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Original Article

Effects of the lncRNA MALAT1 gene region rs664589 site mutation on acute myocardial infarction in Chinese Han

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Abstract

We aimed to study the association between the non-coding region of the lncRNA MALAT1 gene, the non-coding region rs664589 C>G variant, and the risk of acute myocardial infarction (AMI) in the Chinese Han population. 165 NSTEMI and 135 STEMI patients were enrolled in the study. An additional 150 healthy individuals were enrolled as the controls. All subjects were analyzed for the MALAT1 rs664589 locus genotype. The receiver operating curve (ROC) was used to determine the effect of MALAT1 rs664589 single nucleotide polymorphism (SNP) on the diagnosis of AMI by plasma lncRNA MALAT1. The *MALAT1* rs664589 site G allele carrier was 1.39 times more likely to have NSTEMI than the C allele carrier (95% CI: 1.16-1.61, $P = 0.001$) and 1.59 times more likely to have STEMI than the C allele carrier (95% CI: 1.31-1.85, $P < 0.001$). The *MALAT1* rs664589 site C>G mutation resulted in an increase in the area under the ROC curve (AUC) of the plasma lncRNA MALAT1 level for the diagnosis of AMI. The plasma lncRNA MALAT1 levels in AMI patients were negatively correlated with hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p (r = -0.81, -0.75, -0.66, -0.71, and -0.88). The C>G mutation of *MAL6641* rs664589 causes an increased risk of AMI in the Chinese Han population. The SNP at this site affects the value of plasma lncRNA MALAT1 in the diagnosis of AMI. The specific mechanism may indicate that the C>G mutation of the *MALAT1* rs664589 changes the regulation of miRNAs expression by lncRNA MALAT1.

Keywords: Acute myocardial infarction; Human lung adenocarcinoma metastasis-related transcript; Long-chain non-coding RNA; microRNA; Single nucleotide polymorphism

1. Introduction

According to the 2015 China Cardiovascular Disease Report, the mortality rate of cardiovascular disease in China is ranked first and is higher than that of cancer and other diseases [1]. Globally, the number of deaths from cardiovascular disease increased by 41% from 1990 to 2013 [2]. Among them, acute myocardial infarction (AMI) is the highest reason for cardiovascular morbidity and mortality in the world due to acute myocardial tissue necrosis caused by persistent severe ischemia [3], which can lead to heart failure and malignant arrhythmia [4, 5]. Thrombolytic and percutaneous coronary interventions can significantly improve the prognosis of patients with acute myocardial infarction. However, many patients with myocardial infarction lack typical symptoms and do not receive medical treatment in time and therefore develop heart failure or arrhythmia [6]. Thus, early diagnosis and an understanding of candidate gene regulatory targets are critical to improving the prognosis of patients with myocardial infarction.

With the development of genome sequencing technology, more and more non-coding RNA (ncRNA) functions have been identified. Their expression level reflects the essential characteristics of the disease, and therefore, ncRNA has important value in exploring the mechanisms and biomarkers of cardiovascular disease. MicroRNAs have been extensively studied as potential disease biomarkers and therapeutic targets [7]. However, studies of long-chain non-coding RNAs with higher tissue/cell specificity and potential regulatory capacity than protein-encoding genes are lacking. With the development of gene sequencing technology, especially RNA sequencing technology [8], it has been confirmed that lncRNA is involved in various biological processes including cell growth, differentiation, cell proliferation and apoptosis [7]. In addition, several studies have shown that circulating lncRNA is an effective biomarker for the diagnosis of a variety of malignancies [9]. lncRNA also plays an important role in cardiovascular diseases.

The MALAT1 gene is located on the human 11q13 chromosome, which lacks a meaningful open coding region and cannot be ultimately translated into protein, but instead produces lncRNA MALAT1. LncRNA MALAT1 is involved in a variety of biological processes, such as a competitive endogenous RNA (ceRNA), and can regulate proliferation and metastasis of osteosarcoma by competitively binding to miR-34a/c-5p and miR-449a/b [10]. Studies by Sun et al. [10] have shown that MALAT1 may be a carcinogenic lncRNA that promotes the proliferation and metastasis of osteosarcoma and can be considered a therapeutic target for human osteosarcoma. However, few studies have focused on the effects of MALAT1 gene mu-

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tations on disease. This study focused on the MALAT1 non-coding region rs664589 locus C>G mutation because we found that rs664589 locus C>G mutations can cause lncRNA MALAT1 to lose its target site of interaction with miRNAs (as determined through the lncRNASNP2 database; http://bioinfo.life.hust.edu.cn/lncRNASNP#!/), can affect its regulation of miRNAs, and may have a certain impact on the occurrence of disease.

We analyzed the association between the rs664589 locus C>G variant and the risk of AMI in China by case-control study and analyzed the effect of mutation on the diagnosis of AMI by lncRNA MALAT1.

2. Materials and Methods

2.1. Demographic data of the subjects

We selected 300 patients with AMI who were treated in The First People´s Hospital of Yuhang District and The Second Affiliated Hospital of Zhejiang Chinese Medical University from March 2015 to October 2018, including 211 males and 89 females, aged 25-87 years with an average of 62.5±11.5 years. A total of 135 patients developed acute ST-segment elevation myocardial infarction (STEMI), and the diagnostic criteria were referenced to the Fourth universal definition of myocardial infarction (2018) [11]. The control group consisted of 150 healthy subjects with no cardiovascular disease recruited from the physical examination center of The First People´s Hospital of Yuhang District and The Second Affiliated Hospital of Zhejiang Chinese Medical University including 101 males and 49 females, aged 32-82 years with an average of 63.2±11.6 years old. The study was approved by the Medical Ethics Committee of The First People´s Hospital of Yuhang District and The Second Affiliated Hospital of Zhejiang Chinese Medical University, and all subjects signed an informed consent form.

2.2. Genotyping

Genomic DNA was extracted from the peripheral venous blood of all subjects using QIAamp DNA Blood Mini Kit (QIA-GEN, Valencia, Calif), eluted with sterile water, and stored in a refrigerator at -80°C for testing. The fragment of interest containing the rs664589 site was amplified by the polymerase chain reaction (PCR). The PCR amplified primers were: 5'-AGT TTT ATT AAA GGG GAG GGG C-3' (upstream) and 5'-TAA ACC CAC CCC ACC AAT CC-3' (downstream). The PCR reaction mixture was: 1.5 U Taq DNA polymerase (TaKaRa, Dalian, China), 1 µL of each of the forward and reverse primers, 2 µL of 2.5 mM dNTP, 100 ng of genomic DNA, and ddH2O to bring the volume of the mixture to 20 µL. The conditions of the PCR reaction were: 95°C, 3 min; 35 cycles of 95°C, 30 s; 58°C, 30 s, 72°C, 30 s, and then extension at 72°C for 10 min. The PCR reaction product was subjected to Sanger sequencing by the Shanghai Boshang Biotechnology Co., Ltd. (Shanghai, China), and the rs664589 locus genotype was determined for each subject based on the sequencing results and the sequences in the NCBI database.

2.3. qRT-PCR

Total RNA was extracted from the plasma of 150 healthy controls, 165 NSTEMI patients, and 135 STEMI patients using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and according to the manufacturer's instructions. A Quantitative real-time polymerase chain reaction (qRT-PCR) of hsa-miR-6735-3p, hsa-miR-3677-5p, lncRNA MALAT1 and hsa-miR-1972, hsa-miR-194-5p, and hsa-miR-4717-5p was performed on a Bio-Rad CFX96 thermal cycler using SYBR Green Q-PCR Master Mix (TaKaRa, Dalian, China). Based on the primer information in Table 1, U6 was used as an internal control. The expression levels of lncRNA MALAT1 and hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p were normalized to U6. Each sample was tested in triplicate.

2.4. Statistical analysis

Statistical analysis of the data in this study was performed using Statistic Package for Social Science (SPSS) 22.0 (IBM, Armonk, NY, USA). Continuous variables were expressed as the mean ± standard deviation, and the statistical analysis was performed by a one-way analysis of variance or t-test. The categorical variables are represented by n (%), and we used the Chi-square test for statistical analysis. A Hardy-Weinberg equilibrium detection of the rs664589 locus genotype of the non-coding region of the *MALAT1* was assessed by the Chi-square test. Binary logistic regression analysis was used to analyze the association between the rs664589 locus genotype and the

Table 1. RT-PCR primer information.

RNA	Primer sequence (5’ to 3’)
lncRNA MALAT1	Forward primer: 5’-GGC GGA ATT GCT GGT AGT TT-3’; Reverse primer: 5’-AGC ATA GCA GTA CAC GCC TT-3’
hsa-miR-1972	Forward primer: 5’-AAA GTT TTA TTA AAG GGG AGG GGC-3’; Reverse primer: 5’-ACC AAT CCC AAC CGT AAC AGG-3’
hsa-miR-194-5p	Forward primer: 5’-GCG GCG GTG TAA CAG CAA CTC C-3’; Reverse primer: 5’-ATC CAG TGC AGG GTC CGA GG-3’
hsa-miR-4717-5p	Forward primer: 5’-TTT TAT TAA AGG GGA GGG GCA A-3’; Reverse primer: 5’-CCC ACC AAT CCC AAC CGT A-3’
hsa-miR-6735-3p	Forward primer: 5’-GTT TTA TTA AAG GGG AGG GGC T-3’; Reverse primer: 5’-CCA CCA ATC CCA ACC GTA ACA-3’
hsa-miR-3677-5p	Forward primer: 5’-GAG GGG CAA ATA TTG GCA ATT AG-3’; Reverse primer: 5’-TAC CTA AAC CCA CCC CAC CAA-3’
U6	Forward primer: 5’-GCT TCG GCA CAT ATA CTA AAA T-3’; Reverse primer: 5’-CGC TTC ACG AAT TTG CGT GTC AT-3’

NSTEMI and STEMI risk and to adjust for factors such as age, gender, BMI, smoking, and drinking status. All tests were bilateral, and P < 0.05 indicated statistically significant differences.

3. Results

3.1. Demographic characteristics

The 300 patients with AMI enrolled in this study included 165 patients with NSTEMI and 135 patients with STEMI, and the study also included 150 healthy controls. The demographic characteristics of the NSTEMI patients, STEMI patients, and healthy controls are presented in Table 2. The analysis showed that there were no differences in age, gender, BMI, smoking, and drinking status among the three groups (P>0.05). The levels of white blood cells, platelets, neutrophils and high-sensitivity CRP in patients with STEMI were significantly lower than those in STEMI patients, while the proportion of monocytes and lymphocytes was significantly higher than that in patients with STEMI (P < 0.05).

3.2. The MALAT1 rs664589 site SNP is associated with NSTEMI risk

The control group enrolled in this study met the Hardy-Weinberg equilibrium (P>0.05). The correlation between the genotype frequency and allele frequency of the MALAT1 rs664589 locus and NSTEMI risk are presented in Table 3. The analysis showed that the risk of NSTEMI increased by 1.34 times (95% CI: 1.03-1.66, P=0.03) and 1.69 times (95% CI: 1.06-2.04, P=0.03) in subjects with CG and GG genotypes, respectively, based on the CC genotype. The risk of NSTEMI was not significantly increased in the additive and recessive models (P>0.05), whereas the risk of NSTEMI was 1.41 times higher in the dominant model (95% CI: 1.11-1.71, P<0.001). Overall, the MALAT1 rs664589 locus G allele carrier was 1.39 times more likely to have NSTEMI than the C allele carrier (95% CI: 1.16-1.61, P=0.001).

3.3. The MALAT1 rs664589 site SNP is associated with STEMI risk

The control group enrolled in this study met the Hardy-Weinberg equilibrium (P>0.05). The correlation between the genotype frequency and the allele frequency of the MALAT1 rs664589 locus and STEMI risk are presented in Table 4. The analysis shows that the risk of STEMI in subjects with CG and GG genotypes increased by 1.45 times (95% CI: 1.07-1.87, P=0.02) and 2.04 times (95%

Table 2. Demographic characteristics of the study population.

	Healthy control(n=150)	NSTEMI (n=165)	STEMI (n=135)
Age, mean(range)	63.2(24-83)	62.9(27-85)	62.1(25-87)
gender, male, n(%)	101(67.3%)	115(69.7%)	96(71.1%)
BMI, mean(range)	24.1(17.4-31.5)	23.6(17.9-32.5)	23.5(17.2-30.5)
Smoking, n(%)			
Ever	66(44.0%)	77(46.7%)	60(44.4%)
Never	84(56.0%)	88(53.3%)	75(55.6%)
Drinking, n(%)			
Ever	78(52.00%)	83(50.3%)	72(53.3%)
Never	72(48.00%)	82(49.7%)	63(46.7%)
Blood cell counts at admission, median (range)			
White blood cells, $\times10^9$/L		9.4(2.9-28.5)	14.4(4.8-29.3)*
Platelets, $\times10^9$/L		241.7(59.5-733.1)	247.7(71.5-678.5)*
Monocytes, %		6.5(1.2-17.9)	5.8(1.1-23.1)*
Lymphocytes, %		17.8(3.5-46.9)	14.8(2.5-70.5)*
Neutrophils, %		71.8(43.5-98.5)	75.9(23.5-98.5)*
High-sensitivity CRP, mean(range), mg/L		6.1(2.1-16.2)	10.9(2.4-105.0)*

BMI, body mass index; CRP, C-reactive protein; NSTEMI, non–ST-segment–elevation myocardial infarction; NSTEMI, non–ST-segment–elevation myocardial infarction; *Compared with NSTEMI, P<0.05.

Table 3. Correlation between the MALAT1 rs664589 locus genotype and the allele frequency and NSTEMI risk.

	NSTEMI (n=165)	Healthy control(n=150)	HWE *p*	crude OR(95%CI)	crude *p*	adjusted OR(95%CI)	adjusted *p*
CC	111(67.27%)	123(82.00%)	0.17	1.00(reference)			
CG	42(25.45%)	24(16.00%)		1.94(1.10-3.41)	0.02	1.34(1.03-1.66)	0.03
GG	12(7.27%)	3(2.00%)		4.43(1.22-16.12)	0.01	1.69(1.06-2.04)	0.03
Additive model				1.22(0.87-1.71)	0.25	1.10(0.93-1.32)	0.29
Dominant model				2.22(1.31-3.76)	<0.001	1.41(1.11-1.71)	<0.001
recessive model				3.84(1.06-13.90)	0.03	1.57(0.99-1.88)	0.06
C	264(80.00%)	270(90.00%)		1.00(reference)			
G	66(20.00%)	30(10.00%)		2.25(1.42-3.58)	<0.001	1.39(1.16-1.61)	0.001

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; NSTEMI, non–ST-segment–elevation myocardial infarction; HWE, Hardy-Weinberg equilibrium; OR, Odds ratio; CI, Confidence interval.

CI: 1.37-2.40, P=0.001), respectively, based on the CC genotype. The risk of STEMI was not significantly increased in the additive model (P>0.05), while the risk of NSTEMI in the dominant and recessive models increased by 1.59 times (95% CI: 1.23-1.98, P=0.001) and 1.85 times (95% CI: 1.26-2.17, P < 0.001), respectively. Overall, the MALAT1 rs664589 locus G allele carrier was 1.59 times more likely to have STEMI than the C allele carrier (95% CI: 1.31-1.85, P < 0.001).

3.4. Stratified analysis of the demographic characteristics

We stratified the demographic characteristics of the subjects: subjects <60 years old were classified as young subjects and subjects ≥60 years old were classified as elderly subjects. Subjects with a BMI <25 kg/m^2 were classified as non-obese subjects, and subjects with a BMI > 25 kg/m2 were classified as obese subjects. The stratified analysis showed that the MALAT1 rs664589 locus G allele (CG/GG) was carried only in young subjects, male subjects, non-obese subjects, non-smoking subjects, and non-drinking subjects. The risk of NSTEMI was significantly increased (P < 0.05) (Table 5). Similarly, STEMI risk is significant only when the MALAT1 rs664589 G allele (CG/GG) is carried in young subjects, male subjects, non-obese subjects, smoking subjects, and non-drinking subjects (P < 0.05) (Table 6).

3.5. Comparison of RNA expression in the plasma

We detected the differences of lncRNA MALAT1, hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p between NSTEMI and STEMI in the healthy control group plasma by RT-PCR (Figure 1). The results showed that the LncRNA MALAT1 in NSTEMI and STEMI plasma was significantly higher than that in the healthy control group. The level of lncRNA MALAT1 in NSTEMI patients was significantly higher than that in STEMI patients (P<0.05). We found that regardless of the patients with NSTEMI or STEMI, the plasma hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p levels were significantly lower than the healthy control group (P < 0.05). Differences in the plasma levels of hsa-miR-1972 and hsa-miR-194-5p were only observed in NSTEMI and STEMI (P<0.05).

3.6. The detection of plasma miRNAs levels is a potential diagnostic marker for NSTEMI and STEMI

We detected hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p levels in the plasma of subjects by RT-PCR and plotted the receiver operating curve (ROC) for NSTEMI and STEMI (Figures 2A and 2B). The results show that hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p are reliable markers for the diagnosis of NSTEMI and STEMI, and in particular, the HCS of hsa-miR-4717-5p level diagnostic NSTEMI and STEMI both exceeded 0.8 (Table 7).

3.7. The MALAT1 rs664589 site SNP affects plasma lncRNA MALAT1 levels to diagnose ROC values for NSTEMI and STEMI

We performed a receiver operating curve (ROC) of NSTEMI and STEMI for lncRNA MALAT1 levels. The results show that the AUC of the lncRNA MALAT1 level for the diagnosis of NSTEMI was 0.7427 (Figure 3A), and the

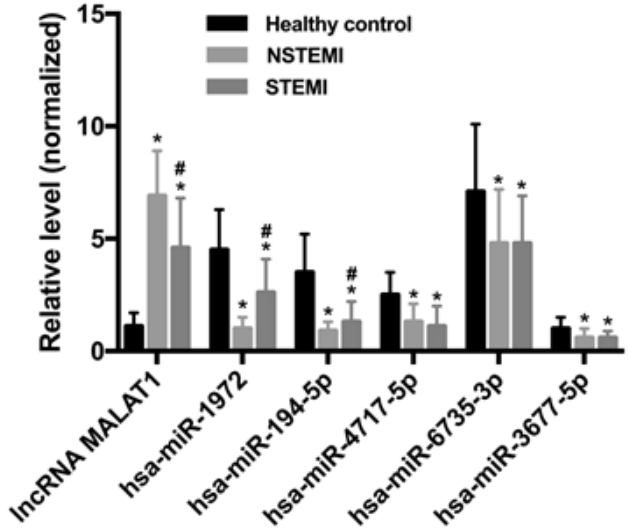

Fig. 1. Comparison of the RNA levels in plasma. *P<0.05 compared with healthy control; #P<0.05 compared with NSTEMI.

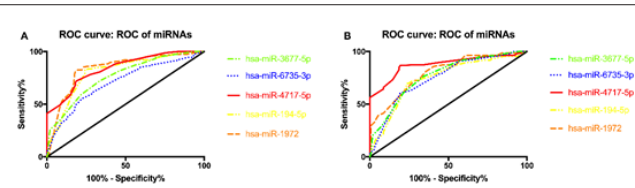

Fig. 2. Plasma miRNAs levels to diagnose ROC curves for NSTEMI and STEMI. (A) plasma miRNAs levels to diagnose the ROC curve of NSTEMI; (B) plasma miRNAs levels to diagnose the ROC curve of STEMI.

Table 4. The correlation between MALAT1 rs664589 locus genotype and the allele frequency and STEMI risk.

	STEMI (n=135)	Healthy control(n=150)	HWE *p*	crude OR(95%CI)	crude *p*	adjusted OR(95%CI)	adjusted *p*
CC	85(62.96%)	123(82.00%)	0.17	1.00(reference)			
CG	35(25.93%)	24(16.00%)		2.11(1.17-3.80)	0.01	1.45(1.07-1.87)	0.02
GG	15(11.11%)	3(2.00%)		7.24(2.03-25.77)	0.001	2.04(1.37-2.40)	0.001
Additive model				1.30(0.91-1.87)	0.15	1.16(0.94-1.44)	0.18
Dominant model				2.68(1.56-4.62)	<0.001	1.59(1.23-1.98)	0.001
recessive model				6.13(1.73-21.65)	<0.001	1.85(1.26-2.17)	<0.001
C	205(75.93%)	270(90.00%)		1.00(reference)			
G	65(24.07%)	30(10.00%)		2.85(1.79-4.56)	<0.001	1.59(1.31-1.85)	<0.001

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; STEMI, ST-segment–elevation myocardial infarction; HWE, Hardy-Weinberg equilibrium; OR, Odds ratio; CI, Confidence interval.

Table 5. Hierarchical analysis of the correlation between the genotype of MALAT1 rs664589 and the risk of NSTEMI.

	NSTEMI (n=165)	Healthy control (n=150)	crude OR(95%CI)	crude *p*	adjusted OR (95%CI)	adjusted *p*
Age						
<60						
CC	60(87.7%)	64(67.4%)	1.00(reference)			
CG/GG	29(12.3%)	9(32.6%)	3.44(1.50-7.86)	<0.01	1.58(1.16-1.95)	<0.01
≥60						
CC	51(76.6%)	59(67.1%)	1.00(reference)			
CG/GG	25(23.4%)	18(32.9%)	1.61(0.79-3.28)	0.19	1.25(0.86-1.72)	0.26
Gender						
Male	78(81.2%)	82(67.8%)	1.00(reference)			
CC	37(18.8%)	19(32.2%)	2.05(1.09-3.86)	0.03	1.36(1.02-1.71)	0.04
CG/GG						
Female						
CC	33(83.7%)	41(66.0%)	1.00(reference)			
CG/GG	17(16.3%)	8(34.0%)	2.64(1.01-6.88)	0.04	1.53(0.96-2.12)	0.07
BMI						
<25						
CC	74(84.6%)	77(70.5%)	1.00(reference)			
CG/GG	31(15.4%)	14(29.5%)	2.30(1.14-4.67)	0.02	1.41(1.04-1.77)	0.03
≥25						
CC	37(78.0%)	46(61.7%)	1.00(reference)			
CG/GG	23(22.0%)	13(38.3%)	2.20(0.98-4.93)	0.06	1.43(0.96-1.99)	0.08
Smoking						
Ever						
CC	51(80.3%)	53(66.2%)	1.00(reference)			
CG/GG	26(19.7%)	13(33.8%)	2.08(0.96-4.48)	0.06	1.36(0.95-1.79)	0.09
Never						
CC	60(83.3%)	70(68.2%)	1.00(reference)			
CG/GG	28(16.7%)	14(31.8%)	2.33(1.13-4.83)	0.02	1.44(1.03-1.87)	0.03
Drinking						
Ever						
CC	56(79.5%)	62(67.5%)	1.00(reference)			
CG/GG	27(20.5%)	16(32.5%)	1.87(0.91-3.82)	0.09	1.32(0.93-1.76)	0.12
Never						
CC	55(84.7%)	61(67.1%)	1.00(reference)			
CG/GG	27(15.3%)	11(32.9%)	2.72(1.24-6.00)	0.01	1.50(1.07-1.92)	0.02

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; NSTEMI, non–ST-segment–elevation myocardial infarction; HWE, Hardy-Weinberg equilibrium; OR, Odds ratio; CI, Confidence interval.

AUC for the diagnosis of STEMI was 0.8435 (Figure 3D). The ROC curves of NSTEMI and STEMI were diagnosed by the plasma lncRNA MALAT1 levels in subjects with different genotypes of MALAT1 rs664589. The results show that when carrying the G allele (CG/GG), the AUC for both NSTEMI and STEMI was significantly greater than the CC genotype for NSTEMI or STEMI (Figure 3B, 3C, 3E, and 3F).

3.8. Correlation between the MALAT1 rs664589 SNP and plasma miRNAs levels

We analyzed the lncRNA MALAT1, hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p level in the plasma of subjects with different genotypes of the MALAT1 rs664589 locus. We observed a significant increase in the plasma lncRNA MALAT1 levels of healthy controls and NSTEMI and STEMI patients with MALAT1 rs664589 locus C>G variants, and the levels of hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p were significantly decreased (P<0.05) (Figure 4A, 4B, and 4C).

3.9. Plasma lncRNA MALAT1 levels are negatively correlated with miRNAs levels

Pearson correlation was used to analyze the correlation between the plasma lncRNA MALAT1 level and the levels of hsa-mir-1972, hsa-mir-194-5p, hsa-mir-4717-5p, hsa-mir-6735-3p, and hsa-mir-3677-5p in patients with AMI. The results show that the plasma levels of lncRNA MALAT1 were negatively correlated with those of hsa-mir-1972, hsa-mir-194-5p, hsa-mir-4717-5p, hsa-mir-6735-3p, and hsa-mir-3677-5p (r= -0.81, -0.75, -0.66,

Table 6. Hierarchical analysis of the correlation between the genotype of MALAT1 rs664589 and the risk of NSTEMI.

	STEMI (n=135)	Healthy control (n=150)	crude OR(95%CI)	crude *p*	adjusted OR (95%CI)	adjusted *p*
Age						
<60						
CC	53(87.7%)	64(63.1%)	1.00(reference)			
CG/GG	31(12.3%)	9(36.9%)	4.16(1.82-9.51)	<0.01	1.71(1.26-2.12)	**<0.01**
≥60						
CC	32(76.6%)	59(62.7%)	1.00(reference)			
CG/GG	19(23.4%)	18(37.3%)	1.95(0.90-4.23)	0.09	1.46(0.90-2.22)	0.14
Gender						
Male						
CC	56(81.2%)	82(58.3%)	1.00(reference)			
CG/GG	40(18.8%)	19(41.7%)	3.08(1.62-5.87)	<0.01	1.67(1.24-2.15)	**<0.01**
Female						
CC	29(83.7%)	41(74.4%)	1.00(reference)			
CG/GG	10(16.3%)	8(25.6%)	1.77(0.62-5.02)	0.28	1.34(0.70-2.11)	0.42
BMI						
<25						
CC	52(84.6%)	77(62.7%)	1.00(reference)			
CG/GG	31(15.4%)	14(37.3%)	3.28(1.59-6.75)	<0.01	1.71(1.23-2.22)	<0.01
≥25						
CC	33(78.0%)	46(63.5%)	1.00(reference)			
CG/GG	19(22.0%)	13(36.5%)	2.04(0.88-4.70)	0.09	1.42(0.90-2.07)	0.14
Smoking						
Ever						
CC	33(80.3%)	53(55.0%)	1.00(reference)			
CG/GG	27(19.7%)	13(45.0%)	3.34(1.51-7.36)	<0.01	1.76(1.19-2.43)	<0.01
Never						
CC	52(83.3%)	70(69.3%)	1.00(reference)			
CG/GG	23(16.7%)	14(30.7%)	2.21(1.04-4.71)	0.04	1.46(0.99-1.97)	0.06
Drinking						
Ever						
CC	47(79.5%)	62(65.3%)	1.00(reference)			
CG/GG	25(20.5%)	16(34.7%)	2.06(0.99-4.29)	0.06	1.41(0.96-1.93)	0.08
Never						
CC	38(84.7%)	61(60.3%)	1.00(reference)			
CG/GG	25(15.3%)	11(39.7%)	3.65(1.61-8.26)	<0.01	1.81(1.23-2.43)	<0.01

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; STEMI, ST-segment–elevation myocardial infarction; HWE, Hardy-Weinberg equilibrium; OR, Odds ratio; CI, Confidence interval.

-0.71, and -0.88) (Figure 5A, 5B, 5C, 5D, and 5E).

4. Discussion

In this study, we demonstrated that lncRNA MALAT1 is highly expressed in NSTEMI patients and STEMI patients, and the C>G mutation in the non-coding region of the MALAT1 gene rs664589 leads to an increased risk of NSTEMI and STEMI in the Chinese Han population. Detection of the level of plasma lncRNA MALAT1 has the potential to diagnose NSTEMI and STEMI. However, the MALAT1 gene rs664589 site C>G variant affects the value of plasma lncRNA MALAT1 in the diagnosis of NSTEMI and STEMI. In addition, the C>G variation at the rs664589 site of *MALAT1* also affects the levels of lncRNA MALAT1, hsa-microRNA-1972, hsa-microRNA-194-5p, hsa-microRNA-4717-5p, hsa-microRNA-6735-3p and hsa-microRNA-3677-5p in plasma, which suggests that the C>G variation at the rs664589 site of *MALAT1* may alter the regulation of lncRNA MALAT1 for the expression of microRNAs, but further evidence is needed.

MALAT1 was originally discovered as a tumor-associated lncRNA, and functional studies determined that MALAT1 can regulate gene expression through splicing and epigenetic control [12-14]. However, the function of lncRNA MALAT1 is currently less studied in cardiovascular diseases. Some researchers have observed an abnormally high expression of MALAT1 in cultured endothelial cells [15]. In addition, studies have shown that MALAT1 attenuates H2O2-induced oxidative stress, lipid peroxidation and DNA damage in HUVECs [16]. In this study, we found that plasma lncRNA MALAT1 was abnormally elevated in NSTEMI and STEMI patients compared with

Table 7. Comparison of the area under the curve (AUC) of NSTEMI and STEMI in the diagnosis of plasma miRNAs.

miRNAs	NSTEMI	STEMI
hsa-miR-1972	0.8362	0.7607
hsa-miR-194-5p	0.8071	0.7431
hsa-miR-4717-5p	0.8403	0.8770
hsa-miR-6735-3p	0.7065	0.7485
hsa-miR-3677-5p	0.7627	0.7711

NSTEMI, non–ST-segment–elevation myocardial infarction; STEMI, ST-segment–elevation myocardial infarction; AUC, Area under the curve.

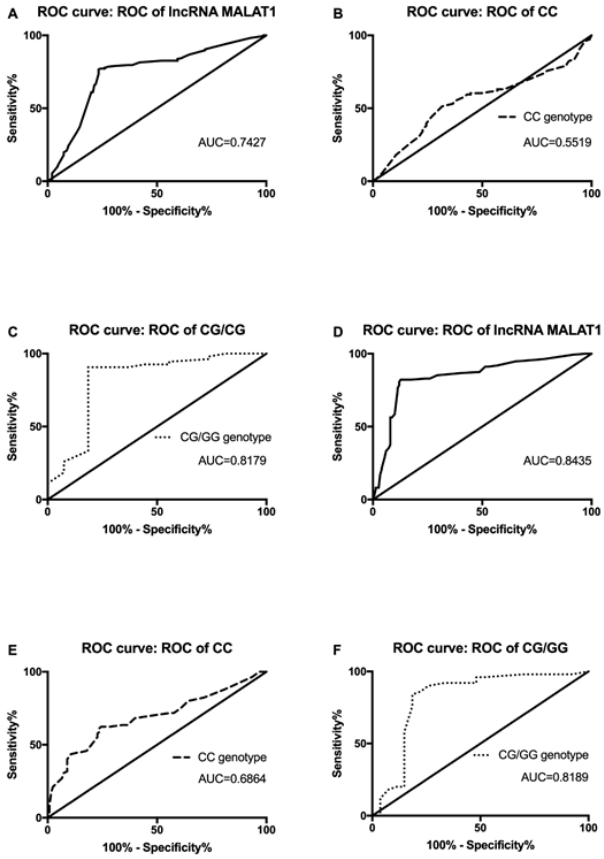

Fig. 3. The plasma lncRNA MALAT1 levels were used to diagnose the ROC curves for NSTEMI and STEMI. (A) ROC curve of the plasma lncRNA MALAT1 level in the diagnosis of NSTEMI; (B) ROC curve of the plasma lncRNA MALAT1 level corresponding to the MALAT1 rs664589 site CC genotype in the diagnosis of NSTEMI; (C) ROC curve of the plasma lncRNA MALAT1 level corresponding to the MALAT1 rs664589 site CG/GG genotype in the diagnosis of NSTEMI; (D) ROC curve of the plasma lncRNA MALAT1 level in the diagnosis of STEMI; (E) ROC curve of the plasma lncRNA MALAT1 level corresponding to the MALAT1 rs664589 site CC genotype in the diagnosis of STEMI; and (F) ROC curve of the plasma lncRNA MALAT1 level corresponding to the MALAT1 rs664589 site CG/GG genotype in the diagnosis of STEMI.

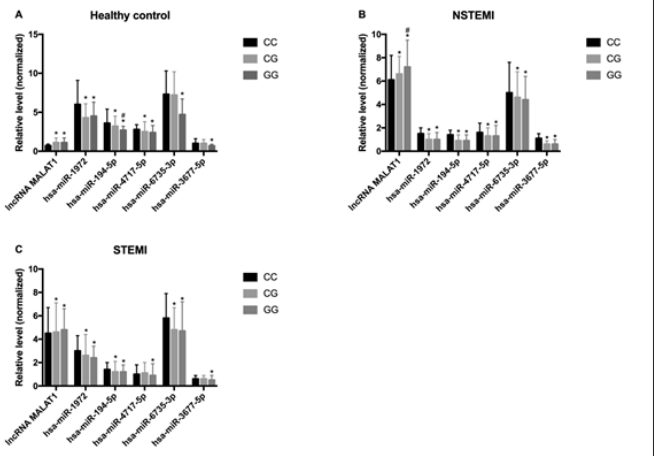

Fig. 4. Correlation of the MALAT1 rs664589 site SNP with plasma RNA levels. Compared with the rs664589 site CC genotype, *P<0.05; compared with the rs664589 site CG genotype, #P<0.05.

the healthy control group, whereas the plasma lncRNA MALAT1 level in NSTEMI patients was significantly higher than that in STEMI patients (P<0.05). Further studies found that the AUC of the NNCEMI at the level of lncRNA MALAT1 was 0.7427, and the AUC for the diagnosis of STEMI was 0.8435, which indicated that plasma lncRNA MALAT1 is a potential marker for NSTEMI and STEMI.

The rs664589 locus is in the 3' non-coding region of the MALAT1 gene. In addition, the C>G mutation affects the binding of lncRNA MALAT1 to various miRNAs, including hsa-miR-1972, hsa-miR-194-5p, hsa-miR-4717-5p, hsa-miR-6735-3p, and hsa-miR-3677-5p (Figure 6), which is why this study selected these miRNAs sites for study. In the present study, we analyzed the effect of the MALAT1 gene C>G variant on the diagnosis of NSTEMI and STEMI using plasma lncRNA MALAT1 levels. The results show that the plasma levels of lncRNA MALAT1 were higher in diagnosing NSTEMI and STEMI when carrying the G allele (CG/GG), which indicated that the plasma levels of lncRNA MALAT1 were more valuable in diagnosing NSTEMI and STEMI in CG/GG genotype subjects.

In addition, we analyzed the genotypes of rs664589 in NSTEMI patients, STMI patients, and healthy controls, and found that the G allele carriers at the MALAT1 rs664589 locus had a 1.59 times higher risk of STEMI than C allele carriers, which suggests that the population carrying the G allele may have a higher risk of AMI and warrants clinical attention. Further stratified analysis showed that there were significant differences in the risk of AMI among subjects with the G allele in different populations. For example, the risk of NSTEMI was significant and only increased in young subjects, male subjects, non-obese subjects, non-smokers and non-drinkers with the G allele at MALAT1 rs664589 (CG/GG) (P < 0.05). The risk of STEMI significantly increased in young subjects, male subjects, non-obese subjects, smokers and non-drinkers with G allele (CG/GG) at MALAT1 rs664589 locus (P < 0.05). As a result, we can conduct targeted interventions to prevent the occurrence of AMI. It is difficult to understand that young subjects with the G allele have a higher risk of AMI than older subjects. We believe that it is related to

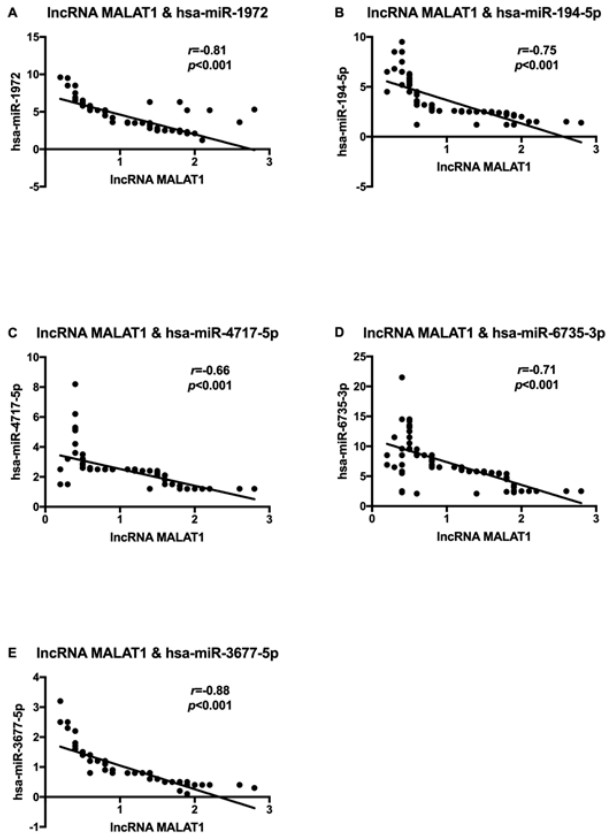

Fig. 5. The correlation between the level of lncRNA MALAT1 and the level of microRNAs in the plasma of patients with AMI. (A) the correlation between the level of lncRNA MALAT1 and the level of hsa-mir-1972 in patients with AMI; (B) the correlation between the level of lncRNA MALAT1 and the level of hsa-mir-194-5p in patients with AMI; (C) the correlation between the level of lncRNA MALAT1 and the level of hsa-mir-4717-5p in patients with AMI; (D) the correlation between the level of lncRNA MALAT1 and the level of hsa-miR-6735-3p in patients with AMI; and (E) the correlation between the level of lncRNA MALAT1 and the level of hsa-miR-3677-5p in patients with AMI.

the increase in life pressure in recent years, the change in young people's diet, and other living habits in some economically developed areas of China as well as the improvement in social and economic status [17].

In addition, our further analysis shows that the plasma levels of lncRNA MALAT1 are negatively correlated with the levels of hsa-mir-1972, hsa-mir-194-5p, hsa-mir-4717-5p, hsa-mir-6735-3p and hsa-mir-3677-5p in patients with AMI. Combined with online prediction tools, we conclude that lncRNA MALAT1 may have negative regulatory effects on the expression of hsa-mir-1972, hsa-mir-194-5p, hsa-mir-4717-5p, hsa-mir-6735-3p and hsa-mir-3677-5p. However, *in vitro* evidence is still needed.

This study also has some shortcomings that need to be further improved. First, there are few collected cases, which may magnify the error of the statistical analysis. It would be necessary to use a larger sample size for further research. Second, there is no effective *in vitro* model to verify the role of lncRNA MALAT1 in AMI and the roles of hsa-mir-1972, hsa-mir-194-5p, hsa-mir-4717-5p, hsa-mir-6735-3p, and hsa-mir-3677-5p in this process. In addition, this study focused only on the Chinese Han population. Determining whether there is a correlation among other ethnic groups is a very interesting topic that is worthy of further study.

5. Conclusion

In conclusion, we found that the C > G mutation at the rs664589 site of the MALAT1 gene is associated with an increased risk of AMI in the Chinese Han population. The C > G mutation at the rs664589 site affects the diagnostic value of plasma lncRNA MALAT1 for AMI. We speculate that the C>G mutation at the rs664589 locus of the MALAT1 gene alters the expression regulation of lncRNA MALAT1 on hsa-mir-1972, hsa-mir-194-5p, hsa-mir-4717-5p, hsa-mir-6735-3p, and hsa-mir-3677-5p but this needs to be confirmed an in vitro model.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of The First People´s Hospital of Yuhang District and The Second Affiliated Hospital of Zhejiang Chinese Medical University.

Informed Consent

All subjects signed an informed consent form.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Study design: Huamin Yu, Shen Wang; Data collection: Huamin Yu, Mingjuan Shi, Jindong Sun, Cairong Li, Jingwen Chen, Dongming Lin; Data analysis: Huamin Yu, Mingjuan Shi, Jindong Sun, Cairong Li; Interpretation of data: Huamin Yu, Jindong Sun, Shen Wang; Draft manuscript: Huamin Yu; Review manuscript: Shen Wang.

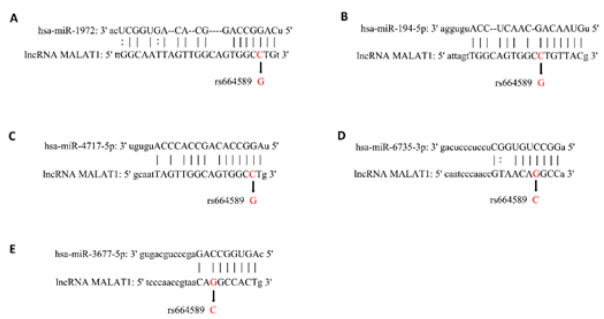

Fig. 6. The rs664589 locus C>G variants and the binding site of lncRNA MALAT1 to miRNAs. (A) the binding site of lncRNA MALAT1 to hsa-miR-1972; (B) the binding site of lncRNA MALAT1 to hsa-miR-194-5p; (C) the binding site of lncRNA MALAT1 to hsa-miR-4717-5p; (D) the binding site of lncRNA MALAT1 to hsa-miR-6735-3p; and (E) the binding site of lncRNA MALAT1 to hsa-miR-3677-5p. For additional information, please refer to the lncRNASNP2 database (http://bioinfo.life.hust.edu.cn/lncRNASNP#!/).

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