

Original Paper

Predictive Effects of Circulating miR-221, miR-130a and miR-155 for Coronary Heart Disease: A Multi-Ethnic Study in China

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Key Words

MicroRNA • Coronary heart disease • Multi-ethnicity

Abstract

Background: Differences in microRNA (miRNA) profiles between patients with and without coronary heart disease (CHD) have not been fully determined. The purpose of the study was to evaluate in a multi-ethnic population in China the predictive value of miRNAs previously suggested to have a role in CHD. **Subject and method:** 932 participants were included, and plasma samples obtained. A quantitative reverse-transcription PCR (RT-qPCR) assay was conducted to confirm the concentration of plasma miRNAs. Circulating levels of miRNAs were quantified using the $2^{-\Delta ct}$ method. The severity of coronary atherosclerosis was evaluated via Gensini Scores. **Result:** The circulating levels of the nine proposed miRNAs were not different among the five main ethnicities examined (all $p > 0.05$). The Spearman correlation analyses indicated that miR-221 and miR-130a were negatively associated with the severity of CHD as indicated by Gensini Scores ($r = -0.106$, $p = 0.001$; $r = -0.073$, $p = 0.026$). Results of the univariate analysis showed that lower circulating miR-221 (OR, 1.663; 95 % CI, 1.255-2.202, $p = <0.001$), miR-155 (OR, 1.520; 95 % CI, 1.132-2.042, $p = 0.005$), and miR-130a (OR, 1.943; 95 % CI, 1.410-2.678, $p = <0.001$) were potential risk factors for CHD. Moreover, miR-130a (OR, 2.405; 95 % CI, 1.691-3.421, $p = <0.001$) remained independently associated with the risk of CHD after adjusting for potential confounding factors. The analysis of the possible positive/negative associations between miR-221, miR-155 and miR-130a were conducted. A positive association between miR-130a and miR-155 was found ($SI = 1.60$, $SIM = 1.21$ and $AP = 0.22$), and in these groups, the proportion of CHD attributable to the interaction between miR-130a and miR-155 was as high as 22 %. A negative interaction was found between miR-221 and miR-130a ($SI = 0.68$, $SIM = 0.60$ and $AP = 0.27$). **Conclusion:** Plasma levels of miR-221, miR-130a and miR-155 decreased in patients with CHD, and miR-130a may be an independent predictor for CHD.

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Introduction

Despite significant improvements in diagnosis and treatment in recent decades, cardiovascular diseases (CVD), particularly coronary heart disease (CHD), remain the leading cause of human morbidity and mortality in the world [1]. Data from the World Health Organization (WHO) shows that approximately 17.5 million people died from CVDs in 2012 (7.4 million caused by CHD), which accounts for 31 % of all global deaths [2]. In China, the age-standardized mortality of CVD in 2002 was 300 persons per 100,000 [3]. Early prevention of CHD is of clinical significance for the overall reduction of CVD mortality. Although significant advancement has been achieved for the early prediction of acute CVD, such as acute coronary syndrome, biomarkers for the detection chronic stable CHD remains a challenge in clinical practice.

CHD is caused by coronary atherosclerosis, an inflammatory disease due to the formation of plaque and subsequent obstruction of the coronary arteries. It has been suggested that during the process of plaque formation, the cellular components of the plaque may release miRNAs into the circulation. These have the potential to serve as new biomarkers for predicting CHD [4-5]. miRNAs are endogenous, small non-coding RNAs which are ~22 nucleotides in length, and are involved in the post-transcriptional regulation of genes. Functional changes, including up-regulation and down-regulation of miRNAs are known to be involved in the pathogenesis of many diseases such as myocardial infarction [6-9], coronary artery calcification (CAC) [10], arrhythmia [11], cardiomyocyte hypertrophy [12-13], heart failure [14], renal ischemia-reperfusion injury [15], non-alcoholic fatty liver disease [16], tuberculosis [17], pancreatic ductal adenocarcinoma [18], and diabetes mellitus [19]. miRNAs are reported to not only exist within the cell but also reside stably in the extracellular compartments such as the circulation, which enables relatively simple detection of miRNAs as disease biomarkers. Another interesting characteristic of disease-specific miRNAs is that they may be ethnicity specific [20]. Moreover, accumulating evidence indicates that some miRNAs may be specific for patients with CHD [21]. However, the potential role of these miRNAs in multi-ethnic CHD patients has rarely been reported. In this study, we aimed to evaluate the predictive value of previously suggested miRNAs for CHD in a multi-ethnic population in China.

Subject and Methods

Study subjects

From March 1, 2010 to April 31, 2015, 932 consecutive adult subjects (681 males and 251 females) aged 32-84 years, who underwent coronary angiography for suspected or known coronary atherosclerosis at the Friendship Hospital of Ili Kazakh Autonomous Prefecture in China, were selected in this study. Among the 932 subjects, 587 subjects were of Han ethnicity, 146 were of Uyghur ethnicity, 91 were of Kazakh ethnicity, 67 were of Hui ethnicity, and 41 were of other ethnicities (5 were of Mongol ethnicity, 26 were of Siwe ethnicity, 2 were of Kyrgyz ethnicity, 2 were of Uzbeks ethnicity, 2 were of Manju ethnicity, 2 were of Daur ethnicity, and 2 were of Russian ethnicity). Subjects with spastic angina pectoris, infectious processes within 2 weeks, heart failure, adrenal dysfunction, and thyroid dysfunction were excluded. The study was performed in accordance with approved guidelines, and all experimental protocols were approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University and the Friendship Hospital of Ili Kazakh Autonomous Prefecture in China. All subjects provided written informed consent.

Coronary Angiography

Coronary arteries were cannulated using either the Judkins technique [22] or through a radial artery approach with 6F catheters and recorded at a rate of 30 frames/s. Significant CHD was defined as at least one major epicardial vessel with > 50 % stenosis; control subjects were defined as all of the major epicardial vessels with < 50 % stenosis [23]. The severity of the coronary atherosclerosis was based on the Gensini scoring system [24-27].

Laboratory measurements

Four millilitres of venous blood was drawn after 12 hours of fasting to perform biochemical assays on the second day of hospitalization; total cholesterol (TCH, mmol/L), triglyceride (TG, mmol/L), fasting blood glucose (FBG, mmol/L), creatinephosphokinase-myoglobin isoenzyme(CK-MB, U/L), fasting high-density lipoprotein cholesterol (HDL-C, mmol/L), fasting low-density lipoprotein cholesterol (LDL-C, mmol/L), apolipoprotein A (Apo-A, g/L) and apolipoprotein B (Apo-B, g/L) were determined by enzymatic procedures on an automated autoanalyzer (AU 2700 Olympus, 1st Chemical Ltd, Japan). Excellent intra-assay and inter-assay CVs of < 5 % were obtained with our assay method.

Selection of miRNAs

Based on previous studies [28], nine CHD-related miRNAs were proposed as candidates in the study. This included miR-122, miR-133b [29], miR-214 [30], miR-21, miR-106a [31], miR-130a, miR-155, miR-221 [32], and miR-125b [33-36].

Plasma preparation and RNA isolation

All samples (5 ml) were collected in EDTA plasma tubes on the morning following arrival. Samples were processed within 4 hours and stored at 4 °C. Plasma was collected after centrifugation (15 min at 1000 ×g) and was stored at -80 °C until further analysis.

For the RT-qPCR assay of plasma, total RNA was extracted using the one-step phenol/chloroform purification protocol, as previously reported [37].

Quantification of miRNAs by RT-qPCR analysis

For miRNA profiling, the RT-qPCR assay was performed using a TaqMan PCR kit according to the manufacturer's instructions (Applied Biosystems, Foster City, USA); a minor modification was made according to the State Key Laboratory of Pharmaceutical Biotechnology (School of Life Sciences, Nanjing University), reported in 2010 [38]. Due to the superior performance of a combination of let-7d, let-7g and let-7i, this combination was chosen as a reference for the normalization of plasma miRNAs rather than the commonly used reference genes U6, RNU44, RNU48 and miR-16 [39]. The resulting threshold cycle (CT) values were determined according to the default threshold settings when the reactions were complete. The relative amount of each miRNA was calculated based on the internal control (i.e., the combination of let-7d, let-7g and let-7i, analysed using the $2^{-\Delta ct}$ method, which is a widely used method for presenting relative gene expression by comparative CT). The calculation formula was as following: $2^{\text{exp} - (\text{mean CT target miRNA} - \text{mean CT internal control})}$ [40-41] for CT < 40 and 2^{-40} in the case Ct ≥ 40 (considered as undetermined) [42]. The rate of undetermined miRNAs (CT > 40) was as following: 47.1 % for miR-125b, 26.7 % for miR-122, 49.1 % for miR-214, 38.5 % for miR-133b, 10.8 % for miR-221, 8.3 % for miR-21, 8.2 % for miR-155, 8.9 % for miR-106a, and 8.0 % for miR-130a.

Gene Ontology and Pathway analysis

Gene Ontology (GO) functional classification was performed by Blast2GO software (<http://www.blast2go.de>), and contigs were divided into biological processes, cellular components and molecular functions according to GO terms. GO analysis was used to analyze the main function of differently expressed genes according to GO, which is the key functional classification of NCBI. This classification system organizes genes into hierarchical categories and identifies the gene regulatory network based on biological process, cellular components and molecular functions. A two-tailed Fisher's exact test and X^2 test were used to classify the GO category, and the FDR was calculated to correct the *P* value. A smaller FDR indicated a smaller error in judging the *P* value. Pathway analysis was used to determine significant pathways based on differential gene expression according to the KEGG. Fisher's exact test was used to select the significant pathway, and the threshold of significance was defined by the *P* value and FDR.

Statistical analysis

Data were statistically analysed using Statistics Package for Social Sciences (ver. 16.0; SPSS Incorporated, Chicago, IL, USA). Subjects were classified into five groups according to ethnicity, two groups according to CHD status and four groups according to the quartile of the Gensini score. Data for age, TG, TCH, HDL-C, LDL-C, FBG, CK-MB, Apo-A, Apo-B, Gensini scores, miR-122, miR-133b, miR-214, miR-21,

miR-106a, miR-130a, miR-155, miR-221, and miR-125b were skewed distributed parameters and were expressed as median and quartile ranges, with comparisons performed using the Mann-Whitney U test and Kruskal-Wallis H test. The categorical variable of gender was compared between or among the groups of patients using a chi-squared analysis. The Spearman two-way test was used to assess the relationship between Gensini scores with miRNAs and classical risk factors. The receiver operating characteristic (ROC) curve was performed, and the area under the ROC curve (AUC) was calculated to evaluate the specificity and sensitivity of CHD prediction for each miRNA [43]. ORs regarding the presence of obstructive CHD were determined via either a univariate or a multivariate logistic regression analysis and are presented with 95 % CIs. Differences were considered to be significant if the null hypothesis could be rejected with > 95 % confidence. All P-values were two-tailed. To further explore the synergistic effect of miRNA on the risk of CHD, a 4×2 table approach was conducted to calculate odds ratios (ORs), 95 % confidence intervals (CIs) and two-tailed P-values, and multiple indexes (SI, SIM, RERI, and AP) were used to evaluate the synergistic effect between miRNAs [44, 45].

Results

Baseline characteristics of subjects grouped according to ethnicity

A total of 932 subjects (681 males and 251 females) with a median age of 60- years were selected in the study. The baseline characteristics of the subjects grouped according to ethnicity are presented in Table 1. Significant differences in age ($p = 0.020$), CK-MB ($p < 0.001$), LDL-C ($p = 0.003$), apolipoprotein A ($p = 0.007$), apolipoprotein B ($p = 0.018$), and FBG ($p = 0.028$) were observed among the various ethnicities, with the frequency distribution in CHD status ($p = 0.012$) significantly differing among the various ethnicities. However, there was no statistical difference in gender ($p = 0.063$), TCH ($p = 0.093$), triglyceride ($p = 0.067$), HDL-c ($p = 0.337$), miR-125b ($p = 0.561$), miR-122 ($p = 0.589$), miR-214 ($p = 0.197$), miR-133b ($p = 0.486$), miR-221 ($p = 0.737$), miR-21 ($p = 0.410$), miR-155 ($p = 0.783$), miR-106a ($p = 0.112$), and miR-130a ($p = 0.714$) among the various ethnicities.

Demographic, clinical and biochemical characteristics of subjects according to Gensini Score

Gensini Score of the subjects in the present study ranged from 0 to 240.00, with a median of 16.00 (quartile range, 3.00–45.00). Table 2 shows the demographic, clinical and

Table 1. Baseline characteristics of the subjects grouped according to ethnicity. Data are summarized by 50th (25th/75th) percentiles for continuous variables and N_1/N_2 for binary variables. CK-MB, MB isoenzyme of creatine kinase; TCH, total cholesterol; TG, triglyceride; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; Apo-A, apolipoprotein A; Apo-B, apolipoprotein B; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by $2^{-\Delta ct}$ method

Variable	Ethnicity (n=932)					F or chi-square test	P
	Han (n = 587)	Hui (n = 67)	Uygur (n = 146)	Kazakh (n = 91)	Others (n = 41)		
Age (years)	62.00(52.00-70.00)	58.00(54.00-64.00)	60(53.75-66.25)	57.00(50.00-64.00)	64.00(54.50-68.50)	11.727	0.020
Gender (M/F)	414/173	55/12	108/38	75/16	29/12	89.12	0.063
CK-MB (U/L)	16.00(12.50-21.00)	16.00(13.40-22.00)	18.00(14.00-24.25)	19.00(15.00-24.00)	16.00(12.50-21.50)	25.227	<0.001
TCH (mmol/L)	4.61(3.90-5.45)	4.35(3.58-5.33)	4.88(4.09-5.69)	4.61(3.80-5.28)	4.72(3.91-5.48)	7.957	0.093
TG (mmol/L)	1.82(1.25-2.48)	1.72(1.08-2.53)	1.71(1.24-2.43)	1.52(1.07-2.05)	2.03(1.26-2.77)	8.775	0.067
HDL-C (mmol/L)	1.36(1.14-1.62)	1.32(1.14-1.61)	1.46(1.16-1.70)	1.29(1.06-1.62)	1.39(1.17-1.60)	4.550	0.337
LDL-C (mmol/L)	2.68(2.14-3.31)	2.68(1.97-3.54)	3.05(2.45-3.86)	2.85(2.22-3.53)	2.96(2.07-3.42)	16.331	0.003
Apo-A (g/L)	1.29(1.17-1.46)	1.33(1.19-1.51)	1.25(1.12-1.42)	1.25(1.10-1.38)	1.20(1.10-1.48)	14.240	0.007
Apo-B (g/L)	0.89(0.76-1.04)	0.87(0.71-1.06)	0.97(0.80-1.12)	0.89(0.70-1.05)	0.87(0.74-1.00)	11.870	0.018
FBG (mmol/L)	5.07(4.62-6.03)	5.21(4.75-7.07)	5.08(4.56-5.94)	4.95(4.49-5.55)	4.97(4.76-5.55)	10.891	0.028
miR-125b	0.04(0.00-0.24)	0.00(0.00-0.18)	0.04(0.00-0.21)	0.02(0.00-0.28)	0.15(0.00-0.27)	2.982	0.561
miR-122	0.15(0.00-1.77)	0.27(0.01-2.17)	0.29(0.00-1.85)	0.31(0.00-1.54)	1.00(0.02-1.88)	2.815	0.589
miR-214	0.00(0.00-0.23)	0.01(0.00-0.28)	0.00(0.00-0.22)	0.00(0.00-0.26)	0.13(0.00-0.36)	6.023	0.197
miR-133b	0.17(0.00-0.44)	0.16(0.00-0.49)	0.20(0.00-0.53)	0.14(0.00-0.41)	0.15(0.00-0.42)	3.444	0.486
miR-221	0.10(0.03-0.18)	0.09(0.05-0.24)	0.09(0.03-0.17)	0.11(0.02-0.22)	0.10(0.04-0.30)	0.992	0.737
miR-21	1.82(0.62-4.60)	1.84(0.75-6.23)	2.06(0.64-4.51)	2.13(0.44-5.94)	0.93(0.46-3.65)	3.973	0.410
miR-155	200.25(50.21-335.013)	188.71(74.03-236.890)	150.18(42.44-213.868)	121.94(45.89-168.671)	158.68(40.79-297.156)	1.740	0.783
miR-106a	1.61(0.83-3.16)	1.65(0.95-3.36)	1.48(0.68-2.47)	1.28(0.62-2.77)	1.22(0.78-1.82)	7.492	0.112
miR-130a	3.18(1.56-5.70)	3.43(1.52-13.93)	2.93(1.41-5.26)	2.99(1.06-5.94)	3.43(1.62-12.96)	2.116	0.714

Table 2. Characteristics of the study population categorized by the quartile of Gensini Scores: results of K independent sample test. CK-MB, MB isoenzyme of creatine kinase; TCH, total cholesterol; TG, triglyceride; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; Apo-A, apolipoprotein A; Apo-B, apolipoprotein B; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by $2^{-\Delta ct}$ method

Characteristics	Gensini Scores				Statistical parameter	P value
	0 (n = 189)	0.1-3.0 (n = 63)	3.5-4.4 (n = 445)	4.5 (n = 235)		
Age (years)	57.00 (49.00-65.00)	58.00 (49.00-68.00)	62.00 (54.00-70.00)	62.00 (55.00-69.00)	30.634	<0.001
Gender (M/F)	118/71	48/15	330/115	185/50	15.261	0.002
CK-MB (U/L)	16.00 (13.00-20.00)	17.00 (14.00-20.00)	16.00 (13.00-21.00)	18.00 (13.00-26.00)	11.453	0.010
TCH (mmol/L)	4.58 (3.91-5.27)	4.47 (3.60-5.22)	4.66 (3.90-5.50)	4.69 (3.96-5.67)	5.124	0.163
TG (mmol/L)	1.71 (1.14-2.36)	1.59 (1.13-2.09)	1.80 (1.26-2.52)	1.83 (1.24-2.50)	5.727	0.126
HDL-C (mmol/L)	1.41 (1.20-1.64)	1.35 (1.17-1.64)	1.36 (1.12-1.62)	1.32 (1.08-1.66)	4.941	0.176
LDL-C (mmol/L)	2.71 (2.16-3.38)	2.62 (1.98-3.23)	2.74 (2.15-3.42)	2.90 (2.26-3.55)	8.548	0.036
Apo-A (g/L)	1.30 (1.18-1.48)	1.35 (1.18-1.47)	1.29 (1.15-1.46)	1.25 (1.11-1.41)	12.639	0.005
Apo-B (g/L)	0.89 (0.77-1.03)	0.87 (0.68-1.02)	0.90 (0.74-1.07)	0.91 (0.77-1.06)	2.505	0.474
FBG (mmol/L)	4.84 (4.50-5.23)	5.00 (4.72-5.50)	5.13 (4.63-6.04)	5.34 (4.62-6.37)	29.441	<0.001
miR-125b	0.01 (0.00-0.23)	0.15 (0.00-0.32)	0.04 (0.00-0.25)	0.02 (0.00-0.20)	5.220	0.156
miR-122	0.28 (0.01-1.35)	0.48 (0.01-2.83)	0.13 (0.00-1.69)	0.35 (0.00-1.95)	4.782	0.188
miR-214	0.01 (0.00-0.26)	0.05 (0.00-0.29)	0.00 (0.00-0.23)	0.00 (0.00-0.23)	3.809	0.283
miR-133b	0.16 (0.00-0.40)	0.28 (0.00-0.58)	0.17 (0.00-0.46)	0.14 (0.00-0.48)	4.742	0.192
miR-221	0.12 (0.04-0.26)	0.11 (0.05-0.30)	0.09 (0.03-0.17)	0.08 (0.03-0.16)	14.597	0.002
miR-21	1.72 (0.61-4.48)	2.00 (0.54-6.15)	1.93 (0.64-4.53)	1.78 (0.55-4.76)	0.283	0.963
miR-155	252.48 (64.22-3395.90)	205.07 (72.00-382.68)	149.09 (45.89-1944.54)	190.02 (39.95-4182.07)	4.490	0.213
miR-106a	1.61 (0.87-3.34)	1.62 (0.92-3.20)	1.44 (0.71-3.08)	1.52 (0.80-2.66)	3.637	0.303
miR-130a	3.61 (1.70-16.77)	2.95 (2.01-7.84)	3.10 (1.42-5.52)	2.95 (1.49-5.21)	8.194	0.042

Table 3. Spearman correlations between Gensini Scores and features of the study population. CK-MB, MB isoenzyme of creatine kinase; TCH, total cholesterol; TG, triglyceride; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; Apo-A, apolipoprotein A; Apo-B, apolipoprotein B; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by $2^{-\Delta ct}$ method

Variables	Relationship coefficient	P value
Age (years)	0.152	<0.001
CK-MB (U/L)	0.144	<0.001
TCH (mmol/L)	0.076	0.021
TG (mmol/L)	0.052	0.110
HDL-C (mmol/L)	-0.056	0.088
LDL-C (mmol/L)	0.086	0.009
Apo-A (g/L)	-0.114	0.001
Apo-B (g/L)	0.060	0.068
FBG (mmol/L)	0.187	<0.001
miR-125b	-0.032	0.334
miR-122	<0.001	0.985
miR-214	-0.056	0.089
miR-133b	0.002	0.940
miR-221	-0.106	0.001
miR-21	-0.011	0.746
miR-155	-0.032	0.325
miR-106a	-0.060	0.066
miR-130a	-0.073	0.026

biochemical characteristics of patients according to Gensini Score; quartile values were used as cut-off points. The results suggest that distributions of age ($p = <0.001$), CK-MB ($p = <0.001$), LDL-c ($p = 0.036$), apolipoprotein A ($p = 0.005$), fasting glucose ($p = <0.001$), miR-221 ($p = 0.002$), and miR-130a ($p = 0.042$) were significantly different among the various groups, and the gender distribution ($p = 0.002$) was significantly different among the groups. In addition, no difference was found regarding the distributions of TCH ($p = 0.163$), TG ($p = 0.126$), HDL-C ($p = 0.176$), apolipoprotein B ($p = 0.474$), miR-125b ($p = 0.156$), miR-122 ($p = 0.188$), miR-214 ($p = 0.283$), miR-133b ($p = 0.192$), miR-21 ($p = 0.963$), miR-155 ($p = 0.213$), and miR-106a among the various groups.

Correlations of Gensini Score with clinical characteristics and circulating miRNAs

As shown in Table 3, results of the Spearman correlation analyses indicated that Gensini Score was positively associated with age ($r = 0.152$, $p = <0.001$), CK-MB ($r = 0.144$, $p = 0.002$), TCH ($r = 0.076$, $p = 0.021$), LDL-C ($r = 0.086$, $p = 0.009$), and FBG ($r = 0.187$, $p = <0.001$). Gensini Score was negatively associated with apolipoprotein A ($r = -0.092$, $p = 0.005$), miR-221 ($r = -0.106$, $p = 0.001$), and miR-130a ($r = -0.073$, $p = 0.026$).

Table 4. Correlations between miRNAs and features of the study population: results of Spearman analyses. CK-MB, MB isoenzyme of creatine kinase; TCH, total cholesterol; TG, triglyceride; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by 2^{-Δct} method

miRNAs	Age (years)		CK-MB (U/L)		TCH (mmol/L)		TG (mmol/L)		HDL-C (mmol/L)		LDL-C (mmol/L)		Apo-A (g/L)		Apo-B (g/L)		FBG (mmol/L)	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
miR-125b	-0.033	0.314	0.062	0.060	-0.065	0.046	-0.031	0.341	-0.024	0.456	-0.057	0.084	0.001	0.968	0.018	0.575	0.009	0.791
miR-122	0.045	0.167	0.051	0.122	0.043	0.193	0.041	0.212	0.099	0.002	0.040	0.225	<0.001	0.987	0.074	0.024	0.024	0.457
miR-214	0.012	0.712	0.020	0.544	-0.047	0.149	-0.077	0.019	-0.017	0.607	-0.039	0.238	0.049	0.138	0.064	0.050	0.024	0.462
miR-133b	0.067	0.042	0.051	0.122	-0.026	0.422	<0.001	0.989	0.082	0.012	0.015	0.651	0.090	0.006	0.094	0.004	0.024	0.462
miR-221	-0.045	0.172	0.037	0.261	-0.052	0.110	-0.018	0.576	-0.063	0.055	-0.082	0.012	0.018	0.576	0.081	0.013	0.022	0.507
miR-21	-0.052	0.112	-0.058	0.078	-0.013	0.702	0.018	0.589	-0.007	0.835	-0.017	0.603	-0.023	0.492	0.117	<0.001	0.006	0.846
miR-155	-0.035	0.291	-0.006	0.044	-0.014	0.680	0.086	0.008	0.020	0.545	-0.034	0.304	-0.025	0.446	0.104	0.001	-0.004	0.896
miR-106a	-0.081	0.014	-0.060	0.065	-0.032	0.325	0.004	0.893	-0.058	0.078	-0.057	0.084	0.038	0.245	0.044	0.175	0.049	0.134
miR-130a	-0.059	0.073	-0.005	0.888	-0.018	0.575	-0.006	0.853	-0.034	0.293	-0.023	0.477	0.054	0.102	0.063	0.055	0.059	0.070

Table 5. Correlations between miRNAs and miRNAs: results of the Spearman analyses. The value of each miRNA means the relative amount calculated by 2^{-Δct} method

miRNAs	miR-125b		miR-122		miR-214		miR-133b		miR-221		miR-21		miR-155		miR-106a		miR-130a	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
miR-125b	1	-	0.281	<0.001	0.534	<0.001	0.440	<0.001	0.377	<0.001	0.036	0.279	-0.070	0.320	0.206	<0.001	0.281	<0.001
miR-122	0.281	<0.001	1	-	0.252	<0.001	0.256	<0.001	0.266	<0.001	0.058	0.078	0.069	0.036	0.018	0.589	0.059	0.073
miR-214	0.534	<0.001	0.252	<0.001	1	-	0.408	<0.001	0.416	<0.001	0.008	0.814	-0.131	<0.001	0.199	<0.001	0.322	<0.001
miR-133b	0.440	<0.001	0.256	<0.001	0.408	<0.001	1	-	0.338	<0.001	0.140	<0.001	-0.038	0.246	0.174	<0.001	0.247	<0.001
miR-221	0.377	<0.001	0.266	<0.001	0.416	<0.001	0.338	<0.001	1	-	0.390	<0.001	0.319	<0.001	0.551	<0.001	0.695	<0.001
miR-21	0.036	0.279	0.058	0.078	0.008	0.814	0.140	<0.001	0.390	<0.001	1	-	0.617	<0.001	0.618	<0.001	0.486	<0.001
miR-155	-0.070	0.032	0.069	0.036	-0.131	<0.001	-0.038	0.246	0.319	<0.001	0.617	<0.001	1	-	0.510	<0.001	0.430	<0.001
miR-106a	0.206	<0.001	0.018	0.589	0.199	<0.001	0.174	<0.001	0.551	<0.001	0.618	<0.001	0.510	<0.001	1	-	0.715	<0.001
miR-130a	0.281	<0.001	0.059	0.073	0.322	<0.001	0.247	<0.001	0.695	<0.001	0.486	<0.001	0.430	<0.001	0.715	<0.001	1	-

Table 6. Receiver operating characteristic curve analyses for the predicting of CHD prevalence. CI, confidence interval; AUC, area under the receiver operating characteristic curve; CHD, coronary heart disease; CK-MB, MB isoenzyme of creatine kinase; TCH, total cholesterol; TG, triglyceride; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; Apo-A, apolipoprotein A; Apo-B, apolipoprotein B; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by 2^{-Δct} method. AUC, the closer it is to 0.5, the less predictive it is

Variables	AUC (95 %CI)	P value
Age (years)	0.587 (0.547-0.626)	<0.001
CK-MB (U/L)	0.560 (0.522-0.599)	0.003
TCH (mmol/L)	0.523 (0.483-0.562)	0.268
TG (mmol/L)	0.530 (0.489-0.571)	0.145
HDL-C (mmol/L)	0.555 (0.515-0.594)	0.008
LDL-C (mmol/L)	0.534 (0.494-0.574)	0.095
Apo-A (g/L)	0.534 (0.503-0.583)	0.036
Apo-B (g/L)	0.525 (0.486-0.564)	0.224
FBG (mmol/L)	0.612 (0.574-0.649)	<0.001
miR-125b	0.510 (0.470-0.550)	0.633
miR-122	0.527 (0.488-0.566)	0.186
miR-214	0.519 (0.478-0.559)	0.363
miR-133b	0.513 (0.474-0.553)	0.517
miR-221	0.569 (0.529-0.609)	0.001
miR-21	0.502 (0.463-0.542)	0.909
miR-155	0.504 (0.501-0.579)	0.049
miR-106a	0.533 (0.494-0.573)	0.105
miR-130a	0.566 (0.525-0.606)	0.001

Spearman correlations of miRNAs with clinical characteristics and the other miRNAs

As shown in Table 4 and 5, using nonparametric Spearman correlation tests for all collected variables, we found that in all subjects, miR-221 was associated with LDL-C ($r = -0.082, p = 0.012$), apolipoprotein B ($r = 0.081, p = 0.013$), miR-125b ($r = 0.377, p = <0.001$), miR-122 ($r = 0.266, p = <0.001$), miR-214 ($r = 0.416, p = <0.001$), miR-133b ($r = 0.338, p = <0.001$), miR-21 ($r = 0.390, p = <0.001$), miR-155 ($r = 0.319, p = <0.001$), miR-106a ($r = 0.551, p = <0.001$), miR-130a ($r = 0.695, p = <0.001$). miR-155 was associated with CK-MB ($r = -0.006, p = 0.044$), TG ($r = 0.086, p = 0.008$), apolipoprotein B ($r = 0.104, p = 0.001$), miR-125b ($r = -0.070, p = 0.032$), miR-155 ($r = 0.069, p = 0.036$), miR-214 ($r = -0.131, p = <0.001$), miR-221 ($r = 0.319, p = <0.001$), miR-21 ($r = 0.617, p = <0.001$), miR-106a ($r = 0.510, p = <0.001$), miR-130a ($r = 0.430, p = <0.001$); miR-130a were associated with miR-125b ($r = 0.281, p = <0.001$), miR-214 ($r = 0.322, p = <0.001$), miR-133b ($r = 0.247, p = <0.001$), miR-221 ($r = 0.695, p = <0.001$), miR-21 ($r = 0.486, p = <0.001$), miR-155 ($r = 0.430, p = <0.001$), miR-106a ($r = 0.715, p = <0.001$).

Predictors of CHD prevalence: results of ROC analyses

To further explore the applicability of circulating miRNAs and classical risk factors as potential diagnostic biomarkers of CHD, subsequent ROC analyses were performed, and the

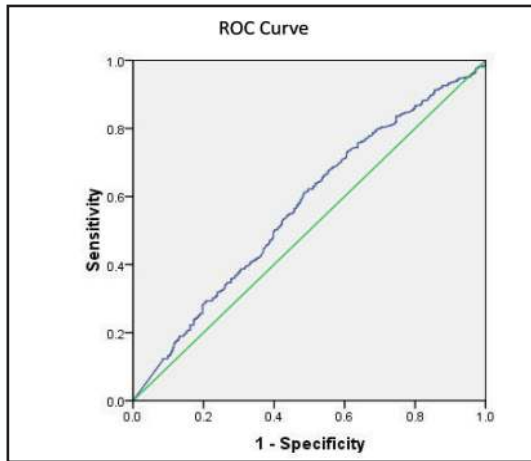


Fig. 1. The receiver operating characteristic curve for the predictive efficacy of miR-221 for CHD prevalence.

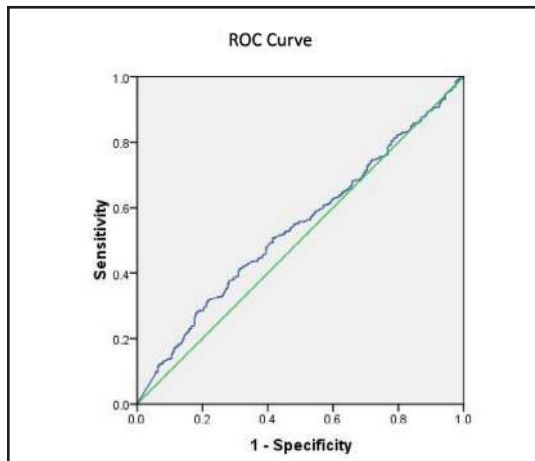


Fig. 2. The receiver operating characteristic curve for the predictive efficacy of miR-155 for CHD prevalence.

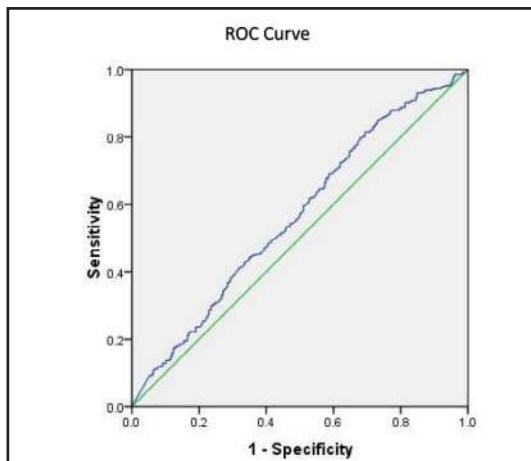


Fig. 3. The receiver operating characteristic curve for the predictive efficacy of miR-130a for CHD prevalence.

Table 7. The optimal cut-off and the Youden index. CK-MB, MB isoenzyme of creatine kinase; HDL-C, fasting high-density lipoprotein cholesterol; Apo-A, apolipoprotein A; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by $2^{-\Delta\text{ct}}$ method

Variables	Cutoff	Sensitivity	Specificity	Youden index
Age (years)	59.5	0.574	0.556	0.130
CK-MB (U/L)	25.9	0.202	0.940	0.142
HDL-C (mmol/L)	1.255	0.697	0.418	0.115
Apo-A (g/L)	1.155	0.778	0.287	0.065
FBG (mmol/L)	5.445	0.423	0.799	0.222
miR-155	100.427	0.687	0.414	0.101
miR-221	0.114	0.514	0.613	0.127
miR-130a	6.521	0.306	0.815	0.121

results are shown in Table 6. The *AUC* for the predicting of CHD prevalence was 0.587 for age (95 % *CI*: 0.547-0.626, $p < 0.001$); 0.560 for CK-MB (95 % *CI*: 0.522-0.599, $p = 0.003$); 0.555 for HDL-C (95 % *CI*: 0.515-0.594, $p = 0.008$); 0.534 for Apo-A (95 % *CI*: 0.503-0.583, $p = 0.036$); and 0.612 for FBG (95 % *CI*: 0.574-0.649, $p < 0.001$). Of all the miRNAs, miR-221 had an *AUC* of 0.569 (95 % *CI*: 0.529-0.609, $p = 0.001$) (Fig. 1), miR-155 had an *AUC* of 0.504 (95 % *CI*: 0.501-0.579, $p = 0.049$) (Fig. 2) and miR-130a had an *AUC* of 0.566 (95 % *CI*: 0.525-0.606, $p = 0.001$) (Fig. 3). The optimal cut-off value, the sensitivity, the specificity and Youden index of age, CK-MB, HDL-C, Apo-A, FBG, miR-221, miR-155 and miR-130a are shown in Table 7.

Predictors of CHD prevalence: results of logistic regression analyses

We performed a miRNAs score analysis to evaluate the associations between the combination of the plasma miRNAs and CHD. Briefly, the miRNAs score of each miRNA (miR-221, miR-155 and miR-130a), was set to 1 if its concentration was lower than the optimal cut-off value for the corresponding miRNA. Otherwise the score was set to 0. Thus, the miRNAs score was the sum of the score of miR-221, miR-155 and miR-130a. The characteristics of the study population categorized by the miRNAs score were shown in Table 8.

Table 8. Characteristics of the study population categorized by the miRNAs score: results of K independent sample test. CK-MB, MB isoenzyme of creatine kinase; TCH, total cholesterol; TG, triglyceride; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; Apo-A, apolipoprotein A; Apo-B, apolipoprotein B; FBG, fasting blood glucose

Characteristics	miRNAs score				Statistical parameter	P value
	0 (n = 150)	1 (n = 194)	2 (n = 341)	3 (n = 247)		
Age (years)	59.50 (52.00-67.00)	60.00 (52.00-68.00)	61.00 (51.00-69.00)	61.00 (53.00-70.00)	4.045	0.257
Gender (M/F)	112/38	141/53	256/85	172/75	2.385	0.496
CK-MB (U/L)	16.50 (13.00-21.70)	17.00 (13.68-21.03)	16.00 (13.00-21.00)	17.00 (13.00-22.00)	3.254	0.354
TCH (mmol/L)	4.51 (3.68-5.37)	4.74 (4.07-5.45)	4.63 (3.84-5.43)	4.72 (3.94-5.67)	5.253	0.154
TG (mmol/L)	1.73 (1.24-2.61)	1.67 (1.16-2.47)	1.84 (1.28-2.48)	1.75 (1.17-2.42)	3.950	0.267
HDL-C (mmol/L)	1.29 (1.10-1.57)	1.36 (1.14-1.64)	1.33 (1.10-1.62)	1.45 (1.18-1.69)	9.549	0.023
LDL-C (mmol/L)	2.63 (2.07-3.39)	2.75 (2.27-3.34)	2.73 (2.15-3.32)	2.95 (2.15-3.69)	6.775	0.079
Apo-A (g/L)	1.29 (1.14-1.46)	1.29 (1.17-1.46)	1.28 (1.13-1.43)	1.28 (1.14-1.48)	2.023	0.568
Apo-B (g/L)	0.86 (0.74-1.01)	0.97 (0.83-1.09)	0.90 (0.75-1.06)	0.85 (0.73-1.03)	22.115	<0.001
FBG (mmol/L)	5.06 (4.71-5.93)	5.00 (4.58-5.84)	5.10 (4.62-5.99)	5.08 (4.50-5.94)	1.184	0.757
Gensini score	7.50 (0.00-36.00)	12.00 (1.50-36.25)	19.00 (4.00-50.50)	5.08 (4.50-5.94)	19.016	<0.001

Table 9. Univariate logistic regression analysis for the predictors of CHD prevalence. CHD, coronary heart disease; CK-MB, MB isoenzyme of creatine kinase; TCH, total cholesterol; TG, triglyceride; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; Apo-A, apolipoprotein A; Apo-B, apolipoprotein B; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by $2^{-\Delta\text{ct}}$ method

Characteristics	β -coefficient	OR	95 % CI	P value
Age (years)(< 59.5 v.s. \geq 59.5)	0.525	1.690	1.276, 2.239	<0.001
Gender(F/M)	0.606	1.832	1.352, 2.483	<0.001
CK-MB (U/L) (< 25.9 v.s. \geq 25.9)	1.381	3.980	2.351, 6.737	<0.001
TCH (mmol/L)(< 4.63 v.s. \geq 4.63)	0.120	1.127	0.853, 1.490	0.400
TG (mmol/L)(< 1.76 v.s. \geq 1.76)	0.203	1.225	0.926, 1.620	0.155
HDL-C (mmol/L)(> 1.255 v.s. \leq 1.255)	0.504	1.655	1.229, 2.228	0.001
LDL-C (mmol/L)(< 2.75 v.s. \geq 2.75)	0.168	1.183	0.895, 1.565	0.237
Apo-A (g/L)(> 1.155 v.s. \leq 1.155)	0.345	1.412	1.018, 1.960	0.039
Apo-B (g/L)(< 0.90 v.s. \geq 0.90)	0.084	1.087	0.823, 1.437	0.556
FBG (mmol/L)(< 5.445 v.s. \geq 5.445)	1.071	2.918	2.098, 4.057	<0.001
miR-125b (> 0.034 v.s. \leq 0.034)	0.026	1.027	0.777, 1.357	0.853
miR-122 (> 0.191 v.s. \leq 0.191)	0.162	1.176	0.889, 1.555	0.255
miR-214 (> 0.003 v.s. \leq 0.003)	0.130	1.138	0.861, 1.505	0.363
miR-133b (> 0.167 v.s. \leq 0.167)	0.217	1.242	0.939, 1.643	0.128
miR-221 (> 0.114 v.s. \leq 0.114)	0.508	1.663	1.255, 2.202	<0.001
miR-21 (> 1.840 v.s. \leq 1.840)	-0.087	0.916	0.693, 1.211	0.540
miR-155 (> 100.427 v.s. \leq 100.427)	0.419	1.520	1.132, 2.042	0.005
miR-106a (> 1.542 v.s. \leq 1.542)	0.162	1.176	0.889, 1.555	0.255
miR-130a (> 6.521 v.s. \leq 6.521)	0.664	1.943	1.410, 2.678	<0.001
miRNAs score	0.326	1.386	1.208, 1.589	<0.001
0				
1	0.752	1.566	1.010, 2.426	0.045
2	0.990	2.121	1.422, 3.164	<0.001
3	0.214	2.691	1.738, 4.167	<0.001

Table 10. Multivariate logistic regression analysis for the predictors of CHD prevalence. CHD, coronary heart disease; CK-MB, MB isoenzyme of creatine kinase; HDL-C, fasting high-density lipoprotein cholesterol; FBG, fasting blood glucose. The value of each miRNA means the relative amount calculated by $2^{-\Delta\text{ct}}$ method

Characteristics	β -coefficient	OR	95 % CI	P value
Age (years)(< 59.5 v.s. \geq 59.5)	0.650	1.916	1.411, 2.601	<0.001
Gender(F/M)	0.652	1.918	1.375, 2.676	<0.001
CK-MB (U/L) (< 25.9 v.s. \geq 25.9)	1.317	3.730	2.150, 6.472	<0.001
HDL-C (mmol/L)(> 1.255 v.s. \leq 1.255)	0.595	1.814	1.315, 2.502	<0.001
FBG (mmol/L)(< 5.445 v.s. \geq 5.445)	0.997	2.711	1.915, 3.837	<0.001
miR-130a (> 6.521 v.s. \leq 6.521)	0.878	2.405	1.691, 3.421	<0.001

The results of the univariate logistic regression analysis for the predictors of CHD prevalence are shown in Table 9. Older age, male, higher CK-MB, lower HDL-C, and lower Apo-A level were potential predictors of CHD. Moreover, lower circulating miR-221 (OR, 1.663; 95 % CI, 1.255-2.202, $p = <0.001$), miR-155 (OR, 1.520; 95 % CI, 1.132-2.042, $p = 0.005$), and miR-130a (OR, 1.943; 95 % CI, 1.410-2.678, $p = <0.001$) were significantly associated with CHD in the univariable analysis.

It was assumed that subjects with a miRNA score of 0 have a background risk of CHD (OR is assumed to be 1); the results of the univariate logistic regression analysis indicated that the relative risk of CHD among the subjects with miRNAs scores of 1 and 2 and were

Table 11. The relationship between miR-221, miR-155, and miR-130a. *SI*, synergy index for interaction in an additive model; *SI*, *SI*, synergy index for interaction in a multiplicative model; *REI*, relative excess risk due to interaction; *AP*, proportion of disease attributable to interaction

miR-221	miR-155	CHDs	Controls	OR (95 %CI)	P value
0	0	178	114	1	
0	1	74	32	1.481 (0.919-2.386)	0.106
1	0	202	80	1.617 (1.140-2.294)	0.007
1	1	194	58	2.142 (1.471-3.120)	<0.001
SI=1.04 SIM=0.89 REI=0.04 AP=0.02					
miR-221	miR-130a	CHDs	Controls	OR (95%CI)	P value
0	0	99	79	1	
0	1	153	67	1.822 (1.207-2.752)	0.004
1	0	21	8	2.095 (0.881-4.982)	0.094
1	1	375	130	2.302 (1.611-3.288)	<0.001
SI=0.68 SIM=0.60 REI=-0.62 AP=-0.27					
miR-130a	miR-155	CHDs	Controls	OR (95 %CI)	P value
0	0	100	74	1	
0	1	280	120	1.138 (0.532-2.435)	0.738
1	0	20	13	1.727 (1.194-2.497)	0.004
1	1	248	77	2.383 (1.606-3.536)	<0.001
SI=1.60 SIM=1.21 REI=0.52 AP=0.22					

1.566 (OR, 1.566; 95 % CI, 1.010-2.426, $p = 0.045$), 2.121 (OR, 2.121; 95 % CI, 1.422-3.164, $p = <0.001$) and 2.691 (OR, 2.691; 95 % CI, 1.738-4.167, $p = <0.001$).

A multivariate logistic regression analysis (Forward: Conditional method) was used to identify the risk factors for CHD among the entire population. In the multivariate model involving the entire population, we included the following variables: age, gender, TCH, TG, FBG, HDL-C, LDL-C, CK-MB, Apo-A, Apo-B, miR-122, miR-133b, miR-214, miR-21, miR-106a, miR-130a, miR-155, miR-221, and miR-125b. The results are reported as adjusted ORs and 95 % confidence intervals in Table 10. Older age, male, higher CK-MB, lower HDL-C, higher FBG, and lower miR-130a (OR, 2.405; 95 % CI, 1.691-3.421, $p = <0.001$) remained independently associated with the risk of CHD.

Interaction between miR-221, miR-155 and miR-130a

The analysis of the possible positive/negative associations between miR-221, miR-155 and miR-130a are expressed in Table 11. According to the results of the ROC analysis, subjects with a miR-221, miR-155 and miR-130a concentration below 0.114, 100.427, and 6.521 were considered to have an elevated risk of CHD, respectively. Regarding the baseline risk for subjects unexposed to risk expression of miR-221, miR-155 or miR-130a (reference category, 1.0), the OR estimating the joint effect of miR-221 with miR-155, miR-221 with miR-130a, and miR-155 with miR-130a was significantly higher than the ORs estimating the effects of each factor in the absence of the other respectively. A positive association between miR-130a and miR-155 was found ($SI = 1.60$, $SIM = 1.21$ and $AP = 0.22$), and in these groups, the proportion of CHD attributable to the interaction between miR-130a and miR-155 was as high as 22 %. A negative interaction was found between miR-221 and miR-130a ($SI = 0.68$, $SIM = 0.60$ and $AP = 0.27$).

Functional annotation of the target genes for miR-221

To further identify the putative function of the predicted target genes for miR-221, GO analysis was performed. Eighteen different biological processes, four cellular components and six different molecular functions were predicted (Fig. 4). The most significantly enriched GO terms were involved in three main categories: protein transport, protein localization, and establishment of protein localization. These results suggested that regulation of protein transport and localization played a vital role in the process of the CHD for miR-221. In addition, KEGG analysis was further conducted to elucidate the biological pathways of the miR-221 target genes (Fig. 5). The categories of neurotrophin signaling pathway, T cell receptor signaling pathway, and ErbB signaling pathway were the most enriched pathways, which were all in the group of "signaling pathways". This suggested that miR-221 regulation of signaling pathway genes played crucial roles in the development of CHD.

Fig. 4. GO analysis of the target genes for miR-221.

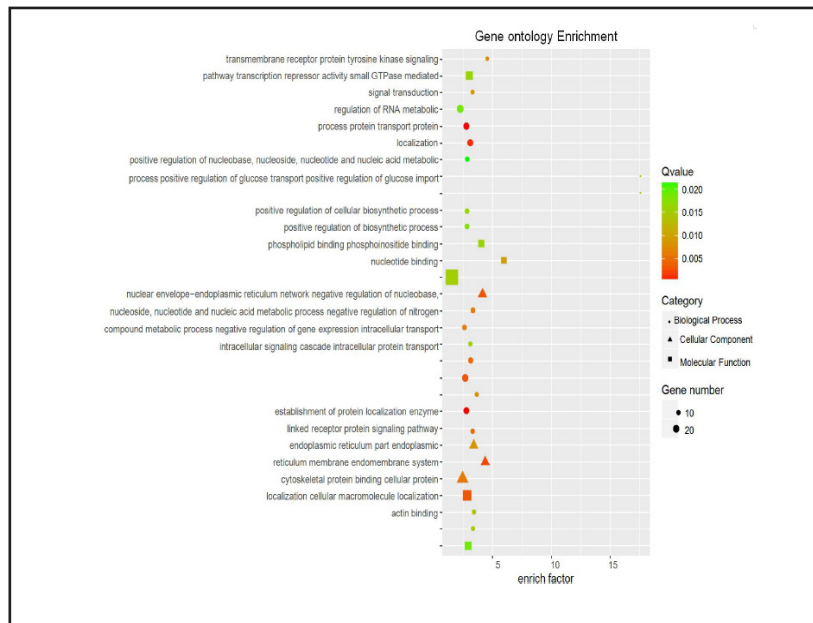
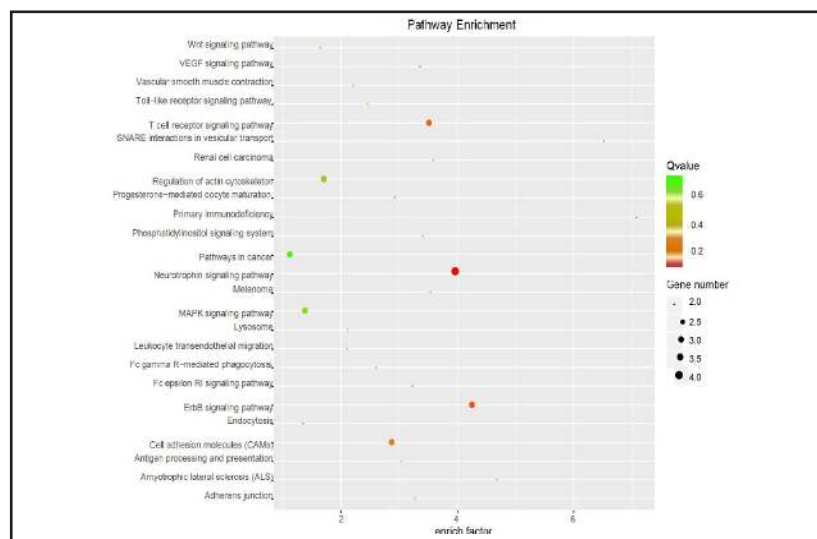


Fig. 5. KEGG pathways of the target genes for miR-221.



Functional annotation of the target genes for miR-155

The GO/pathway analysis results for miR-155 are presented in Fig. 6 and Fig. 7. The most significantly enriched GO terms for miR-155 involved three main categories, including enzyme binding, transmembrane receptor protein serine/threonine kinase activity, and transforming growth factor beta receptor activity. Moreover, KEGG analysis suggests that the categories of pathways in cancer, pancreatic cancer, and colorectal cancer were the most enriched pathways, which were all in the group of cancer pathways.

Functional annotation of the target genes for miR-130a

Fig. 8 indicates that the most significantly enriched GO terms for miR-130a were the three main categories of regulation of transcription, regulation of cell cycle, and regulation of protein catabolic process. These results suggest that regulation of transcription played a vital role in the process of the CHD for miR-221. In addition, KEGG analysis presented in Fig. 9 suggest that the categories of viral myocarditis, endocytosis, and neuroactive ligand-receptor interaction were the most enriched pathways.

Fig. 6. GO analysis of the target genes for miR-155.

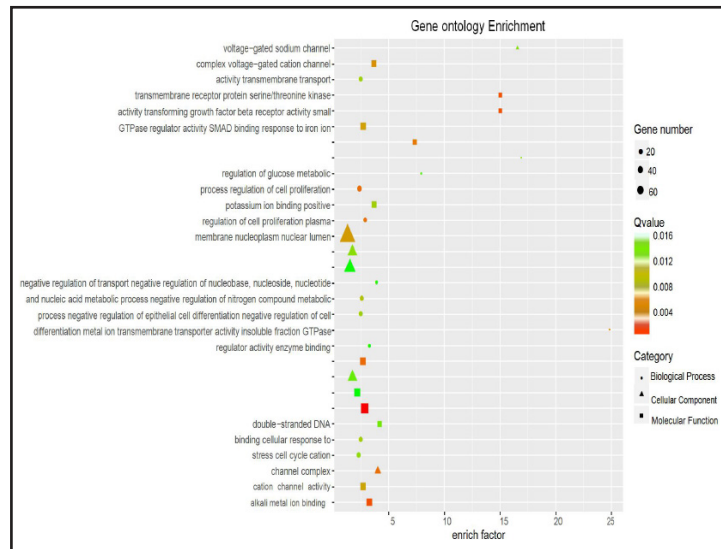
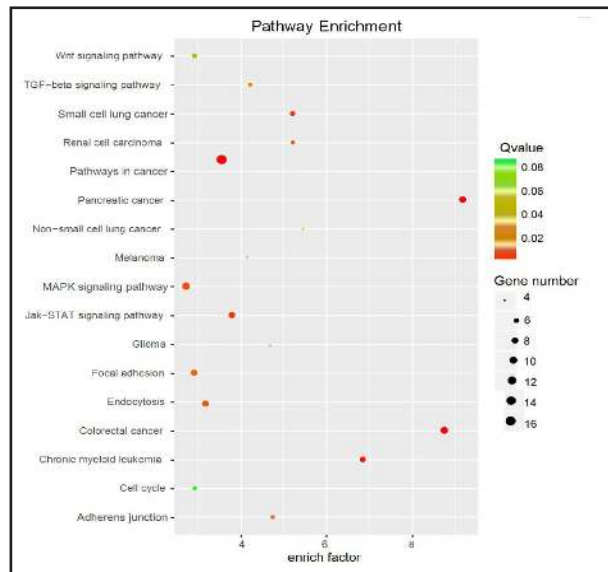


Fig. 7. KEGG pathways of the target genes for miR-155.



Discussion

In this study, we evaluated the predictive role of circulating miRNAs for CHD in a multi-ethnic population in China. We found that serum miR-155, miR-221 and miR-130a were significantly reduced in patients with CHD as compared with controls. More importantly, lower circulating miR-155, miR-221 and miR-130a were all significant predictors of CHD prevalence in this population. More importantly, miR-130a remained the independent predictor for CHD prevalence after adjustment for conventional confounding factors. To the best of our knowledge, ours is the first study that evaluates circulating miRNAs in a multi-ethnic population of documented CHD patients and control subjects. These results suggest that miRNAs, particularly miR-130a, may be novel biomarkers for CHD risk in this multi-ethnic population. Cohort studies are needed to confirm our results, and mechanism analyses are needed to determine the role of miRNAs in the pathogenesis of CHD.

miRNAs are endogenous, non-coding, and small (18–22 nucleotides) RNA molecules. miRNAs are recruited to the RNA-induced silencing complex (RISC) and regulate the output

Fig. 8. GO analysis of the target genes for miR-130a.

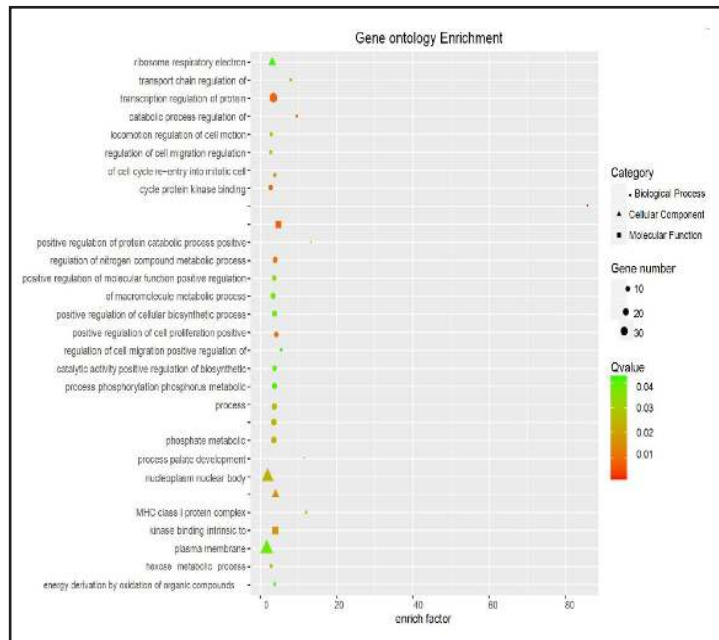
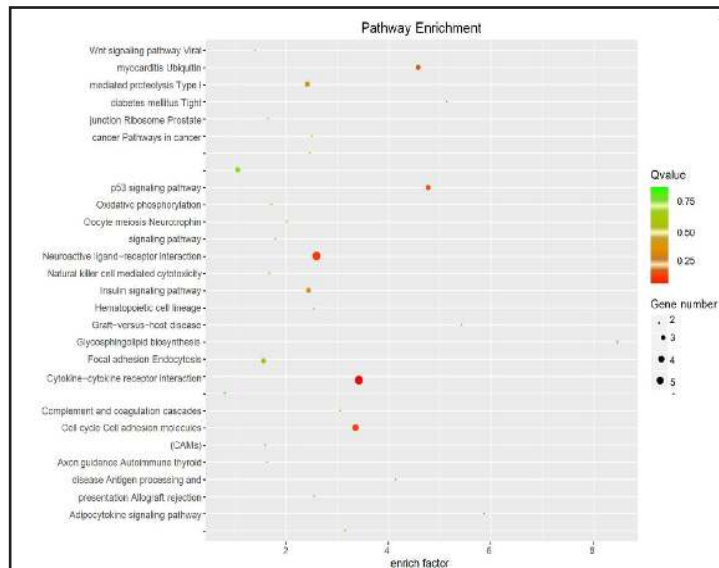


Fig. 9. KEGG pathways of the target genes for miR-130a.



of protein-coding genes through diverse mechanisms [46]. The interaction of miRNAs with the 3'untranslated region (3'UTR) of protein-coding genes is considered as the main mechanism of action, which usually leads to a decrease in protein output either by mRNA degradation or by translational repression [47]. Recent studies have shown that miRNAs are key regulators of gene expression. The expression levels of miRNAs have been shown to be associated with CVD [48], cancer [49], and other diseases [50]. In addition, miRNAs have been used as potential biomarkers for both non-communicable and communicable diseases [51, 52]. However, to date, miRNA expression differences have not been reported in a multi-ethnic population. In the present study, we systematically explored nine CHD-related miRNAs in plasma from 932 CHD patients and non-CHD controls, with the results suggesting that the expression level of the miRNAs was not different among the various ethnic groups.

Results from our study indicate that miRNAs, particularly miR-155, miR-221 and miR-130a may be novel markers of CHD. As a specific miRNA identified in human umbilical vein endothelial cells (HUVECs), miR-221 participates in the regulation of angiogenesis

[53]. Previous findings demonstrate that miR-221 inhibits the proliferation of endothelial progenitor cells (EPC) via interactions with the MEK/ERK pathway, thereby indicating a potential role of miR-221 for maintaining the integrity of the endothelium [54]. Interestingly, treatment with atorvastatin increased EPC numbers and decreased miR-221/222 levels in patients with CHD [55]. As for miR-155, it has been demonstrated that miR-155 exerts an antiangiogenic but proarteriogenic function in the regulation of neovascularization via the suppression of divergent cell-specific target genes in mice [56]. In fact, a previous study has concluded that circulating miR-155 is lower in CHD patients, and inversely associated with the extent of coronary stenosis as indicated by the Gensini Scores [57]. Also, miR-130a has also been implicated in the angiogenic process [58, 59], demonstrating that the overexpression of miR-130a may contribute importantly to gap junction remodeling and to the pathogenesis of atrial and ventricular arrhythmias [60]. Moreover, miR-130a has been implicated in the process of cardiac development, perhaps via regulation of FOG-2 (also known as zfp2)[61]. Results from our study further confirmed the potential association between the expression of the above three miRNAs and CHD. Moreover, expression of these miRNAs also correlated with the severity of coronary lesions as indicated by Gensini Scores. These results should be verified in future cohort studies, and the exact mechanisms underlying the role of the above miRNAs in the pathogenesis of CHD deserves further investigation.

To comprehensively investigate miR-221, miR-155, miR-130a and their targets and provide some information for further understanding the miRNA-mediated regulation network in the development of CHD, GO/pathway enrichment analysis was conducted in this study. Our analysis showed that miR-221, miR-155, and miR-130a contribute to various biological processes, including the regulation of protein transport and localization, cancer related pathways, endocytosis, and neuroactive ligand-receptor interactions. Taken together, these results represented partial information on the biological function of miRNA in the occurrence and development of CHD. However, the exact mechanisms underlying the association of miRNA and CHD require further study.

Our study has limitations which should be considered when interpreting the results. Firstly, our study is cross-sectional in design. Although we adjusted for conventional risk factors of CHD, the potential association between the aforementioned miRNAs and CHD prevalence may be confounded by residue factors. Moreover, our study only provides evidence for the potential association between the miRNAs and CHD; whether changes in the miRNAs identified are causative to the pathogenesis of CHD requires further evaluation. Finally, the results may be different according to the different populations included. Therefore, further studies are needed to confirm our results.

In summary, expression levels of miR-221, miR-155, and miR-130a were decreased in CHD patients compared with controls and may be predictive of CHD risk in this population. Our findings suggested that miR-221, miR-155, and miR-130a might play an important role in the development of CHD, serving as a potential biomarker for predicting CHD. Cohort studies are needed to confirm our results, and mechanism analyses are needed to determine the role of miRNAs in the pathogenesis of CHD.

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Disclosure Statement

There was not any conflict of interest existing in this manuscript.

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