Association of ANRIL polymorphisms with coronary artery disease
A systemic meta-analysis
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Abstract
Background: The long noncoding RNAs have gradually been reported to be an important class of RNAs with pivotal roles in the development and progression of myocardial infarction (MI). In this study, we hypothesized that genetic variant of cyclin-dependent kinase inhibitor 2B antisense RNA (ANRIL) may affect the prognosis of MI patients.

Methods: A systematic review and meta-analysis of studies including 11,269 cases and 10,707 controls on the association of 5 ANRIL single nucleotide polymorphism and the overall risk of MI or coronary artery disease (CAD) was performed.

Results: In the meta-analysis, rs4977574 A > G, rs1333040 C > T, rs1333042 A > G and rs10757274 A > G ANRIL polymorphisms were correlated with overall MI or CAD risk. No significant associations were found between ANRIL rs1333049 G > C polymorphism and CAD risk.

Conclusions: The results indicated that ANRIL polymorphism (rs4977574, rs1333040, rs1333042, and rs10757274) were more generally associated with CAD or MI risk. Further experimental studies to evaluate the limits of this hypothesis are warranted, and future functional studies are required to clarify the possible mechanisms.

Abbreviations: ANRIL = cyclin-dependent kinase inhibitor 2B antisense RNA, CAD = coronary artery disease, IncRNAs = long non-coding RNAs, MI = myocardial infarction, SNP = single nucleotide polymorphism.

Keywords: cyclin-dependent kinase inhibitor 2B antisense RNA, single nucleotide polymorphism, myocardial infarction, coronary artery disease, meta-analysis

1. Introduction
Morbidity and mortality associated with coronary artery disease (CAD) result in significant economic and social burdens.\textsuperscript{11} Hypertension, smoking, hypercholesterolemia, and diabetes are estimated to contribute to 50% to 60% of disease susceptibility and genetic variation may account for predisposition in 40% to 50% of sporadic cases.\textsuperscript{2–5} Atherosclerosis contributes to the pathophysiology of CAD, but the effects of variation in the molecular and genetic determinants are not yet clear.\textsuperscript{6,16} Recent evidence of the association of single nucleotide polymorphisms (SNPs) and increased risk of CAD indicates that genetic polymorphisms have a key role in the pathogenesis of CAD.\textsuperscript{7}

Long noncoding (lnc) RNAs are transcripts of at least 200 base pairs in length that do not code for proteins.\textsuperscript{18} Molecular studies have shown that long non-coding RNAs (lncRNAs) play important roles in cell cycle regulation and affect proliferation, differentiation, and apoptosis. LncRNAs are also important regulators of tissue atherosclerosis and disease processes related to CAD. Studies of the functions of lncRNAs in disease, including CAD, are ongoing.\textsuperscript{9–12} Cyclin-dependent kinase inhibitor 2B antisense RNA (ANRIL), also known as CDKN2B antisense RNA overlaps with CDKN2B on human chromosome 9p21,\textsuperscript{11} a locus associated with genetic susceptibility for cardiovascular diseases. It spans 50 kb of DNA that express the ANRIL transcripts,\textsuperscript{14} which modulate expression of CDKN2A/B and influence cellular activities. ANRIL expression is also modulated by several CAD-associated SNPs in the 9p21 locus. The reduction of ANRIL expression leads to inhibition of vascular smooth muscle cell growth, which in turn increases the risk of atherosclerotic vascular disease.\textsuperscript{13}

Numerous studies have demonstrated that ANRIL SNPs have been investigated as potential CAD susceptibility loci. However, the results remain controversial possibly due to the fact that independent studies are underpowered and biased, especially for small cohorts. Here, a systematic meta-analysis of eligible studies conducted before May 10, 2020 was performed in order to obtain more precise and comprehensive insight into the impact of...
The results indicated that ANRIL polymorphisms are associated with increased CAD risk.

2. Materials and methods

2.1. Selection of relevant studies

Study reports published before May 10, 2020 were retrieved from PubMed and the Web of Science using the search terms (coronary artery disease, coronary heart disease, or myocardial infarction [MI]), (“ANRIL” or “CDKN2B-AS”), and (polymorphism, variant, or mutation). Case-control studies of the relationship between ANRIL polymorphism and CAD or MI were eligible. At least 2 studies of ANRIL polymorphism reporting the genotype frequencies of each included ANRIL SNP (ie, rs4977574, rs1333040, rs1333042, rs10757274 or rs1333049) were desired. Only studies published in English were eligible. Studies were excluded if they were not investigations of ANRIL SNPs (rs4977574, rs1333040, rs1333042, rs10757274 or rs1333049), were duplicate publications of the same population, or did not include a control group. Seventeen articles including 11,269 cases and 10,707 controls were selected as shown in Figure 1. Among the seventeen eligible articles, eleven studies examined Asian populations, 4 studies examined Caucasian populations and 1 examined Turkish Cypriot populations, and the characteristics of the included studies are listed in Table 1.

2.2. Statistical analysis

Revman 5.3 software (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark) was used to for the meta-analysis. ORs and their 95% CIs were calculated to determine the significance of associations between ANRIL allele genotypes and susceptibility to MI or CAD. Heterogeneity was tested with the χ²-based Q and I² tests. The pooled OR was calculated using a fixed effect model in the absence of heterogeneity (P > .05, I² < 50%). Otherwise, a random effect model was used. The stability of the pooled ORs was determined by 1-way sensitivity analysis. Publication bias was estimated by funnel plot.

3. Results

3.1. Study characteristics

Several SNPs in the ANRIL have been associated with susceptibility to CAD, although the evidence is inconsistent. This systematic meta-analysis was carried out to evaluate the association between ANRIL and MI or CAD reported in the published literature. Seventeen articles evaluating a total of 11,269 cases and 10,707 controls were selected for analysis as shown in Figure 1. Among the seventeen eligible articles, eleven studies examined Asian populations, 4 studies examined Caucasian populations and 1 examined Turkish Cypriot populations, and the characteristics of the included studies are listed in Table 1.

3.2. Meta-analysis of ANRIL rs4977574 A > G polymorphism and MI or CAD risk

The association of ANRIL rs4977574 A > G polymorphism and MI or CAD risk was investigated in eleven studies including 9,169 patients and 8,566 healthy controls. The variant A allele was found to be protective against CAD development (OR = 0.82, 95% CI = 0.76-0.88, P < .00001, I² = 51%, Fig. 2). Sensitivity analysis by successive omission of individual studies to examine the stability of the pooled ORs, revealed a significant change when the data reported by Tang et al, Huang et al, Sakalar et al or Sehime et al was removed (Supplementary Figure 1A-D, http://links.lww.com/MD/E981, http://links.lww.com/MD/E982, http://links.lww.com/MD/E983, http://links.lww.com/MD/E984). Publication bias was detected by funnel plot. The symmetric funnel plot presented no significant publication bias in this meta-analysis (Fig. 3).
Table 1
Characteristics of included studies on lncRNA ANRIL polymorphisms and MI or CAD risk included in the meta-analysis.

| Cite | First author | Year | Ethnicity | Country/Region | Case | Control |
|------|--------------|------|-----------|----------------|------|---------|
| 18   | Tang         | 2017 | Asian     | China          | 289  | 338     |
| 22   | Cao          | 2016 | Asian     | China          | 565  | 541     |
| 23   | Matsui       | 2015 | Asian     | Japan          | 1822 | 2284    |
| 24   | Wang         | 2014 | Asian     | China          | 2317 | 2584    |
| 25   | Lee          | 2014 | Asian     | China          | 925  | 634     |
| 19   | Huang        | 2014 | Asian     | China          | 590  | 482     |
| 27   | Saade        | 2011 | Asian     | Lebanon        | 1520 | 423     |
| 18   | Şehime       | 2019 | Turkish   | Turkey         | 71   | 153     |
| 29   | Golabgir     | 2016 | Caucasian | Iran           | 200  | 110     |
| 22   | Cao          | 2016 | Asian     | China          | 565  | 541     |
| 26   | Qi           | 2012 | Asian     | China          | 142  | 192     |
| 18   | Şehime       | 2019 | Turkish   | Turkey         | 71   | 153     |
| 30   | Arne         | 2009 | Caucasian | Germany        | 817  | 670     |
| 31   | Xu           | 2018 | Asian     | China          | 884  | 907     |
| 20   | Mafi         | 2017 | Caucasian | Iran           | 103  | 102     |
| 22   | Cao          | 2016 | Asian     | China          | 526  | 513     |
| 30   | Arne         | 2009 | Caucasian | Germany        | 817  | 670     |
| 31   | Xu           | 2018 | Asian     | China          | 884  | 907     |
| 32   | Saleem       | 2018 | Asian     | Pakistan       | 204  | 219     |
| 1    | Ahmed        | 2013 | Asian     | Pakistan       | 290  | 294     |
| 26   | Qi           | 2012 | Asian     | China          | 142  | 192     |
| 31   | Xu           | 2018 | Asian     | China          | 884  | 907     |

| Genotype distribution | Case | Control |
|-----------------------|------|---------|
| rs4097574 A > G       | AA   | AG      | GG       |
| rs1333040 C > T       | CC   | TT      |
| rs1333042 A > G       | AA   | AG      | GG       |
| rs10757274 A > G      | AA   | AG      | GG       |

3.3. Meta-analysis of ANRIL rs1333040 C > T polymorphism and MI or CAD risk

The association of lncRNA ANRIL rs1333040 C > T polymorphism and CAD risk was investigated in 4 studies including 978 cases and 996 controls. Significant overall associations were identified in the allele genotype model (OR = 0.82, 95% CI = 0.71–0.95, P = .007, I² = 33%, Fig. 4). No publication bias was observed in the funnel plot, indicating that the results are statistically robust. (Supplementary Figure 2, http://links.lww.com/MD/E985).

3.4. Meta-analysis of ANRIL rs1333042 A > G polymorphism and MI or CAD risk

A total of 4 studies involving 2329 cases and 2192 controls examined the association between rs1333042 A > G polymorphism and MI or CAD risk. Significant associations with the likelihood of CAD or MI were detected for rs1333042 (OR = 0.77, 95% CI = 0.63–0.94, P = .01, I² = 78%, Fig. 5), a slight bias was found when the data from Mafi et al’s study in a Caucasian patient population was removed by sensitivity analysis (Supplementary Figure 3, http://links.lww.com/MD/E986). Publication bias was examined by the visual inspection of funnel plot (Supplementary Fig. 4, http://links.lww.com/MD/E987).

3.5. Meta-analysis of ANRIL rs10757274 A > G polymorphism and MI or CAD risk

The association between rs10757274 (OR = 0.77, 95% CI = 0.63–0.94, P = .010, I² = 65%, Fig. 6) A > G polymorphism and MI or CAD risk was examined in 4 studies involving 1676 cases and 1874 controls. A study by Cheng et al that focused on MI was the major contributor of heterogeneity. After removing this study, heterogeneity was significantly reduced (Supplementary Figure 5, http://links.lww.com/MD/E988). Funnel plot was constructed to evaluate the publication bias for study of rs10757274. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Supplementary Figure 6, http://links.lww.com/MD/E989).
3.6. Meta-analysis of ANRIL rs1333049 G > C polymorphism and MI or CAD risk

The evaluations of the associations between rs1333049 and MI or CAD risk are displayed in Figure 7. Overall, the G variant allele of rs1333049 exhibited no significant association with MI or CAD risks (OR = 0.95, 95% CI = 0.57–1.59, P = .85, I² = 94%, Fig. 7). The visual inspection of funnel plot proved that almost no apparent publication bias existed in our meta-analysis about rs1333049 (Supplementary Figure 7, http://links.lww.com/MD/E990).

4. Discussion

In the current study, we performed a systemic meta-analysis by pooling 17 studies with totals of 11,269 cases and 10,707 controls. The lncRNA were chosen for study because ANRIL is the best replicated genetic risk locus of CAD and regulates genes involved in glucose and fatty acid metabolism. The variants within this locus have been significantly associated with an increased risk of CAD and diabetes in European and some other populations.[4] Our results revealed that the lncRNA ANRIL rs4977574 A > G polymorphism might be an important risk factor for developing MI or CAD. Huang et al. previously conducted a meta-analysis assessing the association between ANRIL polymorphisms and CAD risk.[19] However, the literature search was performed before 2012, with few data sets combined. A lot more case–control studies on this topic emerged since then, with 9 more studies eligible by the literature search until July 2019. Huang et al. found that ANRIL polymorphism was significantly
Figure 4. Odds ratios and 95% confidence intervals of the association of ANRIL rs1333040 C>T polymorphism and MI or CAD in the allele model. ANRIL = cyclin-dependent kinase inhibitor 2B antisense RNA, CAD = coronary artery disease, MI = myocardial infarction.

Figure 5. Odds ratios and 95% confidence intervals of the association of ANRIL rs1333040 C>T polymorphism and MI or CAD in the allele model. ANRIL = cyclin-dependent kinase inhibitor 2B antisense RNA, CAD = coronary artery disease, MI = myocardial infarction.

Figure 6. Odds ratios and 95% confidence intervals of the association of ANRIL rs10757274 A>G polymorphism and MI or CAD in the allele model. ANRIL = cyclin-dependent kinase inhibitor 2B antisense RNA, CAD = coronary artery disease, MI = myocardial infarction.

Figure 7. Odds ratios and 95% confidence intervals of the association of ANRIL rs1333049 G>C polymorphism and MI or CAD in the allele model. ANRIL = cyclin-dependent kinase inhibitor 2B antisense RNA, CAD = coronary artery disease, MI = myocardial infarction.
associated with higher CAD risk, which was consistent with our finding. However, the lack of sensitivity analysis indicated that the pooled results were unstable and incredible. Our sensitivity analysis by successive omission of individual studies to examine the stability of the pooled ORs, revealed a significant change when the data reported by Tang et al, Huang et al, Sakalar et al or Sehine et al was removed. Considering the more studies included and larger sample size combined, pooled ORs from our study were more robust.

The results of our analysis indicated that the rs1333040 T allele of polymorphism is a potential genetic marker for CAD susceptibility, especially in Asians. A possible mechanism underlying the association of these alleles to increased susceptibility is through the regulation of the expression of ANRIL itself. Polymorphism rs1333040 resides in an intronic enhancer region, which was found to influence the activity of this enhancer and expression of ANRIL gene. A similar finding was made for polymorphism rs1333042, regulated ANRIL expression originated from the G (relative to the A) allele, which was found to influence the activity of proteins bound, such as CEBPβ and STAT3. For rs10757274, the polymorphism showed much linkage disequilibrium with the reported MI- associated SNPs (eg, rs153737, rs4977575, rs10757272, etc) and contribute to the activation or inhibition of expression of related genes.

As for rs1333049 polymorphism, there is no significant association was found in our meta-analyses. However, the results are inconsistent with Waqas et al study. Their study demonstrated a strong association of the rs1333049 with MI and it could be used as a useful genetic marker for the screening of MI in the general Pakistani population. The differences in these study findings may be explained by ethnic, environmental, or lifestyle differences that affected the development of MI. The way in which these SNP loci contribute to susceptibility to MI requires further study.

The strength of our meta-analysis stems from systematically reviewing the relationships between 5 ANRIL polymorphisms (rs4977574, rs1333040, rs1333042, rs10757274 and rs1333049) and CAD or MI susceptibility. In addition, the well-designed search and selection methods significantly increased the statistical power of this meta-analysis. However, there are also some limitations that need to be addressed. First, significant heterogeneity between the studies was observed for the analyses of rs4977574, rs1333042 and rs10757274. Among the 17 published studies contained in our meta-analysis, some studies were population-based, while others were hospital-based. Second, few studies involving the 5 SNP loci examined were identified; the small number sizes might decrease the reliability of the results and increases the probability of random errors, thus affecting the assessment of associations between these lncRNA ANRIL polymorphisms and CAD or MI susceptibility. Finally, some loci in our analysis was limited to individuals of Asian descent so that it remains unclear as to whether these results can be generalized to other populations.

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Author contributions
All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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