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Association of lncRNA Polymorphisms with Myocardial Infarction: A Case-Control Study and a Meta-Analysis

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Abstract

Background: The long noncoding RNAs (lncRNAs) 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) and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) may affect the prognosis of MI patients.

Methods: The study included 401 Han Chinese MI patients and 409 controls. Four lncRNA tag single nucleotide polymorphisms (SNPs)-ANRIL rs9632884 and rs1537373, MALAT1 rs619586 and rs3200401were selected. SNP genotyping was performed by an improved multiplex ligation detection reaction assay. A systematic review and meta-analysis of studies including 9,807 cases and 9.326 controls on the association of five ANRIL SNPs and the overall risk of MI or coronary artery disease (CAD) was performed.

Results: rs9632884 and rs3200401 SNPs were significantly associated with lipid levels in both controls and MI patients (P<0.003-0.046). Several SNPs interacted with sex and age to modify total cholesterol, low-density lipoprotein cholesterol, and creatinine levels to modify the risk of MI. No association between the lncRNAs SNPs and susceptibility to MI was found (P>0.05 for all). In the meta-analysis, rs4977574 A>G and rs1333049 G>C ANRIL polymorphisms, but not rs1333040, rs1333042 or rs10757274, were correlated with overall MI or CAD risk.

Conclusions: Taken together, this study provides additional evidence that genetic variation of the ANRIL rs9632884 and MALAT1 rs3200401 can mediate lipid levels in MI patients.

Keywords: Cyclin-dependent kinase inhibitor 2B antisense RNA; Metastasis associated lung adenocarcinoma transcript 1; Single nucleotide polymorphism; Myocardial infarction; Coronary artery disease

Introduction

Morbidity and mortality associated with coronary artery disease (CAD) result in significant economic and social burdens [1]. Hypertension, smoking, hypercholesterolemia, and diabetes are estimated to contribute to 50%-60% of disease susceptibility and genetic variation may account for predisposition in 40%-50% of sporadic cases [2-5]. Atherosclerosis contributes to the pathophysiology of CAD, but the effects of variation in the molecular and genetic determinants are not yet clear [6]. 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 [7].

Long noncoding (lnc)RNAs are transcripts of at least 200 base pairs in length that do not code for proteins [8]. Studies of the functions of lncRNAs in disease, including CAD, are ongoing [9-12]. ANRIL, also known as CDK2BAS (CDKN2B antisense RNA) overlaps with CDKN2B on human chromosome 9p21 [13], a locus associated with genetic susceptibility for cardiovascular diseases (CVD). It spans 50 kb of DNA that express the ANRIL transcripts [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 [13]. MALAT1, a transcript first described in metastatic lung adenocarcinoma, regulates hyperglycemia-induced inflammation in endothelial cells. In several studies, MALAT1 inhibition was shown to decrease endothelial cell proliferation both *in vitro* and *in vivo*. MALAT1 sequesters serine/arginine splicing factors in nuclear speckle domains, thereby regulating alternative splicing [15], and it may also have immune-regulatory activity [16]. This is the first cardiovascular study to describe MALAT1 polymorphisms. In this study, the association of four tag SNPs (ANRIL rs9632884 and rs1537373, MALAT1 rs619586 and rs3200401) and myocardial infarction (MI) was investigated in a case-control study of 401 Han Chinese patients with MI [9].

Methods and Methods

Sample collection

A total of 401 hospitalized MI patients were enrolled at the Second Affiliated Hospital, Harbin Medical University (China) between September 2016 and November 2017. The study protocol was approved by the local ethics review board; all participants provided written informed consent. The diagnosis of MI was based on the Pakistan Risk of Myocardial Infarction Study (PROMIS) international guideline. MI was diagnosed by symptoms within 24 h of hospital admission, an electrocardiogram consistent with MI, and positive troponin-I. Patients with recent illnesses or infections were not eligible [17,18]. A group of 409 age-(5-year bands) and sex-matched medical center patients without a history of CAD or symptoms of MI were selected as

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controls. Patients with cerebrovascular, neurological, or kidney disease, blood disorders, cancer, peripheral vascular disease, or autoimmune diseases were excluded from the control group. The controls were free of MI by questionnaires, history-taking, and clinical examination. The examination comprised physical examination, blood sampling, electrocardiography, chest X-ray, and Doppler echocardiography. Participant age, sex, blood pressure, lipid profile, fasting glucose, medical, drug, smoking, and alcohol histories were collected.

SNP selection

Four ANRIL and MALAT1 lncRNA loci were selected by a tagSNP method using Haploview version 4.2 bioinformatics software (Broad Institute, Cambridge, MA, USA); assuming a minor allele frequency >0.05 and a squared correlation between genotypes (r^2)>0.8 for the SNPs in the Han Chinese population (CHB+CHS). The SNP information was retrieved from the 1000 Genomes Project and included those associated with cardiovascular disease in recent studies.

SNP genotyping

The genomic DNA was extracted using a GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, USA) as per the product instruction. The SNP genotyping work was performed using an improved multiplex ligation detection reaction (iMLDR) technique developed by Genesky Biotechnologies Inc. (Shanghai, China). A multiplex PCR-ligase detection reaction method was used in the iMLDR. For each SNP, the alleles were distinguished by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNPs were further distinguished by different extended lengths at the 3'end. Two negative controls were set: one with double-distilled water as template and the other with DNA sample without primers while keeping all other conditions the same in one plate. Duplicate tests were designed and the results were consistent. A random sample accounting for ~5% (n=40) of the total DNA samples was directly sequenced using Big Dye-terminator version 3.1 and an ABI3730XL automated sequencer (Applied Biosystems) to confirm the results of iMLDR.

Selection of relevant studies

Study reports published before April 25, 2018 were retrieved from PubMed and the Web of Science using the search terms (coronary artery disease, coronary heart disease, or myocardial infarction), ("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 two studies of ANRIL polymorphism reporting the genotype frequencies of each included ANRIL SNP (i.e. 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. Thirteen articles including 9,807 cases and 9.326 controls were selected (Figure 1).

Statistical analysis

All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). All tests were two-sided and P-values <0.05 were considered significant. Between-group differences in demographic characteristics and genotype frequencies of the four SNPs were evaluated by Student's t-test for continuous variables and χ^2 tests for categorical variables. The Hardy-Weinberg equilibrium was assessed for controls using the goodness-of-fit χ^2 test. Associations of genotypes and alleles and the risk of MI were estimated by odds ratios (ORs) and 95% confidence intervals (CIs). Haploview software (version 4.2) was used for the analysis of pairwise linkage disequilibrium, haplotype

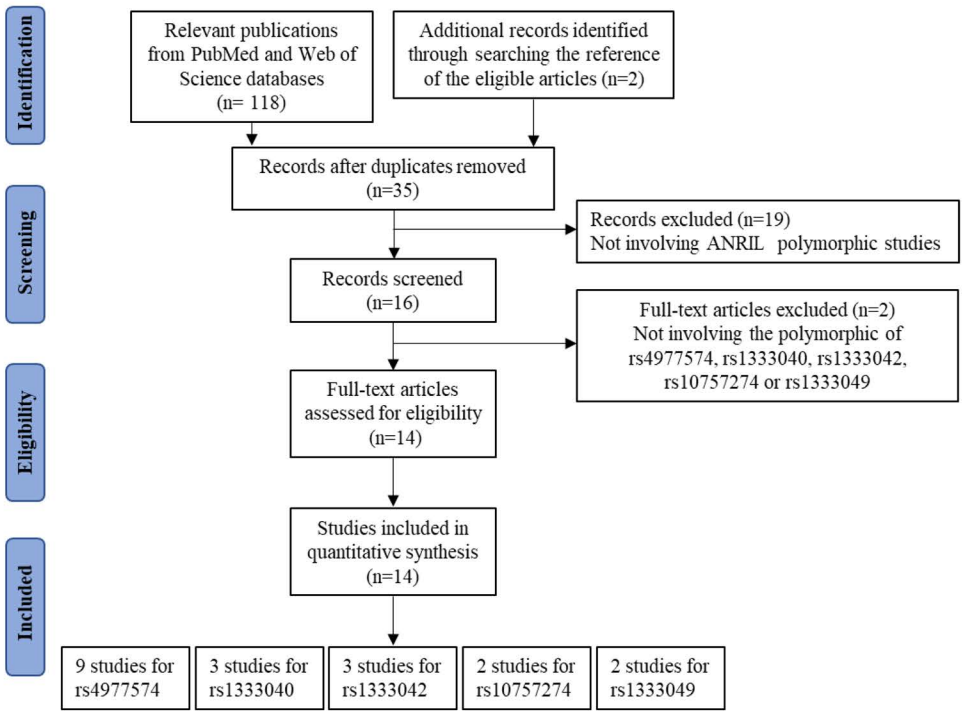

Figure 1: Thirteen articles including 9,807 cases and 9.326 controls were selected for analysis.

structure, and genetic association of polymorphism loci. Differences of lipid levels and genotypes were determined by the Kruskal-Wallis test. Significant interactions of the four SNPs with alcohol consumption, cigarette smoking, age, sex, and hypertension with lipid levels and the risk of MI were detected by the independent-samples t-test for categorical variables and linear regression analysis for continuous variables after controlling for potential confounders, a p-value <0.002 after the Bonferroni correction was considered statistically significant.

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 χ^2-based Q and I^2 tests. The pooled OR was calculated using a fixed effect model in the absence of heterogeneity (P>0.05, I^2<50%). Otherwise, a random effect model was used. The stability of the pooled ORs was determined by one-way sensitivity analysis.

Results

Population characteristics

The clinical characteristics of the 810 study participants are shown in Table 1. The majority (82.0%) were men (60.3%) were current smokers, and 26.2% drank alcohol. Except for hypercholesterolemia and triglycerides, the factors associated with increased risk of MI or CAD occurred more frequently in the case than in the control group.

Genotype and allele frequencies in patients and controls

The genotype and allele frequencies of four SNPs selected for study are shown in Table 2. No deviations from Hardy-Weinberg equilibrium were observed in either cases or controls. The genotype and allele frequencies of the rs9632884, rs1537373, rs619586 and rs3200401 SNPs in MI patients and controls were not significantly different (all P>0.05).

Genotypes of the lncRNA SNPs and the risk of MI

Results of the genetic model analysis are shown in Table 3. Comparison of both heterozygous and homozygous carriers of the minor allele (G) with homozygous carriers of the major allele (C) revealed that the ANRIL rs9632884 and rs1537373 SNPs were negatively associated with MI, suggesting a dominant genetic effect. No association of rs619586 and rs3200401 and MI were observed. Similar, but weaker trends were observed for the recessive model, with no significant associations of the four SNPs with MI (all P>0.05).

Variables	MI (n=401)	Controls (n=409)	P-value[a]
Age, years	58.20 (11.65)	56.34 (9.52)	0.223
Male sex, No. (%)	329 (82.0%)	339 (82.9%)	0.753
Smoking, No. (%)	242 (60.3%)	187 (45.7%)	**3.04E-05**[b]
Alcohol, No. (%)	105 (26.2%)	82 (20.0%)	**0.038**
Diabetes, No. (%)	99 (26.7%)	50 (12.2%)	**4.71E-06**
Hypertension, No. (%)	192 (47.9%)	143 (35.0%)	**1.90E-4**
Hypercholesterolemia, No. (%)	55 (13.7%)	39 (9.5%)	0.063
WBC, 10^9/L	11.96 (3.81)	6.84 (1.90)	**1.78E-80**
FBG, mmol/L	7.13 (3.86)	5.93 (1.93)	**1.25E-7**
CRE, μmol/L	85.67 (30.17)	70.83 (13.30)	**3.22E-18**
AST, U/L	80.44 (85.24)	25.12 (9.43)	**2.54E-32**
TC, mmol/L	4.56 (1.05)	4.96 (0.84)	**7.03E-9**
Triglycerides, mmol/L	1.65 (1.12)	1.67 (1.23)	0.764
HDL cholesterol, mmol/L	1.28 (0.43)	1.37 (0.36)	**1.65E-3**
LDL cholesterol, mmol/L	2.77 (0.83)	3.30 (0.73)	**1.92E-20**

Values are means ± SD or n (%). [a]Two-sided chi-square test or independent-samples *t*-test. [b]P-values <0.05 are **bold**. Type 2 diabetes was diagnosed as (1) fasting plasma glucose (FPG) ≥ 7.0 mmol/L; (2) 2 h postprandial glucose ≥ 11.1 mmol/L; or (3) use of antidiabetes medications. Hypertension was defined as systolic/diastolic blood pressure ≥ 140 mmHg or ≥ 90 mmHg or use of antihypertensive medications. Hypercholesterolemia was defined as use of cholesterol-lowering medications or total serum cholesterol >200 mg/dl. WBC: White blood cell; FBG: Fasting blood glucose; AST: Aspartate transaminase; CRE: Creatinine; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Table 1: Baseline characteristics of the study participants.

SNP/group	Genotype[a] (n (%))			χ^2	P	Allele (n (%))		χ^2	P	OR (95% CI)
rs9632884	GG	GC	CC			G	C			
Case	22 (0.05)	141 (0.35)	238 (0.59)			185 (0.23)	617 (0.77)	0.99	0.32	0.89 (0.71-1.12)
Control	24 (0.06)	158 (0.39)	227 (0.56)	0.25	0.61	206 (0.25)	612 (0.75)			
rs1537373	TT	GT	GG			T	G			
Case	37 (0.09)	153 (0.38)	211 (0.53)			227 (0.28)	575 (0.72)	0.89	0.35	0.90 (0.73-1.12)
Control	41 (0.10)	167 (0.41)	201 (0.49)	0.52	0.46	249 (0.30)	569 (0.70)			
rs619586	GG	GA	AA			G	A			
Case	4 (0.01)	53 (0.13)	344 (0.86)			61 (0.08)	741 (0.92)	0.03	0.87	0.97 (0.67-1.40)
Control	1 (0.00)	62 (0.15)	346 (0.85)	1.06	0.30	64 (0.08)	754 (0.92)			
rs3200401	TT	CT	CC			T	C			
Case	12 (0.03)	101 (0.25)	288 (0.72)			125 (0.16)	677 (0.84)	1.49	0.22	0.85 (0.65-1.10)
Control	12 (0.03)	122 (0.30)	275 (0.67)	0.12	0.73	146 (0.18)	672 (0.82)			

[a]All are in Hardy-Weinberg equilibrium. SNP: Single nucleotide polymorphism; MI: Myocardial infarction.

Table 2: Genotypic and allelic frequencies of four SNPs in MI patients and controls.

SNP/group	Genotype		χ^2	P	OR (95% CI)
rs9632884					
Dominant	GG+GC	CC			
Case	163 (0.41)	238 (0.59)	1.22	0.26	0.85 (0.64-1.14)
Control	182 (0.44)	227 (0.56)			
Recessive	GG	GC+CC			
Case	22 (0.05)	379 (0.95)	0.06	0.81	0.93 (0.51-1.69)
Control	24 (0.06)	385 (0.94)			
rs1537373					
Dominant	TT+GT	GG			
Case	190 (0.47)	211 (0.53)	0.98	0.32	0.87 (0.66-1.15)
Control	208 (0.51)	201 (0.49)			
Recessive	TT	GT+GG			
Case	37 (0.09)	364 (0.91)	0.14	0.70	0.91 (0.57-1.46)
Control	41 (0.10)	368 (0.90)			
rs619586					
Dominant	GG+GA	AA			
Case	57 (0.14)	344 (0.86)	0.22	0.63	0.91 (0.62-1.34)
Control	63 (0.15)	346 (0.85)			
Recessive	GG	GA+AA			
Case	4 (0.01)	397 (0.99)	1.87	0.17	4.11 (0.54-31.15)
Control	1 (0.00)	408 (1.00)			
rs3200401					
Dominant	TT+CT	CC			
Case	113 (0.28)	288 (0.72)	2.01	0.16	0.80 (0.60-1.09)
Control	134 (0.33)	275 (0.67)			
Recessive	TT	CT+CC			
Case	12 (0.03)	389 (0.97)	0.002	0.96	1.02 (0.45-2.30)
Control	12 (0.03)	397 (0.97)			
SNP: Single nucleotide polymorphism; MI: Myocardial infarction.					

Table 3: Genetic model analysis of the association of four SNPs and MI susceptibility.

Genotype and lipid levels

We expected that genetic risk associated with the SNPs would be reflected by established CAD risks, including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (CRE), or fasting blood glucose (FBG). As shown in Table 4, the minor G allele of rs9632884 was associated with high TG concentrations (P=0.030) and the rs3200401 variants were associated with increased TC in MI patients compared with the control group (P=0.046). None of the four SNPs were associated with LDL-C, HDL-C, CRE, or FBG in MI patients (P>0.05).

Interactions of the four SNPs and drinking, smoking, age, sex and hypertension on lipid levels and the risk of MI

The interactions of the four SNPs and drinking, smoking, age, sex and hypertension on lipid levels and the risk of MI are shown in Table 5. The SNP of rs9632884 interacted with sex to modulate CRE levels. Several SNPs interacted with age to influence TC (rs9632884) and LDL-C (rs1537373) levels.

Association of haplotypes and MI

Haplotype analysis of the rs9632884 and rs1537373 ANRIL SNPs did not find significant differences in the MI and control groups (P=0.307). The MALAT1 the haplotypes were not associated with incident MI (P=0.223; Supplementary Tables 1 and 2) (Supplementary Figures 1A and 1B).

Meta-analysis of ANRIL in MI or CAD

Several SNPs in the ANRIL have been associated with susceptibility to CAD [19], 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. Thirteen articles evaluating a total of 9,807 cases and 9.326 controls were selected for analysis as shown in Figure 1, and the characteristics of the included studies are listed in Table 6. The association of ANRIL rs4977574 A>G polymorphism and MI or CAD risk was investigated in eight studies including 6,694 patients and 7,183 healthy controls. The variant G allele was found to be protective against CAD development (OR=0.81, 95% CI=0.75-0.87, P<0.00001, I^2=47%, Figure 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. or Sakalar et al. was removed (Supplementary Figures 2A and 2B) [20, 21]. The association of lncRNA ANRIL rs1333040 C>T polymorphism and CAD risk was investigated in three studies including 907 cases and 843 controls. No significant overall associations were identified in the allele genotype model (OR=0.82, 95% CI=0.64-1.04, P=0.11, I^2 =51%, Figure 3); rs1333049 G>C polymorphism was found to be associated with increased overall risk of CAD (OR=1.49, 95% CI=1.23-1.80, P<0.0001, I^2=0%; Supplementary Figure 3); and rs1333042 (OR =0.85, 95% CI =0.65-1.11, P=0.24, I^2 =79%) and rs10757274 (OR =0.75, 95% CI =0.41-1.38, *P*=0.36, I^2 =85%) polymorphisms were found not to be associated with CAD risk (Supplementary Figures 4 and 5).

SNP	Genotype (Counts)	TC mmol/L	TG mmol/L	HDL-C mmol/L	LDL-C mmol/L	CRE μmol/L	FBG mmol/L
rs9632884							
case	GG (22)	4.61 ± 0.95	1.19 ± 0.55	1.39 ± 0.43	2.87 ± 0.78	81.03 ± 30.51	7.44 ± 2.47
	GC (141)	4.57 ± 1.06	1.57 ± 1.01	1.28 ± 0.29	2.79 ± 0.85	87.74 ± 30.63	7.65 ± 3.33
	CC (238)	4.55 ± 1.05	1.74 ± 1.22	1.27 ± 0.48	2.74 ± 0.83	86.33 ± 28.02	7.70 ± 3.61
	P	0.991	**0.030**	0.234	0.731	0.249	0.834
control	GG (24)	5.02 ± 0.87	1.64 ± 0.80	1.34 ± 0.25	3.33 ± 0.81	75.71 ± 13.01	5.93 ± 2.36
	GC (158)	4.95 ± 0.86	1.75 ± 1.30	1.37 ± 0.33	3.29 ± 0.70	71.39 ± 14.24	5.87 ± 1.77
	CC (227)	4.94 ± 0.84	1.61 ± 1.21	1.38 ± 0.40	3.27 ± 0.77	69.95 ± 12.55	5.98 ± 1.97
	P	0.768	0.352	0.935	0.819	0.090	0.643
rs1537373							
case	TT (37)	4.68 ± 1.01	1.50 ± 1.30	1.35 ± 0.42	2.87 ± 0.83	82.96 ± 27.37	7.82 ± 3.37
	GT (153)	4.55 ± 1.09	1.60 ± 0.98	1.24 ± 0.28	2.81 ± 0.88	89.72 ± 33.24	7.68 ± 3.35
	GG (211)	4.55 ± 1.03	1.71 ± 1.19	1.30 ± 0.50	2.72 ± 0.80	84.86 ± 25.87	7.63 ± 3.55
	P	0.838	0.210	0.321	0.501	0.297	0.544
control	TT (41)	4.96 ± 1.07	1.59 ± 0.76	1.38 ± 0.28	3.44 ± 0.69	72.51 ± 13.09	5.94 ± 2.00
	GT (167)	4.98 ± 0.83	1.73 ± 1.32	1.38 ± 0.34	3.28 ± 0.74	70.23 ± 13.97	5.85 ± 1.85
	GG (201)	4.92 ± 0.82	1.63 ± 1.23	1.37 ± 0.40	3.25 ± 0.76	71.02 ± 12.79	6.01 ± 1.96
	P	0.618	0.599	0.529	0.223	0.373	0.788
rs619586							
case	GG (4)	4.42 ± 0.80	1.18 ± 0.33	1.36 ± 0.34	2.83 ± 0.77	83.50 ± 17.62	4.82 ± 2.21
	GA (53)	4.54 ± 1.09	1.73 ± 1.15	1.33 ± 0.78	2.79 ± 0.77	83.68 ± 18.32	7.22 ± 3.61
	AA (344)	4.57 ± 1.05	1.64 ± 1.13	1.27 ± 0.34	2.76 ± 0.85	87.01 ± 30.51	7.78 ± 3.42
	P	0.950	0.333	0.603	0.842	0.950	0.354
control	GG (1)	5.71	1.99	1.27	4.06	53	10.22
	GA (62)	5.03 ± 0.93	1.72 ± 1.36	1.41 ± 0.35	3.31 ± 0.75	71.55 ± 15.05	5.77 ± 1.77
	AA (346)	4.93 ± 0.83	1.66 ± 1.21	1.37 ± 0.37	3.27 ± 0.75	70.77 ± 12.96	5.95 ± 1.93
	P	0.503	0.687	0.528	0.455	0.356	0.282
rs3200401							
case	TT (12)	4.92 ± 0.96	1.89 ± 0.92	1.20 ± 0.37	2.97 ± 0.78	90.10 ± 23.24	7.21 ± 3.50
	CT (101)	4.55 ± 1.10	1.63 ± 1.24	1.25 ± 0.34	2.75 ± 0.89	85.23 ± 33.48	7.79 ± 3.38
	CC (288)	4.55 ± 1.030	1.64 ± 1.09	1.29 ± 0.45	2.76 ± 0.82	86.83 ± 27.68	7.65 ± 3.48
	P	0.465	0.384	0.848	0.306	0.241	0.819
control	TT (12)	5.50 ± 0.78	1.78 ± 1.11	1.40 ± 0.30	3.71 ± 0.73	68.17 ± 16.39	6.21 ± 2.71
	CT (122)	4.86 ± 0.85	1.57 ± 1.25	1.41 ± 0.44	3.20 ± 0.78	72.91 ± 13.48	5.82 ± 1.68
	CC (275)	4.96 ± 0.84	1.70 ± 1.22	1.36 ± 0.33	3.30 ± 0.73	70.04 ± 13.01	5.98 ± 1.98
	P	**0.046**	0.169	0.732	0.430	0.160	0.799

SNP: Single nucleotide polymorphism; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; CRE: Creatinine; FBG: Fasting blood glucose. Results are mean ± SD. Significance (P<0.05) was determined by the Kruskal-Wallis test.

Table 4: Lipid level and genotype in MI patients and controls.

SNP	Factor	TC	TG	HDL-C	LDL-C	CRE	FBG
rs9632884	Drinking	0.544	0.113	0.094	0.464	0.440	0.306
	Smoking	0.711	0.978	0.279	0.335	0.741	0.371
	Age	**0.005**	0.082	0.466	0.016	0.197	0.130
	Sex	0.292	0.433	0.017	0.699	**3.17E-5**	0.864
	Hypertension	0.304	0.907	0.873	0.676	0.242	0.257
rs1537373	Drinking	0.684	0.989	0.010	0.239	0.197	0.096
	Smoking	0.166	0.271	0.306	0.654	0.296	0.350
	Age	**0.042**	**0.016**	0.647	**0.007**	0.864	0.907
	Sex	0.668	0.990	0.058	0.788	**0.033**	0.649
	Hypertension	0.295	0.319	0.645	0.394	0.739	0.588
rs619586	Drinking	0.288	0.846	0.911	**0.040**	0.100	0.429
	Smoking	0.608	0.274	0.108	0.310	0.249	0.182
	Age	**0.027**	0.342	0.625	0.103	0.899	0.304
	Sex	0.165	0.012	0.783	0.634	0.078	0.477
	Hypertension	0.286	0.245	0.125	0.898	0.465	0.492
rs3200401	Drinking	0.692	0.970	0.816	0.519	0.067	0.353
	Smoking	0.170	0.393	0.197	0.429	0.879	0.831
	Age	0.054	**0.028**	0.057	0.213	0.440	0.051
	Sex	0.629	0.574	0.552	0.640	0.302	0.102
	Hypertension	0.345	0.509	0.634	0.301	0.621	0.471

SNP: Single nucleotide polymorphism; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; FBG: Fasting blood glucose; CRE: Creatinine; MI: Myocardial infarction. Significant difference, P<0.002 after the Bonferroni correction.

Table 5: Interaction of gene polymorphism with modifiable risk factors, blood lipids, creatinine, and fasting glucose.

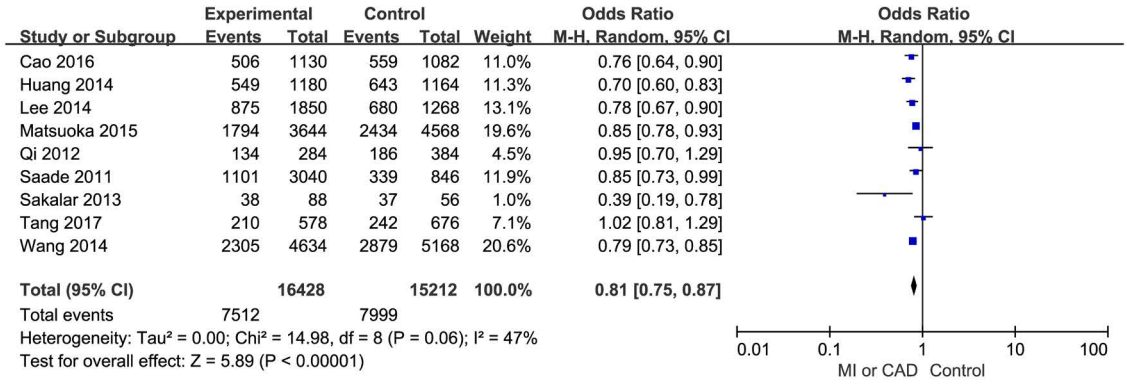

Figure 2: The variant G allele was found to be protective against CAD development.

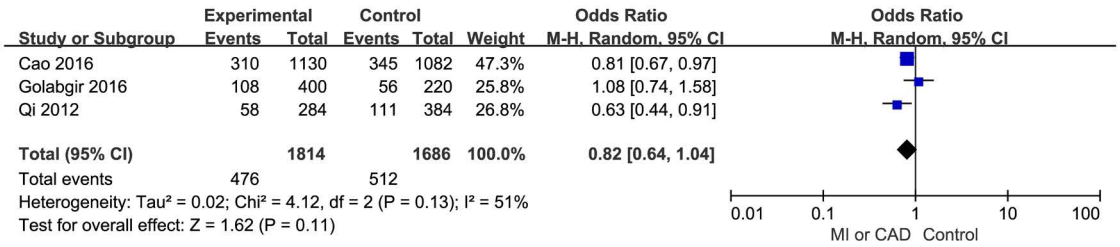

Figure 3: The association of lncRNA ANRIL rs1333040 C>T polymorphism and CAD risk was investigated in three studies including 907 cases and 843 controls. No significant overall associations were identified in the allele genotype model.

Discussion

The study investigated the influence of genetic variation in two lncRNAs (ANRIL and MALAT1) on MI risk in a series of Han Chinese patients and controls. The two rs9632884 and rs3200401 SNPs identified in the study population were significantly associated with lipid levels in both controls and MI patients (P<0.003-0.046). However, no associations of the lncRNA SNPs and susceptibility to MI were found (P>0.05 for all). The two lncRNAs 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 [22,23]. The variants within this locus have been significantly associated with an increased risk of CAD and diabetes in European and some other populations [24-26]. MALAT1, recently renamed nuclear-enriched noncoding transcript 2 because it accumulates in the nucleus, was selected because it regulates alternative splicing that can result in gene expression of multiple proteins in healthy and diseased states [27]. MALAT1 is highly abundant in mammalian cells, but MALAT1 polymorphism has not been associated with MI [28].

In the study patients, rs9632884 was associated with TG levels but not with MI. In previous studies in Asian patients Song et al. found that ANRIL expression increased serum lipids and the atherogenic index [29], and Chen et al. showed that coronary atherosclerosis patients had elevated total LDLs and TGs [30]. We did not find a significant association of ANRIL rs9632884 and rs1537373 SNPs with MI in Han Chinese, but rs9632884 has been associated with the risk of CAD in European studies [31], which is inconsistent with our findings. To avoid false-negative results, we performed a systematic meta-analysis to confirm the results. SNP rs4977574 on chromosome 9p21.3 is located in ANRIL. This region has been considered as the most widely and consistently replicated risk locus for CHD and MI. We demonstrated that the variant G allele of rs4977574 exhibited a significant decreased risk for developing CAD, and the C allele of rs1333049 was associated with a significant increased CAD risk in Asians. In contrast, the rs1333040, rs1333042 and rs10757274 variant alleles exhibited no significant association with CAD risks. The differences in these study findings may be explained by ethnic, environmental, or lifestyle differences that affected the development of MI. Another explanation is that the participants in this study had experienced their first MI, and their risk factor profiles may differ from the profiles of patients with recurrent CAD. The way in which these SNP loci contribute to susceptibility to MI requires further study.

To the best of our knowledge, no previous studies have investigated the association of MALAT1 lncRNA polymorphisms and acute MI. rs3200401 was associated with TC levels, but the nature of any interaction was not determined. Some lncRNAs act as competing endogenous RNAs and function as micro (mi) RNA sponges that sequester miRNA regulates transcript expression by sharing common miRNA response elements [32]. Variant miRNA binding sites could result in gain or loss of function of miRNA-lncRNA interactions, ultimately affecting expression of other miRNA-targeted mRNAs [33,34]. According to the lncRNASNP [35], the C>T of the MALAT1 rs3200401 SNP results in a 1.62 kcal/mol minimal free energy (MFE, ΔG) change that may accompany loss of hsa-miR-1324 miRNA binding sites on MALAT1, leading to loss of function of miRNA-lncRNA interactions (Supplementary Figure 6). However, we did not find any associations of MALAT1 rs619586 and rs3200401 SNP and the risk of MI. Further study is needed to draw any conclusions about the role of MALAT1 rs619586 and rs3200401 in atherogenesis, and since this study included only Han Chinese participants, further studies in other ethnic groups are needed to confirm the relationships.

Cite	First author	Year	Ethnicity	Country /Region	Case	Control	Genotype distribution						Genotyping methods	Disease	P for HWE[a]
							Case			Control					
							rs4977574 A>G								
							AA	AG	GG	AA	AG	GG			
[20]	Tang	2017	Asian	China	289	338	37	136	116	38	166	134	PCR	CHD	0.208
[36]	Cao	2016	Asian	China	565	541	117	272	176	152	255	134	PCR-RFLP	CHD	0.191
[37]	Matsuoka	2015	Asian	Japan	1822	2284	448	898	476	651	1132	501	PCR	MI	0.831
[38]	Wang.	2014	Asian	China	2317	2584	583	1139	595	777	1325	482	TaqMan	CAD	0.047
[39]	Lee	2014	Asian	China	925	634	198	479	248	181	318	135	TaqMan	CAD	0.831
[40]	Huang	2014	Asian	China	590	582	122	305	163	138	367	77	PCR	CHD	0.000
[21]	Sakalar	2013	Caucasian	Turkey	44	28	8	22	14	13	11	4	PCR-RFLP	MI	0.513
[41]	Qi	2012	Asian	China	142	192	35	64	43	46	94	52	PCR	MI	0.783
[42]	Saade	2011	Asian	Lebanese	1520	423	208	685	627	72	195	156	PCR	CAD	0.409
							rs1333040 C>T								
							CC	CT	TT	CC	CT	TT			
[43]	Golabgir	2016	Caucasian	Iran	200	110	5	98	97	3	50	57	PCR	CAD	0.038
[36]	Cao	2016	Asian	China	565	541	39	232	294	60	225	256	PCR-RFLP	CHD	0.323
[41]	Qi	2012	Asian	China	142	192	7	44	91	9	93	90	PCR	MI	0.013
							rs1333042 A>G								
							AA	AG	GG	AA	AG	GG			
[44]	Mafi	2017	Caucasian	Iran	103	102	19	46	38	12	46	44	TaqMan	CAD	0.997
[36]	Cao	2016	Asian	China	525	513	43	227	255	67	219	227	PCR-RFLP	CHD	0.22
[45]	Arne	2009	Caucasian	Germany	817	670	174	380	263	208	306	156	TaqMan	CHD	0.036
							rs10757274 A>G								
							AA	AG	GG	AA	AG	GG			
[44]	Mafi.	2017	Caucasian	Iran	103	102	16	47	40	29	51	22	TaqMan	CAD	0.962
[46]	Cheng	2017	Asian	China	286	646	6	76	204	11	175	460	PCR-LDR	MI	0.221
							rs1333049 G>C								
							GG	GC	CC	GG	GC	CC			
[2]	Ahmed	2013	Asian	Pakistan	290	294	87	180	23	65	166	63	PCR	MI	0.027
[41]	Qi	2012	Asian	China	142	153	21	79	42	4	99	50	PCR	MI	0.000

[a]HWE in control; MI: Myocardial infarction; CAD: Coronary artery disease; CHD: Coronary heart disease.

Table 6: Characteristics of included studies on lncRNA ANRIL polymorphisms and MI or CAD risk included in the meta-analysis.

The study limitations include the relatively small sample size and inclusion of only one Chinese ethnicity. Second, there were differences in the clinical characteristics of the patients and controls. The statistical analyses adjusted for several confounders, but not all of the potential influences on the results were eliminated. Finally, the biological activities of the genetic variants of the two lncRNAs were not investigated. Larger studies are warranted to assess the potential association of the SNPs with more complex, clinical endpoints of MI and CAD.

Taken together, this study provides additional evidence that genetic variation of the ANRIL rs9632884 and MALAT1 rs3200401 can mediate lipid levels in MI patients, although the four SNPs in the cardiovascular-related lncRNAs were not associated with the risk of MI in this Han Chinese population. Those with the rs9632884 GG genotype had lower FBG than those with rs12762303 CC and rs12762303 GC genotypes. Those with the rs3200401 TT genotype had higher TC levels than those with rs3200401 CT and rs3200401 CC genotypes. The SNP of rs9632884 interacted with sex to modify CRE levels and to modify the risk of MI. Several SNPs interacted with age to influence TC (rs9632884) and LDL-C (rs1537373) levels. However, this study was designed as a pilot study and further investigations are needed to confirm our results and to elucidate unresolved questions. The contribution of other genetic variants of these vascular-related genes to CAD and MI cannot be excluded.

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References

1. AbdulAzeez S, Al-Nafie AN, Al-Shehri A, Borgio JF, Baranova EV, et al. (2016) 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 17: 395.
2. Ahmed W, Ali IS, Riaz M, Younas A, Sadeque A, et al. (2013) Association of ANRIL polymorphism (rs1333049:C>G) with myocardial infarction and its pharmacogenomic role in hypercholesterolemia. Gene 515: 416-420.
3. Roberts R, Stewart AF (2012) Genetics of coronary artery disease in the 21st century. Clin Cardiol 35: 536-540.
4. Severino P, D'Amato A, Netti L, Pucci M, De Marchis M, et al. (2018) Diabetes Mellitus and Ischemic Heart Disease: The Role of Ion Channels. Int J Mol Sci 19: E802.
5. Liu X, Jiang C, Yang P (2017) Association of single nucleotide polymorphisms in the 5' upstream region of the C4BPA gene with essential hypertension in a northeastern Han Chinese population. Mol Med Rep 16: 1289-1297.

6. Zhao Q, Liao S, Wei H, Liu D, Li J, et al. (2016) CDKN2BAS polymorphisms are associated with coronary heart disease risk a Han Chinese population. Oncotarget 7: 82046-82054.

7. Tang SS, Cheng J, Cai MY, Yang XL, Liu XG, et al. (2016) Association of lincRNA-p21 Haplotype with Coronary Artery Disease in a Chinese Han Population. Dis Markers 2016: 9109743.

8. Ballantyne RL, Zhang X, Nunez S, Xue C, Zhao W, et al. (2016) Genome-wide interrogation reveals hundreds of long intergenic noncoding RNAs that associate with cardiometabolic traits. Hum Mol Genet 25: 3125-3141.

9. Yang C, Tang R, Ma X, Wang Y, Luo D, et al. (2015) Tag SNPs in long non-coding RNA H19 contribute to susceptibility to gastric cancer in the Chinese Han population. Oncotarget 6: 15311-15320.

10. Zhang F, Zhang L, Zhang C (2016) Long noncoding RNAs and tumorigenesis: genetic associations, molecular mechanisms, and therapeutic strategies. Tumour Biol 37: 163-175.

11. Wang KC, Chang HY (2011) Molecular mechanisms of long noncoding RNAs. Mol Cell 43: 904-914.

12. Tsai MC, Spitale RC, Chang HY (2011) Long intergenic noncoding RNAs: new links in cancer progression. Cancer Res 71: 3-7.

13. Ma Y, Ma W, Huang L, Feng D, Cai B (2015) Long non-coding RNAs, a new important regulator of cardiovascular physiology and pathology. Int J Cardiol 188: 105-110.

14. Greco S, Salgado Somoza A, Devaux Y, Martelli F (2017) Long Noncoding RNAs and Cardiac Disease. Antioxid Redox Signal.

15. Lorenzen JM, Thum T (2016) Long noncoding RNAs in kidney and cardiovascular diseases. Nat Rev Nephrol 12: 360-373.

16. Bar C, Chatterjee S, Thum T (2016) Long Noncoding RNAs in Cardiovascular Pathology, Diagnosis, and Therapy. Circulation 134: 1484-1499.

17. Howson JMM, Zhao W, Barnes DR, Ho WK, Young R, et al. (2017) Fifteen new risk loci for coronary artery disease highlight arterial-wall-specific mechanisms. Nat Genet 49: 1113-1119.

18. Saleheen D, Zaidi M, Rasheed A, Ahmad U, Hakeem A, et al. (2009) The Pakistan Risk of Myocardial Infarction Study: a resource for the study of genetic, lifestyle and other determinants of myocardial infarction in South Asia. Eur J Epidemiol 24: 329-338.

19. Lopez-Reyes A, Rodriguez-Perez JM, Fernandez-Torres J, Martinez-Rodriguez N, Perez-Hernandez N, et al. (2014) The HIF1A rs2057482 polymorphism is associated with risk of developing premature coronary artery disease and with some metabolic and cardiovascular risk factors. The Genetics of Atherosclerotic Disease (GEA) Mexican Study. Exp Mol Pathol 96: 405-410.

20. Tang O, Lv J, Cheng Y, Qin F (2017) The Correlation Between 9p21 Chromosome rs4977574 Polymorphism Genotypes and the Development of Coronary Artery Heart Disease. Cardiovasc Toxicol 17: 185-189.

21. Sakalar C, Gurbuz E, Kalay N, Kaya MG (2013) Higher frequency of rs4977574 (the G Allele) on chromosome 9p21.3 in patients with myocardial infarction as revealed by PCR-RFLP analysis. Tohoku J Exp Med 230: 171-176.

22. Consortium CAD, Deloukas P, Kanoni S, Willenborg C, Farrall M, et al. (2013) Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 45: 25-33.

23. Bochenek G, Hasler R, El Mokhtari NE, Konig IR, Loos BG, et al. (2013) The large non-coding RNA ANRIL, which is associated with atherosclerosis, periodontitis and several forms of cancer, regulates ADIPOR1, VAMP3 and C11ORF10. Hum Mol Genet 22: 4516-4527.

24. Wellcome Trust Case Control C (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447: 661-678.

25. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, et al. (2007) Genomewide association analysis of coronary artery disease. N Engl J Med 357: 443-453.

26. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, et al. (2007) A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 316: 1491-1493.

27. Hutchinson JN, Ensminger AW, Clemson CM, Lynch CR, Lawrence JB, et al. (2007) A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. BMC Genomics 8: 39.

28. Vausort M, Wagner DR, Devaux Y (2014) Long noncoding RNAs in patients with acute myocardial infarction. Circ Res 115: 668-677.

29. Song CL, Wang JP, Xue X, Liu N, Zhang XH, et al. (2017) Effect of Circular ANRIL on the Inflammatory Response of Vascular Endothelial Cells in a Rat Model of Coronary Atherosclerosis. Cell Physiol Biochem 42: 1202-1212.

30. Chen G, Cai L, Chen B, Liang J, Lin F, et al. (2011) Serum level of endogenous secretory receptor for advanced glycation end products and other factors in type 2 diabetic patients with mild cognitive impairment. Diabetes Care 34: 2586-2590.

31. Zanetti D, Carreras-Torres R, Esteban E, Via M, Moral P (2015) Potential Signals of Natural Selection in the Top Risk Loci for Coronary Artery Disease: 9p21 and 10q11. PLoS One 10: e0134840.

32. Yu SY, Dong B, Tang L, Zhou SH (2018) LncRNA MALAT1 sponges miR-133 to promote NLRP3 inflammasome expression in ischemia-reperfusion injured heart. Int J Cardiol 254: 50.

33. Paraskevopoulou MD, Hatzigeorgiou AG (2016) Analyzing MiRNA-LncRNA Interactions. Methods Mol Biol 1402: 271-286.

34. Wang JZ, Xiang JJ, Wu LG, Bai YS, Chen ZW, et al. (2017) A genetic variant in long non-coding RNA MALAT1 associated with survival outcome among patients with advanced lung adenocarcinoma: a survival cohort analysis. BMC Cancer 17: 167.

35. Miao YR, Liu W, Zhang Q, Guo AY (2018) lncRNASNP2: an updated database of functional SNPs and mutations in human and mouse lncRNAs. Nucleic Acids Res 46: D276-D280.

36. Cao XL, Yin RX, Huang F, Wu JZ, Chen WX (2016) 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 17: 586.

37. Matsuoka R, Abe S, Tokoro F, Arai M, Noda T, et al. (2015) Association of six genetic variants with myocardial infarction. Int J Mol Med 35: 1451-1459.

38. Wang Y, Wang L, Liu X, Zhang Y, Yu L, et al. (2014) Genetic variants associated with myocardial infarction and the risk factors in Chinese population. PLoS One 9: e86332.

39. Lee IT, Goodarzi MO, Lee WJ, Rotter JI, Chen YD, et al. (2014) The chromosome 9p21 variant not predicting long-term cardiovascular mortality in Chinese with established coronary artery disease: an eleven-year follow-up study. Biomed Res Int 2014: 626907.

40. Huang Y, Ye H, Hong Q, Xu X, Jiang D, et al. (2014) Association of CDKN2BAS polymorphism rs4977574 with coronary heart disease: a case-control study and a meta-analysis. Int J Mol Sci 15: 17478-17492.

41. Qi L, Li JM, Sun H, Huang XQ, Lin KQ, et al. (2012) Association between gene polymorphisms and myocardial infarction in Han Chinese of Yunnan province. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 29: 413-419.

42. Saade S, Cazier JB, Ghassibe-Sabbagh M, Youhanna S, Badro DA, et al. (2011) Large scale association analysis identifies three susceptibility loci for coronary artery disease. PLoS One 6: e29427.

43. Golabgir Khademi K, Foroughmand AM, Galehdari H, Yazdankhah S, Pourmahdi Borujeni M, et al. (2016) Association Study of rs1333040 and rs1004638 Polymorphisms in the 9p21 Locus with Coronary Artery Disease in Southwest of Iran. Iran Biomed J 20: 122-127.

44. Mafi Golchin M, Ghaderian SMH, Akhavan-Niaki H, Jalalian R, Heidari L, et al. (2017) Analysis of Two CDKN2B-AS Polymorphisms in Relation to Coronary Artery Disease Patients in North of Iran. Int J Mol Cell Med 6: 31-37.

45. Schaefer AS, Richter GM, Groessner-Schreiber B, Noack B, Nothnagel M, et al. (2009) Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. PLoS Genet 5: e1000378.

46. Cheng J, Cai MY, Chen YN, Li ZC, Tang SS, et al. (2017) Variants in ANRIL gene correlated with its expression contribute to myocardial infarction risk. Oncotarget 8: 12607-12619.