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Meta Analysis

ANRIL rs2383207 polymorphism and coronary artery disease (CAD) risk: a meta-analysis with observational studies

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Abstract: Some studies investigated the association of antisense non-coding RNA in the INK4 locus (ANRIL) rs2383207 polymorphism with coronary artery disease (CAD) risk. However, the result was still inconsistent. The aim of this study was to investigate whether there is an association between the ANRIL rs2383207 polymorphism and CAD risk. We carried out a PubMed (Medline), EMBASE database search covering all published articles. The strength of association between ANRIL rs2383207 polymorphism and CAD risk was assessed by calculating OR with 95% CI. A total of 13 case-control studies involving 6796 cases and 9956 controls were included in this meta-analysis. ANRIL rs2383207polymorphism was associated with a significantly an increased risk of CAD (OR=1.47; 95%CI, 1.33-1.62). We also found that this polymorphism increased CAD risk in Caucasians (OR=1.51; 95%CI, 1.28-1.77) and Asians (OR=1.42; 95%CI, 1.26-1.61). In the subgroup analysis according to gender, both women and men were significantly associated with the increased risk of CAD (OR=1.36; 95%CI, 1.03-1.79 and OR=1.58; 95%CI, 1.20-2.09). In the subgroup analysis by age, ANRIL rs2383207 polymorphism showed significant results in old CAD patients and young CAD patients (OR=1.32; 95%CI, 1.20-1.44 and OR=1.53; 95%CI, 1.32-1.77). Furthermore, this polymorphism also influenced myocardial infarction risk (OR=1.75; 95%CI, 1.24-2.47). Even the studies with adjustment for age, gender, smoking were included, the significant association was also observed (OR=1.43; 95%CI, 1.26-1.62). In conclusion, this meta-analysis suggested that ANRIL rs2383207 polymorphism is associated with CAD risk.

Key words: Coronary heart disease, ANRIL, meta-analysis, polymorphism.

Introduction

Cardiovascular diseases have become the leading cause of death in the world, among which coronary artery disease (CAD) stands out due to its high morbidity and mortality. Many studies have identified some risk factors for CAD, including age, gender, hypertension, diabetes, and smoking. However, these conventional risk factors can only explain minority of the etiology of CAD, indicating that genetic factors play a pivotal role in the development of CAD.

Recently, antisense non-coding RNA in the INK4 locus (ANRIL) has garnered substantial attention. ANRIL is transcribed as a 3.8-kb lncRNA in the antisense orientation of the INK4b/ARF/INK4a gene cluster (1). ANRIL is a genetic risk factor for several conditions with inflammatory components in Caucasians, and is the strongest genetic susceptibility locus for periodontitis (2). It has been shown that the disease-associated single nucleotide polymorphisms (SNPs) of chromosome 9p21 have been associated with the expression of ANRIL (3). In particular, the CAD-associated polymorphisms within the core risk haplotype region have been shown to regulate ANRIL expression in vitro (4) and also in vivo (5). Some studies investigated the association of ANRIL rs2383207 polymorphism with CAD risk (6-18). However, the result was still inconsistent. The aim of this study was to investigate whether there is an association between the ANRIL rs2383207 polymorphism and CAD risk.

Methods

Publication search

We carried out a PubMed (Medline), EMBASE database search covering all published articles with a combination of the following key words: “CAD or coronary artery disease” and “ANRIL”. In addition, we searched for potentially relevant studies by checking the titles and abstracts to retrieve any other eligible studies.

Inclusion criteria

The following criteria were used to select the eligible studies: (a) evaluation of the association between ANRIL rs2383207 polymorphism and CAD risk; (b) an unrelated case–control study in which family members were excluded; (c) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI). When authors reported two or more publications on the same patient population, only the largest study was selected. Additionally, when a study reported the results on different subpopulations, we treated them as a separate study.

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Data extraction

The following information was extracted from all eligible studies independently by two investigators: first author's name, year of publication, ethnicity, age, gender, sample size, Hardy-Weinberg equilibrium (HWE) in controls, and adjustment. As regard to disagreements, the two investigators negotiated with each other to reach a consensus finally.

Quality assessment

Quality assessment was conducted for each article according to strengthening the Reporting of Genetic Association studies (STREGA) containing eleven items associated with valid data reported in the study. For each item, there are three degrees, "yes"(scored 2),"can't tell"(scored 1) or "no"(scored 0), after evaluating each item, a total score from 0 to 22 was reported for each article. Studies would be divided into 3 grades: Grade A (scored 15-22, high quality), Grade B (scored 8-14, medium quality), or Grade C (scored 0-7, inferior quality). Only the studies of Grade A or B would be included in the final analysis.

Statistical analysis

The strength of association between ANRIL rs2383207 polymorphism and CAD risk was assessed by calculating OR with 95% CI. The pooled ORs were performed in additive model. A statistical test for heterogeneity was performed based on the Q statistic. The P>0.10 of the Q-test indicated a lack of heterogeneity among studies. The summary OR estimate of each study was calculated by the random-effects model or the fixed-effects model. Stratified analysis was performed by race, age, and gender. Potential publication bias was examined by Egger's test. All statistical tests were performed with the STATA version 11.0 (Stata Corporation, College station, TX, USA). A P value <0.05 was considered statistically significant.

Results

Study characteristics

The flow chart in **Figure 1** summarizes this literature review process. In this current study, a total of 13 eligible studies met the inclusion criteria. Finally, a total of 13 case-control studies involving 6796 cases and 9956 controls were included in this meta-analysis. There were 7 studies performed using Asians and 6 studies using Caucasians. Characteristics of studies are presented in **Table 1**.

Results of meta-analysis

The results of the association between ANRIL rs2383207 polymorphism and CAD risk are listed in **Table 2**. ANRIL rs2383207polymorphism was associated with a significantly an increased risk of CAD (OR=1.47; 95%CI, 1.33-1.62; **Figure 2**). We also found that this polymorphism increased CAD risk in Caucasians (OR=1.51; 95%CI, 1.28-1.77) and Asians (OR=1.42; 95%CI, 1.26-1.61). In the subgroup analysis according to gender, both women and men were significantly associated with the increased risk of CAD (OR=1.36; 95%CI, 1.03-1.79 and OR=1.58; 95%CI, 1.20-2.09). In the subgroup analysis by age, ANRIL rs2383207 polymorphism showed significant results in old CAD patients and young CAD patients (OR=1.32; 95%CI, 1.20-1.44 and OR=1.53; 95%CI, 1.32-1.77). Furthermore, this polymorphism also influenced myocardial infarction risk (OR=1.75; 95%CI, 1.24-2.47). Even the studies with adjustment for age, gender, smoking were included, the significant association was also observed (OR=1.43; 95%CI, 1.26-1.62).

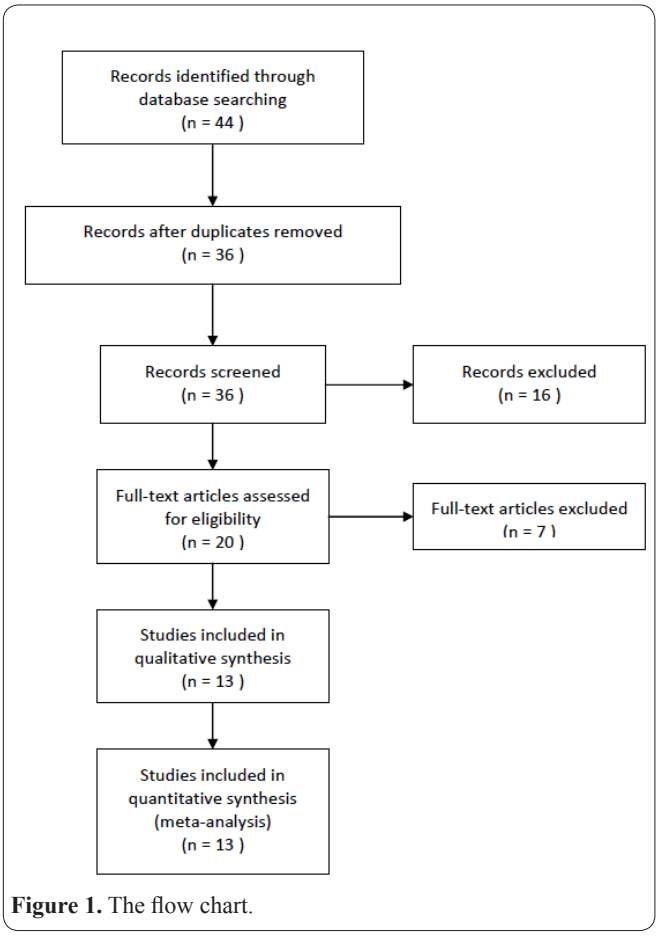

Figure 1. The flow chart.

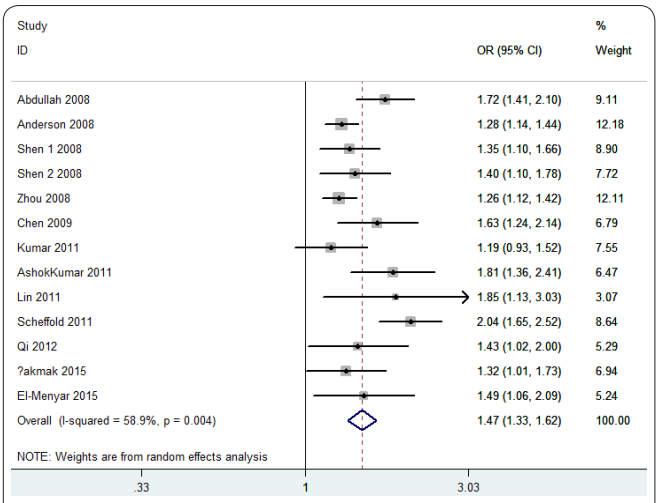

Figure 2. Meta-analysis for the association between ANRIL rs2383207 polymorphism and CAD risk.

The shape of the funnel plots did not reveal any evidence of obvious asymmetry (**Figure 3**). The Egger test also did not display any evidence of publication bias (P=0.07).

Discussion

This present meta-analysis investigating the rela-

Table 1. Characteristics of the studies included in this meta-analysis.

Study	Year	Ethnicity	Age (y)	Gender	No. of case	No. of control	Quality score	Hardy–Weinberg equilibrium	Adjustment
Abdullah	2008	Caucasian	40.3	Mixed	310	560	18	Yes	NA
Anderson	2008	Caucasian	51	Mixed	1011	1010	19	Yes	NA
Shen 1	2008	Caucasian	60	Mixed	416	308	17	Yes	Gender, age, smoking, body mass index, hypertension, diabetes,total cholesterol, low-density lipoprotein cholesterol, and triglyceride level
Shen 2	2008	Asian	63	Mixed	611	294	18	Yes	Gender, age, hypertension, and diabetes
Zhou	2008	Asian	60	Mixed	1360	1360	17	Yes	NA
Chen	2009	Asian	47	Mixed	232	212	17	Yes	NA
Kumar	2011	Asian	48	Mixed	443	311	18	Yes	Gender, age, diet, diabetes, hypertension, body mass index, smoking and levels of homocysteine
AshokKumar	2011	Asian	53	Mixed	414	408	19	Yes	Gender, age, diabetes, hypertension, smoking, and familial history
Lin	2011	Asian	63	Mixed	425	1377	19	Yes	Gender, age, hypertension, diabetes, hypercholesterolemia, and current smoking
Scheffold	2011	Caucasian	52	Mixed	976	3532	19	Yes	NA
Qi	2012	Asian	39-82	Mixed	142	192	17	Yes	NA
Çakmak	2015	Caucasian	53	Mixed	220	240	18	Yes	NA
El-Menyar	2015	Caucasian	56	Mixed	236	152	18	Yes	Gender, age, smoking, body mass index

NA, not available.

Table 2. Results of this meta-analysis.

	Test of association			Heterogeneity	
	OR (95% CI)	P Value	Model	I^2 (%)	P Value
Overall	1.47 (1.33-1.62)	<0.00001	R	59	0.004
Caucasian	1.51 (1.28-1.77)	<0.00001	R	73	0.003
Asian	1.42 (1.26-1.61)	<0.00001	F	39	0.13
Old	1.32 (1.20-1.44)	<0.00001	F	0	0.45
Young	1.53 (1.32-1.77)	<0.00001	R	70	0.002
Male	1.36 (1.03-1.79)	0.03	F	29	0.23
Female	1.58 (1.20-2.09)	0.001	F	0	0.63
MI	1.75 (1.24-2.47)	0.001	F	67	0.08
Adjustment for age, gender, smoking	1.43 (1.26-1.62)	<0.00001	F	35	0.19

NA, not available.

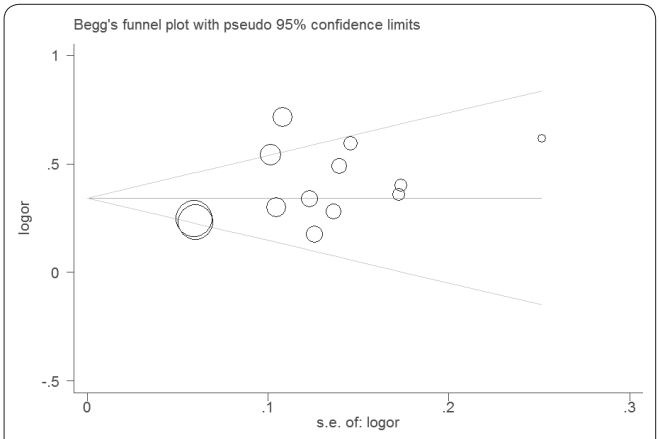

Figure 3. Funnel plot for the association between ANRIL rs2383207 polymorphism and CAD risk.

tionship between ANRIL rs2383207 polymorphism and CAD risk. Thirteen studies with a total of 6796 cases and 9956 controls were included. At the overall analysis, the ANRIL rs2383207 polymorphism was significantly associated with CAD risk. In the subgroup analysis by ethnicity, we found that Caucasians and Asians with ANRIL rs2383207 polymorphism had an increased CAD risk. In the subgroup analysis according to gender, both women and men were significantly associated with the increased risk of CAD. In the subgroup analysis by age, ANRIL rs2383207 polymorphism showed significant results in old CAD patients and young CAD patients. Furthermore, this polymorphism also influenced myocardial infarction risk.

The underlying molecular mechanisms are still not well understood. Congrains et al. suggested that ANRIL rs2383208 polymorphism affected the expression of ANRIL, which, in turn modulate cell growth, possibly via CDKN2A/B regulation (3). Teeuw et al. showed that a leading SNP in ANRIL was explanatory for inter-individual variation in C-reactive protein (CRP) levels (19). However, whether ANRIL rs2383207 polymorphism influence the expression of ANRIL or CRP was not determined. Thus, this issue should be studied in the future.

Recent findings showed the potential role of ANRIL in directing cellular fates leading to cardiovascular disease. Nonetheless, a recent study revealed that ANRIL association with CAD susceptibility can be related to its capability of regulating gene expression in trans (20), leading to decreased apoptosis and increased cell proliferation and cell adhesion, characteristic and essential alterations of atherogenesis (21).

This present study had some limitations that should be acknowledged. First, the sample size was relatively small in subgroup analyses by age and gender. Second, significant heterogeneity was detected in included studies and the accuracy of results would be affected in spite of utilizing the random-effects model to calculate pooled ORs. Third, we didn't explore gene-gene and gene-environment interactions because of the insufficient data.

In conclusion, this meta-analysis suggested that ANRIL rs2383207 polymorphism is associated with CAD risk. However, considering the above-mentioned limitations, larger well-designed studies with more consideration of gene-gene and gene-environment interactions are warranted in future.

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