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ANRIL polymorphism rs4977574 is associated with increased risk of coronary artery disease in Asian populations

A meta-analysis of 12,005 subjects

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Abstract

Background: Several studies have shown that *ANRIL* polymorphism may be associated with the risk of coronary artery disease (CAD). However, these studies do not provide a clear consensus in Asian population. Thus, this meta-analysis was aimed to evaluate the relationship between the common variant rs4977574 in *ANRIL* and CAD risk in Asian population.

Methods: We conducted a systematic literature search of PubMed, Embase and the Cochrane Library and 2 Chinese databases. A total of 12,005 subjects from 6 independent studies were included. The pooled odds ratio (OR) and their corresponding 95% confidence intervals (CIs) were used to assess the association between rs4977574 and CAD using random effects model.

Results: A significant association was observed between rs4977574 and CAD risk under the allelic (OR: 1.18, 95% CI: 1.04–1.34, $P = .010$), recessive (OR: 1.27, 95% CI: 1.01–1.60, $P = .04$), dominant (OR: 1.28, 95% CI: 1.13–1.44, $P = .002$), homozygous (OR: 1.46, 95% CI: 1.15–1.86, $P = .002$), and heterozygous model (OR: 1.17, 95% CI: 1.07–1.28, $P = .0004$), especially in the Chinese subgroup and the myocardial infarction (MI) subgroup ($P < .05$).

Conclusion: The *ANRIL* polymorphism rs4977574 is associated with CAD risk in Asian population. The rs4977574 with G allele may confer to a higher risk of CAD, especially MI.

Abbreviations: CAD = coronary artery disease, CI = confidence interval, HWE = Hardy–Weinberg equilibrium, MI = myocardial infarction, OR = odds ratio.

Keywords: *ANRIL*, coronary artery disease, myocardial infarction, polymorphism, rs4977574

1. Introduction

Coronary artery disease (CAD) is a chronic multifactorial disease and a leading contributor to global morbidity and mortality.[1] Both genetic and environmental factors are involved in the pathogenesis of CAD; genetic factors could explain 30% to 60% of the variations in CAD risk.[2,3] To date, several common variants on chromosome 9p21.3 associated with CAD have been identified by genome-wide association studies (GWASs).[4–6]*ANRIL*, also known as *CDKN2BAS* and a potential candidate gene at chromosome 9p21.3 that encodes a large antisense non–coding RNA, is located adjacent to the *CDKN2A-CDKN2B* gene cluster.[4,7,8] The promoter region of *ANRIL* affects the transcription of *CDKN2A* and *CDKN2B* by binding to zinc-finger proteins.[9]*ANRIL* is expressed in cardiac tissues, which may affect development of CAD via regulating vascular cell proliferation with reduced expression of *CDKN2A* and *CDKN2B*.[10]*ANRIL* knockdown in vascular smooth muscle was shown to change the expression of genes involved in extracellular matrix remodeling, suggesting that *ANRIL* plays a role in vascular structure and function.[11]

Several GWASs have revealed that the single nucleotide polymorphism (SNP) rs4977574 of *ANRIL* was associated with increased CAD risk in different ethnicities.[12–19] A meta-analysis by Huang et al of 23 studies among 36,452 cases and 39,781 controls showed a strong association between rs4977574 and the risk of CAD ($P < .0001$, odds ratio [OR] = 1.27, 95% CI 1.22–1.31). A meta-analysis of 13 case-control studies involving 6796 cases and 9956 controls by Wang et al found that *ANRIL* rs2383207 polymorphism was associated with a significantly increased risk of CAD (OR = 1.47; 95% CI 1.33–1.62), including Caucasians (OR = 1.51; 95% CI 1.28–1.77) and Asians (OR = 1.42; 95% CI 1.26–1.61). These findings implicate *ANRIL* in CAD development.

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BX and ZF contributed equally to this work.

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However, the associations between rs4977574 and the risk of CAD are likely to be varied across different ethnicities. To date, most of the studies on *ANRIL* rs4977574 polymorphism focused on Caucasians,[17,20,21] and few studies are available on Asians. Huang et al carried out a case-control study of 590 Chinese CAD patients and 482 non-CAD patients subjects and found that *ANRIL* rs4977574 polymorphism was associated with a significantly increased risk of CAD in females ($\chi^2 = 10.29$, $P = .003$, OR = 2.14, 95% CI 1.31–2.77).[22] Additional studies on Chinese, Japanese and people from other Asian countries also suggested that *ANRIL* rs4977574 polymorphism was associated with an increased CAD risk,[16,17,21,23–24] but hitherto, no meta-analysis of *ANRIL* rs4977574 polymorphism and CAD risk is available. In the present study, we carried out a meta-analysis of the overall effect of *ANRIL* polymorphism rs4977574 (A>G) on CAD risk in Asian subjects.

2. Methods

2.1. Literature search

We conducted a systematically literature search of PubMed, Embase, and the Cochrane Library as well as 2 Chinese databasesWanfang and CNKI (China National Knowledge Infrastructure) for publications up to April 1, 2018 by using the following keywords: "rs4977574" AND ("polymorphism" OR "variant") AND ("CAD" OR "CAD" OR "myocardial infarction [MI]" OR "coronary heart disease" OR "CAD" OR "MI"). The publications were limited to English and Chinese. All eligible studies and their references were evaluated for their eligibility for inclusion. Only original researches were included. Case reports, editorials and reviews were excluded.

2.2. Inclusion criteria

Publications were eligible if

(1) the study examined the association ofrs4977574 with CAD including MI;
(2) it was a case-control study;
(3) the OR together with 95% confidence interval (CI) was obtained.

Major exclusions were

(1) non-Asian population;
(2) publications other than English or Chinese;
(3) duplicated publications based on the same data.

2.3. Data extraction

We extracted the following characteristics: first author, publication year, region, genotyping, number of genotypes, and total number of cases and controls. All data were extracted from each literature by 2 authors (BX and ZF) independently. Disagreement was resolved by consensus of these 2 authors. If they could not reach a consensus, the result was reviewed by a third author (SH).

2.4. Quality assessment

To assess the quality of each study, the Newcastle–Ottawa scale (NOS) was used to evaluate all the observational studies. NOS score ranges from 4 to 9. If a study score was higher than 7, it was a high-quality study. Two investigators (BX and ZF) independently assessed the quality of these studies, and the third investigator (SH) resolved the disagreement between the first 2 investigators.

2.5. Ethical statement

All results and analyses were from previous published studies; thus, no ethical approval and patient consent are required.

2.6. Statistical analysis

We assessed the strength of the association between rs4977574 and CAD risk by ORs together with 95% CIs. *Z* test was used to determine the significance of the pooled ORs, and *P* values <.05 were considered significant. We evaluated the association between rs4977574 and CAD risk under the allelic (distribution of G allelic frequency of ANRIL gene polymorphism), recessive (GG versus AG+AA), dominant (GG+AG versus AA), homozygous (GG versus AA) and heterozygous (AG versus AA) genetic models. The heterogeneity of the ORs was checked by *Q* test or I^2. A significant heterogeneity across studies was considered if *P* was less than .05 or I^2 was greater than 50%. When a significant heterogeneity exists, we pooled ORs with the random-effects model. Otherwise, the fixed-effects model was selected.[25] Sensitivity analyses were performed to test the reliability of results. Hardy–Weinberg equilibrium (HWE) was assessed using Fisher exact test and significance was defined as $P < .05$. Potential publication bias was measured by the funnel plot and funnel plot asymmetry was assessed by Egger test with significance set at $P < .05$. All statistical tests were performed with STATA 11.0 (StataCorp, College Station, Texas) and Review Manager 5.0.

3. Results

3.1. Eligible studies

The study flowchart is shown in Figure 1. Thirteen relevant articles were identified from our initial search and 6 articles met the inclusion criteria. In total, data were extracted from 5683 CAD patients and 6322 controls. The study subjects came from China, Japan and Iran, and were categorized into Chinese or non-Chinese Asians (Table 1). Several studies carried out subgroup analysis of different genetic ethnicities. Seven studies were excluded; among them, 3 papers reported data of Caucasians and 4 papers did not report genotype data. By quality assessment with NOS, 3 studies scored higher than or equal to 7 and were considered high quality and 2 studies scored 6and were close to the high-quality standard (Table S1, http://links.lww.com/MD/C528).

3.2. Meta-analysis

Overall, significant associations were found between rs4977574 and CAD risk when all studies were pooled into the meta-analysis under the allelic model (OR = 1.18, 95% CI 1.04–1.34, $P = .010$), the dominant model (OR = 1.28, 95% CI 1.13–1.44, $P < .0001$), the recessive model (OR = 1.27, 95% CI 1.01–1.60, $P = .04$),the homozygous model (OR = 1.46, 95% CI 1.15–1.86, $P = .002$), and the heterozygous model (OR = 1.17, 95% CI 1.07–1.28, $P = .0004$) (Figs. 2 and 3). Under the dominant and heterozygous genetic model, no heterogeneity was detected in the whole population ($P_{heterogeneity} = .18$ and .67, respectively). In addition, the whole population displayed significant heterogeneity under another 3 genetic models ($P_{heterogeneity} < .05$). When Chinese subjects were analyzed as a subgroup, heterogeneity was still detected under the allelic, recessive and homogeneity models ($P_{heterogeneity} < .05$).In contrast, no heterogeneity was shown under all the genetic models when non-Chinese Asians were analyzed as a subgroup ($P_{heterogeneity} < .05$) (Figs. 2 and 3). For

Figure 1. The study flowchart.

Table 1

Characteristics of included studies on association between the *ANRIL* polymorphism rs4977574 and CAD.

					CAD genotype			Control genotype			Sample Size	
First author	Year	Region	Ethnicity	MI/Non-MI	AA	AG	GG	AA	AG	GG	CAD/control	P_{HWE}
Huang [22]	2014	China	Chinese	Non-MI	122	305	163	138	267	77	590/482	.006
Wang [16]	2014	China	Chinese	MI	583	1139	595	777	1325	482	2317/2584	.047
Matsuoka [17]	2015	Japan	Non-Chinese	MI	448	898	476	651	1132	501	1822/2284	.831
Cao [21]	2016	China	Chinese	Non-MI	117	272	176	152	255	134	565/541	.191
Beigi [23]	2016	Iran	Non-Chinese	Non-MI	22	44	34	17	44	32	100/93	.784
Tang [24]	2017	China	Chinese	Non-MI	37	136	116	38	134	166	289/338	.172

CAD genotype = genotype of the patients with coronary artery disease (CAD), Control genotype = genotype of the controls, MI = patients with only myocardial infarction, Non-MI = patients with all kinds of CAD including myocardial infarction, P_{HWE} = *P* value for Hardy–Weinberg equilibrium of controls.

patients with MI as a subgroup, no heterogeneity was detected under all the genetic models ($P_{heterogeneity} > .05$). In contrast, no heterogeneity was demonstrated under only the dominant and heterozygous models when non-MI CAD patients were analyzed as a subgroup ($P_{heterogeneity} > .05$) (Supplementary Figure S1–5, http://links.lww.com/MD/C528).

In the Chinese subgroup, a significant association between rs4977574 and CAD risk was found under the dominant model (OR = 1.33, 95% CI = 1.12–1.57, $P = .0009$), the homozygous model (OR = 1.55, 95% CI 1.10–2.19, $P = .01$), and the heterozygous model (OR = 1.19, 95% CI 1.07–1.33, $P = .002$). In the non-Chinese Asian subgroup, a significant association between rs4977574 and CAD risk was found under only the recessive model (OR = 1.24, 95% CI 1.08–1.43, $P = .002$) (Table S2, http://links.lww.com/MD/C528).

In the MI subgroup, a significant association between rs4977574 and CAD risk was found under the allelic model (OR = 1.23, 95% CI 1.14–1.32, $P < .00001$), the recessive model (OR = 1.38, 95% CI 1.16–1.65, $P = .0004$), the dominant model (OR = 1.25, 95% CI 1.14–1.38, $P < .00001$), the homozygous model (OR = 1.51, 95% CI 1.27–1.80, $P < .00001$), and the heterozygous model (OR = 1.15, 95% CI 1.04–1.27, $P = .006$). In the non-MI CAD subgroup, a significant association between rs4977574 and CAD risk was found under only the heterozygous model (OR = 1.25, 95% CI 1.04–1.51, $P = .02$) (Table S3, http://links.lww.com/MD/C528).

3.3. Sensitivity analysis and publication bias

Sensitivity analysis revealed that the significance of ORs was not materially changed by omitting any single study in the current meta-analysis. The shape of funnel plot and Egger test ($P = .352$) showed no evidence of obvious asymmetry under the allelic model (Fig. 4).

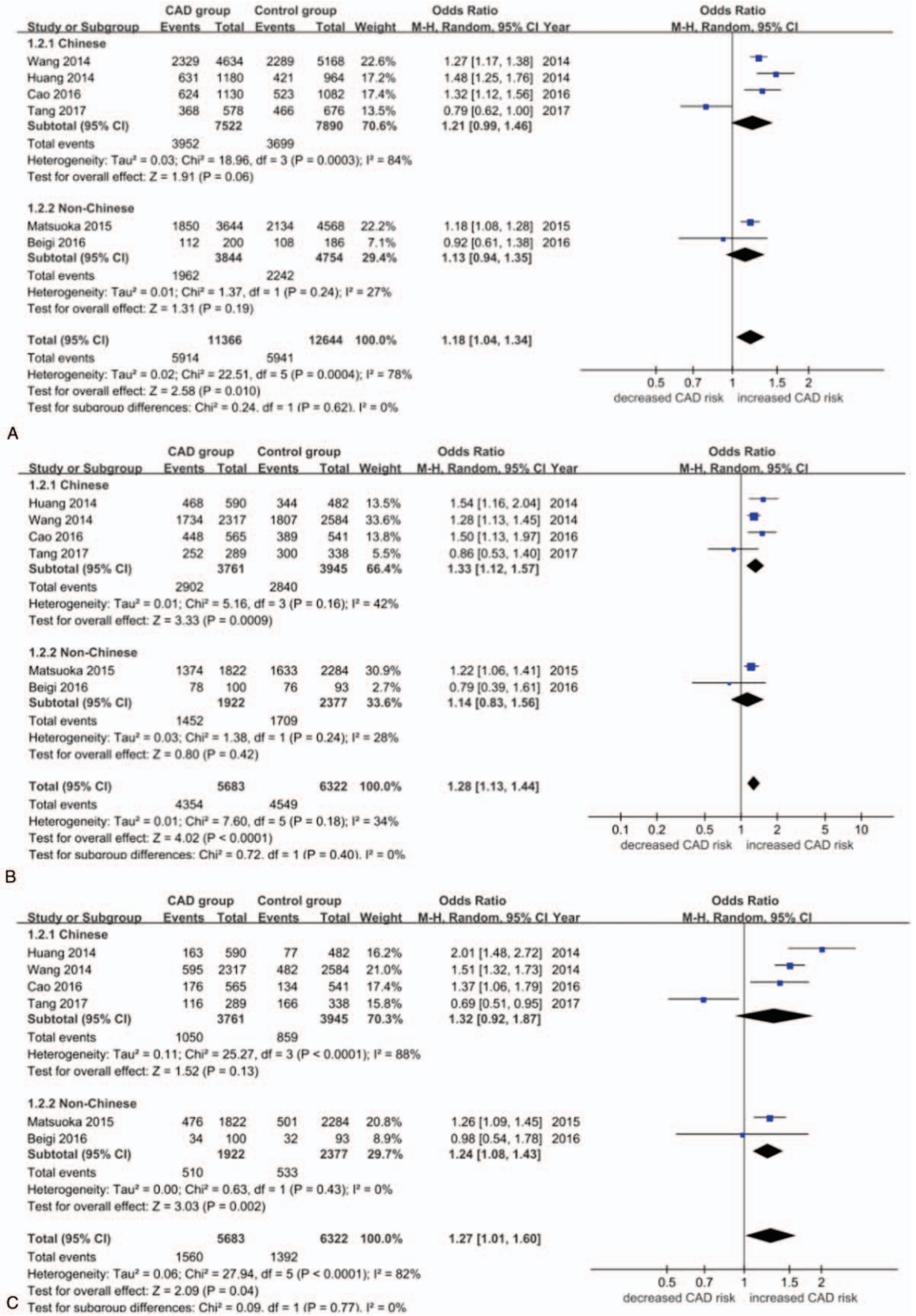

Figure 2. A. Forest plot (random effects model) for the association between *ANRIL* polymorphism rs4977574 and CAD stratified by ethnicity under the allelic genetic model (distribution of G allelic frequency of ANRIL gene polymorphism). G, guanine;CAD, coronary artery disease. B. Forest plot (random effects model) for the association between *ANRIL* polymorphism rs4977574 and CAD stratified by ethnicity under the dominant genetic model (GG+AG versus AA).A, adenine; G, guanine;CAD, coronary artery disease.C. Forest plot (random effects model) for the association between *ANRIL* polymorphism rs4977574 and CAD stratified by ethnicity under the recessive genetic model (GG versus AA+AG).A, adenine; G, guanine.CAD, coronary artery disease. CAD=coronary artery disease.

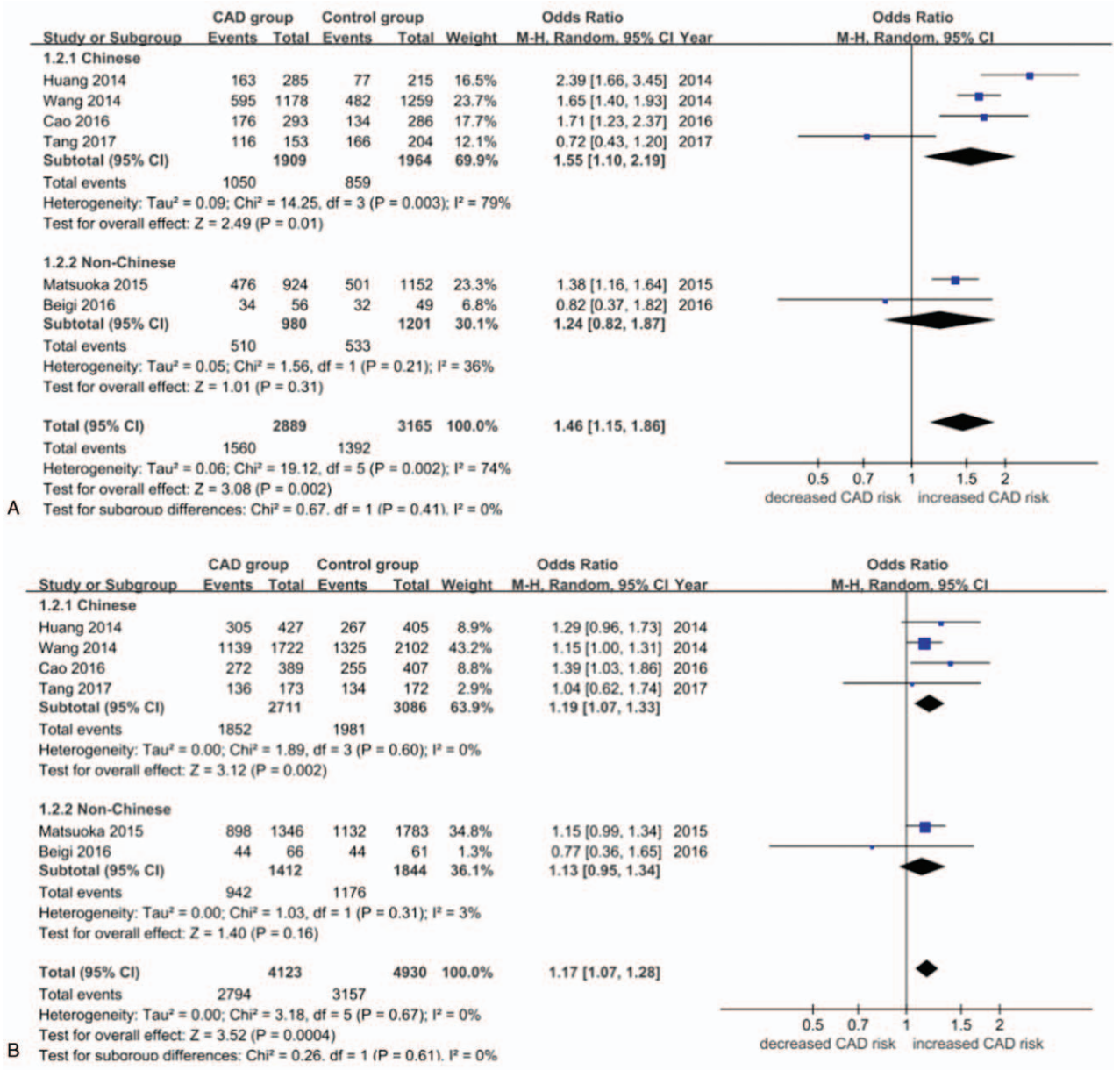

Figure 3. A. Forest plot (random effects model) for the association between *ANRIL* polymorphism rs4977574 and CAD stratified by ethnicity under the homozygous genetic model (GG versus AA).A, adenine; G, guanine; CAD, coronary artery disease. B. Forest plot (random effects model) for the association between *ANRIL* polymorphism rs4977574 and CAD stratified by ethnicity under the heterozygous genetic model (AG versus AA).A, adenine; G, guanine; CAD, coronary artery disease. CAD = coronary artery disease.

4. Discussion

Though several studies have demonstrated that rs4977574 of *ANRIL* is associated with increased CAD risk in different ethnicities, no meta-analysis of *ANRIL* rs4977574 polymorphism and CAD risk is available in Asian populations. Our current meta-analysis revealed significant correlation between rs4977574 and CAD risk under the allelic, recessive, dominant, homozygous, and heterozygous genetic models in the whole Asian population. When the Chinese subjects were analyzed as a subgroup, rs4977574 was significantly associated with CAD under the recessive, dominant, homozygous, and heterozygous genetic models. Moreover, the association strength grew higher with higher ORs under different models. When MI patients were analyzed as a subgroup, rs4977574 was also significantly associated with CAD under the allelic, recessive, dominant, homozygous, and heterozygous genetic models. In contrast, when non-MI CAD patients were analyzed as a subgroup, 4977574 was not significantly associated with CAD under the allelic, recessive, dominant, and homozygous genetic models, except for the heterozygous genetic model. The results suggested that *ANRIL* polymorphism rs4977574 with G-allele is associated with a higher CAD risk in Asians, especially in Asians of Chinese descent. Moreover, 4977574 with G-allele may be more evidently correlated with MI susceptibility when compared with other types of CAD.

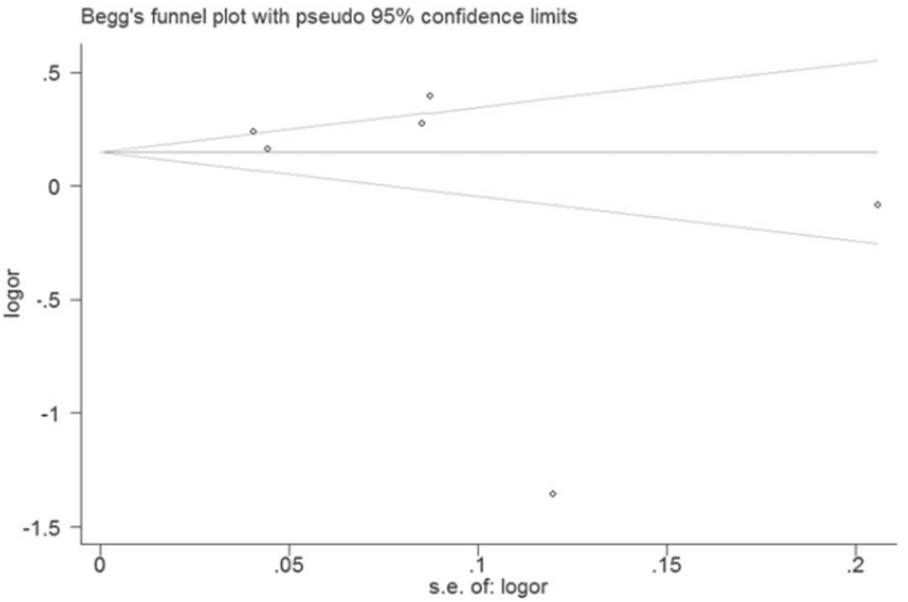

Figure 4. Funnel plot for eligible studies of association between *ANRIL* polymorphism rs4977574 and CAD under an allelic genetic model (distribution of G allelic frequency of ANRIL gene polymorphism). CAD = coronary artery disease, G = guanine, OR = odds ratio.

Several common variants of *ANRIL* have been reported to associate with MI in European whites and Hispanic populations. The rs4977574 in *ANRIL* was first found to be associated with CAD in European and American-Caucasian population.[13,14]

Although Huang et al investigated the association between rs4977574 and CAD in a meta-analysis, the majority of the researches in their included studies were derived from subjects of European ancestry. Over the recent 3 years, an increasing number of GWASs were conducted in Asian countries, especially in China. So this meta-analysis was designed to summarize the latest findings from these studies. The results demonstrated a significant association between rs4977574 and CAD in Asians, which is consistent with previous studies in European countries. MI is the most severe entity of CAD with most severe atherosclerosis, which may lead to sudden death. Our meta-analysis proved that rs4977574 was associated with susceptibility of MI.

ANRIL, encoding a large antisense non–coding RNA, is adjacent to the *CDKN2A-CDKN2B* locus. *CDKN2A-CDNK2B*, coding for 2 cyclin-dependent kinase inhibitors, play an important role in regulation of the cell cycle, suggesting that these 2 genes may be involved in the pathogenesis of atherosclerosis. A functional analysis conducted by Jar Inova et al found risk allele within the 9p21.3 locus enhancer, which alters the transcriptional activity of *ANRIL* and then regulates cellular proliferation by interfering with adjacent protein-coding genes.[10] Congrains et al found abnormal expression of *CDKN2A/B* and reduced cell growth when *ANRIL* was knocked down in vascular smooth muscle cell.[26] So the genetic variants of *ANRIL* influence atherosclerosis process including thrombogenesis, vascular remodeling or repair, and plaque stability through altering *ANRIL* expression and modulating cell proliferation.[10,26]*ANRIL* changes the expression of these coding-related genes by multiple mechanisms including RNA interference, gene silencing, chromatin remodeling, or DNA methylation.[27] Besides, *ANRIL* is expressed in multiple cell types such as endothelial cells, smooth muscle cells, and inflammatory cells stimulated by atherosclerosis.[28]

The study also has several limitations. First, specific data such as genotype frequency were not obtained from some studies performed in Asian populations. In fact, the included studies in this meta-analysis only included 3 ethnicities, Chinese, Japanese and Iranians. They are not representative of all Asians. Chinese is also a broad category as it may include many minority groups apart from Han Chinese. The studies representative of minority groups is limited. Second, some included studies were not in accordance with HWE. Third, our search strategy limited publications to English and Chinese which may lead to some eligible studies being not taken into account.

5. Conclusion

We carried out this meta-analysis to identify the role of *ANRIL* polymorphism rs4977574 in the development of CAD in Asian population. More specifically, G allele may confer to a higher susceptibility of CAD, especially MI. It may be an effective prediction tool of CAD combined with environmental factors in the future. However, more functional studies are warranted for providing more evidence.

Author contributions

Conceptualization: Xiangjun Yang.
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Formal analysis: Zhen Fang.
Funding acquisition: Xiangjun Yang.
Investigation: Bing Xu, Zhen Fang.
Methodology: Bing Xu.
Software: Shenghu He.
Supervision: Shenghu He, Xiangjun Yang.
Writing – original draft: Zhen Fang.
Writing – review & editing: Junhong Wang.

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