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Original Article

Association of rs1333040 SNPs with susceptibility, risk factors, and clinical characteristics of acute myocardial infarction patients in a Chinese Han population

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Abstract: This study aimed to examine the association of rs1333040 SNPs and several risk and environmental factors with acute myocardial infarction (AMI). The association of rs1333040 single nucleotide polymorphisms (SNPs) within the cyclin-dependent kinase inhibitor 2B antisense RNA1 (CDKN2B-AS1) gene with AMI has been confirmed in some European populations. However, at the time this study was initiated, no rs1333040 SNPs had been associated with AMI in Chinese individuals. Genotypes of rs1333040 were determined in 334 AMI patients and 334 healthy controls from a Chinese Han population by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP), and then confirmed by direct sequencing. The TT genotype of rs1333040 was positively correlated with AMI risk ($P < 0.001$). The frequency of the C allele of rs1333040 in patients with diagnosis time (DT) > 12 h was lower than that in patients with shorter DT ($P < 0.05$), with no differences in typical symptoms, serious complications, and infarction location ($P > 0.05$ for each). There were interactions between the rs1333040 SNP genotype (TT, TC, or CC), and patients who smoked ≥ 20 cigarettes/day ($P < 0.017$). The rs1333040 TT genotype was positively correlated with the risk of AMI. For the first time, we discovered that the C allele of rs1333040 was significantly correlated with DT ≤ 12 h of AMI. Also, the interaction between the minor C allele of rs1333040 and smoking appears to increase the risk of AMI.

Keywords: Acute myocardial infarction (AMI), single nucleotide polymorphism (SNPs), susceptibility, risk factor, gene-environment interaction

Introduction

In recent years, genome-wide association studies (GWAS) have focused on genomic factors contributing to the development of coronary artery disease (CAD) and myocardial infarction (MI). Throughout the world, CAD and MI are the leading causes of mortality [1]. Acute myocardial infarction (AMI) is caused when plaque that has built up in the walls of coronary arteries erodes or ruptures, leading to transient, partial, or complete arterial occlusion. AMI is a complicated condition brought about by multiple genetic and environmental factors as well as their interactions [2, 3]. Rs1333040 SNPs, which are located on the 9p21.3 locus and are in genetic disequilibrium, were independently confirmed to be related to MI [4]. Although the association of rs1333040 SNPs with CAD has been confirmed in Italian [5], Iranian [6], and North Indian [7] populations, genetic variants related to AMI in Chinese Han populations have not been definitively identified. Thus, our investigation evaluated whether rs1333040 was associated with susceptibility to, risk factors for, and/or clinical characteristics of AMI in the Chinese Han population.

Materials and methods

Study population

Participants in this study included 334 AMI patients and 334 healthy subjects, all of whom were enrolled in the study from January 1, 2012 to December 8, 2016. The study took place in the First Affiliated Hospital, Guangxi Medical University, in Guangxi Zhuang Autonomous Region, People's Republic of China. The AMI patients were comprised of 258 (77.25%) ma-

les and 76 (22.75%) females, ranging in age between 32 and 86 years old, with an average age of 61.63±10.65 years. The control subjects consisted of 233 (69.76%) males and 101 (30.24%) females, ranging in age between 34 and 84 years, with a mean age of 57.79±10.85 years. Ethical approval for the present study was obtained from the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University, Guangxi province, People's Republic of China. Informed consent was obtained from all participants after they received a full description of the investigation.

Subgroups

To evaluate the relationship between rs1333040 and clinical characteristics, the AMI patients (334 cases) were subdivided as follows: (1) Subjects were subdivided into four subgroups on the basis of time until diagnosis (DT): DT ≤ 2 h (n = 56), 2 h < DT ≤ 6 h (n = 117), 6 h < DT ≤ 12 h (n = 112), and DT > 12 h (n = 49). (2) Subjects were subdivided into two subgroups according to severity of complications: those with no severe complications (n = 293) and those with severe complications (n = 41). (3) Subjects were divided into two subgroups on the basis of symptoms: those with atypical symptoms (n = 73) and those with typical symptoms (n = 261). (4) Subjects were divided into six subgroups according to infarction location: extensive anterior wall (n = 170), inferior wall (n = 112), anteroseptal wall (n = 24), lateral wall (n = 6), right ventricle (n = 13), and multivessel lesion (n = 9).

Epidemiological survey

Information on demographics, socioeconomic status, past medical history, and lifestyle factors was collected using standardized questionnaires. The intake of alcohol was quantified by the number of liang (about 50 g) of rice wine, corn wine, rum, beer, or liquor consumed during the preceding twelve months. Alcohol consumption was categorized into groups according to grams of alcohol consumed per day: ≤ 250 g and > 250 g. Smoking status was categorized into groups according to cigarettes smoked per day: ≤ 20 and > 20. In the physical examination, several parameters such as height, weight, and waist circumference, were measured under the supervision of two people. Sitting blood pressure was measured using a mercury sphygmomanometer at three separate intervals after the subjects had a five-minute rest. The average of the three measurements was used as the blood pressure level. Body mass index (BMI) was calculated as weight in kg divided by the square of height in meters (kg/m^2).

Biochemical analysis

The present survey was carried out using internationally standardized methods [8]. Levels of creatine kinase-MB (CK-MB), cardiac troponin I (cTnI), serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were obtained from samples analyzed by the Biochemical Laboratory of the First Affiliated Hospital, Guangxi Medical University, Guangxi Zhuang Autonomous Region, China.

DNA amplification and genotyping

The phenol-chloroform method was used to extract genomic DNA from peripheral blood leukocytes [9]. The isolated DNA was stored at 4°C until analysis. Genotyping of rs1333040 SNPs was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR amplification was performed using the following pair of primers: forward primer, 5'-GCCTAATCCTGGGGTTCTGT-3'; reverse primer, 5'-GTTCTTGAACCTGGCAGAGC-3' (Sangon, Shanghai, People's Republic of China). Each amplification reaction was carried out in a total volume of 25 μL, which consisted of 2 μL of genomic DNA, 1 μL of each primer (10 pmol/L), 12.5 μL of 2 × Taq PCR Mastermix (constituents: 0.1 U Taq polymerase/μL, 500 μM of each dNTP, and PCR buffer), and 8.5 μL of nuclease-free water. The program began with a pre-denaturing step that was followed by 30 cycles at 95°C for 30 s, 59°C for 30 s, 72°C for 35 seconds, then a final extension at 72°C for 7 minutes. The amplification products were visualized under ultraviolet light after electrophoresis on a 2.0% agarose gel with 0.5 μg/mL ethidium bromide staining. The size of the amplification products for rs1333040 was 674 bp. Next, 5 μL of PCR products and 5 U of BsmI FastDigest enzymes were digested at 37°C for 30 minutes. After restriction enzyme digestion of the amplified DNA, the fragments were separated by electrophoresis on 2% agarose gels and visualized with ethidium-bromide staining and ultraviolet illumination in order to identify the genotypes. For six samples, the genotypes de-

Table 1. Differences in general characteristics and serum lipid levels between the AMI and control groups

Parameter	AMI group	Control group	t (X^2)	P
Number	334	334	-	-
Male/female	258/76	233/101	4.804	0.028
Age (years)	61.63±10.65	57.79±10.85	4.612	< 0.001
Body mass index (kg/m^2)	23.75±3.45	22.49±3.15	4.929	< 0.001
Cigarette smoking (n %)	-	-	63.673	< 0.001
Non-smoker	168 (50.30)	254 (76.00)	-	-
≤ 20 cigarettes/day	38 (11.40)	40 (12.00)	-	-
> 20 cigarettes/day	128 (38.30)	40 (12.00)	-	-
Alcohol consumption [n (%)]	-	-	58.030	< 0.001
Non-drinker	253 (75.70)	271 (81.10)	-	-
≤ 25 g/day	31 (9.30)	62 (18.60)	-	-
> 25 g/day	50 (15.00)	1 (0.30)	-	-
Total cholesterol (mmol/L)	4.54±1.247	4.15±0.84	4.828	< 0.001
Triglycerides (mmol/L)	1.66±1.126	1.41±1.062	3.056	0.002
LDL-C (mmol/L)	2.69±0.986	2.51±0.56	3.031	0.003
HDL-C (mmol/L)	1.13±0.333	1.71±0.398	-20.404	< 0.001

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

tected by PCR-RFLP (2 TT, 2 TC, and 2 CC) were confirmed by direct sequencing. The PCR products were purified by low melting point gel electrophoresis and phenol extraction, then the DNA was sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of China.

Diagnostic criteria

The diagnostic criteria and study protocol followed the guidelines of the European Resuscitation Council [10-12]. The diagnosis of ST-segment elevation myocardial infarction (STEMI), which was a requirement for inclusion in the patient group for this study, was defined as follows: ST-segment elevation of 1 mm or greater in at least two contiguous leads in a 12-lead electrocardiogram; continued chest discomfort typical of myocardial ischemia; cardiac biomarkers, and creatine kinase-MB (CK-MB) or troponin (or both) elevated to more than twice the upper limit of normal laboratory reference values; with confirmation by coronary artery angiography. Ventricular fibrillation (VF) was determined on the basis of the following atypical electrocardiogram patterns: chaotic irregular deflections of varying amplitude; no identifiable *P* waves, QRS complexes, or T waves; and heart rate between 150 and 500 beats/min. Shock was defined as systolic blood pressure < 90 mm Hg; high heart rate (> 120 beats/min); skin pale and clammy; and confusion. Heart failure (HF) was diagnosed according to the brain natriuretic peptide (BNP) and heart ultrasound. The normal values for serum TC, TG, LDL-C, HDL-C, CK-MB, and cTnI in our Clinical Science Experiment Center were 3.10-5.17 mmol/L, 0.56-1.70 mmol/L, 2.70-3.20 mmol/L, 0.91-1.81 mmol/L, 0-25 u/L, and 0-0.014 ng/mL, respectively. Hypertension was diagnosed according to the criteria of the 2003 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [13]. BMI values of less than 24, 24 to 28, and greater than 28 kg/m^2 were defined as normal weight, overweight, and obese, respectively [14].

Statistical analyses

The Hardy-Weinberg equilibrium (HWE) test was applied to confirm the independent segregation of the alleles. The statistical software package SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA) was used to complete the statistical analyses. Qualitative variables were presented as raw count and percentage. Mean ± SD was used for the expression of quantitative variables. Genotype and frequency of the alleles was determined by direct counting. Differences in genotype distribution and sex ratio between the groups were analyzed by Pearson's χ^2 test. The Student's unpaired t-test was used to evaluate the difference in general characteristics between the AMI group and the control group. The risk factors and gene-environment interaction in AMI were estimated using unconditional binary logistic regression. A few confounders such as sex, age, BMI, alcohol consumption, and cigarette smoking were adjusted for the statistical analysis. For all data, two-tailed $P < 0.05$ was considered to indicate statistical significance. When we assessed the interactions between the rs1333040 SNPs and BMI, ciga-

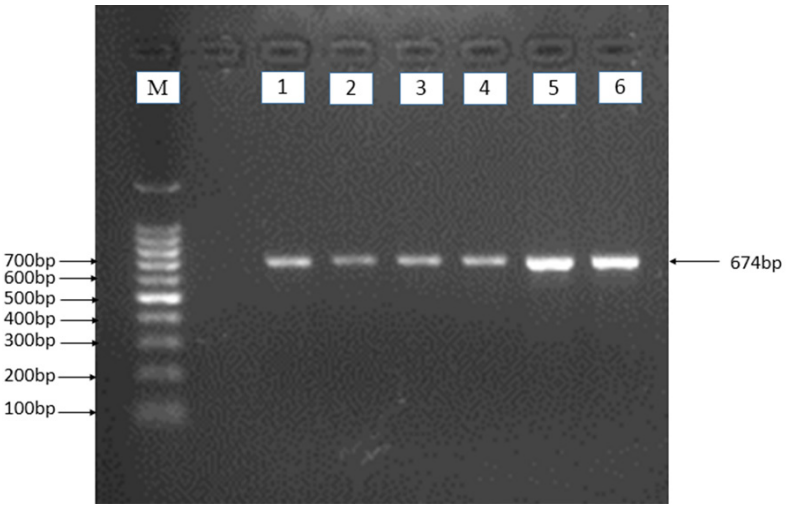

Figure 1. Electrophoresis of polymerase chain reaction products of the samples. Lane M is the 100 bp marker ladder; Lanes 1-6 are samples, the 674 bp bands are the target genes.

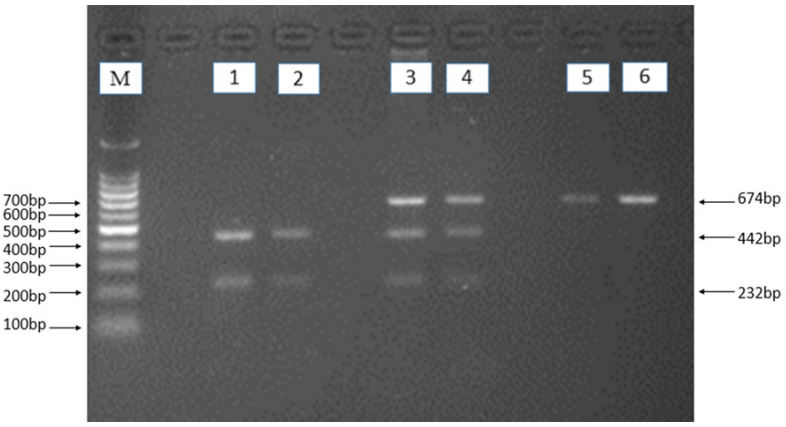

Figure 2. Genotyping of the *CDKN2B-AS1* rs1333040 SNP. Lane M is the 100 bp marker ladder; lanes 1 and 2, CC genotype (232 bp and 442 bp); lanes 3 and 4, TC genotype (674 bp, 442 bp, and 232 bp); and lanes 5 and 6, TT genotype (674 bp).

rette smoking, and alcohol consumption, a value of $P < 0.017$ (corresponding to $P < 0.05$ after adjusting for 3 independent tests by the Bonferroni correction) was considered statistically significant. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CI) were also calculated.

Results

General characteristics and serum lipid levels

Comparison of generalized features and lipid levels are summarized in **Table 1**. Comparing the patient group with the control group, the mean age (61.63±10.65 years vs. 57.79±10.85 years) and BMI (23.75±3.45 kg/m^2 vs. 22.49±3.15 kg/m^2) were higher in the AMI group than in the control group. The numbers (percentages) of subjects with high cigarette and alcohol consumption were 166 (49.70%) and 81 (24.30%), respectively, in the AMI group, and 80 (24.00%) and 63 (18.90%), respectively, in the control group. Significantly, more subjects in the AMI group were heavy cigarette smokers and alcohol drinkers than in the control group ($P <$ 0.001 for each). There were also significant differences in sex ratio between the two groups ($P = 0.028$). In the AMI group, the levels of TC, TG, and LDL-C were 4.54±1.247 mmol/L, 1.66 ±1.126 mmol/L, and 2.69± 0.986 mmol/L, respectively; in the control group, the levels of TC, TG, and LDL-C were 4.15±0.84 mmol/L, 1.41±1.062 mmol/L, and 2.51±0.56 mmol/L, respectively. These levels of serum TC, TG, and LDL-C were significantly higher in the AMI group than in the normal group ($P < 0.05$ for each). However, the serum HDL-C level was lower in the AMI group than in the control group (1.13±0.333 mmol/L vs. 1.71± 0.398 mmol/L, $P < 0.001$).

Electrophoresis, genotyping, and sequencing

Amplification of genomic DNA yielded PCR products of 674 bp (**Figure 1**). The genotypes identified were named according to the absence (C allele) or presence (T allele) of the enzyme restriction sites. Thus, the TT genotype is a wild-type homozygote for the absence of the site (bands at 674 bp, lanes 5 and 6; **Figure 2**). The TC genotype is a heterozygote for the absence and presence of the site (bands at 674, 442, and 232 bp, lanes 3 and 4; **Figure 2**). The CC genotype is a homozygote for the presence of

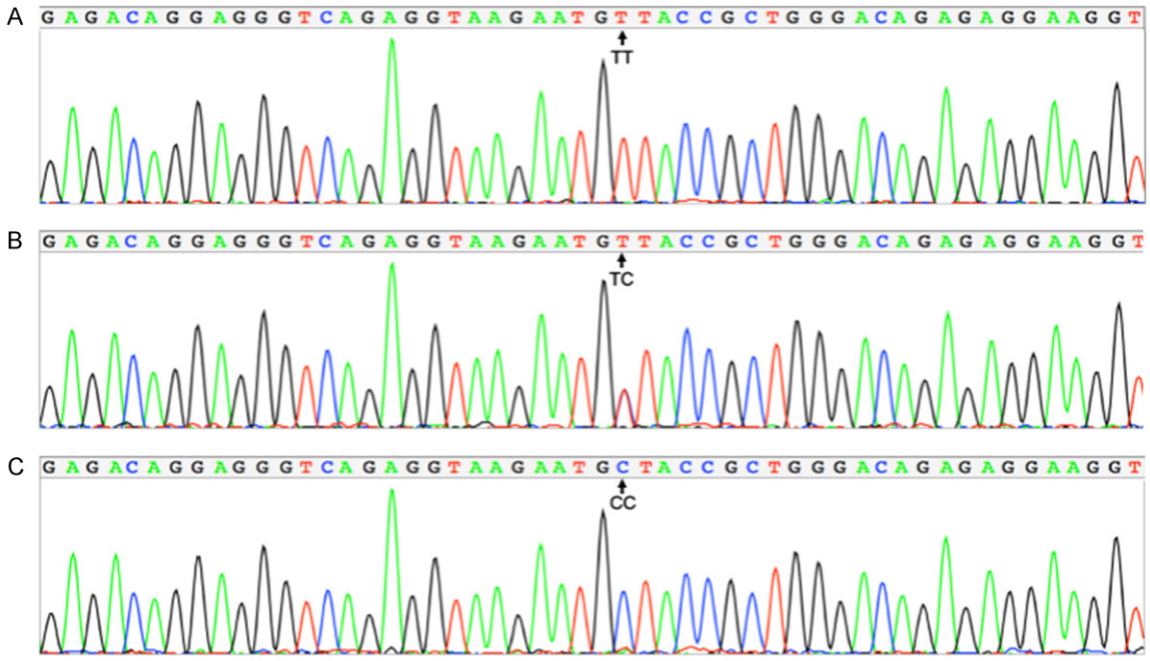

Figure 3. A part of the nucleotide sequence of the CDKN2B-AS1 rs1333040 SNP. A: TT genotype; B: TC genotype; C: CC genotype.

the mutated site (bands at 442 and 232 bp; lanes 1 and 2, **Figure 2**). In a subset of six samples, the genotypes of TT, TC, and CC, as detected by the PCR-RFLP, were also confirmed by sequencing (**Figure 3**).

Genotypic and allelic frequencies

The genotype frequencies in the controls were in Hardy-Weinberg equilibrium ($X^2 = 1.845$, $P = 0.174$). The genotypic and allelic frequencies of the rs1333040 SNPs are presented in **Table 2**. The wild-type TT genotype and T allele frequencies, respectively, were 58.10% and 76.00% in the AMI group, which were higher than those in the control group, 41.90% and 66.00%. The frequencies of the TC and CC genotypes and the minor C allele were 35.90%, 6.00%, and 24.00%, respectively, in the AMI group; these frequencies were lower than the 48.20%, 9.90%, and 34.00% seen in the control group. The differences in both genotypic and allelic frequencies between the AMI and control groups were statistically significant ($P < 0.001$ for each).

Risk factors for AMI

As shown in **Table 3**, non-conditional binary logistic regression analysis showed that diabetes, high blood pressure, smoking, age, and TC were strongly associated with AMI risk, with OR values of 66.165, 12.371, 3.012, 2.080, and 3.297, respectively. In contrast, HDL-C and rs1333040 CC genotype were negatively correlated with AMI risk, with OR values of 0.029 and 0.524 ($P < 0.05$ for each). However, no significant differences were seen between the AMI group and control group in terms of correlation of BMI, sex, TG, alcohol consumption, and LDL-C with AMI risk ($P > 0.05$ for each).

Frequencies of rs1333040 and clinical characteristics

Next, patients were grouped according to time until diagnosis (DT). The frequency of the C allele of rs1333040 in patients with DT > 12 h was significantly lower than in other diagnosis time groups ($P < 0.05$ for each). There were no significant differences in the genotype of rs1333040 between the controls and the AMI subgroups, including subgroups divided according to typical symptoms, serious complications, and infarction location ($P > 0.05$ for each) (**Table 4A-D**).

Interaction between rs1333040 and BMI, smoking, and alcohol consumption

In **Table 5**, the subjects who smoked ≥ 20 cigarettes/day and had the TT genotype were at a

Table 2. Differences in the distribution of the rs1333040 genotypes and allele frequency between the AMI and control groups

Parameter	AMI group [n (%)]	Control group [n (%)]	X^2	*P*
Number (n = 668)	334 (50.00)	334 (50.00)	-	-
Genotypes	-	-	17.901	< 0.001
TT	194 (58.1)	140 (41.90)	-	-
TC	120 (35.90)	161 (48.20)	-	-
CC	20 (6.00)	33 (9.90)	-	-
Allele	-	-	16.330	< 0.001
T	508 (76.00)	441 (66.00)	-	-
C	160 (24.00)	227 (34.00)	-	-

Table 3. Risk factor analysis for Acute Myocardial Infarction

Parameter	B	SE	Wald	Sig	Exp (B)/OR
Diabetes	4.192	1.039	16.273	0.000	66.165
High blood pressure	2.515	0.274	84.281	0.000	12.371
Smoking	1.103	0.148	55.565	0.000	3.012
Age	0.732	0.235	9.670	0.002	2.080
TC	1.193	0.540	4.876	0.027	3.297
HDL-C	-3.555	0.598	35.377	0.000	0.029
Rs1333040 CC genotype	-0.647	0.173	13.960	0.000	0.524
BMI	0.465	0.247	3.555	0.059	1.592
Sex	0.400	0.270	2.185	0.139	1.491
TG	0.437	0.245	3.168	0.075	1.547
Alcohol consumption	0.151	0.193	0.615	0.433	1.163
LDL-C	0.170	0.467	0.133	0.716	1.185

TC, total cholesterol; HDL-C; high-density lipoprotein cholesterol; BMI, body mass index; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol.

600.8% increased risk for AMI ($P < 0.001$). The subjects who smoked ≥ 20 cigarettes/day carrying the minor C allele had an increased risk for AMI of 525.0% ($P < 0.001$). No interaction was seen among any of the genotypes and BMI or alcohol consumption ($P > 0.017$ for each). In addition, no interaction was seen between the presence of the minor C allele and BMI or alcohol consumption ($P > 0.017$ for each).

Discussion

In this study, the frequency of the TT genotype and the T allele of rs1333040 were found to be higher in AMI patients compared with controls. This suggested that there was positive correlation between the TT genotype and T allele of the cyclin-dependent kinase inhibitor 2B antisense RNA1 (CDKN2B-AS1) gene rs1333040 SNP and AMI morbidity. However, the mechanism underlying the involvement of the CDKN2B-AS1 gene in elevated AMI risk is still not clear.

Rs1333040 is found in the 9p21.3 locus, and has been found to be associated with MI [4]. Many promising risk variants for CAD and MI have been discovered, with the most consistent of these being in the locus on chromosome 9p21 [15, 16]. The association of rs1333040 SNPs with CAD has been confirmed in Italy and China [17, 18]. Rs1333040 is located deep in the 12th intron of the CDKN2BAS gene, a non-coding RNA gene, or antisense noncoding RNA in the INK4 locus (ANRIL). ANRIL was first confirmed in melanoma and neural system tumors [19]. The CDKN2A/2B gene has also been found to be related to ischemic stroke [20], type 2 diabetes [21], myocardial infarction, and intracranial aneurysms [22]. Some studies have shown that CAD and type 2 diabetes have the overlapped interval in the 3' end of the non-coding gene, CDKN2BAS, and they may have the same pathogenic pathways [23, 24]. The ANRIL locus has been emphasized as the strongest genetic susceptibility locus for CAD, and it has been directly associated with an increased risk of developing CAD [25-29]. Rs1333040 SNPs can affect the expression of ANRIL and neighboring genes. One study reported that ANRIL can alter the expression of associated protein-coding genes through multiple mechanisms, such as RNA interference, gene silencing, chromatin remodeling [30], and DNA methylation [31]. Another study, by Broad-bent et al. [32], showed that ANRIL is expressed in many cell types and tissues that are affected by atherosclerosis. ANRIL is expressed in vascular smooth muscle cells, and the proliferation and migration of these types of cells play an important role in atherosclerosis [33]. Apart from this, pro-oncogenic Ras can induce vascular smooth muscle cell (VSMC) senescence and expression of pro-inflammatory cytokines in atherosclerosis progression [34], by inhibiting ANRIL expression and activating p15 INK4b, which

Table 4A. Comparison between rs1333040 genotypes and alleles in subgroups based on diagnosis time

Parameter	Subgroups [n (%)]				X^2	P
	DT ≤ 2 h	2 h < DT ≤ 6 h	6 h < DT ≤ 12 h	DT > 12 h		
Number (n = 334)	56 (16.77)	117 (35.03)	112 (33.53)	49 (14.67)	-	-
Genotype	-	-	-	-	12.017	0.062
TT	31 (55.36)	68 (58.12)	57 (50.89)	38 (77.55)	-	-
TC	21 (37.50)	40 (34.19)	48 (42.86)	11 (22.45)	-	-
CC	4 (7.14)	9 (7.69)	7 (6.25)	0 (0.00)	-	-
Allele	-	-	-	-	10.744	0.013
T	83 (74.11)	176 (75.21)	162 (72.32)	87 (88.78)	-	-
C	29 (25.89)	58 (24.79)	62 (27.68)	11 (11.22)	-	-

DT, diagnosis time (time until diagnosis).

Table 4B. Comparison of genotypes and alleles in the severe complications group and in the non-severe complications group

Parameter	Subgroups [n (%)]		X^2	P
	Severe Complications	Non-severe Complications		
Number (n = 334)	293 (87.72)	41 (12.28)	-	-
Genotype	-	-	1.056	0.590
TT	169 (57.68)	25 (60.98)	-	-
TC	105 (35.84)	15 (36.59)	-	-
CC	19 (6.48)	1 (2.43)	-	-
Allele	-	-	0.532	0.466
T	443 (75.60)	65 (79.27)	-	-
C	143 (24.40)	17 (20.73)	-	-

Table 4C. Comparison of genotypes and alleles in the typical-symptom group and the atypical-symptom group

Parameter	Subgroups [n (%)]		X^2	P
	Typical symptoms	Atypical symptoms		
Number (n = 334)	261 (78.14)	73 (21.86)	-	-
Genotype	-	-	1.752	0.416
TT	150 (57.47)	44 (60.27)	-	-
TC	93 (35.63)	27 (36.99)	-	-
CC	18 (6.90)	2 (2.74)	-	-
Allele	-	-	0.758	0.384
T	393 (75.29)	115 (78.77)	-	-
C	129 (24.71)	31 (21.23)	-	-

suggests a potential negative regulation of p15 INK4b by ANRIL [35]. Additionally, Visel et al. [36], found that deletion of the 70 kb noncoding interval on mouse chromosome 4, which is orthologous to the human 9p21.3 locus, resulted in a severe reduction in expression of cardiac CDKN2A/B, suggesting that the presence of risk alleles in the 9p21.3 region may affect progression of CAD by altering vascular cell proliferation. Some researchers have observed that platelet reactivity and IFN-γ can also alter the expression of CDKN2A/2B and result in atherosclerosis [37, 38]. In addition, other researchers have demonstrated that platelet CD40L plays a vital role in atherosclerosis, through activating leukocytes, enhancing the interaction between platelets and leukocytes, and disrupting T-cell homeostasis [37, 39]. At present, it is unknown whether rs1333040 SNPs will be helpful in the early diagnosis of AMI. However, our study presents the first discovery that the C allele of rs1333040 is significantly correlated with AMI when DT ≤ 12 h. The relevant mechanism underlying this phenomenon is not known, but it is possible that related gene regulation affects the stability of atherosclerotic plaques.

In the present study, we also assessed the association between rs1333040 SNPs and several environmental factors. There were interactions indicating that subjects of all genotypes (TT, TC, and CC genotypes) who smoked ≥ 20 cigarettes/day may have an increased risk of AMI. Diabetes, high blood pressure, smoking, age, and TC were all risk factors for AMI, while rs1333040 mutation genotype and HDL-C were negatively correlated with AMI risk. AMI is a multifactorial disease with a compli-

Table 4D. Comparison of genotypes and alleles in groups with different infarct sites

Parameter	Subgroups [n (%)]						X^2	*P*
	Extensive anterior	Inferior	Anteroseptal	Lateral	Right ventricular	Multivessel lesion		
Number (n = 334)	170 (50.90)	112 (33.5)	24 (7.20)	6 (1.80)	13 (3.90)	9 (2.70)	-	-
Genotype	-	-	-	-	-	-	8.504	0.580
TT	101 (59.41)	66 (58.93)	14 (58.33)	3 (50.00)	7 (53.85)	3 (33.33)	-	-
TC	57 (33.53)	38 (33.93)	10 (41.67)	3 (50.00)	6 (46.15)	6 (66.67)	-	-
CC	12 (7.06)	8 (7.14)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	-
Allele	-	-	-	-	-	-	1.279	0.937
T	259 (76.18)	170 (75.9)	38 (79.17)	9 (75.00)	20 (76.92)	12 (66.67)	-	-
C	81 (23.82)	54 (24.10)	10 (20.83)	3 (25.00)	6 (23.08)	6 (33.33)	-	-

Table 5. Interaction between rs1333040 genotypes and environmental factors, and their impact on AMI

Genotypes	Environment factor	B	SE	Wald	Sig	Exp (B)/OR	95.0% CI for OR	
							Lower	Upper
-	BMI (kg/m^2)	-	-	-	-	-	-	-
TT	19-24	-	-	-	-	-	-	-
TT	≥24	-0.016	0.669	0.001	0.051	0.984	0.265	3.647
TC+CC	19-24	-	-	-	-	-	-	-
TC+CC	≥24	0.205	0.661	0.096	0.757	1.227	0.336	4.486
-	Smoking (n/d)	-	-	-	-	-	-	-
TT	0	-	-	-	-	-	-	-
TT	0-20	0.116	0.342	0.116	0.773	1.124	0.575	2.196
TT	≥20	1.793	0.332	29.240	< 0.001	6.008	3.137	11.509
TC+CC	0	-	-	-	-	-	-	-
TC+CC	0-20	0.880	0.405	4.724	0.030	2.411	1.090	5.330
TC+CC	≥20	1.658	0.306	29.308	< 0.001	5.250	2.880	9.569
-	Alcohol (g/d)	-	-	-	-	-	-	-
TT	0	-	-	-	-	-	-	-
TT	0-250	0.752	1.158	0.421	0.516	2.120	0.219	20.522
TT	≥250	19.057	7338.199	< 0.001	0.998	1.889E8	-	-
TC+CC	0	-	-	-	-	-	-	-
TC+CC	0-250	1.405	1.171	1.422	0.230	4.077	0.411	40.436
TC+CC	≥250	19.756	10048.243	< 0.001	0.998	3.800E8	-	-

BMI, body mass index; n/d, number of cigarettes smoked per day; g/d, grams of alcohol consumed per day.

cated pathogenesis, which involves individual genetic background, lifestyle, and environmental risk factors [40]. Well-known risk factors for AMI include obesity, smoking, excessive alcohol intake, high blood pressure, diabetes, and high blood cholesterol [41-44]. However, the combined genetic influence of rs1333040 together with environmental factors is not well-known. At the same time, telomere shortening may be a risk factor for AMI. Some studies have found telomere shortening to be related to the pathogenesis of atherosclerosis and acute vascular syndromes [45, 46] and the other studies have suggested that short telomere length is associated with an increased risk of myocardial infarction [47, 48]. Apart from this, it has been confirmed that lifestyle is one of the strongest predictors of CAD risk, and that this increase in risk may stem from effects on telomere length [49]. For example, a sedentary lifestyle may accelerate the aging process [50]. Various cardiovascular risk factors, such as smoking, sex, and obesity, can be related to regulation of telomere length [51, 52]. Some studies have shown

that smoking can be associated with shorter telomeres in normal weight as well as in lean and obese women [53]. In addition, telomere shortening has been demonstrated in patients with hypertension and diabetes [54-56]. In general, the etiology of gene-environment interactive effects is poorly understood, although various environmental factors have been confirmed to influence biological mechanisms. Therefore, further studies are necessary to elucidate the underlying cause of the associations identified in this study.

In conclusion, this study demonstrates that the TT genotype of rs1333040 was positively correlated with AMI risk. In addition, we discovered that the C allele of rs1333040 was significantly correlated with AMI DT ≤ 12 h. Also, the interaction between the minor C allele of rs1333040 and smoking appears to increase the risk of AMI. Finally, we confirmed that diabetes, high blood pressure, age, smoking, and TC are risk factors for AMI.

Limitations

In our study, there were several potential limitations. First, it is important to note that the number of patients in the study was small, which might limit some potential for discovery. Second, the early diagnosis time of AMI lacked objectivity and supporting materials. In addition, SNP-SNP interactions associated with AMI were not examined in our study. Third, the study evaluated the association of the gene with susceptibility and clinical characteristics of AMI, but did not address the mechanism of gene-AMI interaction.

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Disclosure of conflict of interest

None.

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