci 2016; 17: 586.

[19] Abdulazeez S, Alnafie AN, Alshehri A, Borgio JF, Baranova EV, Almadan MS, Alali RA, Almuhanna F, Alali A and Almansori M. Intronic polymorphisms in the CDKN2B-AS1 gene are strongly associated with the risk of myocardial

infarction and coronary artery disease in the Saudi population. Int J Mol Sci 2016; 17: 395.
[20] Zheng Y, Li Y, Huang T, Cheng HL, Campos H and Qi L. Sugar-sweetened beverage intake, chromosome 9p21 variants, and risk of myocardial infarction in Hispanics. Am J Clin Nutr 2015; 103: 1179.
[21] Tang O, Lv J, Cheng Y and Qin F. The correlation between 9p21 chromosome rs4977574 polymorphism genotypes and the development of coronary artery heart disease. Cardiovasc Toxicol 2017; 17: 185-189.
[22] Matsuoka R, Abe S, Tokoro F, Arai M, Noda T, Watanabe S, Horibe H, Fujimaki T, Oguri M and Kato K. Association of six genetic variants with myocardial infarction. Int J Mol Med 2015; 35: 1451.
[23] Hindy G, Ericson U, Hamrefors V, Drake I, Wirfält E, Melander O and Orhomelander M. The chromosome 9p21 variant interacts with vegetable and wine intake to influence the risk of cardiovascular disease: a population based cohort study. BMC Med Genet 2014; 15: 1-10.
[24] Wang Y, Wang L, Liu X, Zhang Y, Yu L, Zhang F, Liu L, Cai J, Yang X, Wang X. Genetic variants associated with myocardial infarction and the risk factors in Chinese population. PLoS One 2014; 9: e86332.
[25] Shanker J, Arvind P, Jambunathan S, Nair J and Kakkar V. Genetic analysis of the 9p21.3 CAD risk locus in Asian Indians. Thrombosis & Haemostasis 2014; 111: 960-969.
[26] Huang Y, Ye H, Hong Q, Xu X, Jiang D, Xu L, Dai D, Sun J, Gao X and Duan S. Association of CDKN2BAS polymorphism rs4977574 with coronary heart disease: a case-control study and a meta-analysis. Int J Mol Sci 2014; 15: 17478.
[27] Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA and Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. JAMA 2000; 283: 2008-2012.
[28] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-605.
[29] Lee JY, Lee BS, Shin DJ, Woo PK, Shin YA, Joong KK, Heo L, Young LJ, Kyoung KY and Jin KY. A genome-wide association study of a coronary artery disease risk variant. J Hum Genet 2013; 58: 120.
[30] Tragante V, Doevendans PA, Nathoe HM, van der Graaf Y, Spiering W, Algra A, de Borst GJ, de Bakker PI, Asselbergs FW; SMART study group. The impact of susceptibility loci for coronary artery disease on other vascular domains and recurrence risk. Eur Heart J 2013; 34: 2896.
[31] Qi L, Li JM, Sun H, Huang XQ, Lin KQ, Chu JY and Yang ZQ. [Association between gene polymorphisms and myocardial infarction in Han Chinese of Yunnan province]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2012; 29: 413-419.
[32] Qi L, Ma J, Qi Q, Hartiala J, Allayee H and Campos H. Genetic risk score and risk of myocardial infarction in Hispanics. Circulation 2011; 123: 374.
[33] Peden JF, Hopewell JC, Saleheen D, Chambers JC, Hager J, Soranzo N, Collins R, Danesh J, Elliott P and Farrall M. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet 2011; 43: 339-344.
[34] Erdmann J, Willenborg C, Nahrstaedt J, Preuss M, König IR, Baumert J, Linselnitschke P, Gieger C, Tennstedt S and Belcredi P. Genome-wide association study identifies a new locus for coronary artery disease on chromosome 10p11.23. Eur Heart J 2011; 32: 158-168.
[35] Saade S, Cazier JB, Ghassibesabbagh M, Youhanna S, Badro DA, Kamatani Y, Hager J, Yeretzian JS, Elkhazen G and Haber M. Large scale association analysis identifies three susceptibility loci for coronary artery disease. PLoS One 2011; 6: e29427.
[36] Davies RW, Dandona S, Stewart AF, Chen L, Ellis SG, Tang WH, Hazen SL, Roberts R, Mcpherson R and Wells GA. Improved prediction of cardiovascular disease based on a panel of single nucleotide polymorphisms identified through genome-wide association studies. Circ Cardiovasc Genet 2010; 3: 468.
[37] Kathiresan S, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ and Schunkert H. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009; 41: 334-341.
[38] Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, Jonasdottir A, Sigurdsson A, Baker A and Palsson A. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007; 316: 1491-1493.
[39] Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P and Wichmann HE. Genomewide association analysis of coronary artery disease. N Engl J Med 2007; 357: 443.