



***MALAT1* gene rs600231 polymorphism positively associated with acute coronary syndrome in Chinese population: a case-control study**

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Background: Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) has been recognized as a major player in the pathogenesis of coronary artery disease (CAD). The aim of the study was to determine the association between polymorphisms of the *MALAT1* gene and acute coronary syndrome (ACS) in a Chinese population in Xinjiang.

Methods: In the case-control study, we genotyped three nucleotide polymorphisms (rs3200401, rs4102217, rs600231) of the *MALAT1* gene using SNPscanTM typing assays (1,053 controls and 929 ACS patients). Furthermore, we explored a predictive model using *MALAT1* rs600231 and clinical variables to predict the risk of ACS. Finally, the relative expression of long noncoding RNA (lncRNA) *MALAT1* was also measured in 92 ACS patients and 92 controls using quantitative real-time polymerase chain reaction (qRT-PCR).

Results: The prevalence of the GG genotype of rs600231 in ACS group was higher than that in control group (15.7% vs. 14.7%, P=0.048). The dominant model differed (AG + GG vs. AA) and the G allele of rs600231 in ACS group was higher than that in control group (for dominant model: 66.2% vs. 60.9%, P=0.014; for allele: 41.0% vs. 37.8%, P=0.042). Multivariate logistic regression analysis and the predictive nomogram model showed that the dominant model of rs600231 remained an independent risk factor for ACS [odds ratio (OR)=1.32, 95% confidence interval (CI): 1.07–1.63, P=0.009]. The area under the receiver operating characteristic (ROC) curve (AUC) for the nomogram model for the prediction of ACS was 0.738 (95% CI: 0.716–0.761). In addition, in the AG and GG phenotypes, the relative expression of lncRNA *MALAT1* was significantly higher in ACS patients than in controls with the same phenotypes (P<0.05). Among ACS group, compared to other genotype carriers, the relative expression level of *MALAT1* in GG genotype carriers was higher (P<0.05).

Conclusions: The present study suggested that the AG and GG genotype of rs600231 in *MALAT1* gene was independently associated with ACS, and could be a risk genetic marker of ACS in a Chinese population in Xinjiang.

Keywords: Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*); polymorphism; acute coronary syndrome (ACS)

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Introduction

Acute coronary syndrome (ACS) is the leading cause of cardiac mortality worldwide (1) and is characterized by myocardial ischaemia or necrosis, with an instantaneous decrease in coronary blood flow. Hence, it is critical that early detection and effective interventions reduce mortality and improve the prognosis of ACS (2). The current conventional myocardial injury biomarkers for ACS are creatine kinase MB (CK-MB) and cardiac troponin T (cTnT), which serve as the 'gold standard'. However, these markers have limitations in terms of clinical sensitivity and specificity. For example, cTnT could be increased among individuals with other cardiovascular diseases (CVDs), such as severe heart failure, sepsis, or chronic renal insufficiency (3). The level of CK-MB also be increased when the skeletal muscle is damaged (4). In addition, circulating cTnT and CK-MB reach detectable levels after 4 hours and remain detectable for several days (5). Therefore, the identification of novel biomarkers is necessary for predicting ACS and improving clinical practice.

With the rapid development of high-throughput platforms, it has been shown that only 2% of genomic transcripts are in charge of coding proteins. Remarkably, a large portion of RNAs are grouped as long noncoding RNAs (lncRNAs). In recent years, lncRNAs >200 nucleotides in length have become valuable in research, and expanding evidence has indicated their biological function in the regulation of CVDs. For example, lncRNA LIPCAR is identified to be related to the severity of coronary artery disease (CAD) and used as a potential marker for heart failure (6,7). In addition, lncRNA UCA1 which is detectable in plasma, decreased in acute myocardial infarction (AMI) patients at 2 hours after the onset of symptoms (8). More importantly, our previous article (9,10) quantified that MIAT and H19 were increased in peripheral blood mononuclear cells (PBMCs) in AMI patients.

Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*), also named nuclear-enriched abundant transcript 2 (*NEAT2*), an 8,778 bp lncRNA, was found to be located at chromosome region 11q13 by Ji *et al.* (11) in patients with early-stage non-small-cell lung cancer (NSCLC). Among the prevalent lncRNAs, *MALAT1* has attracted special attention, as it is obviously differentially expressed in cardiomyocytes and endothelial cells (ECs) under the risk conditions of hypoxia, oxidative stress and increased cytokines (12-14). Furthermore, *MALAT1* was

found to play a role in regulating cardiac development and in the pathogenesis of CVD. For example, Matkovich *et al.* (15) reported that *MALAT1* played central roles in cardiac contractility, hypertrophy and failure, which correlated with miR-133. Although *MALAT1* is highly conserved (16) in mammals, the single nucleotide polymorphisms (SNPs) were numerous within the region of lncRNA *MALAT1* according to the dbSNP database (<https://www.ncbi.nlm.nih.gov/snp/>). More studies have presented that SNPs in *MALAT1* participate in lung cancer (17), hepatocellular carcinoma (18), colorectal cancer (19), and pulmonary arterial hypertension (20). Wang *et al.* (21) investigated the relationship between *MALAT1* rs619586 and the potential risks of CAD and indicated that it could protect against the occurrence of CAD.

However, no data related to SNPs in *MALAT1* and ACS risks have been reported. We carried out a retrospective case-control study to analyse the association between SNPs in *MALAT1* and ACS risk in a Chinese population.

We present the following article in accordance with the STROBE reporting checklist (available at <http://dx.doi.org/10.21037/cdt-20-906>).

Methods

The protocol of this study was approved by the Human Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China) (approval ID: 20141201-03) and complied with the standards of the Declaration of Helsinki (22) (as revised in 2013). All participants provided written informed consent.

Study design and population

This single-centre, retrospective case-control study was designed to evaluate whether variants of the *MALAT1* gene predispose patients to ACS. We consecutively recruited adult patients with ACS who attended the First Affiliated Hospital of Xinjiang Medical University from 2014 to 2018. Total ACS patients experienced chest pain within 12 h before undergoing percutaneous coronary intervention (PCI). The diagnostic criteria of case patients complied with the 2012 ACCF/AHA guidelines for ACS (23). The controls patients were consecutively recruited from the cardiovascular risk survey study in our hospital at the same time period, and they had no records of CAD based on the details of physical examination, ECG, blood tests, radiographic examination or coronary angiography. For all the subjects, the exclusion

criteria were as follows: (I) thrombolysis therapy and other heart diseases (such as congenital heart disease, cardiomyopathy or severe valvular abnormalities); (II) active and chronic inflammation; and (III) renal or hepatic dysfunction, autoimmune diseases or malignancy. All the recruited participants were from Urumqi, excluding the surrounding and prefecture area. The design scheme of the study has been previously reported (24).

Blood collection and DNA extraction

For ACS patients, the time point of collection was strictly guaranteed to be within 12 hours after chest pain, before coronary angiography. Blood samples must be collected at the first time when the patient was admitted to the emergency department. Then, placed in EDTA-containing tubes immediately. To avoid affecting gene expression, samples must be separated and frozen within 2 hours. As previously described (25), DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (Beijing Bioteke Corporation, Beijing, China). To make the subsequent experiments more convenient, DNA samples were diluted to 50 ng/ μ L and stored at -80°C until use. We also randomly selected 92 ACS patients and 92 healthy controls to test the relative expression of lncRNA *MALAT1*. Total RNA was extracted by TRIzol reagent (Invitrogen), then detected the absorbance at 260 and 280 nm (A_{260}/A_{280}) by NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). If A_{260}/A_{280} was between 1.8 and 2.1, 11 μ L purified RNA was reverse transcription using the Qiagen Kit (Qiagen, China), then measured by quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR Green Master Mix (Applied Biosystems, USA) according to the manufacturer's specifications at Xinjiang Key Laboratory of Cardiovascular Disease Research.

Biochemical analysis

All blood biochemical analyses were performed using a commercially automated biochemical analyser (Dimension AR/AVL Clinical Chemistry System, Newark, NJ, USA) in the Central Laboratory of the First Affiliated Hospital of Xinjiang Medical University. The tests included total triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and high-density

lipoprotein-cholesterol (HDL-C).

SNP selection

Using the HapMap human SNP database (www.hapmap.org) and the 1000G database (<https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/>), we received 12 SNPs in the 10 kb region of the *MALAT1* gene based on the standard of minor allele frequency greater than 0.1 (with $r^2 \geq 0.8$ as a cut-off in linkage disequilibrium pattern analysis). Based on the tagged SNPs in the Chinese Han population and published references (19,21), we finally selected three SNPs (rs3200401, rs4102217, rs600231) in *MALAT1*.

Genotyping

Three sites of *MALAT1* polymorphisms (rs3200401, rs4102217, rs600231) were genotyped using SNPscanTM typing assays with double ligation and multiplex fluorescence PCR (Cat#: G0104; Genesky Biotechnologies Inc., Shanghai, China). The technology was assisted via the Center for Genetic and Genomic Analysis. To ensure success rates of the genotyping in the study, repeat genotyping was performed in a random 5% of the sample, and the concordance rates of SNPs were more than 99%. The probe sequences of *MALAT1* SNPs are shown in Table S1. In our study, the design scheme has been previously reported (26).

Definition of cardiovascular risk factors

Cardiovascular risk factors were defined according to current national guidelines. Hypertension (27) was defined as a history of hypertension or antihypertensive drug use, and/or systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg among repeated measurements. Diabetes (28) was diagnosed as fasting plasma glucose (FPG) ≥ 7.0 mmol/L and/or a history of antidiabetic drug use. Current smokers were considered those who reported tobacco use in the previous 6 months. Dyslipidaemia (29) was defined according to Chinese dyslipidaemia guidelines, and TG ≥ 1.71 mmol/L, TC ≥ 5.20 mmol/L, LDL-C ≥ 3.40 mmol/L, and HDL-C < 1.04 mmol/L were defined as hypertriglyceridemia, hypercholesterolemia, hyper-LDL-C and low HDL-C,

Table 1 Baseline characteristics of study participants

Characteristics	Control (n=1,053)	ACS (n=929)	P value
Age (years)	56.02±9.02	56.78±10.60	0.086
Male, n (%)	461 (43.8)	681 (73.3)	<0.001
BMI (kg/m ²)	25.78±3.74	25.89±3.37	0.654
Smoking, n (%)	254 (24.1)	437 (47.0)	<0.001
Drinking, n (%)	216 (20.5)	298 (32.1)	<0.001
Hypertension, n (%)	465 (44.2)	453 (48.8)	0.040
Diabetes, n (%)	136 (12.9)	232 (25.0)	<0.001
Glucose (mmol/L)	5.65±2.16	8.25±3.83	<0.001
TG (mmol/L)	1.44 (1.02–2.07)	1.61 (1.09–2.44)	<0.001
TC (mmol/L)	4.17±0.93	4.46±1.30	<0.001
HDL-C (mmol/L)	1.12±0.31	0.98±0.28	<0.001
LDL-C (mmol/L)	2.64±0.77	2.85±1.00	<0.001

ACS, acute coronary syndrome; BMI, body mass index; TG, total triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.

respectively.

Predictive nomogram for ACS

The predictive nomogram model for ACS was based on the results of multivariate logistic regression analysis and Akaike information criterion (AIC), including risk genotype and clinical variables. An individual patient's value was located on each variable axis, and a line was drawn upward to determine the number of points received for each variable value. The sum of these numbers was located on the "total points" axis, and a line was drawn downward to the "risk" axis to determine the likelihood of ACS presence.

Statistical analysis

IBM SPSS Statistics 22.0 software (Chicago, IL, USA) was used for data analysis. Continuous variables are expressed as the mean ± standard deviation (SD) or median (quartile) and were compared using an independent-sample *t*-test or nonparametric rank test between ACS patients and controls. Numbers and percentages (%) were used to present the categorical variables, including the distribution of genotypes and models, and were compared using the chi-square test or Fisher's exact test. Hardy-Weinberg equilibrium (HWE) was calculated with online SNP Stats (available online at

<http://bioinfo.iconcologia.net/SNPstats>) to compare ACS patients and control subjects.

Logistic regression analyses were performed to assess the association between *MALAT1* gene polymorphisms and the risk of ACS. The predictive nomogram model was built based on the stepwise regression screening of independent variables. Odds ratios (ORs) were calculated along with 95% confidence intervals (CIs). Statistical significance was set at $P < 0.05$.

Results

Clinical characteristics of study participants

Table 1 shows the clinical characteristics of all study participants. In the study, 929 ACS patients (73.3% men and mean age 56.78±10.60 years) and 1,053 healthy controls (43.8% men and mean age 56.02±9.02 years) were recruited. There was no significant difference in age or body mass index (BMI) between the controls and ACS patients, which indicated that the study was an age-matched case-control study. In the ACS patients, the prevalence of diabetes and hypertension, the status of smoking and alcohol consumption, and the levels of glucose, TC, TG and LDL-C, were significantly higher compared to control subjects ($P < 0.05$). However, the level of HDL-C was lower than that in the controls ($P < 0.05$).

The genotype distribution of selected MALAT1 SNPs in ACS and controls

The distribution of alleles and genotypes of the selected SNPs of *MALAT1* (rs3200401, rs600231, rs4102217) in all ACS patients and healthy controls are showed in *Table 2*. The distribution of genotype or allele rates in rs3200401 and rs4102217 showed no significant difference between control and ACS groups ($P>0.05$). Nevertheless, compared to control subjects, the frequency of the GG genotype was higher in ACS patients (15.7% *vs.* 14.7%, $P=0.048$). The dominant model of rs600231 (AG + GG *vs.* AA) was significantly different between ACS and control participants (66.2% *vs.* 60.9%, $P=0.014$). In ACS patients, the frequency of the G allele was higher than that in controls (41.0% *vs.* 37.8%, $P=0.042$).

The MALAT1 gene polymorphism rs600231 was positively associated with ACS

Univariate regression analysis presented that the dominant model of rs600231 was a risk factor for ACS (AG + GG *vs.* AA, OR =1.26, 95% CI: 1.05–1.51, $P=0.014$) (*Table 3*). Furthermore, multivariate logistic regression analysis was conducted to evaluate the relationship between susceptibility to ACS and rs600231 polymorphisms. After adjusting for confounding factors of ACS, such as sex, smoking, alcohol consumption, hypertension, diabetes and the plasma levels of TG, TC, LDL-C and HDL-C, the dominant model of rs600231 remained an independent risk factor for ACS (AG + GG *vs.* AA, OR =1.32, 95% CI: 1.07–1.63, $P=0.009$). However, there was no difference in the other two SNPs.

Predictive nomogram for ACS

Based on their risk factors of the individuals, we add up all the scores to calculate their total score. The model was as follows: $[-1.384 + (0.284 \times \text{rs600231 AG + GG genotype}) + (0.859 \times \text{diabetes}) + (0.458 \times \text{smoking}) + (0.370 \times \text{TC}) + (1.053 \times \text{gender}) - (0.348 \times \text{alcohol consumption}) - (1.299 \times \text{HDL-C})]$ (*Table 4*). As shown in *Figure 1A*, the score of each risk factor for ACS is listed in the nomogram. The calibration curve displayed in *Figure 1B* indicates good calibration of the nomogram. The area under the receiver operating characteristic (ROC) curve (AUC) for the nomogram model for the prediction of ACS was 0.738 (95% CI: 0.716–0.761) (*Figure 1C*). The proposed nomograms

can be used for the prediction of ACS in this population.

The expression of lncRNA MALAT1 was significantly associated with MALAT1 gene rs600231 polymorphisms

The relationship between lncRNA *MALAT1* levels and *MALAT1* gene rs600231 polymorphisms (AA, AG and GG genotypes) was further evaluated. In the AA genotype, no significant differences were found in lncRNA *MALAT1* between controls and ACS patients ($P>0.05$). However, in AG and GG phenotypes, the relative expression of lncRNA *MALAT1* was significantly higher in ACS patients than in controls carrying the same phenotypes ($P<0.05$). More importantly, the relative expression varied in the ACS patients carrying different genotypes: the highest was in those with the GG genotype, followed by those with the AG genotype, and the lowest was in those with the AA genotype ($P<0.05$) (*Figure 2*). The clinical characteristics of 92 healthy controls and 92 ACS patients are shown in *Table 5*.

Discussion

In the present case-control study, it was worth mentioning that we found a link between *MALAT1* genetic variants (rs3200401, rs4102217, rs600231) and ACS in the Xinjiang population. Xinjiang, located in the northwest part of China, is a multi-ethnic co-populated region. It is well known that the lifestyles and habits of the Xinjiang population vary compared to those of populations in other regions of China. In the Chinese Han population in Xinjiang, our team's previous studies have shown that the prevalence of hypertension, diabetes, dyslipidaemia and obesity was 33.4%, 9.26%, 53.3% and 18.8%, respectively, which was higher than that in other regions (30–34). After a multivariate adjustment analysis for potential confounding factors, there was a significant difference between the polymorphism (rs600231) of *MALAT1* gene and ACS. To our knowledge, this was the first study of the relationship between the *MALAT1* gene and ACS patients in Xinjiang, China.

Atherosclerosis, inflammation and vascular dysfunctions are the main risk factors for coronary heart disease, including in the pathogenesis of ACS (35–37). Previous studies (38,39) have reported that *MALAT1* not only participates in the development of atherosclerosis but also indicates a relationship with endothelial dysfunction. Yan *et al.* (40,41) reported that *MALAT1*, through improving *CD36* expression induced by ox-LDL in macrophages

Table 2 The genotype distribution of selected *MALAT1* SNPs in ACS patients and controls

Polymorphisms	Control (n=1,053), n (%)	ACS (n=929), n (%)	P value
rs3200401			
Genotype			0.297
CC	755 (71.7)	638 (68.7)	
CT	272 (25.8)	269 (29.0)	
TT	26 (2.5)	22 (2.4)	
Dominant model			0.142
CC	755 (71.7)	638 (68.7)	
TT + CT	298 (28.3)	291 (31.3)	
Recessive model			0.884
TT	26 (2.5)	22 (2.4)	
CC + CT	1,027 (97.5)	907 (97.6)	
Additive model			0.119
CT	272 (25.8)	269 (29.0)	
CC + TT	781 (74.2)	660 (71.0)	
Allele			0.211
C	1,782 (84.6)	1,545 (83.2)	
T	324 (15.4)	313 (16.8)	
rs600231			
Genotype			0.048
AA	412 (39.1)	314 (33.8)	
AG	486 (46.2)	469 (50.5)	
GG	155 (14.7)	146 (15.7)	
Dominant model			0.014
AA	412 (39.1)	314 (33.8)	
AG + GG	641 (60.9)	615 (66.2)	
Recessive model			0.538
GG	155 (14.7)	146 (15.7)	
AG + AA	898 (85.3)	783 (84.3)	
Additive model			0.054
AG	486 (46.2)	469 (50.5)	
AA + GG	567 (53.8)	460 (49.5)	
Allele			0.042
A	1,310 (62.2)	1,097 (59.0)	
G	796 (37.8)	761 (41.0)	

Table 2 (continued)

Table 2 (continued)

Polymorphisms	Control (n=1,053), n (%)	ACS (n=929), n (%)	P value
rs4102217			
Genotype			0.550
GG	768 (72.9)	657 (70.7)	
GC	262 (24.9)	250 (26.9)	
CC	23 (2.2)	22 (2.4)	
Dominant model			0.274
GG	768 (72.9)	657 (70.7)	
CG + CC	285 (27.1)	272 (29.3)	
Recessive model			0.784
CC	23 (2.2)	22 (2.4)	
CG + GG	1,030 (97.8)	907 (97.6)	
Additive model			0.303
CG	262 (24.9)	250 (26.9)	
CC + GG	791 (75.1)	679 (73.1)	
Allele			0.294
G	1,798 (85.4)	1,564 (84.2)	
C	308 (14.6)	294 (15.8)	

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; SNP, single nucleotide polymorphism; ACS, acute coronary syndrome.

Table 3 Logistic regression analysis for ACS risk factors

Factors	COR (95% CI)	P value	AOR (95% CI)	P value
Dominant model (AG + GG vs. AA)	1.26 (1.05–1.51)	0.014	1.32 (1.07–1.63)	0.009
Age (years)	1.01 (1.00–1.02)	0.083		
Male	3.53 (2.92–4.26)	<0.001	2.88 (2.19–3.78)	<0.001
Smoking	2.79 (2.31–3.38)	<0.001	1.60 (1.20–2.13)	0.001
Drinking	1.83 (1.49–2.24)	<0.001	0.72 (0.54–0.95)	0.021
Hypertension	1.20 (1.01–1.44)	0.04	1.19 (0.97–1.46)	0.105
Diabetes	2.24 (1.78–2.84)	<0.001	2.33 (1.78–3.06)	<0.001
TG	1.19 (1.11–1.28)	<0.001	0.92 (0.84–1.01)	0.075
TC	1.26 (1.16–1.37)	<0.001	1.50 (1.22–1.85)	<0.001
HDL-C	0.20 (0.14–0.28)	<0.001	0.24 (0.16–0.37)	<0.001
LDL-C	1.32 (1.18–1.47)	<0.001	1.01 (0.80–1.28)	0.944

Univariate and multivariate analysis between *MALAT1* SNP rs600231 and the risk factors of ACS. ACS, acute coronary syndrome; COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval; TG, total triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; *MALAT1*, metastasis-associated lung adenocarcinoma transcript 1; SNP, single nucleotide polymorphism.

Table 4 Stepwise regression screening of independent variables to build a simplified model

Factors	Estimate	Std error	OR (95% CI)	P value
Dominant model (AG + GG vs. AA)	0.284	0.107	1.328 (1.077–1.639)	0.008
Male	1.053	0.138	2.865 (2.185–3.757)	<0.001
Smoking	0.458	0.145	1.581 (1.191–2.100)	0.002
Drinking	-0.348	0.144	0.706 (0.533–0.937)	0.016
Diabetes	0.859	0.137	2.361 (1.806–3.087)	<0.001
TC	0.370	0.050	1.447 (1.311–1.597)	<0.001
HDL-C	-1.299	0.196	0.273 (0.186–0.401)	<0.001
Constant	-1.384	0.294	0.251 (0.141–0.446)	<0.001

OR, odds ratio; CI, confidence interval; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol.

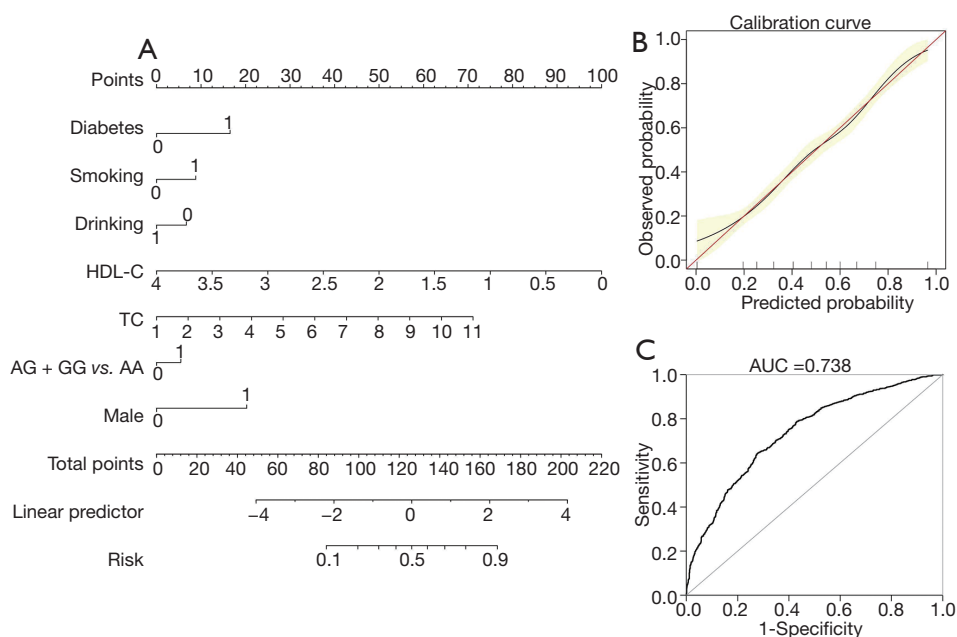


Figure 1 Predictive nomogram for ACS. (A) Nomogram of ACS risk for patients. (B) Calibration plot of the nomogram model. (C) ROC curve analysis of predictive model. ACS, acute coronary syndrome; ROC, receiver operating characteristic; HDL-C, high-density lipoprotein-cholesterol; TC, total cholesterol.

or enhancing the stability of SREBP-1C protein, could increase hepatic lipid accumulation. Several studies (42,43) documented that the relative level of *MALAT1* was higher in high-fat food-fed ApoE^{-/-} mice treated with ox-LDL, which could enhance the effect of EndMT and activate Wnt/ β -catenin signalling. Inflammatory cytokines may also induce ECs injury by oxidative damage, and *MALAT1* not only enhances oxidative stress in ECs but can also be upregulated by IL-6 via the mechanism of an extracellular

regulated protein kinases (ERK) signal (44). In addition, *MALAT1* was increased in a hypoxia-reoxygenation (H/R) cell or ischaemia/reperfusion (I/R) mouse model and could inhibit the expression of PTEN by sponging miR-320 in various physiological processes, including cell migration, differentiation and apoptosis (45). Considering the underlying biological function, we analysed the effects about SNPs in *MALAT1* among ACS patients.

In our study, the result showed that rs600231 AG and

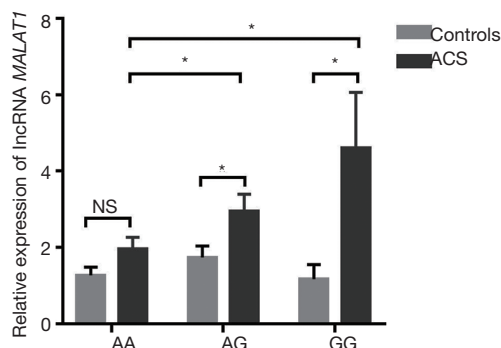


Figure 2 Influence of the *MALAT1* gene polymorphism rs600231 on lncRNA *MALAT1* expression in ACS patients and healthy controls in PBMCs. *, $P < 0.05$. *MALAT1*, metastasis-associated lung adenocarcinoma transcript 1; lncRNA, long noncoding RNA; ACS, acute coronary syndrome; PBMC, peripheral blood mononuclear cell.

Table 5 Clinical characteristics of 92 ACS patients and 92 healthy controls

Characteristics	Control (n=92)	ACS (n=92)	P value
Age (years)	56.74±9.73	58.42±10.91	0.271
Male, n (%)	61 (66.3)	65 (70.7)	0.526
BMI (kg/m ²)	26.16±3.61	25.99±2.84	0.723
Smoking, n (%)	30 (32.6)	41 (44.6)	0.096
Drinking, n (%)	29 (31.5)	33 (35.9)	0.533
Hypertension, n (%)	41 (44.6)	43 (46.7)	0.767
Diabetes, n (%)	8 (8.8)	26 (28.3)	0.001
TG (mmol/L)	1.57±0.87	1.81±1.00	0.084
TC (mmol/L)	3.96±0.89	4.82±1.16	<0.001
HDL-C (mmol/L)	1.31±0.52	0.99±0.24	<0.001
LDL-C (mmol/L)	2.57±0.81	3.04±0.94	<0.001

ACS, acute coronary syndrome; BMI, body mass index; TG, total triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

GG genotypes carriers were more susceptible to ACS than AA genotypes carriers. The dominant model (AG + GG vs. AA) and G allele of rs600231 presented distinct differences among ACS patients and controls ($P < 0.05$). After adjusted the covariates, including age, sex, hypertension, diabetes, smoking, alcohol consumption, TC, TGs, HDL-C and HDL-C, the significant difference in the polymorphism (rs600231) of the *MALAT1* gene and ACS remained. These findings suggested that rs600231 in *MALAT1* is a risk factor for ACS (AG + GG vs. AA, OR = 1.32, 95% CI: 1.07–1.63, $P < 0.05$). Furthermore, using above clinical variables and rs600231 of *MALAT1*, we firstly developed a diagnostic nomogram model which was of high discrimination and calibration to predict the risk of ACS. The calibration curve displayed in nomogram model indicates good calibration, which the area under ROC curve for the evaluating of ACS was 0.738 (95% CI: 0.716–0.761). *MALAT1* rs600231 was selected because of its regulation of gene expression, which could remotely control the promoter region (46–48). However, rs600231 was explored in the context of its association with cancer, and we found no related results for CAD. Previous studies have also explored whether rs3200401 and rs4102217 of *MALAT1* are involved in the prediction of CAD. Similar findings (49,50) that the two SNPs were not associated with the onset of CAD were reported, but these studies recognized the linking with the CAD risk factors, such as diabetes, the status of smoking and lipids (TC and TG).

In addition, the relative expression of the lncRNA *MALAT1* was measured in PBMCs from parts of controls and ACS patients. We revealed that enrolled participants carrying GG phenotype demonstrated the highest lncRNA *MALAT1* expression. The relative expression of lncRNA *MALAT1* in ACS who carried AG or GG phenotype was obvious higher than that in controls who carried the same phenotypes, but no difference was found in the AA phenotype. The first study (51) on circulating lncRNAs in 414 AMI patients and 86 healthy controls tested the relative

expression of lncRNA *MALAT1*. As a result, it was obvious that the lncRNA *MALAT1* was more highly expressed in AMI patients than in controls. Indeed, in 132 AMI patients and 104 controls, our previous research (9) found that the relative expression of lncRNA *MALAT1* was higher in AMI patients than in controls.

However, there are some limitations to our study. First, our present study evaluated only the relationship between three SNPs of the *MALAT1* gene (rs3200401, rs4102217 and rs600231) and ACS risks among the Chinese population in the Xinjiang region, and the other SNPs of the *MALAT1* gene were not evaluated. Second, the enrolled ACS subjects in our present study were from one hospital because this was not a multicentre large-scale study, and thus, the sample size limitation and selection bias could not be avoided. Finally, we measured only the lncRNA *MALAT1* in a small portion of unmatched participants, so the analysis might not have had enough power to determine the real effect.

In conclusion, our study provides the first evidence between the risk factor of rs600231 in *MALAT1* gene and the susceptibility of ACS in the Chinese population (Xinjiang). *MALAT1* gene polymorphisms may present predictive value for the risk of ACS patients, while the combination of *MALAT1* gene polymorphisms (AG and GG genotypes) and the expression of lncRNA *MALAT1* may improve the ability to predict the severity of ACS. Further studies also need to be performed to elucidate the potential molecular mechanism of rs600231 in the *MALAT1* gene in ACS.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and was approved by the Human Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China) (approval ID: 20141201-03). All participants provided written informed consent.

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Table S1 The probes sequence of *MALAT1* SNPs

SNP	Probes sequence
rs3200401_CR	TGCATTTACTTGCCAACAGAACAGAAAG
rs3200401_TR	TGCATTTACTTGCCAACAGAACAGAGAA
rs3200401_3R	ACCTGAAGTCAAGACAACTGCATTC
rs4102217_GR	CCTGCTGCCTCCCTTCCTATC
rs4102217_CR	CCTGCTGCCTCCCTTCCTATG
rs4102217_3R	CAGCACTTCTGTCAGTCTCTCCAA
rs600231_AR	TGAAACCCAGCAGACAGGACT
rs600231_GR	TGAAACCCAGCAGACAGGACC
rs600231_3R	GTCACTTCACAGAGAGCTGAGGGC

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; SNP, single nucleotide polymorphism.