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# Predictive value of miR-636 in patients with acute myocardial infarction undergoing percutaneous coronary intervention and its bioinformatics analysis

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## Abstract

**Background** MicroRNAs (miRNAs) play an important role in the pathogenesis of cardiovascular diseases such as acute myocardial infarction (AMI). Percutaneous coronary intervention (PCI) is currently the most direct and effective procedure to treat AMI, but the occurrence of postoperative cardiovascular events (MACE) affects patients' quality of life. The objective of this study was to identify a new biomarker that could provide a theoretical basis for the prevention of MACE in patients with AMI undergoing PCI.

**Methods** 142 AMI patients who underwent PCI and 130 healthy volunteers were selected as study subjects. Detection of miR-636 expression level by fluorescence quantitative PCR. ROC, Kaplan-Meier and Cox regression analyses were applied to evaluate the diagnostic and prognostic value of miR-636 for AMI. The miR-636 target genes were predicted and enriched for GO function and KEGG pathway.

**Results** MiR-636 expression levels were elevated in patients with AMI. ROC curve analysis showed that miR-636 had a feasible diagnostic value in distinguishing AMI patients from healthy controls miR-636 expression levels were elevated in patients who developed MACEs. ROC results showed that miR-636 had significant diagnostic value in differentiating AMI patients with and without MACEs after PCI treatment. GO and KEGG enrichment analyses showed that miR-636 may transmit information to vesicles formed by the cell membrane.

**Conclusions** MiR-636 expression serves as a biomarker for diagnosing AMI and predicting the occurrence of MACE after PCI.

**Keywords** AMI, miR-636, MACE

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## Background

Acute myocardial infarction (AMI) is a serious cardiovascular disease that primarily affects middle-aged and older individuals. Its primary cause is the reduction of blood flow within the coronary arteries, which supply blood to the heart. This reduction can be caused by the occurrence of coronary artery obstruction, which leads to persistent, unremitting ischemia and hypoxia in cardiomyocytes. These conditions can irreversibly damage cardiomyocytes and result in necrosis, or the death of, these cells. This, in turn, can further impair cardiac function. More than one-tenth of the world's population dies from coronary heart disease, and about 700 million people worldwide suffer from myocardial infarction every year [1]. The aging of population is becoming more and more serious, and the prevalence of cardiovascular and cerebrovascular diseases is getting higher and higher every year, and the prevalence and mortality of myocardial infarction account for a large proportion. AMI is an acute and critical condition, and according to the China Chest Pain Center Quality Control Report (2023), the in-hospital mortality rate of patients with AMI in China is as high as 10%, which is the largest cause of death in coronary heart disease [2]. Studies have shown that in myocardial infarction, cardiomyocytes die in large numbers and fail to regenerate, leading to ventricular remodeling and reduced cardiac function. The main pathological change in the pathogenesis of myocardial infarction is cardiomyocyte apoptosis, which is also a form of cardiomyocyte death [3–5]. In addition to thrombolysis, percutaneous coronary intervention (PCI) is currently the most direct and effective procedure for the treatment of AMI [6], which can significantly restore correct coronary arteries and reduce the infarct size, but post-procedure multiple cardiovascular events (MACE) affect the quality of life of patients. The China PWACE study demonstrated a high rate of recurrent infarctions in early stages after discharge from the hospital in AMI patients, and a 1-year The 1-year mortality rate of recurrent patients was 25.42 times higher [7]. Electrocardiography and specific myocardial biomarkers are two of the most important tools for identifying and diagnosing acute myocardial infarction [8]. Measurement of continuous troponin (cTnI) levels in the blood is commonly used in the clinic to diagnose myocardial infarction, and elevated troponin is often delayed in the onset of acute coronary ischemia [9]. This poses a major obstacle to the diagnosis of AMI. The identification of reliable and consistent biomarkers is particularly important for the diagnosis and evaluation of MACE. MicroRNA (miRNA), which is easily detectable and quantifiable, is a major indicator and ideal candidate for early diagnosis of AMI [10].

MiRNA is a class of small non-coding RNAs containing 18–24 nucleotides [11]. It has been found to play an important role in the process of cardiovascular disease mechanisms. MiRNA gene expression is closely related to the regulation of cardiac function, including angiogenesis, cardiomyocyte development, and cardiac failure. MiRNAs are the main indicators and ideal candidates for early diagnosis of AMI [10], for example, miRNA-1, miRNA-499, miRNA-208 and miRNA-133 are considered as biological markers of myocardial infarction, and the expression levels of all these miRNAs are altered myocardial infarction [12, 13]. MiRNA-636 plays an important role in the pathological process of myocardial infarction, and studies have shown that its expression is upregulated in cardiomyopathy [14] and myocardial fibrosis [15]. However, studies on the molecular function of miR-636 are scarce. Clinical studies on miRNA-636 in PCI in patients with AMI are even more rarely reported. The aim of this experiment was to investigate the predictive value of miRNA-636 and its bioinformatics analysis in the diagnosis and adverse prognostic outcomes of patients undergoing PCI for AMI, and to provide a basis for the health recovery and timely monitoring of the condition of patients with AMI after surgery.

## Methods

### Ethical statement

All subjects signed an informed consent form before enrollment. The study strictly adhered to the principles of the Declaration of Helsinki. Approval was obtained from the ethics committee of Affiliated Jinhua Hospital, Zhejiang University School of Medicine.

### Study subjects

142 AMI patients who underwent PCI in Affiliated Jinhua Hospital, Zhejiang University School of Medicine from 2020 to 2023 were selected as the experimental group. All patients with AMI were first-onset, confirmed the need for transcoronary intervention by coronary angiography, and underwent PCI for the first time with a successful procedure. Among them, 59 cases were male and 83 cases were female; another 130 volunteers who matched the age and gender of AMI patients and underwent health checkups in the hospital were selected as the control group.

Patients with myocardial infarction referred to the 2019 guidelines for the diagnosis of myocardial infarction. Increased and/or regressed cardiac biomarkers that were higher than the upper limit of normal on at least 1 occasion, along with clinical evidence of acute myocardial ischemia, including (1) symptoms of acute myocardial ischemia; (2) new ischemic electrocardiographic changes; (3) new onset of physiologic Q-waves; (4) new imaging of loss of surviving myocardium or abnormalities of ventricular wall segmental motion evidence; (5) coronary

angiography or intracavitary imaging or autopsy confirmation of coronary thrombus.

Exclude patients with other cardiac diseases; patients with severe inflammatory diseases, sepsis, severe hepatic or renal failure, and severe burns; patients with combined immunodeficiency diseases; patients with combined chronic systemic diseases and tumors; and pregnant and lactating women.

In this study, the Gensini score was used as a method to quantify the overall severity of coronary artery stenosis, taking into account the location of the lesion. The lesion sites included were categorized into five major vessels: left main, left anterior descending, left circumflex, and right coronary artery. Intraoperative adequate balloon pre-dilatation of the target lesion and balloon post-dilatation after stent placement were performed. A new generation drug-eluting stent was used for the stent, and the imaging showed good stent release with residual stenosis of less than 5%.

### Clinical samples collection and measurement of clinical indicators

On the second day after PCI, 5 mL of venous blood was drawn on an empty stomach, centrifuged at 4 °C and 3000 g for 15 min. The supernatant was then placed in an EP tube without ribonuclease and stored at -80 °C. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and cardiac troponin I (cTnI) were measured using a Roche automated biochemistry analyzer. Detection of high-sensitivity C-reactive protein (hs-CRP) by immunochromatographic assay.

### Real time PCR

Detecting the relative expression of miRNA in serum. Total RNA was extracted from serum of all research subjects using RNA extraction kit. RNA concentration and quality using the Nano▪Drop2000 instrument. RNAs with OD260/280 values between 1.9 and 2.1 and with OD260/230 values above 2.0 were used for subsequent experiments. Total RNA was reverse transcribed into cDNA using a reverse transcription kit and used as a template. The relative expression levels of miRNAs in the sera of all subjects were sequentially measured using a fluorescence quantitative PCR instrument. The reaction conditions were pre-denaturation at 95℃ for 5 min, cycling conditions were denaturation at 95℃ for 10 s, annealing at 60℃ for 30 s, and extension at 72℃ for 34 s, with a total of 40 cycles. The relative expression level of miR-636 in serum was calculated using the $2^{-\Delta\Delta Ct}$ method. The internal reference was U6. U6 forward primer: 5'-GCTTCGGCAGCACATATACTAAAAT-3'; reverse primer: 5'-CGCTTCACGAATTTGCGTGTCA T-3'. MiR-636 forward primer: 5'-ACACTCCAGCTGGG TGTGC-3'; reverse primer: 5'-TGGTGTCGTGGAGTC G-3'. The primers were synthesized by Beijing RuiBiotech Biotechnology Co.

### Prognostic follow-up

AMI patients who underwent PCI were followed up by telephone and outpatient follow-up 1 week after discharge for 6 months. The start of follow-up was 1 week after each patient was discharged from the hospital, and the last follow-up time was up to the occurrence of Major adverse cardiovascular events (MACEs) or half a year after the patient was discharged from the hospital, and the occurrence and time of major MACE were recorded. MACE includes: unstable angina, malignant arrhythmia, cardiac death, severe heart failure, nonfatal myocardial infarction, target site revascularization, in-stent thrombus formation, and all-cause mortality.

### Functional enrichment analysis of target genes regulated by miRNAs

The miR-636 target genes were predicted using miRWalk, miRDB, EVmiRNA and TargetScan databases. The predicted results from simultaneous analysis of the four databases were intersected online via Jvenn. Gene Ontology (GO) and Kyoto Encyclopedia of the Genome (KEGG) pathway enrichment analyses were performed on the predicted target genes using Funrich software. $P<0.05$.

### Data analysis

SPSS 23.0 was used to analyze the experimental data. Measurement information was expressed using mean±standard deviation ($\bar{x}\pm s$). Independent samples t-test was used to compare the data between the two groups. Count data were expressed using examples and the role of miR-636 in the development of AMI was assessed using the $\chi 2$ test. The predictive efficacy of miR-636 levels for the occurrence of MACE in AMI patients was evaluated using subject operating characteristic (ROC) curves. Yuden index was calculated to obtain the cutoff, sensitivity, and specificity of the ROC. Kaplan-Meier was used to analyze the probability of developing MACE in AMI patients with different serum miR-636 levels. Cox unifactorial and multifactorial analyses were used to analyze the risk factors associated with the occurrence of MACE in AMI patients. $P<0.05$ was considered a statistically significant difference.

## Results

### Comparison of baseline data of enrolled subjects

The enrolled healthy controls included 65 males and 65 females with a mean age of 57.40±10.13 years. The included AMI patients included 59 males and 83 females with a mean age of 57.13±9.11 years. There was no

**Table 1** General information of the enroll participants

| Parameters | Controls ($n=130$) | AMI ($n=142$) | *P* values |
|---|---|---|---|
| Age (years) | 57.40 ± 10.13 | 57.13 ± 9.11 | 0.180 |
| Gender (male/female) | 65/65 | 59/83 | 0.162 |
| BMI (kg/m$^2$) | 25.32 ± 2.02 | 24.96 ± 1.97 | 0.837 |
| TC (mmol/L) | 4.01 ± 0.46 | 4.10 ± 0.56 | 0.015 |
| TG (mmol/L) | 1.76 ± 0.25 | 1.74 ± 0.25 | 0.571 |
| LDL (mmol/L) | 2.30 ± 0.24 | 2.36 ± 0.24 | 0.399 |
| HDL (mmol/L) | 1.24 ± 0.24 | 1.17 ± 0.16 | 0.000 |
| cTnI(ng/mL) | 0.00 ± 0.00 | 1.28 ± 0.35 | 0.000 |
| NT-proBNP (pg/mL) | 101.51 ± 19.17 | 475.04 ± 131.32 | 0.000 |
| hs-CRP (pg/L) | 2.29 ± 0.53 | 11.95 ± 3.69 | 0.000 |

Note: BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; cTnI, cardiac troponin I; hs-CRP, high-sensitivity C-reactive protein

significant difference in age, gender composition, BMI, TG and LDL between the healthy control group and the AMI patient group ($P>0.050$, Table 1). However, TC, cTnI, NT-proBNP and hs-CRP were significantly higher in the AMI patient group than in the healthy control group, while HDL was significantly lower ($P<0.050$).

#### Serum miR-636 has high diagnostic value for AMI

In this study, the expression level of serum miR-636 was examined by fluorescence quantitative PCR in all groups of enrollees, and the results showed that in patients with AMI, an elevated expression level of miR-636 was observed compared with healthy volunteers (Fig. 1A), with a significant difference ($P<0.0001$). The results of ROC curve analysis showed that miR-636 had a feasible diagnostic value in terms of distinguishing AMI patients from healthy controls, with an AUC of 0.932 (95% CI=0.903–0.960), and with a critical value of 1.460, the sensitivity and specificity for discriminating AMI patients from controls were 0.789 and 0.923, respectively (Fig. 1B).

Based on the average serum miR-636 expression level of AMI patients, the patients were divided into high expression group and low expression group to analyze the relationship between miR-636 expression level and clinicopathological characteristics of AMI patients. The results revealed that the expression level of miR-636 was significantly correlated with Genisini score ($P=0.044$) and cTnI ($P=0.028$). This suggests that the expression level of miR-636 may directly reflect the severity of the disease. (Table 2).

#### Predictive significance of serum miR-636 for MACE after PCI treatment in AMI patients

AMI patients were categorized into No-MACEs group and MACEs group according to whether they developed MACEs after PCI. 92 patients without MACE were included with a mean age of (56.94±9.78) years. 50 patients with MACE were included with a mean age of (56.94±9.78) years. There were no significant differences between the No-MACE group and the group of patients with MACE in terms of age, gender composition, BMI,

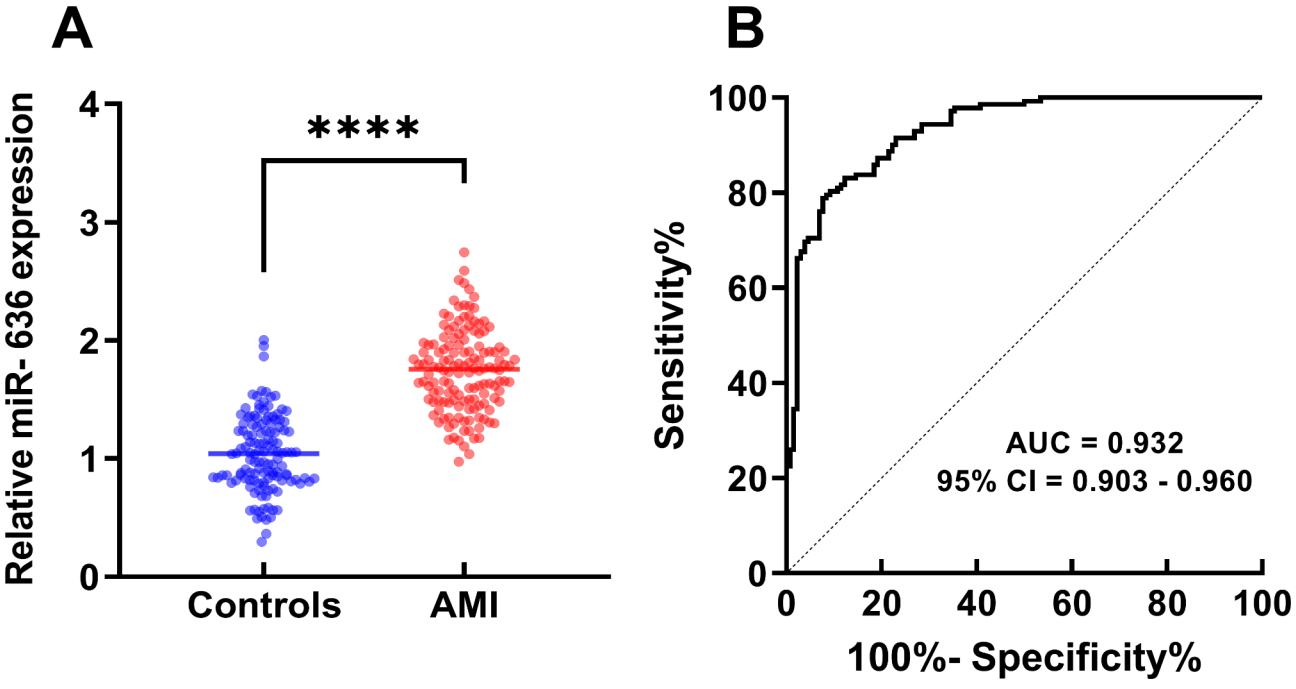


**Fig. 1** (**A**) MiR-483-5p expression levels in patients with AMI and healthy individuals. (**B**) MiR-636 has a significant diagnostic value for discriminating AMI patients and healthy individuals with an AUC value of 0.932. ****$P<0.0001$

**Table 2** Association between AMI patients' clinicopathological features and miR-636 expression levels

| Parameters | Total ($n=142$) | Low miR-636 group ($n=68$) | High miR-636 group ($n=74$) | *P* values |
|---|---|---|---|---|
| LDL (mmol/L) | | | | |
| < 2.36 | 70 | 39 | 31 | 0.066 |
| ≥ 2.36 | 72 | 29 | 43 | |
| HDL (mmol/L) | | | | |
| < 24.96 | 83 | 36 | 47 | 0.202 |
| ≥ 24.96 | 59 | 32 | 27 | |
| Genisini score | | | | |
| < 70.70 | 71 | 40 | 31 | 0.044 |
| ≥ 70.70 | 71 | 28 | 43 | |
| cTnI(ng/mL) | | | | |
| < 1.28 | 72 | 41 | 31 | 0.028 |
| ≥ 1.28 | 70 | 31 | 43 | |
| NT-proBNP (pg/mL) | | | | |
| < 475.04 | 67 | 36 | 31 | 0.188 |
| ≥ 475.04 | 75 | 32 | 43 | |
| hs-CRP (pg/L) | | | | |
| < 11.95 | 72 | 37 | 35 | 0.397 |
| ≥ 11.95 | 70 | 31 | 39 | |

**Table 3** General information of the enroll participants with AMI after PCI were compared and divided into the No-MACEs and MACEs groups

| Parameters | No-MACEs ($n=92$) | MACEs ($n=50$) | *P* values |
|---|---|---|---|
| Age (years) | 57.23 ± 8.78 | 56.94 ± 9.78 | 0.375 |
| Gender (male/female) | 39/53 | 20/30 | 0.782 |
| BMI (kg/m$^2$) | 25.01 ± 2.03 | 24.87 ± 1.88 | 0.241 |
| TC (mmol/L) | 4.13 ± 0.52 | 4.05 ± 0.633 | 0.264 |
| TG (mmol/L) | 1.73 ± 0.27 | 0.74 ± 0.27 | 0.130 |
| LDL (mmol/L) | 2.33 ± 2.67 | 2.41 ± 1.78 | 0.001 |
| HDL (mmol/L) | 1.20 ± 0.18 | 1.11 ± 0.08 | 0.000 |
| Genisini score | 67.42 ± 11.77 | 76.72 ± 14.90 | 0.012 |
| cTnI(ng/mL) | 1.20 ± 0.35 | 1.42 ± 0.30 | 0.179 |
| NT-proBNP (pg/mL) | 458.10 ± 138.68 | 506.23 ± 111.23 | 0.143 |
| hs-CRP (pg/L) | 11.25 ± 3.37 | 13.23 ± 3.93 | 0.335 |

TC, TG, cTnI, NT-proBNP and hs-CRP ($P>0.050$). However, LDL and HDL were significantly lower in the MACE group than in the No-MACE group, and the Genisini score was significantly higher ($P<0.050$). (Table 3). The expression levels of serum miR-636 were examined in patients with AMI who underwent PCI. The results demonstrated that the expression levels of serum miR-636 were higher in the group with MACEs than in the group with No-MACEs, with a statistically significant difference ($P<0.0001$) (Fig. 2**A**). In addition, the results of ROC curve analysis showed that miR-636 had a significant diagnostic value in distinguishing AMI patients occurred MACE with the cutoff of 1.784 and the AUC of 0.901. The sensitivity and specificity were 0.880 and 0.793, respectively (Fig. 2**B**).

Kaplan-Meier analysis confirmed the same results: AMI patients with high miR-636 levels had a worse prognostic profile (Fig. 3). As shown in Table 4, Cox regression analysis indicated that miR-636 (HR = 3.400, 95% CI = 1.673–6.911, $P=0.001$) could serve as an independent predictor of MACE occurrence.

### Prediction of miR-636 target genes and analysis of GO function and KEGG pathway

Downstream target prediction of miR-636 was performed using miRWalk, miRDB, EVmiRNA and TargetScan databases. There were 14,988, 764, 398, and 3,771 predicted targets in the above 4 databases, respectively, and intersecting the predicted target genes from the four databases yielded a total of 117 candidate target genes (Fig. 4**A**). All candidate target genes were analyzed for GO function and KEGG pathway enrichment, and 30 GO-enriched entries and 10 possible pathways were obtained. The GO terms included biological processes (BP), cellular components (CC), and molecular functions (MF).

BP was mainly enriched in Vesicle-Mediated Transport in Synapse, Glutamatergic Synaptic Transmission, Excitatory Postsynaptic Potential, Postsynaptic Chemotransmission, Acidic Amino Acid Transport, Glutamatergic Regulation of Synaptic Transmission, Modulation of Chemical Synaptic Transmission, Negative Regulation of Synaptic Transmission, Regulation of Trans-Synaptic Signaling, and Synaptic Vesicle Exocytosis (Fig. 4**B**).

CC was mainly enriched in Glutamatergic Synapse, Nuclear Membrane, Presynaptic Membrane, Synaptic Membrane, Nuclear Envelope, Membrane Raft, Membrane Microdomain, Membrane Region, Cell-Cell Junction, and Gap Junction (Fig. 4**C**).

MF was mainly enriched incalcium channel activity, beta-catenin binding, calcium ion transmembrane transporter activity, channel activity, passive transmembrane transporter activity, cation channel activity, ion channel activity, divalent inorganic cation transmembrane transporter activity, ion channel binding, and cadherin binding (Fig. 4**D**).

KEGG enrichment analysis resulted in 10 signaling pathways, mainly including Human cytomegalovirus infection, Glutamatergic synapse, Dilated cardiomyopathy, Cushing syndrome, Thyroid cancer, Human papillomavirus infection, Arrhythmogenic right ventricular cardiomyopathy, Platelet activation, Gap junction and Focal adhesion (Fig. 4**E**).

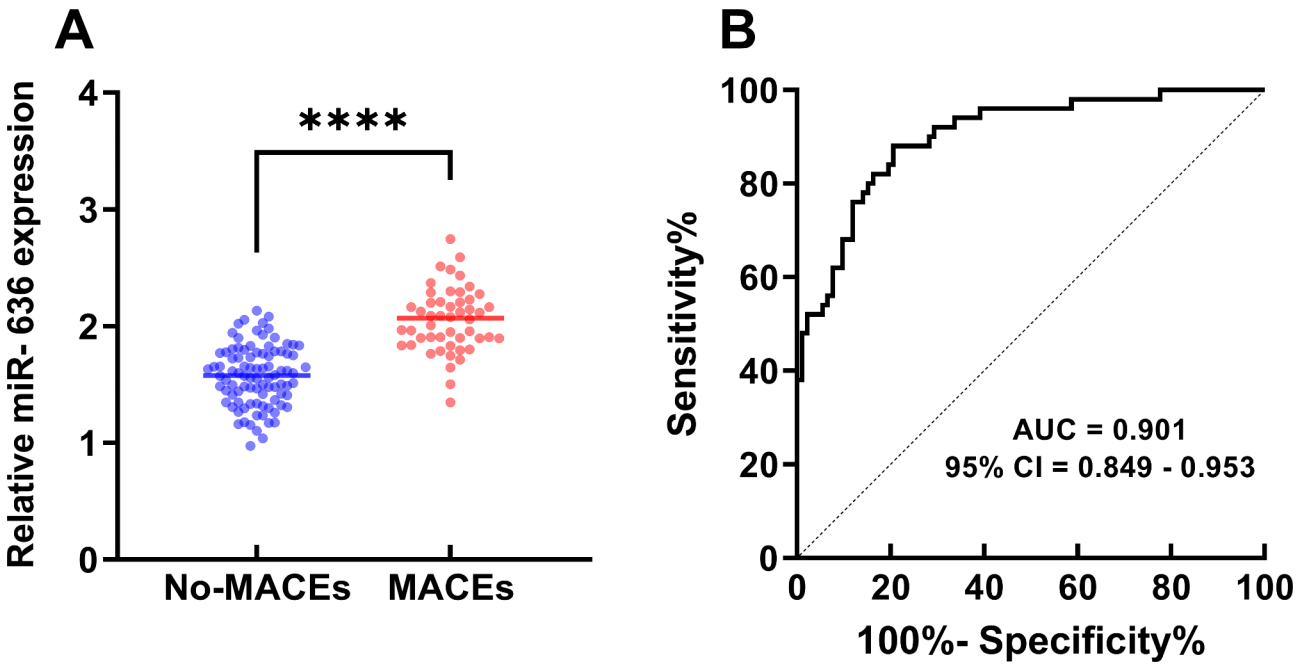


**Fig. 2** (**A**) MiR-483-5p levels after PCI in patients with AMI. (**B**). MiR-636 has a significant diagnostic value in identifying the prognosis of AMI patients with an AUC value of 0.901. ****$P < 0.0001$

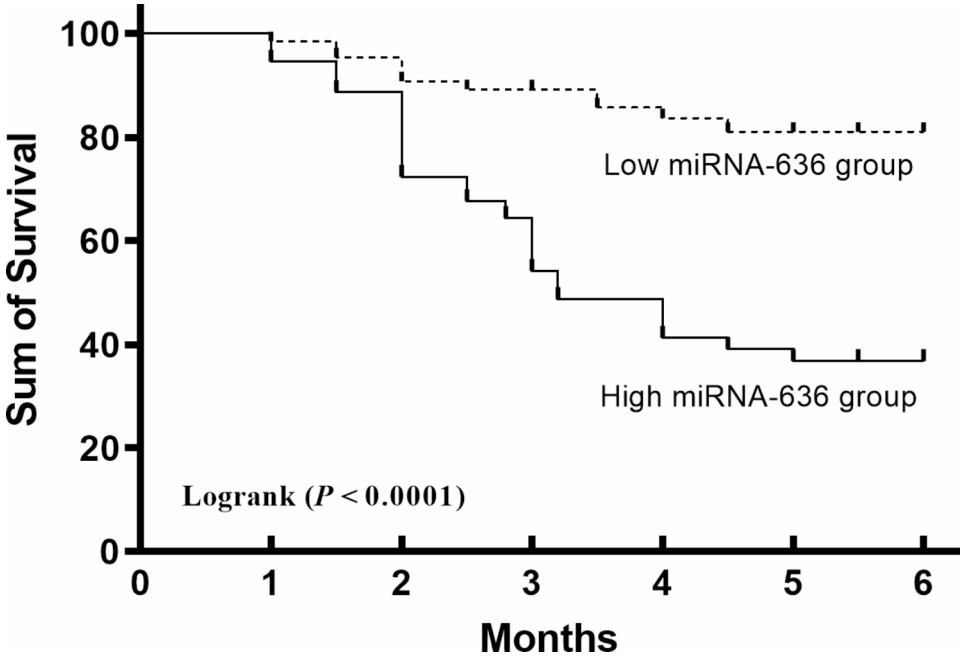


**Fig. 3** Kaplan Meier monitored the correlation between miR-636 levels and the incidence of MACE in AMI patients 6 months after PCI

## Discussion

AMI refers to a series of physiopathological changes on the basis of coronary artery atherosclerosis and stenosis, which leads to myocardial ischemia and hypoxia, and then induces myocardial necrosis and other manifestations. AMI is a common and major cause of death, and one of the main causes of death-related factors globally [16, 17]. According to the China Cardiovascular Health and Disease Report 2021, the overall AMI mortality rate in China is on the rise, and the recurrence rate of early myocardial infarction in AMI patients after discharge from the hospital is high. MiRNAs play a significant role in the pathological process of myocardial infarction. It has been demonstrated that miR-1 is positively correlated

**Table 4** Cox regression analysis of potential influences factors affecting the occurrence of MACE after PCI in patients with AMI was performed

| Parameters | *P* values | Hazard ratio (95% CI) |
|---|---|---|
| miR-636 | 0.001 | 3.400 (1.800–7.349) |
| LDL (mmol/L) | 0.637 | 1.157 (0.632–2.117) |
| HDL (mmol/L) | 0.206 | 0.664 (0.353–1.252) |
| Genisini score | 0.050 | 1.916 (1.001–3.669) |
| cTnI(ng/mL) | 0.044 | 1.963 (0.718–2.370) |
| NT-proBNP (pg/mL) | 0.383 | 1.305 (0.718–2.370) |
| hs-CRP (pg/L) | 0.155 | 1.555 (0.847–2.857) |

with the onset of arrhythmia subsequent to myocardial infarction [12, 13]. As mentioned earlier, miR-636 was significantly up-regulated in cardiomyopathy [14] and myocardial fibrosis [15], so we hypothesized that miR-636 plays an important role in the pathological process of AMI. Focusing on the role of miR-636 as a potential biomarker for the early detection of AMI and recovery after PCI in AMI patients.

In this study, firstly, by comparing the baseline data of AMI patients and control group of healthy volunteers, it was found that TC, cTnI, NT-proBNP and hs-CRP were significantly higher in the AMI patient group than in the control group of healthy volunteers, and HDL was significantly lower. Serum samples from all the experimenters were tested for miR-636 expression level and found that the serum of AMI patients had significantly higher miR-636 expression level than the control group to healthy volunteers. ROC is widely used to determine the accuracy of diagnostic biomarkers. The results of this study showed that serum miR-636 could significantly differentiate between the control group of healthy volunteers and the experimental group of AMI patients. The Gensini scoring system is a widely used system for evaluating the severity of coronary artery lesions, and the more severe the lesion, the higher the Gensini score [9]. The results revealed that the expression level of miR-636 was significantly correlated with Genisini score and cTnI ($P<0.05$). The cTnI is a specific marker for myocardial injury [18], and the Genisini score can assess the degree of coronary artery stenosis and the severity of atherosclerosis [9]. This suggests that the expression level of miR-636 may directly reflect the severity of AMI.

PCI is the most effective treatment for AMI, significantly reducing infarct size, cardiovascular mortality and disability [19]. Results from a Danish study showed that direct PCI significantly reduced the incidence of cardiovascular endpoint events in patients with myocardial infarction compared with pharmacologic thrombolytic

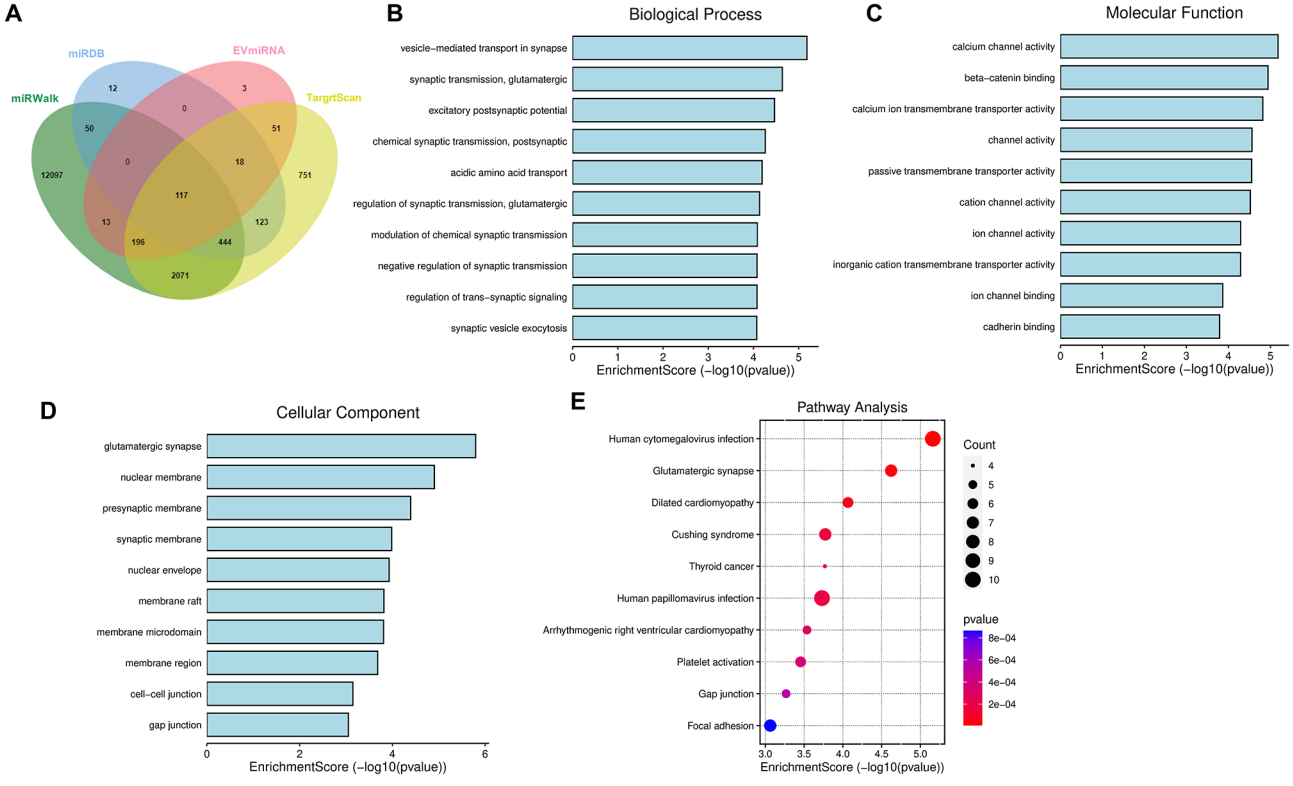


**Fig. 4** Screening of target genes of interest. Using miRDB, miRWalk, miRTarBase and TargetScan databases, the corresponding downstream target genes of miRNA-636 were predicted and made into Venn diagrams (**A**), the results of GO enrichment analysis of miRNA-636 target genes (**B-D**) and the results of KEGG pathway analysis of miRNA-636 target genes (**E**)

therapy [20]. Identification of AMI risk factors is important to promote secondary prevention and reduce future cardiovascular events [21]. Among AMI complications, recurrent myocardial infarction impairs cardiac function with a high risk of death, predisposes to heart failure, and severely affects the quality of life of patients, and MACE is an important indicator for post-PCI evaluation [22]. Identifying patients at risk of recurrent AMI after PCI can effectively implement active monitoring and management of these patients. Currently, it is crucial to explore new prognostic predictors for the clinical management of AMI patients [23, 24]. It was found that the expression level of miR-636 in serum of patients in the MACEs group was significantly higher than that of patients in the No-MACEs group. After ROC analysis, it was confirmed that serum miR-636 had high sensitivity and specificity for distinguishing AMI patients in the No-MACEs group and the MACEs group, and had high diagnostic potential. In this study, most of the patients who had MACEs were patients with high miR-636 expression. cox regression analysis confirmed that miR-636, along with cTnI and Gensini scores, was independent predictors of MACE in patients after coronary intervention.

Finally, in this study, 117 candidate target genes obtained were analyzed by GO enrichment. The results showed that 30 GO-enriched entries were obtained by GO function analysis. Notably, in the BP part of the GO enrichment analysis, the predicted downstream target gene functions of miR-636 were mainly enriched in the vesicle-associated pathway; the CC part of the results showed that the predicted target genes were mainly localized in cell membranes; and the MF part of the results showed that the predicted target genes were mainly involved in the regulation of ion channel activity and other related biological processes. Accordingly, we hypothesized that serum miR-636 may transmit information through the vesicles formed by the cell membrane by regulating related genes located in the cell membrane.

Endothelial Microparticles (EMPs), small extracellular vesicles with a diameter of 0.1–1.0 μm shed by endothelial cells [25], are involved as important mediators in the regulation of endothelial cell function, inflammatory response, oxidative stress, angiogenesis, and vascular permeability [26]. Shah et al. showed that EMPs are important carriers of intercellular substance exchange and signaling, carrying surface antigens, proteins, and various bioactive molecules (cytokines, signaling proteins, miRNAs, etc.), which play a key role in cellular communication [27]. Studies have shown that EMPs have an important role in the development of AMI [28]. MiRNAs are considered to be the main signals that enable EMPs to produce the above effects, and EMPs influence the biological functions of recipient cells by delivering miRNAs. EMPs are secreted less under physiological conditions, and an increase in circulating EMPs may be induced by ischemia and hypoxia, tobacco exposure, chronic inflammation and endothelial cell activation or apoptosis [29]. Currently, EMPs miRNAs are thought to play important roles in cardiovascular and lung diseases [30, 31]. Based on the results of raw letter analysis in this study, we hypothesized that EMPs may act as target genes of miR-636 and be regulated by miR-636 thereby delivering substances and signaling molecules to affect the biological functions of recipient cells.

It is worth to that the *P*-value of the Gensini score in the COX regression analysis was exactly equal to 0.050, which may be due to the insufficient sample size studied. Meanwhile, because of the insufficient sample size, the characteristic electrocardiograms of AMI patients were not classified into ST-segment elevation myocardial infarction and non-ST-segment elevation myocardial infarction in this study, and we will subsequently expand the sample source and sample size, dig into the research arguments as deeply as possible, and further analyze the molecular mechanism of miR-636 in AMI.

## Conclusions

In conclusion, the results of this study showed that miR-636 can be used as an independent predictor for the occurrence of MACE after PCI in AMI patients, and it was concluded that miR-636 may act on the membrane of vascular endothelial cells and play a role as a signaling molecule through the formation of EMPs by endothelial cells to transmit signals and material exchange through the endothelial cells through the biosignaling analysis. This study provides a referable theoretical basis for clinical judgment of the prognosis of AMI patients and for the prevention of AMI after PCI.

#### Acknowledgements

Not applicable.

#### Author contributions

Q. Wang and B. Tang participated in the design of this study, and they both performed the statistical analysis. Q. Tang carried out the study and collected important background information. Z. Jiang contributed to data presentation. Q. Wang drafted the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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#### Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

#### Ethics approval and consent to participate

All subjects signed an informed consent form before enrollment. The study strictly adhered to the principles of the Declaration of Helsinki. Approval was obtained from the ethics committee of Affiliated Jinhua Hospital, Zhejiang University School of Medicine.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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