Journal of the American Heart Association

ORIGINAL RESEARCH

# Prospective Study on Plasma MicroRNA-4286 and Incident Acute Coronary Syndrome

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**BACKGROUND:** Mounting evidence suggests that circulating microRNAs (miRNAs) are critical indicators of cardiovascular disease. However, prospective studies linking circulating miRNAs to incident acute coronary syndrome (ACS) are limited, and the underlying effect of associated miRNA on incident ACS remains unknown.

**METHODS AND RESULTS:** Based on a 2-stage prospective nested case–control design within the Dongfeng-Tongji cohort, we profiled plasma miRNAs from 23 pairs of incident ACS cases and controls by microarray and validated the candidate miRNAs in 572 incident ACS case–control pairs using quantitative real-time polymerase chain reaction. We observed that plasma miR-4286 was associated with higher risk of ACS (adjusted odds ratio according to an interquartile range increase, 1.26 [95% CI, 1.07–1.48]). Further association analysis revealed that triglyceride was positively associated with plasma miR-4286, and an interquartile range increase in triglyceride was associated with an 11.04% (95% CI, 3.77%–18.83%) increase in plasma miR-4286. In addition, the Mendelian randomization analysis suggested a potential causal effect of triglyceride on plasma miR-4286 (β coefficients: 0.27 [95% CI, 0.01–0.53] and 0.27 [95% CI, 0.07–0.47] separately by inverse variance-weighted and Mendelian randomization-pleiotropy residual sum and outlier tests). Moreover, the causal mediation analysis indicated that plasma miR-4286 explained 5.5% (95% CI, 0.7%–17.0%) of the association of triglyceride with incident ACS.

**CONCLUSIONS:** Higher level of plasma miR-4286 was associated with an increased risk of ACS. The upregulated miR-4286 in plasma can be attributed to higher triglyceride level and may mediate the effect of triglyceride on incident ACS.

**Key Words:** acute coronary syndrome ■ microRNA ■ miR-4286 ■ prospective study ■ triglyceride



Coronary heart disease (CHD) is a major disease burden worldwide.[1,2] Acute coronary syndrome (ACS), including acute myocardial infarction (AMI) and unstable angina (UA), is the main fatal subtype of CHD.[3] To date, the precise prevention and management of ACS remains a challenge because of its complicated pathogenic and physiological processes. Previous studies have demonstrated that the development of endothelial dysfunction and subsequent vulnerable plaque rupture or erosion is a major pathophysiological process of ACS.[4] However, the original regulator in ACS progression remains to be elucidated, especially from an epigenetic aspect.

MicroRNAs (miRNAs) are a class of small noncoding RNAs that are involved in posttranscriptional regulation by binding target mRNAs and play important roles in cardiac remodeling, angiogenesis, and vessel development.[5] Given the feature of high stability and sensitivity, circulating miRNAs have been widely characterized as diagnostic and prognostic biomarkers for

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.018999.

For Sources of Funding and Disclosures, see page 11.



*JAHA* is available at: www.ahajournals.org/journal/jaha

## CLINICAL PERSPECTIVE

### What Is New?

- In our study, we found that plasma miR-4286 was associated with higher risk of ACS based on a prospective nested case–control design.
- Further Mendelian randomization analysis revealed that triglyceride was causally associated with a higher level of plasma miR-4286.
- Plasma miR-4286 explained 5.5% of the association of triglyceride with incident ACS, suggesting the mediation effect of miR-4286 in ACS development.

### What Are the Clinical Implications?

- These findings identified a novel risk contributor to incident ACS at the circulating miRNA level and revealed its mediation potential in ACS development, suggesting a potential target for intervention or prevention of ACS.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **FRS** | Framingham Risk Score |
| **IR** | insulin resistant |
| **miRNA** | microRNA |
| **MR** | Mendelian randomization |
| **UA** | unstable angina |

cardiovascular disease (CVD).[6,7] However, prospective clues on circulating miRNAs and risk of ACS are limited and based on Europeans.[8,9] Over the past decades, with the change of lifestyles and westernization of the diet, CVD has been the leading cause of death in China, and among cardiovascular deaths, the majority are attributed to ACS.[10] Thereby, prospective associations of circulating miRNAs with incident ACS and its risk factors among the Chinese need to be investigated.

It is now well-established that hyperlipidemia is a major risk factor for fatal and nonfatal CHD.[11,12] With the development of the genome-wide association study, Mendelian randomization (MR) has become a useful approach to estimating the causal relationship between exposure and outcome.[13] Recent MR studies have demonstrated that abnormal plasma lipid levels were causally associated with CHD, indicating that the genetic variants of lipid traits played a key role in the development of atherosclerosis and vulnerable plaque.[14,15] Previous reviews have summarized that liver- or vessel-enriched miRNAs are involved in regulating cholesterol homeostasis.[16,17] Additionally, current studies have identified several circulating miRNAs that are related to lipids or are impacted by lipid-related dietary interventions.[18,19] However, there is still uncertainty regarding whether the relationships are causal between aberrant circulating miRNAs and lipid levels, as well as the mediation potential of associated miRNAs in ACS progression.

Herein, we conducted a 2-stage nested case–control study in the Dongfeng-Tongji cohort to investigate the associations of plasma miRNAs with incident ACS, and of the associated target miRNA with cardiovascular traits and risk factors. For lipid traits associated with target miRNA, we further evaluated their causal relationships and estimated the mediation effects of target miRNA on the associations between lipids and incident ACS. An overview of the study design is presented in Figure S1.

## METHODS

Details of methods are provided in Data S1. Data and materials supporting the conclusions of the article are available from the corresponding author on reasonable request.

### Study Design and Participants

This 2-stage prospective nested case–control study was performed in the Dongfeng-Tongji cohort,[20] which recruited 27 009 retirees of Dongfeng Motor Corporation during September 2008 and June 2010 in Shiyan, China. The first follow-up was completed in October 2013 and 14 120 individuals were newly recruited. All of the study participants were free of ACS at the baseline of the study (2013). During follow-up, 595 cases were newly diagnosed ACS during follow-up through December 31, 2016, including 147 AMI and 448 UA cases. We randomly selected a control who was free of CVD and cancer at the time of the case event to match each incident ACS case using the incidence density sampling method.[21] Each pair was matched on age (±3 years), sex, and blood drawing time (±1 month). All individuals had completed questionnaires and physical examinations, and provided blood samples in 2013. In the discovery stage, we randomly selected 23 pairs of plasma samples to conduct miRNA microarray detection. In the validation stage, the remaining 572 pairs of plasma samples were used for validation by quantitative real-time polymerase chain reaction. A detailed sampling flowchart is provided in Figure S2A.

The study was approved by the Ethics and Human Subject Committee of Tongji Medical College (2012-10) and Dongfeng General Hospital. Each participant provided written informed consent.

### Discovery and Validation of Candidate miRNAs in Plasma

We used 400 μL plasma to extract RNA by the miRNeasy Serum/Plasma kit (Qiagen, Germany)

according to the instruction handbook. Using the Agilent Human miRNA Microarray, Release 21.0, 8x60K (Agilent Technologies Inc, USA), we selected differentially expressed miRNAs by paired *t* tests in the discovery stage. Candidate miRNAs reaching the significant level ($P<0.05$ and absolute fold change >1.3, no multiple testing correction) were included for quantitative real-time polymerase chain reaction validation. Given that the present study was prospective and the cases were relatively healthy status at baseline, and the expression pattern of circulating miRNAs was complex because of its various sources, the miRNA profiles might not be dramatically altered in comparison with controls at baseline. Therefore, we used the uncorrected *P* value as screening criterion in line with previously published prospective or circulating miRNA-based studies.[9,22] We quantitated candidate miRNAs by TaqMan Advanced miRNA assay according to the protocols of the manufacturer (Applied Biosystems Inc, USA), and the reactions were carried out by QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems Inc, USA). The miRNA levels were measured by the $2^{-\Delta Ct}$ method.

## Bidirectional 2-Sample MR Analyses

We screened 39 and 58 independent loci separately associated with triglyceride and high-density lipoprotein cholesterol (HDL-C) with weak linkage disequilibrium ($r^2<0.2$) as genetic instrumental variables based on the previous reports on Asians ($P<5E-08$). For instrumental variables of miR-4286, we selected 24 single nucleotide polymorphisms of miR-4286 based on the genome-wide association study findings from 340 participants in the Dongfeng-Tongji study. A detailed sampling flowchart is provided in Figure S2B. The proportion of exposure explained by the genetic instruments (adjusted $R^2$) and *F* statistics were computed using linear models that fitted the weighted genetic risk score of exposure and adjusted for age and sex. To estimate causal relationships between lipids and miR-4286, we used the bidirectional 2-sample MR analyses by the "TwoSampleMR" and "MRPRESSO" packages, which provide inverse variance–weighted, MR-Egger, weighted median, and MR-PRESSO methods.[23,24]

## Statistical Analysis

The power estimation for the discovery stage was calculated by the PASS software (version 11.0.10). In the situation of setting 23 pairs of samples, 81% power was obtained when anticipating to achieve minimum significant condition (fold change=1.3, $P=0.05$) based on the test of eligible 408 miRNAs on the array after data filtering. Baseline characteristics of the case and control groups in 2 stages were compared by *t* tests or Mann–Whitney *U* tests for continuous variables, and $\chi^2$ tests or Fisher exact tests for categorical variables.

Data of plasma miRNAs were natural log-transformed to improve normality. To investigate the association of candidate miRNAs with incident ACS, we categorized the individual miRNAs into tertiles based on their distribution among the controls and fitted conditional logistic regressions to estimate the odds ratio (OR) and 95% CI for incident ACS. Similarly, we also calculated the OR (95% CI) for incident ACS per interquartile range (IQR) increase in the miRNA level. The multivariable model adjusted for established potential confounders including age, body mass index (BMI), smoking status, drinking status, education level, metabolic equivalent, family history of CHD, diabetes mellitus, hypertension, HDL-C, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication. Missing data of categorical covariates were replaced with an extra category to indicate missingness. Linear trend *P* value was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in the logistic regression model. We defined the statistical significance as a false discovery rate (FDR) <0.1 using the Benjamini-Hochberg method.[25] We further estimated the risk difference that represented the absolute risk change for ACS attributed to plasma miR-4286 using generalized linear regression models assuming an identity link function.[26] We also used elastic net regression models with 10-fold cross-validation to test the robustness between miRNAs and incident ACS, which combines the Lasso and Ridge penalties to reduce the occurrence of false-positives.[27]

Sensitivity analysis was conducted by excluding ACS events that had been diagnosed within 1 year after collection of blood samples in 2013. In addition, we conducted restricted cubic splines to estimate the associations between target miRNA and incident ACS. We also performed stratified analyses according to age, sex, BMI, current smokers, current drinkers, hypertension, diabetes mellitus, fasting glucose level, and hyperlipidemia. To evaluate the predictive value of ACS-associated miRNA, we compared the area under the receiver-operating characteristic curves between the Framingham Risk Score (FRS) for hard CHD and addition of selected miRNA to the FRS, and also calculated the net reclassification improvement and the integrated discrimination improvement to assess the improvement of predictive ability. The net reclassification improvement was calculated according to low (<10%), moderate (10%–30%), and high (>30%) levels of predicted risk of ACS. To estimate the heterogeneity of association between ACS subtypes, we fitted a multinomial logistic regression model with

the outcomes (AMI, UA, or non-ACS) as the dependent variable and miRNA level as independent variable, and adjusted for age, sex, BMI, smoking status, drinking status, education levels, metabolic equivalent, diabetes mellitus, hypertension, family history of CHD, HDL-C, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication. *P* value for heterogeneity was obtained from multinomial logistic regression model in the comparison between AMI and UA.

The relationships between lipid traits and target miRNA were estimated by linear regression. Plasma levels of HDL-C, total cholesterol, triglyceride, and triglyceride/HDL-C ratio were natural log-transformed and included in linear models and further analyses. Multivariable models adjusted for age, sex, BMI, smoking status, drinking status, education level, metabolic equivalent, and use of lipid-lowering drugs. Calculation of linear trend *P* value, and definition of statistical significance were the same as above. We presented the percent difference in miRNA level when categorizing the participants according to tertiles of each lipid level, which represented the percent change in miRNA in relation to the upper lipid group versus lower lipid group. The percent difference (95% CI) in miRNA level was also estimated in relation to per IQR increase in each lipid level.[28] Furthermore, we used the "mediation" R package,[29] which contained mediator models and outcome models, to estimate the mediation effects of target miRNA between lipids and incident ACS. The mediated proportion reflected the average mediation effect of target miRNA between lipids and incident ACS, estimated by the proportion of indirect effect in total effect.

Analyses were performed with R software (version 3.4.3, R Core Team) or SAS program (version 9.4, SAS Institute).

# RESULTS

## Characteristics of the Study Participants

The cases were newly diagnosed with ACS within 0.5 to 3.6 years since the 2013 baseline, and the median follow-up was 1.8 years. Among the participants, mean±SD age was 67.2±7.6 years, and 57.4% were men. In the validation stage, ACS cases were more likely to have a history of hypertension, with higher BMI, fasting glucose, low-density lipoprotein cholesterol, triglyceride, and lower HDL-C levels. By contrast, a consistent but weak group difference was detected between ACS cases and controls in the discovery stage (Table). In comparison with respective controls, both AMI and UA cases had higher blood pressure, fasting glucose, and triglyceride levels. In addition, AMI cases were more likely to be less educated, and physically inactive, while UA cases had higher BMI and lower HDL-C levels (Table S1).

## Selection of Candidate miRNAs in the Discovery Stage

We screened 33 miRNAs to be significantly associated with incident ACS by paired *t* tests ($P<0.05$, fold change>1.3). Via comparing the 33 candidate miRNAs to the miRmine database, we removed 11 extremely low abundance miRNAs in plasma. Finally, we selected 18 candidate miRNAs that could be detected in pooled plasma samples for quantitative real-time polymerase chain reaction validation (Table S2). Hierarchical clustering showed that the cases and controls were grouped distinctly across the heat map of 18 candidate miRNAs (Figure S3).

## Identification of Plasma miR-4286 Associated With Incident ACS in the Validation Stage

Among 14 miRNAs that were selected for further analyses after quality control, most of them were significantly correlated with each other (Spearman's rank correlation coefficients range from −0.44 to 0.83; Figure S4). After multivariate adjustment, we observed a strong positive association between miR-4286 and incident ACS; participants with plasma miR-4286 in the highest tertile of the distribution had a 1.80-fold increased risk of ACS compared with participants with plasma miR-4286 in the lowest tertile (OR, 1.80; 95% CI, 1.28–2.53; *P*-trend<0.001, FDR=0.01). Corresponding ORs for an IQR increase in plasma miR-4286 were 1.26 (95% CI, 1.07–1.48; Figure 1). In addition, compared with the lowest tertile, the greatest risk difference was 10.56% (95% CI, 3.46%–17.66%) for the highest tertile of plasma miR-4286. Meanwhile, an IQR increase in plasma miR-4286 was associated with an absolute risk increase in incident ACS of 4.43% (95% CI, 0.94%–7.91%; Figure S5). Elastic net model including all 14 candidate miRNAs showed that only miR-4286 had a nonzero coefficient ($\beta=0.02$), indicating a strong association between miR-4286 and incident ACS.

Of note, the significant result for miR-4286 was also confirmed by the global mean normalization strategy, and the association remained significant after excluding cases with ACS diagnosed within 1 year (Tables S3 and S4). Cubic spline regression analysis demonstrated a significant nonlinear association between plasma miR-4286 and incident ACS (nonlinear $P=0.002$; Figure S6). According to the stratified analyses, no significant interaction was observed between miR-4286 and ACS-related risk factors (Table S5). The

**Table 1. Baseline Characteristics of the 2-Stage Nested Case–Control Participants**

| Variables | Discovery Stage | | | Validation Stage | | |
|---|---|---|---|---|---|---|
| | ACS Cases (n=23) | Controls (n=23) | *P* Value | ACS Cases (n=572) | Controls (n=572) | *P* Value |
| Age, y | 68.1±6.7 | 68.1±6.7 | 0.96 | 67.2±7.7 | 67.2±7.6 | 0.91 |
| Male, n (%) | 14 (60.9) | 14 (60.9) | 1.00 | 327 (57.2) | 327 (57.2) | 1.00 |
| BMI, kg/m$^2$ | 24.60±3.45 | 23.90±3.58 | 0.50 | 24.71±3.39 | 24.21±3.04 | 0.009 |
| SBP, mm Hg | 146.83±26.00 | 140.22±22.18 | 0.36 | 148.21±24.44 | 142.78±22.37 | <0.001 |
| DBP, mm Hg | 79.04±11.33 | 79.65±12.54 | 0.86 | 83.28±13.26 | 80.67±12.58 | <0.001 |
| Smoking status, n (%) | | | 1.00 | | | 0.08 |
| Current smoker | 5 (21.7) | 5 (21.7) | | 136 (23.8) | 105 (18.4) | |
| Former smoker | 4 (17.4) | 4 (17.4) | | 119 (20.8) | 110 (19.2) | |
| Never smoker | 14 (60.9) | 14 (60.9) | | 315 (55.1) | 355 (62.1) | |
| Drinking status, n (%) | | | 1.00 | | | 0.11 |
| Current drinker | 9 (39.1) | 10 (43.5) | | 158 (27.6) | 185 (32.3) | |
| Former drinker | 2 (8.7) | 1 (4.3) | | 75 (13.1) | 56 (9.8) | |
| Never drinker | 12 (52.2) | 12 (52.2) | | 336 (58.7) | 330 (57.8) | |
| Education level, n (%) | | | 0.87 | | | 0.22 |
| Primary school or below | 7 (30.4) | 8 (34.8) | | 175 (30.6) | 154 (26.9) | |
| Middle school | 10 (43.5) | 8 (34.8) | | 218 (38.1) | 212 (37.1) | |
| High school or beyond | 6 (26.1) | 7 (30.4) | | 174 (30.4) | 196 (34.3) | |
| Physical activity, MET-h/wk | 21.00 (12.00, 44.00) | 21.00 (14.25, 42.00) | 0.61 | 21.00 (9.25, 42.00) | 23.31 (13.88, 42.00) | 0.05 |
| Family history of CHD, n (%) | 2 (8.7) | 1 (4.3) | 0.61 | 39 (6.8) | 41 (7.2) | 0.91 |
| Lipid-lowering medication, n (%) | 1 (4.3) | 1 (4.3) | 1.00 | 85 (14.9) | 82 (14.3) | 0.87 |
| Antihypertensive medication, n (%) | 12 (52.2) | 7 (30.4) | 0.23 | 231 (40.4) | 193 (33.7) | 0.02 |
| Antidiabetic medication, n (%) | 4 (17.4) | 3 (13.0) | 1.00 | 76 (13.3) | 57 (10.0) | 0.10 |
| Hyperlipidemia, n (%) | 11 (47.8) | 6 (26.1) | 0.22 | 380 (66.4) | 374 (65.4) | 0.76 |
| Hypertension, n (%) | 16 (69.6) | 15 (65.2) | 1.00 | 449 (78.5) | 409 (71.5) | 0.01 |
| Diabetes mellitus, n (%) | 9 (39.1) | 4 (17.4) | 0.19 | 160 (28.0) | 134 (23.4) | 0.09 |
| FG, mmol/L | 5.90 (5.11, 6.54) | 5.68 (5.50, 6.15) | 0.92 | 5.80 (5.30, 6.50) | 5.69 (5.20, 6.30) | 0.003 |
| HDL-C, mmol/L | 1.29 (1.17, 1.66) | 1.44 (1.08, 1.60) | 0.84 | 1.31 (1.16, 1.53) | 1.40 (1.19, 1.65) | <0.001 |
| LDL-C, mmol/L | 2.67±1.02 | 2.60±0.84 | 0.79 | 2.89±0.82 | 2.78±0.86 | 0.03 |
| TC, mmol/L | 4.75 (4.12, 5.39) | 4.34 (3.91, 5.29) | 0.46 | 4.89 (4.29, 5.46) | 4.84 (4.17, 5.45) | 0.15 |
| triglyceride, mmol/L | 1.37 (0.92, 2.19) | 1.36 (1.00, 1.70) | 0.79 | 1.40 (1.04, 2.02) | 1.23 (0.89, 1.68) | <0.001 |

ACS indicates acute coronary syndrome; BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low- density lipoprotein cholesterol; MET, metabolic equivalent; SBP, systolic blood pressure; and TC, total cholesterol.

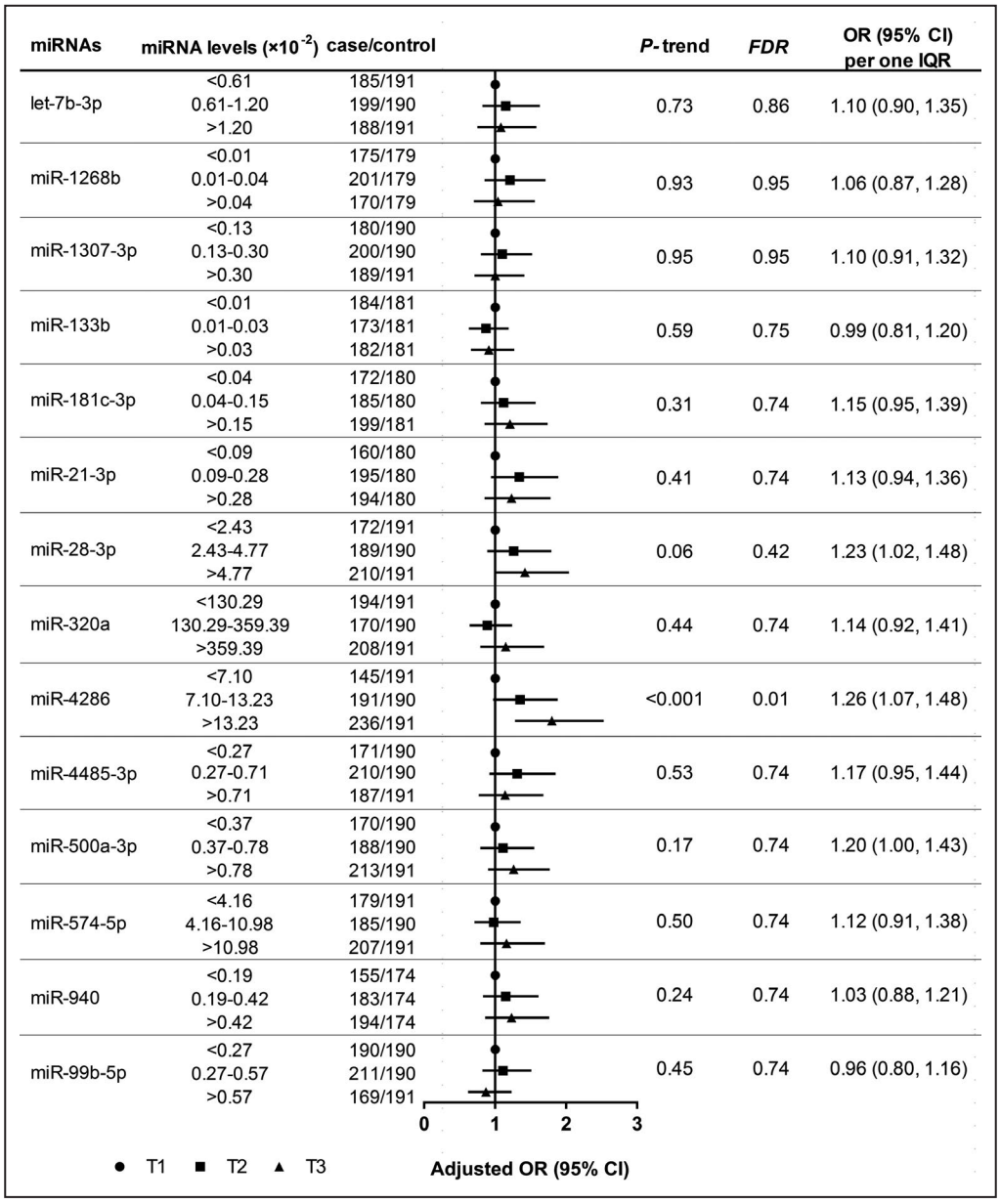



**Figure 1. Associations of candidate miRNAs with incident ACS in the validation stage.**
Plasma miRNA levels were normalized to miR-26b-5p and were expressed as $2^{-\Delta Ct}$. Adjusted OR (95% CI) for incident ACS was obtained using multivariable conditional logistic regression model with adjustment for age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes mellitus, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication. *P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in the logistic regression model. ACS indicates acute coronary syndrome; FDR, false discovery rate; IQR, interquartile range; miRNAs, microRNAs; and OR, odds ratio.

area under the receiver-operating characteristic curves for prediction of incident ACS was 0.55 (95% CI, 0.52–0.59, *P*=0.002), with a sensitivity and specificity being 65.0% and 43.9% at 0.09 of plasma miR-4286 as a cut-off value. In comparison with FRS alone, the addition of miR-4286 increased area under the receiver-operating characteristic curves from 0.64 (95% CI, 0.61–0.68) to 0.66 (95% CI, 0.63–0.69; *P* difference=0.07), the net reclassification improvement (95% CI) was 3.2% (0.9%–5.4%, *P*=0.006), and the integrated discrimination improvement (95% CI) was 1.1% (0.5%–1.8%, *P*<0.001; Figure S7).

In addition, we observed that miR-4286 was associated with higher risk of AMI and UA, with no significant heterogeneity observed for AMI and UA ($P_{heterogeneity}$=0.49; Table S6). It suggested that the

associations of plasma miR-4286 on ACS were consistent across different subtypes. Consequently, we selected miR-4286 as target miRNA associated with incident ACS to further explore its influencing factors and its role in ACS progression.

## Associations of Cardiovascular Traits and Risk Factors With Plasma miR-4286

We observed a higher miR-4286 level in females and the significant correlations between lipid traits and plasma miR-4286 (Tables S7 and S8). Decreased HDL-C level, increased triglyceride level, and elevated triglyceride/HDL-C ratio were significantly associated with higher levels of plasma miR-4286 after multivariable adjustment among all participants and incident ACS cases. According to the results among all participants, comparing the lowest tertiles, the highest tertiles of HDL-C, triglyceride, and triglyceride/HDL-C ratio were significantly associated with 18.08% decrease (95% CI, −28.21% to −6.51%; *P*-trend=0.003, FDR=0.005), 23.89% increase (95% CI, 8.67%–41.24%; *P*-trend=0.001, FDR=0.004), and 23.63% increase (95% CI, 8.37%–41.04%; *P*-trend=0.001, FDR=0.004) in plasma miR-4286, respectively. In addition, an IQR increase in HDL-C, triglyceride, and triglyceride/HDL-C ratio was significantly associated with an 11.05% decrease (95% CI, −16.65% to −5.08%), 11.04% increase (95% CI, 3.77%–18.83%), and 15.01% increase (95% CI, 7.16%–to 23.42%) in plasma miR-4286, respectively (Figure 2). Moreover, we also found the significant associations of triglyceride, HDL-C, and triglyceride/HDL-C ratio with incident ACS (Table S9).

## Causal Relationships Between Lipids and Plasma miR-4286

The genome-wide association study results of miR-4286 are presented in Table S10 and Figure S8, and details of triglyceride and HDL-C associated instrumental variables information are shown in Tables S11 and S12. The *F* statistics of triglyceride, HDL-C, and miR-4286 were 76.50, 83.65, and 105.41, respectively (Table S13). In the MR analyses, triglyceride was causally associated with an increased level of miR-4286, and the adjusted β (95% CI) according to per 1-unit increase was 0.27 (0.01–0.53; *P*=0.04) by conventional inverse variance–weighted test. Although the significance in MR-Egger and weighted median tests was marginal, the significant effect was validated by the MR-PRESSO test (0.27 [0.07–0.47]; *P*=0.01). The HDL-C was negatively associated with miR-4286 in MR-PRESSO test, the adjusted β (95% CI) according to per 1-unit increase was −0.20 (−0.39 to −0.01; *P*=0.04; Figure 3 and Figure S9). Notably, there was no suggestion of a reverse causal effect of miR-4286 on triglyceride or HDL-C (Figures S10 and S11).

## Causal Mediation Effects of Plasma miR-4286 on the Associations of Lipids and Incident ACS

The positive associations of triglyceride and triglyceride/HDL-C ratio with incident ACS were partly mediated through an increasing level of plasma miR-4286, and the mediation proportions (95% CI) were 5.5% (0.7%–17.0%; $P_{mediation}$=0.02) and 6.6% (0.9%–19.5%; $P_{mediation}$=0.02), respectively. Nevertheless, no significant mediation effect was detected for HDL-C as exposure, presumably because of the weak association between HDL-C and incident ACS (Figure 4).

# DISCUSSION

Based on a 2-stage nested case–control design, we found that plasma miR-4286 was positively associated with incident ACS and causally related to triglyceride and HDL-C. Importantly, an increase in plasma miR-4286 also partially mediated the positive association of triglyceride and triglyceride/HDL-C ratio with incident ACS. To summarize, our study identified a novel risk contributor of incident ACS and indicated its mediation potential in ACS development.

According to the prospective findings from the Bruneck cohort and HUNT (Nord-Trøndelag Health Study) cohort, circulating miRNAs associated with risk of AMI were nonoverlapping.[8,9] Interestingly, in the present study, we also found none of the predicted biomarkers as abovementioned to be associated with incident ACS. In comparison with the Bruneck cohort including 47 incident AMI cases and 773 controls, we performed the nested case–control design that used age- and sex-matched cases and controls, which enhanced statistical power and reduced heterogeneity.[8] Although the HUNT study also performed the nested case–control design, they have additionally matched on risk factors including BMI, total cholesterol, and HDL-C. These could be potential explanations for the discrepancy, given that the miRNA identified in the present study was correlated with lipid levels.[9] In addition, the different platform and normalized strategy for miRNA analysis might lead to technical differences. In line with the HUNT study, we also confirmed our result by global mean normalization strategy. Of note, the association between miR-4286 and incident ACS was moderate in this method. This weaker association could be explained by the fact that the closely associated miRNAs cluster might bias the average of cycle thresholds.[8]

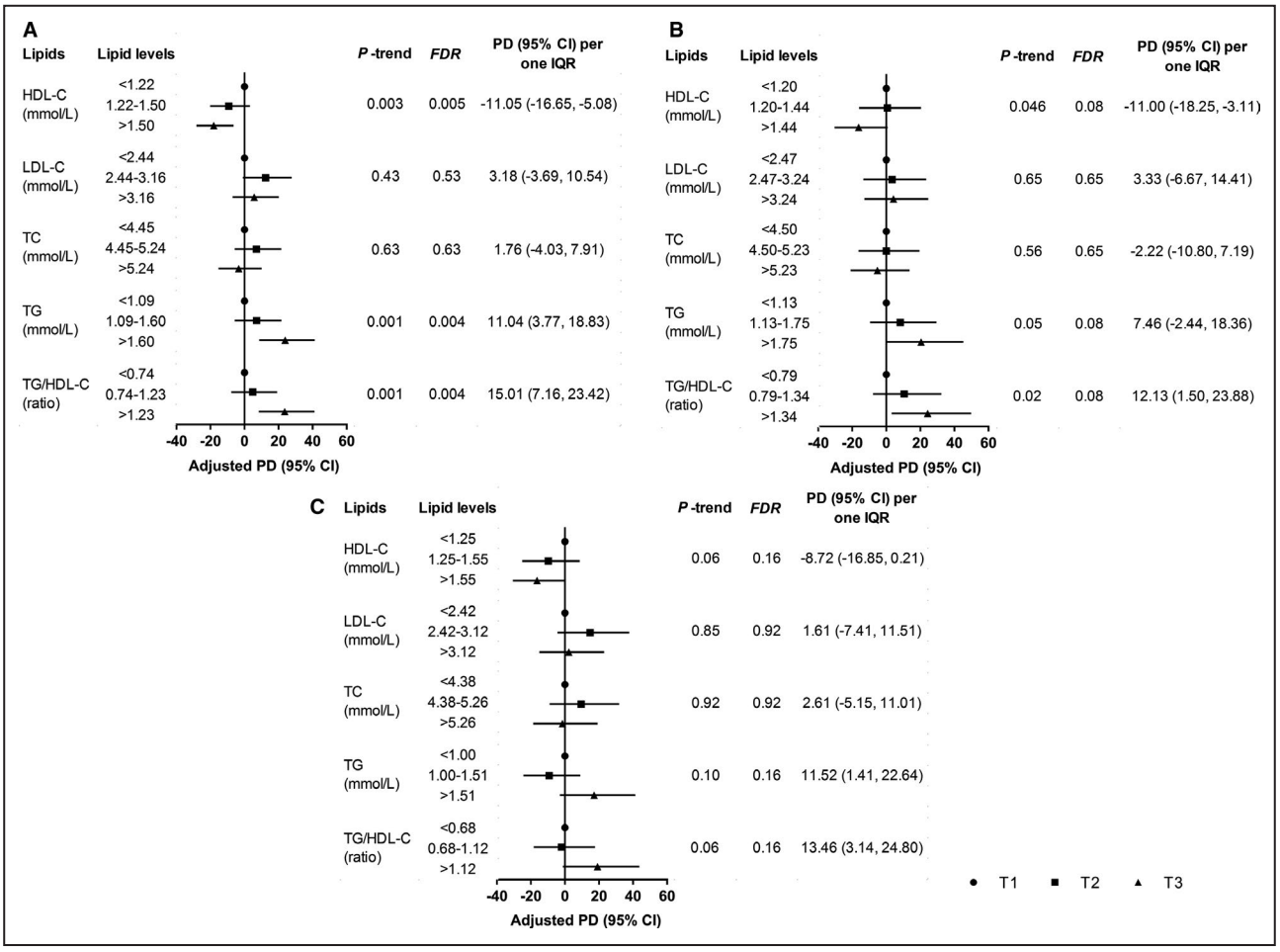



**Figure 2. Associations of lipid traits with plasma miR-4286 in the validation stage.**
Forest plots of **A, B,** and **C** separately show the associations of lipid traits with plasma miR-4286 among all participants, incident ACS cases, and controls. Adjusted PD (95% CI) for plasma miR-4286 was obtained using multivariable linear regression model with adjustment for age, sex, body mass index, smoking status, drinking status, education levels, metabolic equivalent, and use of lipid-lowering medication. *P*-trend was estimated by assigning the median value of lipid to each tertile and using this as a continuous variable in the linear regression model. ACS indicates acute coronary syndrome; FDR, false discovery rate; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; PD, percent difference; TC, total cholesterol; and TG, triglyceride.

As for the predictive potential of plasma miR-4286 for incident ACS, we observed a slight but significant improvement for net reclassification with the FRS plus miR-4286, suggesting that miR-4286 might be a novel ACS risk predictor independent of traditional risk factors. Nevertheless, given that adding plasma miR-4286 to the FRS model could not significantly increase the area under the receiver-operating characteristic curves for incident ACS prediction, the optimal combination of biomarkers including miRNAs or clinical traits in ACS risk prediction should be further explored.

Participants with abnormal levels of plasma triglyceride and HDL-C remain at a high risk of CVD even with normal low-density lipoprotein cholesterol level.[30] Higher triglyceride and lower HDL-C levels, as previously reported, were symbolic of dyslipidemia of insulin resistance (IR), which played an important role in the development of CVD.[31] Other studies have demonstrated that the triglyceride/HDL-C ratio was positively associated with insulin sensitivity, and thus was considered a useful surrogate index to estimate IR.[32] Herein, we identified potentially causal associations of triglyceride and HDL-C with plasma miR-4286, as well as a positive association between triglyceride/HDL-C ratio and miR-4286. Accordingly, we suggested that an increase in plasma miR-4286 might be caused by abnormal triglyceride and HDL-C levels accompanied by impaired insulin sensitivity in the development of ACS. The impact of systematic chronic inflammation caused by impaired insulin sensitivity and abnormal lipid levels might account for the observation, which might stimulate the expression and release of miRNAs involved in the metabolic disturbance and ACS progression.[33]

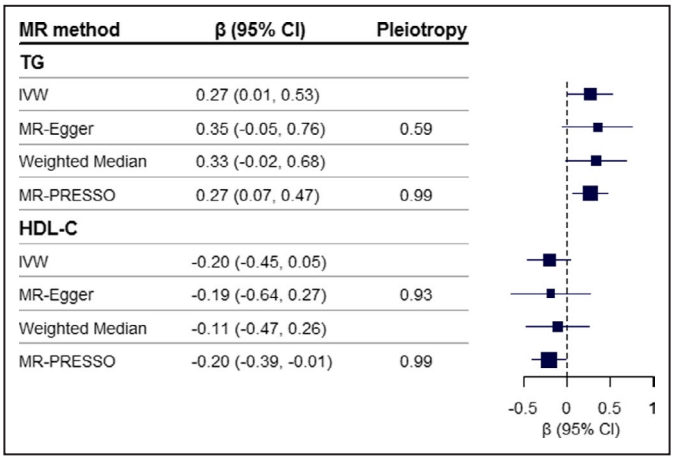



**Figure 3. Causal effects of TG and HDL-C on plasma miR-4286.** The causal effect (95% CI) of TG and HDL-C on miR-4286 was estimated by Mendelian randomization analysis, using 39 TG SNPs and 58 HDL-C SNPs derived from Asian reports, respectively. Pleiotropy *P* value derived from the intercept of MR-Egger test or MR-PRESSO Global test, a small *P* value indicates an unbalanced pleiotropy. HDL-C, high-density lipoprotein cholesterol; IVW, inverse variance-weighted; SNPs, single nucleotide polymorphisms; and TG, triglyceride.

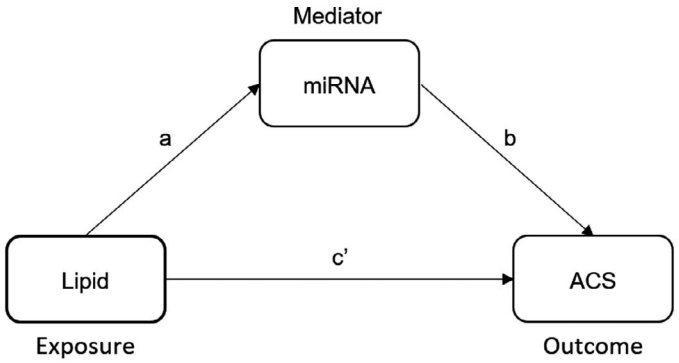
According to pathway enrichment analysis, our result suggested that the highly expressed target genes of miR-4286 in the cardiovascular system were most enriched in the IR pathway (Figure S12). Previous studies have demonstrated the crucial role of IR in endothelial dysfunction.[34] Indeed, proliferation, migration, and dysfunction of endothelial cells is a key point in the development of atherosclerosis and vulnerable plaque.[35] In this study, miR-4286 could partially mediate the impact of triglyceride and triglyceride/HDL-C on incident ACS. It seems possible that endothelium might be a potential target for the mediation effect of miR-4286 on triglyceride-associated ACS. Although the mechanism role of miR-4286 in the IR-related pathway in endothelial cells was lacking, functional study has found that miR-4286 played an important part in cell proliferation and migration via regulating the PI3K/AKT pathway in the development of lung cancer.[36] From a clinical perspective, our findings inferred a possible novel target for miRNA-based therapeutics.[37] Further experimental studies are required to investigate the functional role of miR-4286 in endothelial cells, especially in insulin-related pathways.

As mentioned in a review,[38] the pool of circulating miRNAs is the sum of secreted and leaked miRNAs from different cells. MiRNAs derived from active secretion via microvesicles or binding with HDL or Argonaute2 remain stable in the extracellular environment. On the contrary, passively leaked miRNAs from injured cells were susceptible to degradation. According to the EVmiRNA database[39]—an up-to-date extracellular vesicle miRNA database including 462 sequencing data sets from 17 tissues—we found that miR-4286 was undetectable in the secreted exosomes from endothelial cells or epithelial cells. However, it remains to be investigated whether miR-4286 was passively released from endothelial cells or derived from other cardiovascular cells. In addition, based on the abovementioned database, miR-4286 was also enriched in exosomes from B lymphoblastoid cell lines and lymph. It suggested that lymphocytes might be one of the sources of plasma miR-4286.

There are several strengths in the present study. First, our study was a prospective nested case–control design based on a well-established large prospective cohort, which could provide a relatively strong basis for the associations between plasma miRNAs and incident ACS. Second, we adjusted for traditional risk factors and lipid-lowering medication used in ACS in all association analyses to reduce potential confounding bias. Third, we further investigated the internal causal relationships of plasma miR-4286 and lipids through MR analyses and explored the causal mediation effect of miR-4286 on triglyceride-incident ACS association.

Several limitations in this study should also be acknowledged. First, compared with previous prospective studies, the follow-up period of 3 years was relatively short.[8,9] However, we have excluded incident ACS cases that were diagnosed within 6 months after blood collections, and our findings were largely unchanged after further excluding cases with a follow-up period <1 year. Second, the sample size of the microarray exploration data was limited, which

A




B

| Exposure | Mediator | Total effect (ab + c' path) | | Prop. Mediated | *P* Value |
|---|---|---|---|---|---|
| | | β (95% CI) | *P* Value | | |
| TG | miR-4286 | 0.11 (0.05, 0.16) | <0.001 | 5.5% (0.7%, 17.0%) | 0.02 |
| HDL-C | miR-4286 | -0.11 (-0.23, 0.00) | 0.05 | 12.3% (-10.0%, 84.7%) | 0.06 |
| TG/HDL-C | miR-4286 | 0.09 (0.04, 0.13) | <0.001 | 6.6% (0.9%, 19.5%) | 0.02 |

**Figure 4. Mediation effects of plasma miR-4286 on the associations of lipids with incident ACS.** **A**, Path diagram: Total effect (c)=direct effect (c')+indirect effect (ab). **B**, Total effects and the proportion of mediation effects were obtained by quasi-Bayesian Monte Carlo simulation for 10 000 times in the R package "mediation." Indirect effects were derived from multiple linear regression for the association of lipid with miRNA level adjusting for age, sex, body mass index, smoking status, drinking status, education levels, metabolic equivalent, and use of lipid-lowering medication. Direct effects were calculated by logistic regression for the association of lipid with ACS additional adjusting for miRNA level, diabetes mellitus, hypertension, family history of coronary heart disease, and low-density lipoprotein cholesterol. ACS indicates acute coronary syndrome; HDL-C, high-density lipoprotein cholesterol; miRNA, microRNA; and TG, triglyceride.



may contribute to a relatively high false-positive rate. Although we used a 1:1 matched strategy to improve the statistical power and verified the association by correcting multiple testing in the validation stage, further long-term prospective studies with a larger sample size are needed to replicate our results. Third, there is a lack of established endogenous control to normalize the circulating miRNA levels. In response, we chose 3 eligible miRNAs as candidate endogenous controls and used acknowledged NormFinder to estimate the optimal endogenous control and repeated a global mean normalized strategy to check the stability of our results. Fourth, expression patterns and function of miR-4286 in tissues remain largely unclear. Although there is a lack of evidence for the underlying mechanism, we inferred a potential role of miR-4286 in regulating insulin sensitivity via functional pathway analysis and provided a basis for further mechanism research. Finally, we could not determine the source of miR-4286, given that dysregulation of miRNAs in different tissues might be affected by different factors. Further systematic exploration of the source of circulating miR-4286, as well as its regulatory mechanism, needs to be conducted.

In conclusion, the present study identified a positive association of plasma miR-4286 with incident ACS in a Chinese population. We indicated the causal effect of triglyceride on plasma miR-4286, and highlighted a possible mediation role of plasma miR-4286 in triglyceride-incident ACS association. Further prospective and functional studies are warranted to systemically elucidate the biological mechanisms behind these observations and translate these results into clinical practice for early and precise prevention and treatment.

## ARTICLE INFORMATION

Received August 18, 2020; accepted December 21, 2020.

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## Acknowledgments

We thank all participants and staffs of Dongfeng-Tongji cohort for their cooperation.

## Sources of Funding

This work was supported by the National Key Research and Development Program of China (2016YFC0900800), the National Natural Science Foundation of China (81930092), the Fundamental Research Funds for the Central Universities (2019kfyXMBZ015), the 111 Project, and the Program for Changjiang Scholars and Innovative Research Team in University.

## Disclosures

None.

## Supplementary Material

**Supplemental Methods**
**Tables S1–S13**
**Figures S1–S12**
**References 40–58**

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# SUPPLEMENTAL MATERIAL

**Data S1. Supplementary Methods**

**Study samples of nested case-control study**

Overall, in 2013, a total of 38 295 participants had complete questionnaires and physical examinations information in the Dongfeng-Tongji (DFTJ) cohort. Each participant's medical insurance documents, hospital records, and death certificates could be tracked by Dongfeng Motor Corporation's health-care service system up to 31 December 2016. In further analyses, we excluded participants with cardiovascular disease (CVD), cancer, or severely abnormal electrocardiograms before the date of blood drawing in 2013 (n=10 254), participants without medical insurance record or blood samples (n=3626), as well as participants who were newly diagnosed with coronary heart disease (CHD) <6 months after collection of blood samples (n=490). After exclusion, a total of 23 925 participants were eligible for our analyses. Participants were diagnosed with acute coronary syndrome (ACS) by an expert panel of physicians based on symptoms, clinical examinations according to the guideline of ST-segment elevation or non-ST-segment elevation acute myocardial infarction (AMI) or unstable angina (UA).[40,41] Moreover, we tracked the International Classification of Diseases (ICD) codes to further distinguish AMI (ICD-10 codes I21) and UA (ICD-10 codes I20.0). Finally, we identified 595 incident ACS without stroke or cancer before ACS first-episode, and randomly selected matched controls from the 23 925 eligible participants who were free of ACS at the time of ACS diagnosis.

**Data and sample collection**

Participants were collected information used semi-structured questionnaires by trained

investigators, including sociodemographic factors (e.g., age, sex, and education), health status (e.g., personal and family history of CHD, stroke, and cancer), medical history, and lifestyle, such as smoking status (current, former, never), drinking status (current, former, never) and physical activity. Participants were taken anthropometric examinations (e.g., weight, height, and blood pressure) and clinical examinations (e.g., blood lipid and fasting glucose) after an overnight fast. Fasting blood was drawn with EDTA-anticoagulant tubes and centrifuged immediately, separated into plasma and whole blood, and stored at -80°C until analysis.

Definition of covariates was in line with previously study.[42-44] Current smokers were those who smoked more than one cigarette a day over six months, former smokers were those who quitted smoking for over six months, and never smokers were those who had never smoked in their lifetime. Current drinkers were those who drunk more than once a week over six months, former drinkers were those who quitted drinking for over six months, and never drinkers were those who had never drunk in their lifetime. Physical activity was represented as metabolic equivalent (MET) hours a week multiplied by the coefficient, duration (hours per time) and frequency (times per week) of physical activity. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared, individuals with BMI≥24 were defined as obesity. Hyperlipidemia was defined as individual having self-reported physician-diagnosed hyperlipidemia, or currently taking lipid-lowering medication, or total cholesterol (TC) ≥6.22 mmol/L, or triglyceride (TG) ≥2.26 mmol/L, or high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L, or low-density lipoprotein cholesterol (LDL-C) ≥4.14 mmol/L. Hypertension was defined as individual having self-reported physician-diagnosed hypertension, or currently taking antihypertensive medication, or blood pressure

≥140/90 mmHg. Diabetes was defined as individual having self-reported physician-diagnosed diabetes, or currently taking antidiabetic medication (oral hypoglycemic medication or insulin), or fasting glucose (FG) ≥7.0 mmol/L. Impaired fasting glucose (IFG) was defined as non-diabetic participant whose FG≥5.6 mmol/L and <7.0 mmol/L.

**RNA isolation**

In the discovery stage, 400 μl plasma was used to extract RNA using the miRNeasy Serum/Plasma kit (Qiagen, Germany) according to the instruction handbook. In the validation stage, the method and reagents of RNA isolation were similar to that in the discovery stage, except that we added 2 μl synthetic Caenorhabditis elegans miR-39-3p (cel-miR-39-3p, 50 pmol/L, RiboBio, China) as spiked-in control before chloroform extraction in each plasma sample.[45]

**MiRNA microarray and data preprocessing**

Total RNA from each plasma sample was labeled with the Cyanine 3-pCp and hybridized to the Agilent Human miRNA Microarray, Release 21.0, 8x60K (Agilent Technologies Inc, USA) with 2549 human miRNAs probes according to miRBase database (Release 21.0). The hybridized arrays were scanned on an Agilent Scanner G2565CA after washing. We extracted the array data from the scanned image using the Agilent Feature Extraction software v10.7. After adjusting for background noise by negative control probes, all raw signal values were normalized using the 90th percentile shift and log2-transformed in all statistical analyses. Only the probes with a present or marginal flag passing the 60% detection rate in either ACS or control group were kept for further analysis. Data summarization, quantile-normalization,

and quality control were performed using the Agilent GeneSpring GX software v11.5. Of 23 pairs of plasma samples analyzed by microarrays in the discovery stage, 408 out of 2549 miRNAs on the array were left after data filtering. Only the miRNAs at a significant level ($P$<0.05, absolute fold change [FC] >1.3) were included for further analyses by the paired *t*-tests. To reduce potential false positivity of microarray results, we further removed extremely low abundance miRNAs (an average expression value of reads per million mapped reads <1) in plasma with miRmine database (http://guanlab.ccmb.med.umich.edu/mirmine/help.html), which is an integrated database from 304 high-quality miRNA sequencing experiments of 16 different types of human biospecimen from NCBI-SRA datasets. Finally, candidate miRNAs that could be detected (Ct<37) by Taqman Advanced miRNA assay were selected for the validation stage. Unsupervised hierarchical clustering was used to generate a tree cluster showing distinguishable expression patterns of miRNAs.



**Quality control and analysis of quantitative real-time polymerase chain reaction assays**

The efficiency of amplification in each TaqMan assay was certified to range from 90% ~ 110% by the supplier. Spike-in cel-miR-39-3p was used as an exogenous control to assess RNA extraction and reverse transcription (RT) efficiency. To estimate the inter-assay variations of polymerase chain reaction (PCR) plates, we pooled 20 plasma samples randomly as mix sample and together with validation samples to perform RNA isolation and reverse transcription. We also evaluated hemolysis by calculating the difference in expression level between miR-23a-3p and miR-451a.[46] Plasma samples with a $\Delta Ct_{miR\text{-}23a\text{-}3p\text{-}miR\text{-}451a}$ >7 were defined as hemolysis samples and were removed for further analyses. Assays were carried out in triplicates for each sample. Three miRNAs, miR-16-5p,[47] miR-26b-5p,[48,49] and

miR-423-5p[50] were selected as candidate endogenous controls to normalize the Ct values in each target assay. Not only were these miRNAs reported as endogenous controls and recommended in the manual of TaqMan Advanced miRNA assay, but they were most commonly found in plasma[46] and highly expressed in the discovery stage. To enhance the reliability of the normalization strategy, we also performed the global mean normalization.[51] We used the Normfinder program that combined the intragroup and intergroup variation into a relative stability value to evaluate the expression variability of endogenous controls, of which, the stability value is inversely correlated with the stability of endogenous controls.[52] For PCR data cleaning, each baseline was automatically assigned by the PCR system, and each target set the same threshold. The Ct values above 37 were considered missing. Only the miRNAs whose missing rates were less than 20% in all samples were considered stably expressed and included for differential expression analyses.

Overall, the miRNA isolation and reverse transcription of the samples were successfully completed, as endogenous controls (miR-16-5p, miR-26b-5p, and miR-423-5p) and spike-in (cel-miR-39-3p) indicated good technical performance. In each PCR plate, the same volume of mix sample was tested for cel-miR-39-3p, coefficients of variations for all PCR plates were 2.52%, suggesting good technical performance. All of the samples were free of hemolysis as evaluated by the Ct values difference of miR-23a-3p and miR-451a. However, four miRNAs (miR-142-5p, miR-17-3p, miR-381-3p, and miR-744-3p) were excluded for further analysis as over 20% of participants had an undetected signal in the validation stage. For the endogenous control selection, the most stable endogenous control identified by NormFinder was miR-26b-5p, of which the stability was 0.008, while miR-16-5p, miR-423-

5p, and global mean were 0.011, 0.011 and 0.010, respectively. Thus, we used miR-26-5p as the endogenous control to normalize each candidate miRNA.

**Target genes screening and functional pathway analysis**

Target genes of validated miRNAs were predicted by Targetscan (http://www.targetscan.org/), miRanda (http://www.microrna.org/), miRDB (http://www.mirdb.org/), Pictar (http://pictar.mdc-berlin.de/) and RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/) databases. Target genes successfully imputed by at least three of the above algorithms were considered as predicted genes. We also screened out validated target genes by miRTarBase database that offered the experimental data (http://mirtarbase.mbc.nctu.edu.tw/php/index.php). Furthermore, we extracted the gene expression profiles of coronary artery and heart from the GTEx project (https://gtexportal.org/home/) to filter out low abundance target genes (an average expression value of transcripts per million <5). After target genes screening and filtering, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses (http://www.genome.jp/kegg/) were performed by the clusterProfiler (R Bioconductor).[53] Statistical significance was defined as adjusted *P* value<0.05 based on the Benjamini-Hochberg method.

**Study samples of genome-wide-association analysis of target miRNA**

We conducted a genome-wide association study (GWAS) using the Affymetrix Genome-Wide Human SNP Array 6.0 chips and the Illumina Infinium OminZhongHua-8 chips in 1452 and 7417 Chinese subjects, respectively. Of these subjects, we included 7027 participants who

were enrolled in DFTJ cohort had complete questionnaire information and blood samples in 2013. We further excluded participants with a history of CVD, cancer, or abnormal electrocardiograms (n=2542), as well as participants who were newly diagnosed with CHD<6 months after blood drawing (n=262). To perform bi-directional two-sample Mendelian randomization (MR), we separated two independent populations from the remaining 4223 eligible subjects. Among which, 340 samples were also included in the nested case-control study, thus a GWAS analysis of miR-4286 was performed in these subjects. For the remaining 3883 subjects, after excluding participants using lipid-lowering medication (n=447) or missing TG or HDL-C measurements, we included 3240 subjects who had complete TG measurements and 3238 subjects who had complete HDL-C measurements to estimate the effect sizes of lipid-related SNPs.



**Genome-wide-association analyses of target miRNA**

Genotyping was carried out with Affymetrix Genome-Wide Human SNP Array 6.0 chips and the Illumina Infinium OminZhongHua-8 chips with standard quality control procedures. We performed SNP imputation to merge the two arrays by Minimac3 (v2.0.1) as reported previously.[54] Briefly, we used 1000 Genomes Project ALL Phase 3 Integrated Release Version 5 Haplotypes (February 5, 2013) as reference panel, after filtering missing call rate >5%, minor allele frequency (MAF) <1%, and Hardy-Weinberg equilibrium *P*<1E-05, a total of 549 196 and 703 302 eligible SNPs were detected in the Affymetrix and Illumina dataset, respectively. We performed GWAS analyses of miR-4286 in 340 samples with natural log-transformed data after adjusting for age, sex, and 3 principal components (PCs) to account for population structure by SNPTEST (v2.5.4) using additive models.[55] After a meta-analysis of

the two array dataset by METAL software,[56] we screened 24 independent loci associated with miR-4286 ($P$<5E-06) with weak linkage disequilibrium ($r^2$ <0.2).

**Selection of lipid-related instrumental variables for the two-sample Mendelian randomization analyses**

The genetic instrumental variables (IVs) of TG and HDL-C were selected based on the two larger scale reports in Asian. One is the largest publish GWAS study from the Biobank Japan Project (BBJ) in 162 255 Japanese individuals, the other one is an exome chip study in 47 532 East Asian individuals.[57,58] We included 4280 TG SNPs and 5606 HDL-C SNPs from BBJ, together with 96 TG SNPs and 135 HDL-C SNPs from exome chip study (all $P$<5E-08). Finally, a total of 39 SNPs of TG and 58 SNPs of HDL-C with weakly linkage disequilibrium ($r^2$<0.2) were selected as IVs in MR analyses, after removing low-quality or MAF<1% loci. Of note, we chose a larger effect size of SNP if it was significant in both two studies. Overall, 10 TG SNPs and 10 HDL-C SNPs were selected from the exome chip results, the remaining were derived from the BBJ study.

**Table S1. Baseline characteristics of study participants in the validation stage.**

| Variables | Acute myocardial infarction | | | Unstable angina | | |
|---|---|---|---|---|---|---|
| | Cases (*n*=137) | Controls (*n*=137) | *P* Value | Cases (*n*=435) | Controls (*n*=435) | *P* Value |
| Age, y | 69.9±7.7 | 69.9±7.6 | 0.96 | 66.4±7.5 | 66.3±7.5 | 0.92 |
| Male, No. (%) | 101 (73.7) | 101 (73.7) | 1.00 | 226 (52.0) | 226 (52.0) | 1.00 |
| BMI, kg/m2 | 24.08±3.64 | 23.97±3.17 | 0.79 | 24.91±3.29 | 24.29±3.00 | 0.004 |
| SBP, mmHg | 150.80±27.95 | 142.01±21.93 | 0.004 | 147.41±23.21 | 143.02±22.53 | 0.005 |
| DBP, mmHg | 84.22±13.24 | 80.10±12.60 | 0.009 | 82.99±13.28 | 80.85±12.58 | 0.02 |
| Smoking status, No. (%) | | | 0.07 | | | 0.24 |
| Current smoker | 42 (30.7) | 24 (17.5) | | 94 (21.6) | 81 (18.6) | |
| Former smoker | 33 (24.1) | 37 (27.0) | | 86 (19.8) | 73 (16.8) | |
| Never smoker | 60 (43.8) | 75 (54.7) | | 255 (58.6) | 280 (64.4) | |
| Drinking status, No. (%) | | | 0.43 | | | 0.31 |
| Current drinker | 44 (32.1) | 51 (37.2) | | 114 (26.2) | 134 (30.8) | |
| Former drinker | 23 (16.8) | 16 (11.7) | | 52 (12.0) | 40 (9.2) | |
| Never drinker | 69 (50.4) | 70 (51.1) | | 267 (61.4) | 260 (59.8) | |
| Education level, No. (%) | | | 0.002 | | | 0.31 |
| Primary school or below | 41 (29.9) | 41 (29.9) | | 134 (30.8) | 113 (26.0) | |
| Middle school | 68 (49.6) | 43 (31.4) | | 150 (34.5) | 169 (38.9) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| High school or beyond | 28 (20.5) | 51 (37.2) | | 146 (33.6) | 145 (33.3) | |
| Physical activity, MET-h/week | 21.00 (0.00, 40.25) | 30.00 (14.50, 42.00) | 0.004 | 21.00 (10.50, 42.00) | 21.00 (13.75, 42.00) | 0.52 |
| Family history of CHD, No. (%) | 7 (5.1) | 8 (5.8) | 1.00 | 32 (7.4) | 33 (7.6) | 1.00 |
| Lipid-lowering medication, No. (%) | 14 (10.2) | 19 (13.9) | 0.46 | 71 (16.3) | 63 (14.5) | 0.51 |
| Antihypertensive medication, No. (%) | 54 (39.4) | 42 (30.7) | 0.16 | 177 (40.7) | 151 (34.7) | 0.08 |
| Antidiabetic medication, No. (%) | 20 (14.6) | 15 (10.9) | 0.47 | 56 (12.9) | 42 (9.7) | 0.16 |
| Hyperlipidemia, No. (%) | 86 (62.8) | 85 (62.0) | 1.00 | 294 (67.6) | 289 (66.4) | 0.77 |
| Hypertension, No. (%) | 112 (81.8) | 100 (73.0) | 0.10 | 337 (77.5) | 309 (71.0) | 0.04 |
| Diabetes, No. (%) | 43 (31.4) | 35 (25.5) | 0.35 | 117 (26.9) | 99 (22.8) | 0.18 |
| FG, mmol/L | 5.90 (5.30, 6.70) | 5.60 (5.10, 6.30) | 0.04 | 5.80 (5.30, 6.50) | 5.69 (5.25, 6.26) | 0.02 |
| HDL-C, mmol/L | 1.25 (1.12, 1.51) | 1.36 (1.17, 1.61) | 0.14 | 1.31 (1.16, 1.54) | 1.42 (1.20, 1.67) | <0.001 |
| LDL-C, mmol/L | 2.91±0.85 | 2.71±0.89 | 0.06 | 2.89±0.80 | 2.81±0.85 | 0.15 |
| TC, mmol/L | 4.76 (4.21, 5.39) | 4.76 (4.19, 5.26) | 0.46 | 4.96 (4.33, 5.46) | 4.88 (4.16, 5.50) | 0.28 |
| TG, mmol/L | 1.36 (0.92, 2.09) | 1.23 (0.86, 1.71) | 0.04 | 1.40 (1.07, 1.96) | 1.23 (0.90, 1.67) | <0.001 |

Continuous variables are presented as mean±SD or median (25th, 75th). Categorical variables are presented as numbers (percentage).

*P* Values were estimated using Student t-tests or Mann-Whitney U tests for continuous variables, and Chi-square tests for categorical variables.

Abbreviations: BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride.

**Table S2. Candidate miRNAs associated with incident ACS in the discovery stage.**

| miRNAs | Fold change | Regulation | *P* Value | Chromosome location | miRBase ID | Taqman Advanced assay ID |
|---|---|---|---|---|---|---|
| let-7b-3p | 2.39 | Up | 0.04 | Chr22: 46113686-46113768[+] | MIMAT0004482 | 478221_mir |
| miR-1268b | 1.91 | Down | 0.03 | Chr17: 80098828-80098877[+] | MIMAT0018925 | 480790_mir |
| miR-1307-3p | 1.41 | Up | 0.01 | Chr10: 103394253-103394401[-] | MIMAT0005951 | 483036_mir |
| miR-133b | 2.42 | Down | 0.03 | Chr6: 52148923-52149041[+] | MIMAT0000770 | 480871_mir |
| miR-142-5p | 1.61 | Down | 0.04 | Chr17: 58331232-58331318[-] | MIMAT0000433 | 477911_mir |
| miR-17-3p | 1.56 | Down | 0.04 | Chr13: 91350605-91350688[+] | MIMAT0000071 | 477932_mir |
| miR-181c-3p | 1.83 | Down | 0.02 | Chr19: 13874699-13874808[+] | MIMAT0004559 | 477933_mir |
| miR-21-3p | 1.41 | Down | 0.02 | Chr17: 59841266-59841337[+] | MIMAT0004494 | 477973_mir |
| miR-28-3p | 1.33 | Up | 0.048 | Chr3: 188688781-188688866[+] | MIMAT0004502 | 477999_mir |
| miR-320a | 1.33 | Up | 0.02 | Chr8: 22244966-22245037[-] | MIMAT0000510 | 478594_mir |
| miR-381-3p | 2.60 | Down | 0.048 | Chr14: 101045920-101045994[+] | MIMAT0000736 | 477816_mir |
| miR-4286 | 1.56 | Up | 0.02 | Chr8: 10666978-10667070[+] | MIMAT0016916 | 478096_mir |
| miR-4485-3p | 2.85 | Up | 0.04 | Chr11: 10508270-10508326[-] | MIMAT0019019 | 479430_mir |
| miR-500a-3p | 1.85 | Down | 0.04 | ChrX: 50008431-50008514[+] | MIMAT0002871 | 478951_mir |
| miR-574-5p | 1.37 | Up | 0.04 | Chr4: 38868032-38868127[+] | MIMAT0004795 | 479357_mir |
| miR-744-3p | 2.07 | Up | 0.046 | Chr17: 12081899-12081996[+] | MIMAT0004946 | 479165_mir |
| miR-940 | 1.45 | Up | 0.02 | Chr16: 2271747-2271840[+] | MIMAT0004983 | 479216_mir |

| | | | | | | |
|---|---|---|---|---|---|---|
| miR-99b-5p | 1.59 | Up | 0.01 | Chr19: 51692612-51692681[+] | MIMAT0000689 | 478343_mir |

*P* Values were estimated by paired *t*-tests using microarray normalized data.

Plasma miR-142-5p, miR-17-3p, miR-381-3p, and miR-744-3p were excluded from analysis because of over 20% participants had undetected signal in the validation stage.

Abbreviations: ACS, acute coronary syndrome.

**Table S3. Association of plasma miR-4286 normalized by global mean with incident ACS in the validation stage.**

| miRNA | Tertiles of plasma miR-4286* | | | *P*-trend† | OR (95% CI) per one IQR |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | | |
| miR-4286 | <5.62 | 5.62-8.75 | >8.75 | | |
| *n* (case/control) | 149/191 | 205/190 | 218/191 | | |
| OR (95% CI) | 1 [Ref] | 1.44 (1.03, 2.02) | 1.57 (1.10, 2.24) | 0.02 | 1.17 (1.02, 1.34) |

*Plasma miRNA levels were normalized to the average of all miRNAs levels and were expressed as $2^{-\Delta Ct}$.

†*P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in logistic regression model.

OR (95% CI) was obtained using multivariable conditional logistic regression model with adjustment for age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication.

Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

**Table S4. Association of plasma miR-4286 with incident ACS in the validation stage, excluding ACS cases within the first year of follow-up.**

| miRNA | Tertiles of plasma miR-4286 ($\times 10^{-2}$)* | | | *P*-trend† | OR (95% CI) per one IQR |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | | |
| miR-4286 | <7.06 | 7.06-12.85 | >12.85 | | |
| *n* (case/control) | 110/151 | 153/151 | 191/152 | | |
| OR (95% CI) | 1 [Ref] | 1.49 (1.01, 2.20) | 2.00 (1.34, 2.98) | <0.001 | 1.28 (1.06, 1.55) |

*Plasma miRNA levels were normalized to miR-26b-5p and were expressed as $2^{-\Delta Ct}$.

†*P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in logistic regression model.

OR (95% CI) was obtained using multivariable conditional logistic regression model with adjustment for age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication.

Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

**Table S5. Associations of plasma miR-4286 with incident ACS in subgroups stratified by risk factors in the validation stage.**

| Variables | *n* (case/control) | Tertiles of plasma miR-4286 | | | *P*-trend* | *P*-interaction† |
|---|---|---|---|---|---|---|
| | | T1 | T2 | T3 | | |
| **Age** | | | | | | 0.42 |
| <65 years | 214/213 | 1 [Ref] | 0.99 (0.60, 1.64) | 1.27 (0.78, 2.08) | 0.97 | |
| ≥65 years | 358/359 | 1 [Ref] | 1.55 (1.06, 2.29) | 1.76 (1.20, 2.58) | 0.02 | |
| **Sex** | | | | | | 0.97 |
| Female | 245/245 | 1 [Ref] | 1.58 (0.97, 2.56) | 1.61 (1.01, 2.57) | 0.08 | |
| Male | 327/327 | 1 [Ref] | 1.16 (0.78, 1.73) | 1.55 (1.04, 2.31) | 0.03 | |
| **BMI** | | | | | | 0.10 |
| <24 kg/m$^2$ | 241/277 | 1 [Ref] | 1.34 (0.85, 2.13) | 1.30 (0.84, 2.02) | 0.31 | |
| ≥24 kg/m$^2$ | 331/295 | 1 [Ref] | 1.41 (0.92, 2.14) | 2.04 (1.34, 3.13) | 0.001 | |
| **Current smokers** | | | | | | 0.22 |
| No | 434/465 | 1 [Ref] | 1.38 (0.98, 1.94) | 1.78 (1.27, 2.49) | 0.001 | |
| Yes | 136/105 | 1 [Ref] | 1.23 (0.62, 2.45) | 1.07 (0.55, 2.10) | 0.90 | |
| **Current drinkers** | | | | | | 0.19 |
| No | 411/386 | 1 [Ref] | 1.39 (0.97, 2.00) | 1.78 (1.24, 2.55) | 0.002 | |
| Yes | 158/185 | 1 [Ref] | 1.10 (0.62, 1.96) | 1.19 (0.69, 2.07) | 0.53 | |
| **Hypertension** | | | | | | 0.46 |
| No | 123/163 | 1 [Ref] | 1.06 (0.57, 2.00) | 1.27 (0.67, 2.41) | 0.44 | |
| Yes | 449/409 | 1 [Ref] | 1.40 (0.99, 2.00) | 1.67 (1.19, 2.35) | 0.004 | |
| **Diabetes** | | | | | | 0.88 |
| No | 412/438 | 1 [Ref] | 1.45 (1.02, 2.07) | 1.65 (1.16, 2.34) | 0.008 | |
| Yes | 160/134 | 1 [Ref] | 0.99 (0.53, 1.86) | 1.50 (0.81, 2.76) | 0.15 | |
| **FG level** | | | | | | 0.19 |
| Normal | 198/233 | 1 [Ref] | 1.61 (0.98, 2.68) | 1.35 (0.83, 2.22) | 0.34 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| IFG | 214/205 | 1 [Ref] | 1.32 (0.78, 2.23) | 2.00 (1.20, 3.37) | 0.007 | |
| **Hyperlipidemia** | | | | | | 0.51 |
| No | 192/198 | 1 [Ref] | 0.96 (0.57, 1.63) | 1.43 (0.86, 2.39) | 0.11 | |
| Yes | 380/374 | 1 [Ref] | 1.62 (1.11, 2.36) | 1.75 (1.21, 2.53) | 0.007 | |

Logistic regression models were used in the subgroup analysis with adjustment for age, sex, body mass index, smoking status, drinking status, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, low-density lipoprotein cholesterol, triglyceride, high-density lipoprotein cholesterol, and use of lipid-lowering medication.

[*] *P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in logistic regression model.

[†] *P*-interaction was obtained by continuous indicator*categorical stratifying variable.

Abbreviations: ACS, acute coronary syndrome; BMI, body mass index; FG, fasting glucose; IFG, impaired fasting glucose.

**Table S6. Comparison of the associations between plasma miR-4286 and different subtypes of ACS in the validation stage.**

| ACS subtypes | Tertiles of plasma miR-4286 ($\times 10^{-2}$)* | | | *P*-trend† | OR (95% CI) per one IQR | $P_{heterogeneity}$‡ |
|---|---|---|---|---|---|---|
| | T1 (<7.10) | T2 (7.10-12.23) | T3 (>13.23) | | | |
| AMI | | | | | | |
| *n* (case/control) | 36/109 | 38/190 | 63/191 | | | |
| OR (95% CI) | 1 [Ref] | 1.00 (0.59, 1.69) | 1.74 (1.07, 2.81) | 0.01 | 1.29 (1.02, 1.63) | 0.49 |
| UA | | | | | | |
| *n* (case/control) | 109/191 | 153/190 | 173/191 | | | |
| OR (95% CI) | 1 [Ref] | 1.38 (1.00, 1.91) | 1.51 (1.10, 2.08) | 0.02 | 1.18 (1.01, 1.38) | |

OR (95% CI) was obtained using multinomial logistic regression model with the outcomes (AMI, UA, or non-ACS) as the dependent variable and miRNA level as independent variable, and adjusted for age, sex, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication.

*Plasma miRNA levels were normalized to miR-26b-5p and were expressed as $2^{-\Delta Ct}$.

†*P*-trend was estimated by assigning the median value of miRNA to each tertile and using this as a continuous variable in multinomial logistic regression model.

‡$P_{heterogeneity}$ was obtained from multinomial logistic regression model in the comparison between AMI and UA.

Abbreviations: ACS, acute coronary syndrome; AMI, acute myocardial infarction; CI, confidence interval; IQR, interquartile range; OR, odds ratio; UA, unstable angina.

**Table S7. Adjusted PD (95% CI) for plasma miR-4286 according to categorical cardiovascular traits in the validation stage.**

| Variables | All participants | ACS cases | Controls |
|---|---|---|---|
| **Age** | | | |
| <65 years | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| ≥65 years | 2.72 (-8.46, 15.26) | 3.47 (-12.06, 21.74) | 1.93 (-13.52, 20.14) |
| **Sex** | | | |
| Female | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| Male | -13.37 (-23.62, -1.75) | -12.66 (-27.02, 4.52) | -12.53 (-26.91, 4.68) |
| **BMI** | | | |
| <24 kg/m$^2$ | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| ≥24 kg/m$^2$ | 2.05 (-8.27, 13.53) | 11.11 (-4.39, 29.13) | -7.93 (-20.96, 7.24) |
| **Current smokers** | | | |
| No | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| Yes | -0.53 (-13.55, 14.45) | -4.46 (-21.41, 16.14) | 0.88 (-17.96, 24.06) |
| **Current drinkers** | | | |
| No | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| Yes | 2.73 (-9.09, 16.08) | -0.54 (-16.47, 18.42) | 9.18 (-8.17, 29.81) |
| **Education level** | | | |
| Primary school or below | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| Middle school | -3.98 (-15.74, 9.43) | -7.17 (-22.64, 11.38) | -0.19 (-17.48, 20.72) |
| High school or beyond | -7.46 (-19.21, 6.01) | -5.20 (-21.66, 14.72) | -8.00 (-24.26, 11.74) |
| **MET** | | | |
| <21 h/week | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| ≥21 h/week | -1.56 (-11.69, 9.72) | 1.30 (-12.77, 17.63) | -2.40 (-16.70, 14.35) |
| **Hypertension** | | | |
| No | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| Yes | 4.93 (-5.74, 16.80) | 7.60 (-6.32, 23.58) | -0.15 (-15.75, 18.35) |

| | | | |
|---|---|---|---|
| **Diabetes** | | | |
| No | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| Yes | 3.16 (-8.49, 16.31) | 2.57 (-13.04, 20.99) | 1.25 (-15.17, 20.84) |
| **Hyperlipidemia** | | | |
| No | 0 [Ref] | 0 [Ref] | 0 [Ref] |
| Yes | -3.37 (-13.63, 8.09) | -3.02 (-17.33, 13.78) | -3.42 (-17.66, 13.28) |

Adjusted PD (95% CI) were estimated using linear regression models, including age (<65 years, ⩾65 years), sex (female, male), BMI (<24 kg/$m^2$, ⩾24 kg/$m^2$), current smokers (no, yes), current drinkers (no, yes), education level (primary school or below, middle school, high school or beyond), MET (<21 h/week, ⩾21 h/week), hypertension (no, yes), diabetes (no, yes), and hyperlipidemia (no, yes).

Abbreviations: BMI, body mass index; CI, confidence interval; MET, metabolic equivalent; PD, percent difference.

**Table S8. Adjusted PD (95% CI) for plasma miR-4286 according to linear and tertiles of cardiovascular traits in the validation stage.[*]**

| Variables | Tertiles of cardiovascular traits | | | *P*-trend[†] | PD (95% CI) per one IQR |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | | |
| All participants | | | | | |
| SBP | 0 [Ref] | 3.12 (-9.58, 17.60) | 2.46 (-10.52, 17.33) | 0.74 | -0.73 (-7.50, 6.54) |
| DBP | 0 [Ref] | -0.18 (-12.53, 13.90) | 1.30 (-11.04, 15.35) | 0.83 | -1.36 (-8.06, 5.83) |
| FG | 0 [Ref] | 1.25 (-11.05, 15.24) | 7.86 (-5.57, 23.21) | 0.25 | 1.94 (-2.68, 6.76) |
| HDL-C | 0 [Ref] | -9.28 (-20.24, 3.18) | -18.08 (-28.21, -6.51) | 0.003 | -11.05 (-16.65, -5.08) |
| LDL-C | 0 [Ref] | 12.42 (-1.01, 27.68) | 5.71 (-6.98, 20.13) | 0.43 | 3.18 (-3.69, 10.54) |
| TC | 0 [Ref] | 7.01 (-5.82, 21.59) | -3.46 (-15.32, 10.07) | 0.63 | 1.76 (-4.03, 7.91) |
| TG | 0 [Ref] | 7.11 (-5.74, 21.70) | 23.89 (8.67, 41.24) | 0.001 | 11.04 (3.77, 18.83) |
| TG/HDL-C | 0 [Ref] | 4.84 (-7.75, 19.15) | 23.63 (8.37, 41.04) | 0.001 | 15.01 (7.16, 23.42) |
| ACS cases | | | | | |
| SBP | 0 [Ref] | -15.53 (-29.75, 1.56) | -1.22 (-18.10, 19.14) | 0.96 | 2.16 (-7.40, 12.71) |
| DBP | 0 [Ref] | -9.50 (-24.48, 8.45) | 5.74 (-12.08, 27.17) | 0.49 | -0.75 (-9.85, 9.27) |
| FG | 0 [Ref] | 14.36 (-4.39, 36.80) | 4.99 (-13.17, 26.96) | 0.67 | -1.34 (-7.77, 5.54) |
| HDL-C | 0 [Ref] | 0.58 (-15.97, 20.37) | -16.28 (-30.44, 0.76) | 0.046 | -11.00 (-18.25, -3.11) |
| LDL-C | 0 [Ref] | 3.36 (-13.44, 23.42) | 4.22 (-12.86, 24.64) | 0.65 | 3.33 (-6.67, 14.41) |
| TC | 0 [Ref] | -0.01 (-16.33, 19.49) | -5.26 (-21.01, 13.64) | 0.56 | -2.22 (-10.80, 7.19) |
| TG | 0 [Ref] | 8.18 (-9.58, 29.43) | 20.44 (-0.16, 45.30) | 0.05 | 7.46 (-2.44, 18.36) |
| TG/HDL-C | 0 [Ref] | 10.45 (-7.83, 32.34) | 24.30 (3.13, 49.81) | 0.02 | 12.13 (1.50, 23.88) |
| Controls | | | | | |
| SBP | 0 [Ref] | -1.23 (-18.10, 19.12) | -4.87 (-21.66, 15.53) | 0.61 | -5.04 (-14.43, 5.37) |
| DBP | 0 [Ref] | 9.83 (-8.63, 32.02) | -2.56 (-19.58, 18.06) | 0.78 | -3.11 (-12.74, 7.58) |
| FG | 0 [Ref] | -4.52 (-20.51, 14.70) | 8.19 (-10.35, 30.58) | 0.39 | 4.37 (-2.60, 11.85) |
| HDL-C | 0 [Ref] | -9.80 (-25.13, 8.65) | -16.47 (-30.56, 0.49) | 0.06 | -8.72 (-16.85, 0.21) |
| LDL-C | 0 [Ref] | 14.78 (-4.35, 37.75) | 2.23 (-15.04, 23.02) | 0.85 | 1.61 (-7.41, 11.51) |

| | | | | | |
|---|---|---|---|---|---|
| TC | 0 [Ref] | 9.51 (-8.99, 31.78) | -1.53 (-18.62, 19.15) | 0.92 | 2.61 (-5.15, 11.01) |
| TG | 0 [Ref] | -9.26 (-24.44, 8.97) | 17.09 (-3.01, 41.37) | 0.10 | 11.52 (1.41, 22.64) |
| TG/HDL-C | 0 [Ref] | -2.00 (-18.35, 17.62) | 19.14 (-1.24, 43.73) | 0.06 | 13.46 (3.14, 24.80) |

[*]Adjusted PD (95% CI) corresponds to tertiles of trait or an IQR increase in trait with adjustment for age, sex, body mass index, smoking status, drinking status, education levels, and metabolic equivalent. SBP and DBP additionally adjusted for antihypertensive medication use. FG additionally adjusted for antidiabetic medication use. HDL-C, LDL-C, TC, TG, and TG/HDL-C ratio additionally adjusted for lipid-lowering medication use.

[†]*P*-trend was estimated by assigning the median value of trait to each tertile and using this as a continuous variable in linear regression model.

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; PD, percent difference; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

**Table S9. Associations of plasma lipid traits with incident ACS in the validation stage.[*]**

| Lipids | Tertiles of plasma lipid traits | | | *P*-trend[†] | *FDR* | OR (95% CI) per one IQR |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | | | |
| HDL-C (mmol/L) | <1.25 | 1.25-1.55 | >1.55 | | | |
| *n* (case/control) | 235/189 | 200/186 | 137/197 | | | |
| OR (95% CI) | 1 [Ref] | 0.96 (0.71, 1.30) | 0.66 (0.48, 0.92) | 0.01 | 0.02 | 0.86 (0.73, 1.00) |
| LDL-C (mmol/L) | <2.42 | 2.42-3.12 | >3.12 | | | |
| *n* (case/control) | 175/188 | 175/192 | 222/192 | | | |
| OR (95% CI) | 1 [Ref] | 1.00 (0.74, 1.36) | 1.35 (0.99, 1.83) | 0.05 | 0.06 | 1.26 (1.06, 1.48) |
| TC (mmol/L) | <4.38 | 4.38-5.26 | >5.26 | | | |
| *n* (case/control) | 161/188 | 227/192 | 184/192 | | | |
| OR (95% CI) | 1 [Ref] | 1.53 (1.12, 2.09) | 1.23 (0.88, 1.71) | 0.18 | 0.18 | 1.31 (1.13, 1.52) |
| TG (mmol/L) | <1.00 | 1.00-1.51 | >1.51 | | | |
| *n* (case/control) | 125/190 | 194/190 | 253/192 | | | |
| OR (95% CI) | 1 [Ref] | 1.68 (1.21, 2.34) | 2.06 (1.47, 2.89) | <0.001 | <0.001 | 1.45 (1.21, 1.74) |
| TG/HDL-C (ratio) | <0.68 | 0.68-1.12 | >1.12 | | | |
| *n* (case/control) | 123/191 | 193/190 | 256/191 | | | |
| OR (95% CI) | 1 [Ref] | 1.53 (1.11, 2.12) | 1.99 (1.43, 2.77) | <0.001 | <0.001 | 1.47 (1.22, 1.77) |

[*]Lipid traits were included in the conditional logistic regression models with adjustment for age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, family history of coronary heart disease, diabetes, hypertension, and use of lipid-lowering medication.

[†]*P*-trend was estimated by assigning the median value of lipid to each tertile and using this as a continuous variable in conditional logistic regression models.

Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; TG, triglyceride.

**Table S10. SNPs of miR-4286 selected for the Mendelian randomization analyses based on the genome-wide association analysis (*P*<5E-06).**

| SNPs | Position | Nearest Genes | Allele | EAF | Effect size (SEM) | *P* Value |
|---|---|---|---|---|---|---|
| rs5775955 | 1:88217744 | *RP5-1027O11.1* | CATA/C | 0.88 | -0.65 (0.12) | 4.71E-08 |
| rs185758585 | 2:83971924 | *RNU6-1312P* | T/C | 0.01 | 1.82 (0.37) | 8.97E-07 |
| rs75615431 | 2:157524497 | *RPLP0P7* | C/T | 0.15 | 0.60 (0.12) | 3.55E-07 |
| rs6434150 | 2:186825204 | *RPL21P32* | A/T | 0.90 | 0.64 (0.13) | 6.49E-07 |
| rs17626938 | 3:20080132 | *PP2D1KAT2B* | G/A | 0.08 | -0.68 (0.15) | 4.48E-06 |
| rs6797897 | 3:139620442 | *RP11-166D18.1* | C/G | 0.59 | -0.47 (0.10) | 2.03E-06 |
| rs145356549 | 4:4689685 | *STX18-AS1* | T/C | 0.02 | 1.95 (0.40) | 1.09E-06 |
| rs544792245 | 4:27478020 | *IGBP1P5* | CT/C | 0.01 | 1.96 (0.40) | 9.70E-07 |
| rs372448036 | 4:88553224 | *RP11-742B18.1* | A/ACC | 0.01 | 2.22 (0.43) | 2.09E-07 |
| rs199980924 | 4:113245645 | *ALPK1* | T/G | 0.02 | 1.72 (0.34) | 3.31E-07 |
| rs192431740 | 5:12347309 | *RNU6-679P* | C/T | 0.01 | 1.89 (0.41) | 4.10E-06 |
| rs143142540 | 5:26159829 | *RNU4-43P* | T/C | 0.01 | 1.71 (0.37) | 4.02E-06 |
| rs1057412 | 6:31321752 | *HLA-B* | G/T | 0.12 | 0.57 (0.12) | 3.33E-06 |
| rs606578 | 6:139845597 | *RP11-12A2.3* | A/G | 0.39 | 0.46 (0.10) | 4.51E-06 |
| rs1721018 | 7:17004886 | *AC098592.6* | T/C | 0.29 | -0.42 (0.09) | 1.11E-06 |
| rs28375190 | 7:36431478 | *ANLN* | G/T | 0.18 | -0.50 (0.10) | 1.17E-06 |
| rs7030875 | 9:89180003 | *RP11-359J6.1* | T/A | 0.01 | 2.49 (0.53) | 2.36E-06 |
| rs28374621 | 9:139219818 | *GPSM1, WI2-1959D15.1* | A/G | 0.01 | 1.99 (0.44) | 4.80E-06 |
| rs140233303 | 10:5646881 | *RP13-463N16.6* | AG/A | 0.08 | -0.73 (0.15) | 1.25E-06 |
| rs71399823 | 15:86879954 | *AGBL1* | A/G | 0.05 | 0.88 (0.18) | 1.99E-06 |
| rs61744697 | 17:11672607 | *DNAH9* | T/G | 0.05 | 0.79 (0.17) | 1.66E-06 |
| rs28397896 | 18:10191496 | *RP11-419J16.1* | A/G | 0.32 | 0.43 (0.09) | 5.78E-07 |
| rs34047128 | 19:54266441 | *MIR519A2* | T/C | 0.24 | 0.57 (0.13) | 4.53E-06 |
| rs116437901 | 21:29860677 | *AF131217.1* | T/G | 0.01 | 1.97 (0.36) | 5.14E-08 |

Positions are reported in human genome build hg19. Alleles are listed as effect/reference alleles.

Abbreviations: EAF, effect allele frequency; SEM, standard error of the mean.

**Table S11. SNPs of TG selected for Mendelian randomization analyses based on the previous reports in Asian (*P*<5E-08).**

| SNPs | Position | Nearest Genes | Allele | EAF | Effect size (SEM) | *P* Value |
|---|---|---|---|---|---|---|
| rs35529421 | 1:62965621 | *DOCK7* | A/T | 0.14 | -0.07 (0.006) | 3.57E-32 |
| rs2114273 | 1:93854517 | *RF00019, FNBP1L* | C/T | 0.61 | 0.03 (0.005) | 5.95E-10 |
| rs2144300 | 1:230294916 | *GALNT2* | T/C | 0.19 | -0.03 (0.005) | 4.23E-08 |
| rs12992267 | 2:21215645 | *AC012361.1, AC115619.1* | T/C | 0.09 | 0.05 (0.008) | 1.46E-11 |
| rs13306194 | 2:21252534 | *APOB*[*] | A/G | 0.13 | -0.07 (0.01) | 1.38E-12 |
| rs1260326 | 2:27730940 | *GCKR*[*] | C/T | 0.52 | -0.11 (0.007) | 1.26E-62 |
| rs3749147 | 2:27851918 | *GPN1, ZNF512* | A/G | 0.38 | 0.05 (0.005) | 8.25E-27 |
| rs3752442 | 4:3446883 | *HGFAC* | G/A | 0.43 | -0.03 (0.004) | 1.27E-14 |
| rs1037814 | 4:88049850 | *AFF1* | C/T | 0.56 | 0.03 (0.004) | 9.46E-11 |
| rs154254 | 5:55820584 | *C5orf67* | C/G | 0.39 | 0.03 (0.004) | 7.59E-09 |
| rs6882076 | 5:156390297 | *TIMD4, HAVCR1*[*] | C/T | 0.73 | 0.05 (0.008) | 4.15E-09 |
| rs6905288 | 6:43758873 | *VEGFA, AL157371.2* | A/G | 0.79 | 0.03 (0.006) | 7.90E-09 |
| rs12531645 | 7:73023881 | *MLXIPL, AC005089.1* | A/G | 0.10 | -0.11 (0.007) | 3.23E-49 |
| rs4921914 | 8:18272438 | *NAT2, PSD3*[*] | T/C | 0.49 | -0.05 (0.007) | 4.73E-15 |
| rs1059611 | 8:19824563 | *LPL* | C/T | 0.13 | -0.17 (0.006) | 1.49E-154 |
| rs2954021 | 8:126482077 | *RP11-136O12.2, TRIB1* | G/A | 0.55 | -0.06 (0.004) | 7.43E-42 |
| rs7916868 | 10:64988931 | *JMJD1C* | T/A | 0.73 | -0.03 (0.005) | 1.41E-10 |
| rs4411227 | 10:94831513 | *AL358613.3* | G/C | 0.67 | -0.04 (0.005) | 3.57E-16 |
| rs174551 | 11:61573684 | *FADS2, FADS1* | C/T | 0.37 | 0.04 (0.005) | 8.15E-15 |
| rs7350481 | 11:116586283 | *AP000770.1, BUD13*[*] | C/T | 0.75 | -0.22 (0.008) | 1.90E-160 |
| rs75198898 | 11:116649806 | *ZPR1* | A/G | 0.08 | 0.29 (0.008) | 1.94E-288 |
| rs603446 | 11:116654435 | *ZPR1* | T/C | 0.22 | -0.15 (0.005) | 3.38E-180 |
| rs662799 | 11:116663707 | *APOA5, AP006216.2*[*] | A/G | 0.71 | -0.28 (0.009) | 2.47E-196 |

| | | | | | | |
|---|---|---|---|---|---|---|
| rs9804646 | 11:116665079 | *APOA5, AP006216.2* | T/C | 0.11 | -0.15 (0.007) | 4.99E-106 |
| rs633389 | 11:116667337 | *APOA5, AP006216.2* | T/C | 0.40 | 0.03 (0.004) | 1.30E-12 |
| rs6589567 | 11:116670676 | *ZPR1, APOA5*[*] | C/A | 0.71 | -0.15 (0.01) | 1.36E-51 |
| rs7123454 | 11:116704178 | *APOC3, APOA1* | A/C | 0.61 | -0.08 (0.004) | 2.77E-75 |
| rs7112513 | 11:117037361 | *PAFAH1B2* | G/A | 0.54 | -0.06 (0.004) | 6.23E-49 |
| rs508487 | 11:117075566 | *PCSK7*[*] | T/C | 0.16 | 0.09 (0.01) | 1.03E-13 |
| rs11542139 | 11:117100257 | *PCSK7*[*] | T/C | 0.04 | 0.11 (0.02) | 9.85E-11 |
| rs2075260 | 12:109696838 | *ACACB*[*] | A/G | 0.73 | 0.04 (0.008) | 3.95E-08 |
| rs75766425 | 14:52511911 | *NID2* | C/G | 0.13 | -0.04 (0.007) | 1.27E-08 |
| rs261291 | 15:58680178 | *ALDH1A2* | C/T | 0.51 | 0.05 (0.004) | 2.57E-26 |
| rs1800588 | 15:58723675 | *LIPC, ALDH1A2* | T/C | 0.51 | 0.06 (0.004) | 6.22E-51 |
| rs2679617 | 15:70207077 | *LINC00593, TLE3* | G/A | 0.26 | -0.03 (0.005) | 2.38E-08 |
| rs56156922 | 16:56987369 | *HERPUD1,CETP* | C/T | 0.22 | -0.04 (0.005) | 1.41E-12 |
| rs2278426 | 19:11350488 | *ANGPTL8, DOCK6* | T/C | 0.27 | -0.04 (0.005) | 1.62E-13 |
| rs58542926 | 19:19379549 | *TM6SF2, CILP2* | T/C | 0.08 | -0.06 (0.009) | 9.10E-14 |
| rs75627662 | 19:45413576 | *AC011481.3* | T/C | 0.12 | 0.09 (0.007) | 5.65E-42 |

Positions are reported in human genome build hg19. Alleles are listed as effect/reference alleles.

SNPs of gene marked with asterisk were derived from the East Asian exome meta-analysis study, others were selected from the BioBank Japan Project in Japanese.

Abbreviations: EAF, effect allele frequency; SEM, standard error of the mean; TG, triglyceride.

**Table S12. SNPs of HDL-C selected for the Mendelian randomization analyses based on the previous reports in Asian (*P*<5E-08).**

| SNPs | Position | Nearest Genes | Allele | EAF | Effect size (SEM) | *P* Value |
|---|---|---|---|---|---|---|
| rs6685271 | 1:93634590 | *TMED5* | A/C | 0.60 | -0.04 (0.006) | 1.37E-10 |
| rs2144300 | 1:230294916 | *GALNT2* | T/C | 0.19 | 0.04 (0.007) | 4.25E-08 |
| rs117350179 | 3:12374332 | *PPARG* | G/C | 0.23 | -0.03 (0.006) | 2.96E-08 |
| rs28366301 | 6:32560883 | *HLA-DRB1, HLA-DQA1* | A/G | 0.39 | 0.04 (0.006) | 7.58E-11 |
| rs1358980 | 6:43764551 | *VEGFA, LINC01512* | T/C | 0.54 | -0.03 (0.005) | 2.69E-09 |
| rs1652507 | 6:161082461 | *LPA*[*] | C/T | 0.47 | -0.04 (0.007) | 2.10E-09 |
| rs1026422 | 7:50319807 | *AC020743.3, IKZF1* | A/G | 0.45 | 0.03 (0.005) | 4.84E-10 |
| rs7778167 | 7:127851628 | *MIR129-1, LEP* | A/G | 0.11 | 0.06 (0.008) | 1.99E-11 |
| rs301 | 8:19816934 | *LPL*[*] | C/T | 0.49 | 0.11 (0.008) | 1.72E-40 |
| rs325 | 8:19819328 | *LPL* | C/T | 0.13 | 0.15 (0.008) | 2.09E-79 |
| rs3808447 | 8:116575459 | *TRPS1* | A/G | 0.69 | 0.05 (0.006) | 3.64E-15 |
| rs4743758 | 9:107515814 | *NIPSNAP3A* | T/C | 0.25 | -0.04 (0.006) | 1.94E-10 |
| rs2230808 | 9:107562804 | *ABCA1* | C/T | 0.61 | 0.05 (0.005) | 9.31E-21 |
| rs4149310 | 9:107589134 | *ABCA1* | T/A | 0.69 | 0.06 (0.006) | 2.39E-28 |
| rs1883025 | 9:107664301 | *ABCA1* | T/C | 0.27 | -0.11 (0.006) | 3.03E-76 |
| rs7847628 | 9:123631225 | *PHF19* | G/A | 0.37 | -0.03 (0.006) | 1.62E-08 |
| rs7895716 | 10:94783777 | *EXOC6* | G/C | 0.65 | 0.04 (0.005) | 1.24E-10 |
| rs2297991 | 10:113913222 | *GPAM*[*] | C/T | 0.76 | -0.05 (0.007) | 6.52E-12 |
| rs4917630 | 10:114019830 | *GPAM, TECTB* | A/G | 0.37 | -0.04 (0.005) | 7.56E-13 |
| rs2257129 | 10:122898697 | *RPL19P16, LINC01153* | C/T | 0.70 | -0.04 (0.006) | 1.85E-10 |
| rs174570 | 11:61597212 | *FADS2*[*] | T/C | 0.56 | -0.05 (0.008) | 1.49E-08 |
| rs1263056 | 11:116576415 | *AP000770.1* | G/A | 0.73 | -0.07 (0.006) | 1.40E-29 |
| rs1893460 | 11:116603677 | *AP000770.1* | A/G | 0.19 | 0.11 (0.007) | 1.42E-62 |
| rs10488698 | 11:116633947 | *BUD13*[*] | A/G | 0.07 | 0.09 (0.01) | 2.14E-15 |

| | | | | | | |
|---|---|---|---|---|---|---|
| rs3741297 | 11:116657667 | *ZPR1* | T/C | 0.08 | -0.26 (0.01) | 2.52E-157 |
| rs651821 | 11:116662579 | *APOA5* | T/C | 0.65 | 0.14 (0.005) | 1.07E-153 |
| rs6589567 | 11:116670676 | *ZPR1, APOA5*[*] | C/A | 0.71 | 0.10 (0.01) | 8.86E-24 |
| rs12718465 | 11:116707736 | *APOA1-AS, APOA1* | T/C | 0.07 | -0.09 (0.01) | 7.97E-13 |
| rs4883263 | 12:7649484 | *CD163*[*] | C/T | 0.69 | -0.05 (0.007) | 5.24E-11 |
| rs11067592 | 12:110069190 | *MVK, RN7SKP250* | T/G | 0.08 | -0.09 (0.01) | 5.12E-18 |
| rs11067829 | 12:110116872 | *RN7SKP250, FAM222A* | G/A | 0.46 | 0.04 (0.006) | 5.03E-13 |
| rs28577594 | 12:123895906 | *SETD8, RILPL2* | C/G | 0.74 | -0.04 (0.006) | 1.67E-09 |
| rs61005347 | 12:125325778 | *SCARB1* | T/C | 0.07 | -0.07 (0.01) | 1.22E-11 |
| rs67053123 | 12:125353810 | *SCARB1* | A/T | 0.29 | 0.07 (0.006) | 1.38E-32 |
| rs76213020 | 14:52436005 | *GNG2, AL358333.3* | C/A | 0.14 | 0.05 (0.008) | 1.19E-08 |
| rs118146059 | 15:58544773 | *ALDH1A2* | C/T | 0.03 | -0.16 (0.02) | 2.43E-21 |
| rs12903590 | 15:58577777 | *ALDH1A2* | T/A | 0.54 | 0.07 (0.005) | 8.81E-38 |
| rs261291 | 15:58680178 | *ALDH1A2* | C/T | 0.51 | 0.12 (0.005) | 7.30E-109 |
| rs13329672 | 15:58699937 | *ALDH1A2* | T/C | 0.14 | 0.10 (0.008) | 7.15E-37 |
| rs2070895 | 15:58723939 | *ALDH1A2, LIPC* | A/G | 0.51 | 0.13 (0.005) | 1.13E-140 |
| rs77780456 | 16:56849114 | *NUP93* | G/A | 0.05 | 0.17 (0.01) | 5.93E-39 |
| rs56156922 | 16:56987369 | *HERPUD1, CETP* | C/T | 0.22 | 0.27 (0.006) | 0.00E+00 |
| rs1800775 | 16:56995236 | *HERPUD1, CETP* | A/C | 0.55 | 0.09 (0.005) | 9.95E-71 |
| rs7499892 | 16:57006590 | *CETP* | T/C | 0.16 | -0.16 (0.008) | 3.88E-101 |
| rs116893196 | 16:57010955 | *CETP* | C/G | 0.07 | -0.19 (0.01) | 2.96E-59 |
| rs2303790 | 16:57017292 | *CETP* | G/A | 0.04 | 0.42 (0.01) | 5.00E-198 |
| rs56303487 | 16:68029739 | *DUS2, DPEP2* | T/C | 0.14 | 0.07 (0.008) | 3.09E-19 |
| rs2925979 | 16:81534790 | *CMIP* | C/T | 0.67 | 0.04 (0.006) | 7.40E-13 |
| rs12970066 | 18:47107152 | *LIPG* | G/C | 0.26 | 0.05 (0.006) | 2.44E-14 |
| rs11082764 | 18:47119579 | *LIPG* | G/A | 0.46 | 0.08 (0.005) | 5.63E-51 |
| rs73959590 | 18:47156188 | *LIPG, SMUG1P1* | A/G | 0.09 | -0.09 (0.01) | 9.77E-22 |
| rs4939883 | 18:47167214 | *LIPG*[*] | C/T | 0.80 | 0.06 (0.009) | 1.23E-13 |

| | | | | | | |
|---|---|---|---|---|---|---|
| rs737337 | 19:11347493 | *DOCK6*[*] | C/T | 0.24 | -0.07 (0.008) | 1.50E-18 |
| rs429358 | 19:45411941 | *APOE* | C/T | 0.10 | -0.09 (0.009) | 2.43E-24 |
| rs7412 | 19:45412079 | *APOE*[*] | T/C | 0.10 | 0.10 (0.02) | 3.95E-11 |
| rs5167 | 19:45448465 | *APOC4, APOC2* | G/T | 0.48 | 0.04 (0.005) | 9.06E-13 |
| rs235314 | 21:46271452 | *PTTG1IP* | T/C | 0.41 | -0.03 (0.005) | 3.30E-10 |
| rs7445 | 22:21977047 | *UBE2L3* | T/C | 0.46 | -0.04 (0.005) | 1.25E-14 |

Positions are reported in human genome build hg19. Alleles are listed as effect/reference alleles.

SNPs of gene marked with asterisk were derived from the East Asian exome meta-analysis study, others were selected from the BioBank Japan Project in Japanese.

Abbreviations: EAF, effect allele frequency; HDL-C, high-density lipoprotein cholesterol; SEM, standard error of the mean.

**Table S13. The proportions of genetic explanation of TG, HDL-C, and miR-4286 related SNPs.**

| Traits | #SNP | Beta | SEM | *P* Value | $R^2$ | *F* statistics |
|---|---|---|---|---|---|---|
| TG | 39 | 0.23 | 0.02 | <0.001 | 0.07 | 76.50 |
| HDL-C | 58 | 0.14 | 0.01 | <0.001 | 0.07 | 83.65 |
| miR-4286 | 24 | 0.25 | 0.01 | <0.001 | 0.48 | 105.41 |

Linear regression models adjusted for age and sex were used to examine the effects and F statistics.

Abbreviations: HDL-C, high-density lipoprotein cholesterol; SEM, standard error of the mean; TG, triglyceride.

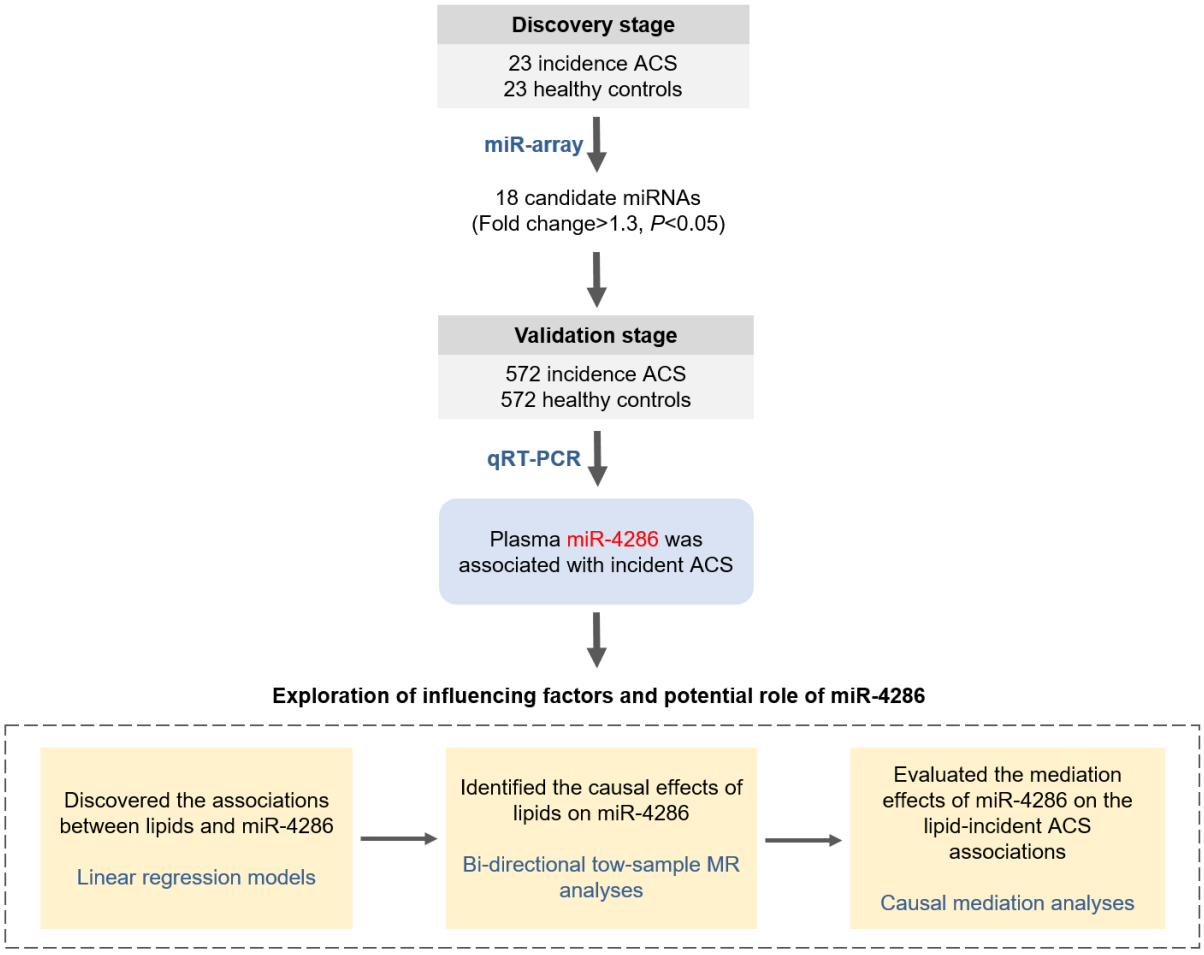


**Figure S1. Overview of the study design.**

ACS, acute coronary syndrome, MR, Mendelian randomization; qRT-PCR, quantitative real-time polymerase chain reaction.

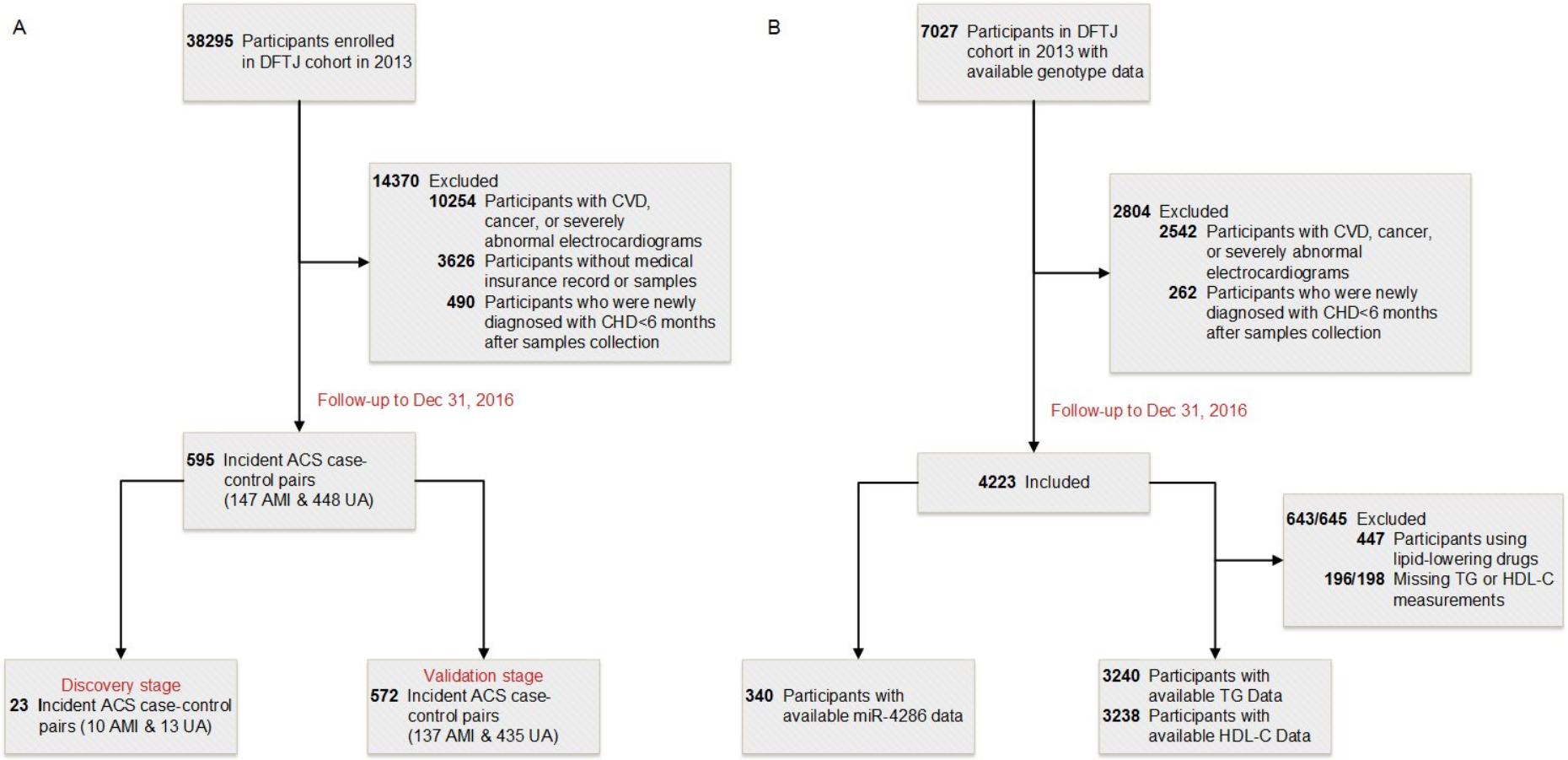


**Figure S2. Flowchart of the study participants.**

**A**, Participants of the nested case-control study; **B**, Participants of the genome-wide association study and Mendelian randomization study.

ACS, acute coronary syndrome; AMI, acute myocardial infarction; CHD, coronary heart disease; CVD, cardiovascular disease; DFTJ,

Dongfeng-Tongji cohort; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, unstable angina.

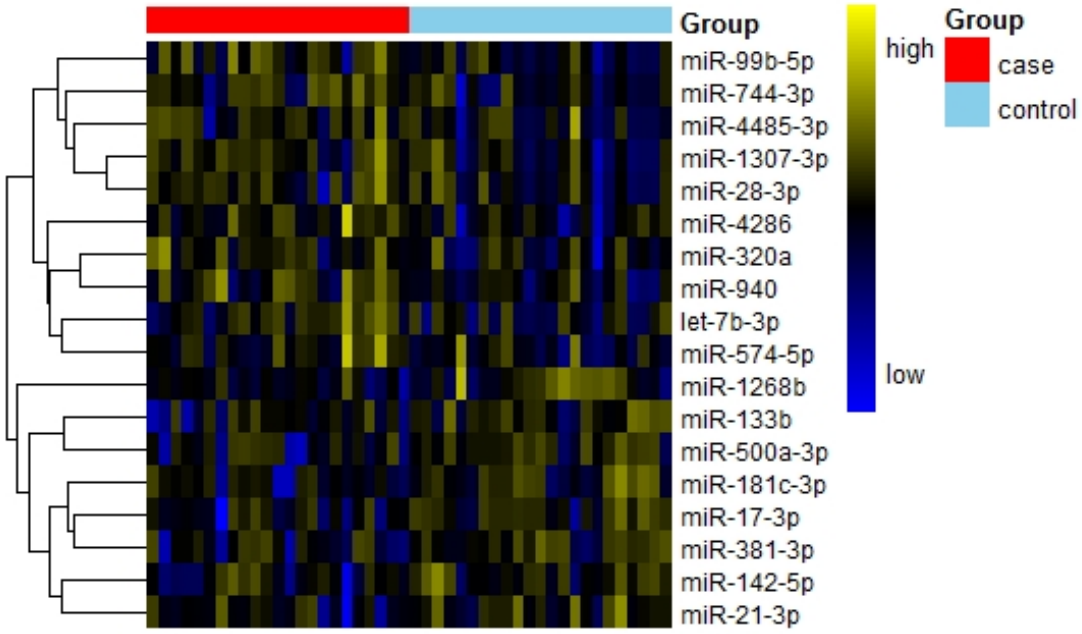


**Figure S3. Hierarchical clustering of 18 differentially expressed candidate miRNAs in the discovery stage.**

The columns of the heatmap represent incident acute coronary syndrome cases (red) and controls (sky blue), and the rows represent miRNAs. The color gradient range from highest (yellow) to lowest (blue) based on the miRNA levels of each sample.

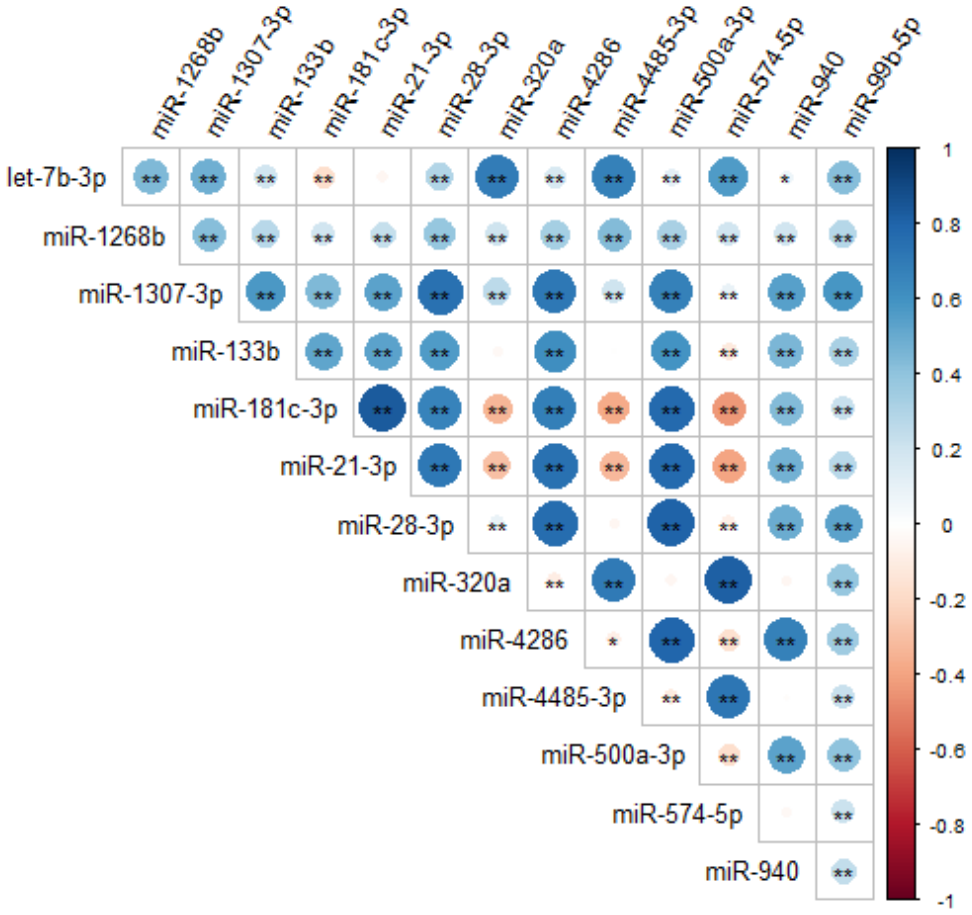


**Figure S4. Correlation patterns of plasma miRNAs in the validation stage.**

Dot size and shade depth represent the sizes of Spearman's rank correlation coefficients. Significant *P* values are marked with asterisk (**P*<0.05; ***P*<0.01).

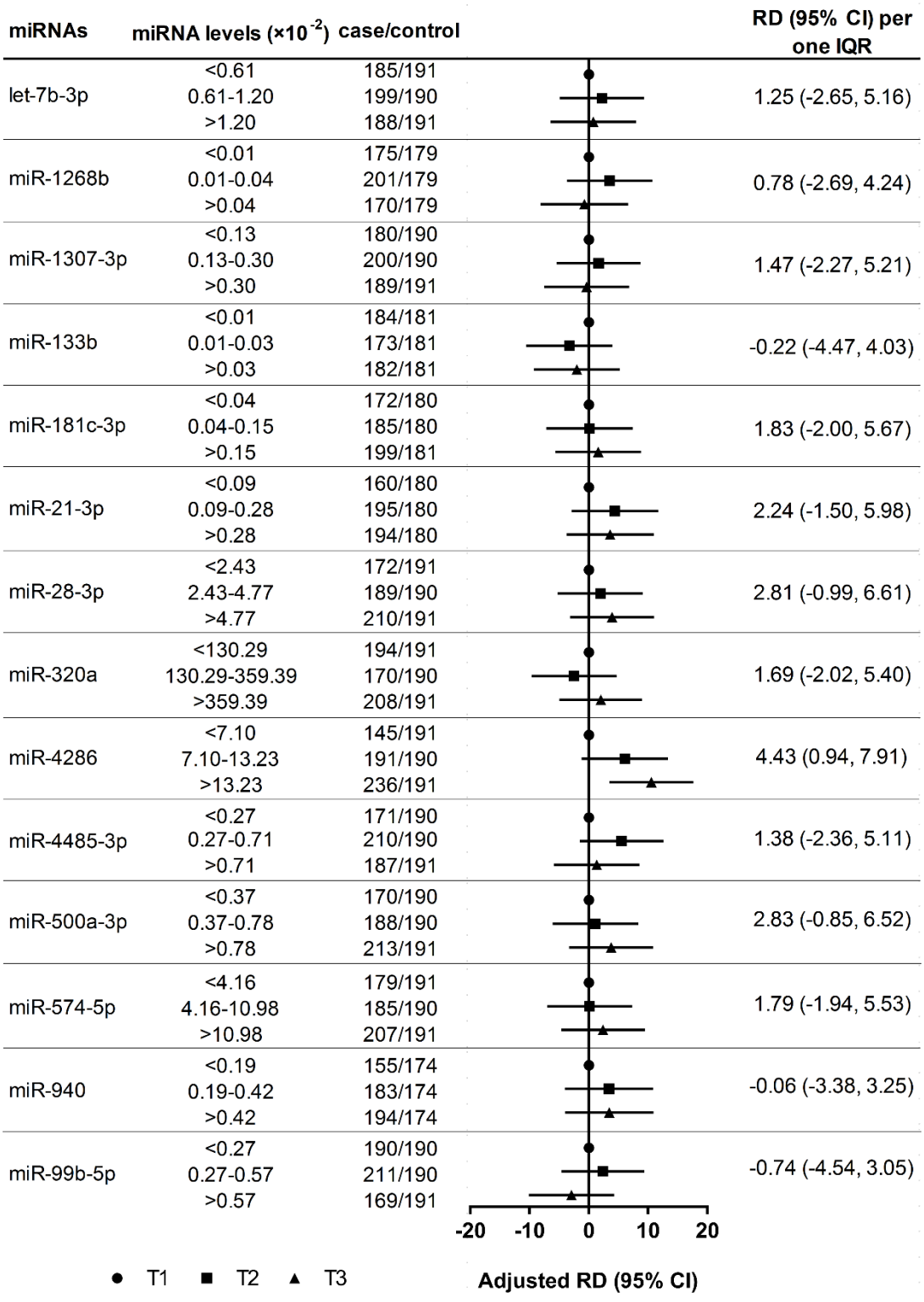


**Figure S5. Adjusted RD (95% CI) for incident ACS according to plasma miRNAs in the validation stage.**

Plasma miRNA levels were normalized to miR-26b-5p and were expressed as $2^{-\Delta Ct}$. Adjusted RD

(95% CI) for incident ACS was obtained using generalized linear regression model with adjustment for age, sex, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and use of lipid-lowering medication. ACS, acute coronary syndrome; CI, confidence interval; IQR, interquartile range; RD, risk difference.

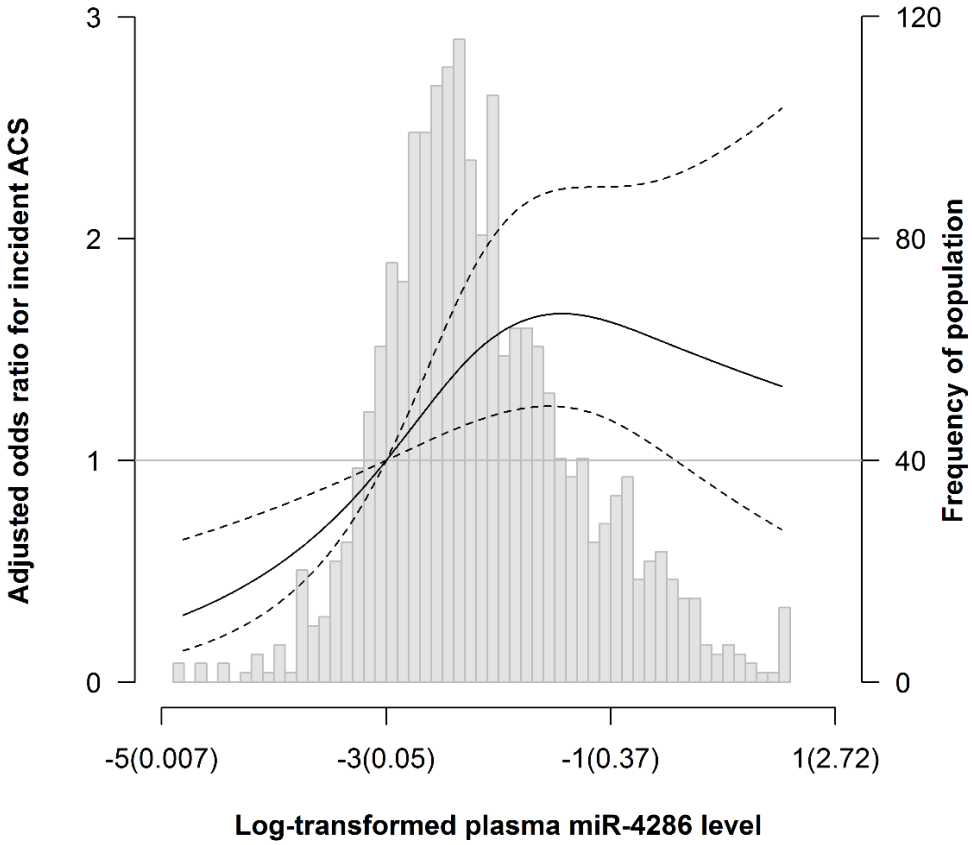


**Figure S6. The restricted cubic spline for the associations between plasma miR-4286 and incident ACS.**

The lines represent adjusted odds ratios based on restricted cubic splines for the log-transformed level of miR-4286 in the conditional logistic model. Knots were placed at the 5th, 50th and 95th percentiles of the distribution, and the reference value was set at the 10th percentile. Adjustment factors were age, body mass index, smoking status, drinking status, education levels, metabolic equivalent, diabetes, hypertension, family history of coronary heart disease, low-density lipoprotein cholesterol, triglyceride, high-density lipoprotein cholesterol, and use of lipid-lowering medication. ACS, acute coronary syndrome.

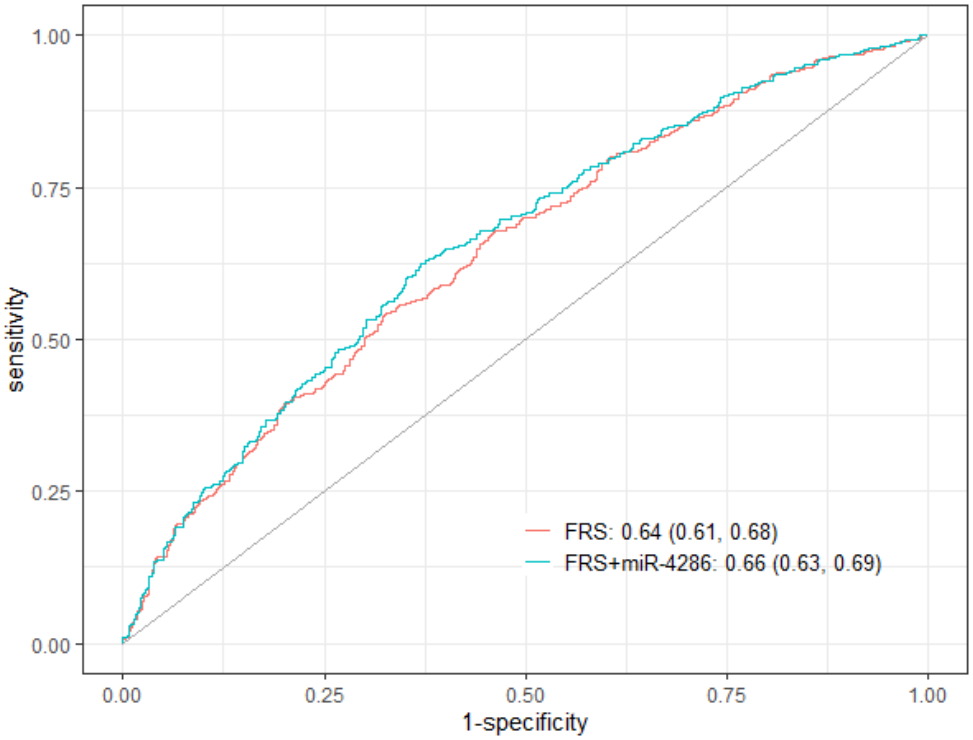


**Figure S7. Receiver-operating characteristic curves for prediction of incident ACS.**

Red curve represents the model of FRS and blue curve represents FRS model plus plasma miR-4286. Annotation shows the area and 95% confidence intervals under the receiver-operating characteristic curves. ACS, acute coronary syndrome; FRS, Framingham Risk Score.

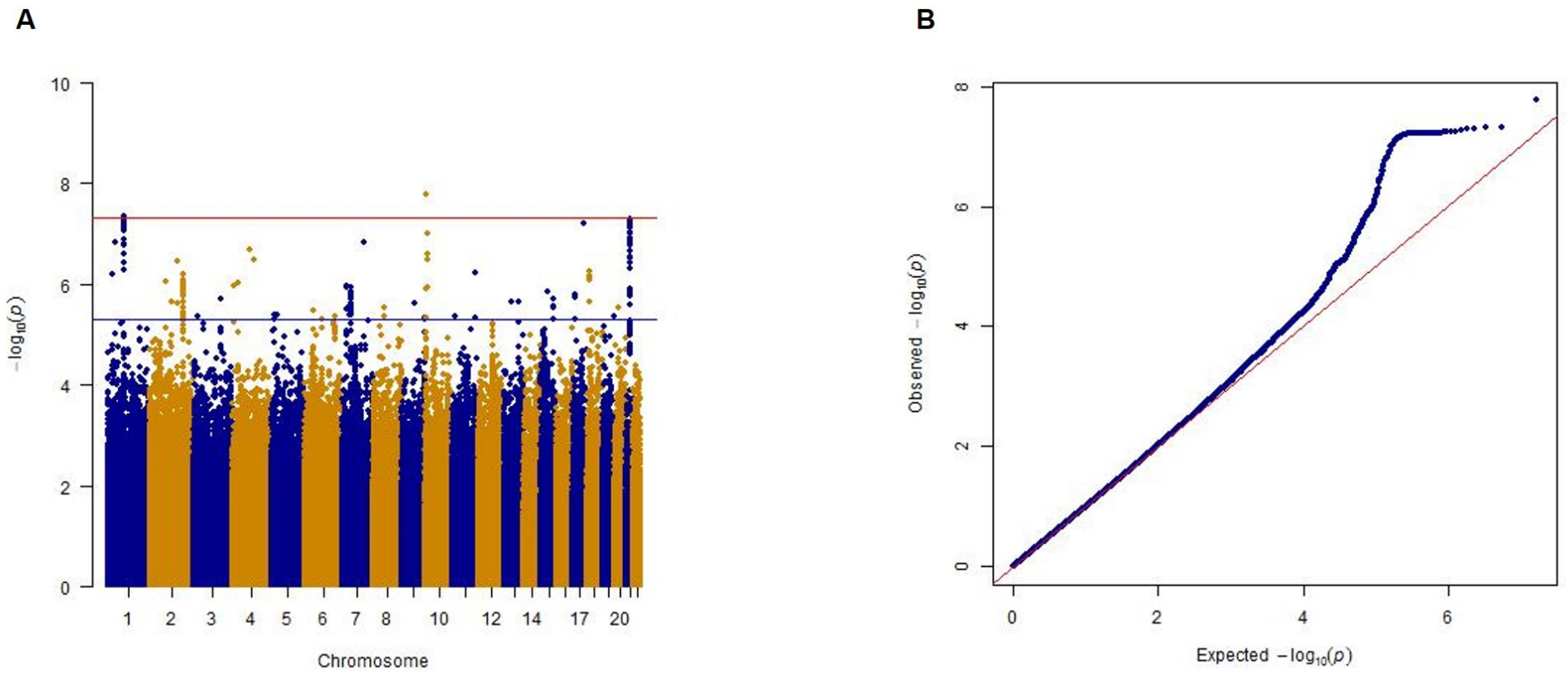


**Figure S8. Manhattan Plot and Q-Q plot.**

The Manhattan Plot and Q-Q plot of the genome-wide association study of miR-4286 are showed separately in **A** and **B**. The inflation factor lambda is 0.99.

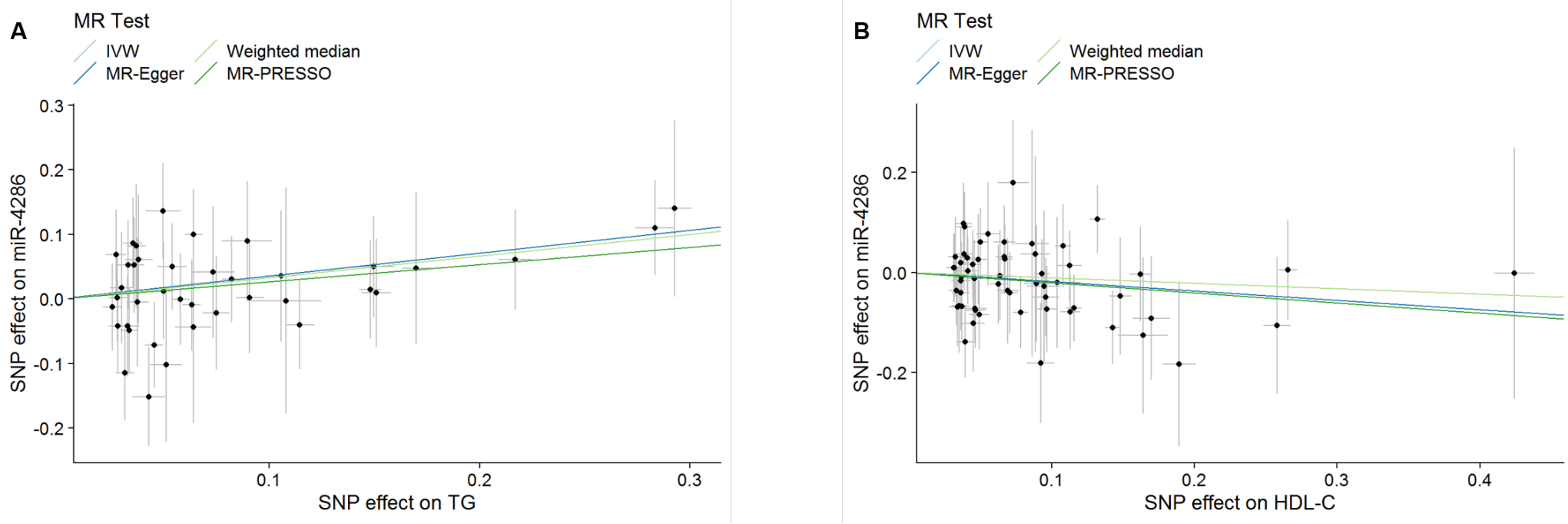


**Figure S9. Scatter plots showing the causal effects of TG and HDL-C on plasma miR-4286 by the Mendelian randomization analyses.**

**A,** Causal effects of TG on miR-4286 based on 39 TG SNPs. **B**, Causal effects of HDL-C on miR-4286 based on 58 HDL-C SNPs. Each point represents a SNP, The x-axis plot shows their effect sizes on TG or HDL-C derived from summary data, and the y-axis shows the effect on miR-4286 as estimated in 340 samples using linear regression model adjusted for age and sex. Error bars represent the 95% confidence intervals of each SNP. The slope of different colored lines represent the causal effects estimated by the IVW test, MR-Egger test, weighted median test, or MR-PRESSO test. IVW, inverse variance-weighted; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

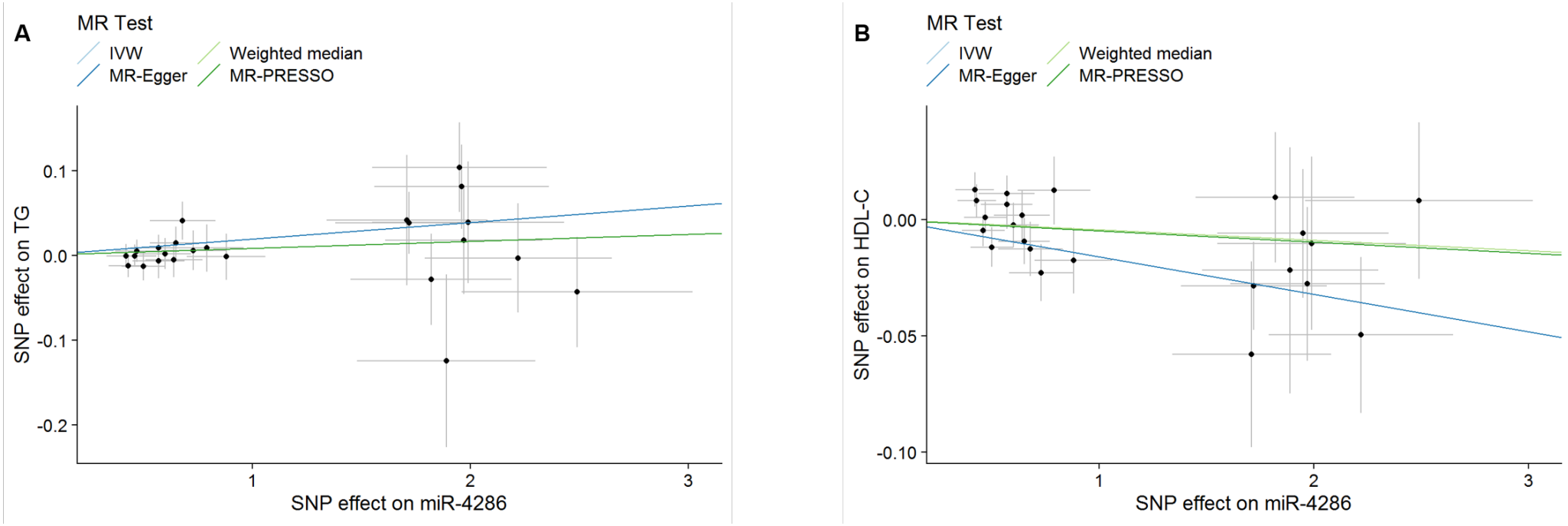


**Figure S10. Scatter plots showing the causal effects of miR-4286 on TG and HDL-C by the Mendelian randomization analyses.**

**A,** Causal effects of miR-4286 on TG based on 24 SNPs of miR-4286. **B,** Causal effects of miR-4286 on HDL-C based on 23 SNPs of miR-4286 after removing 1 pleiotropic SNP (rs544792245) that associated with HDL-C. Each point represents a SNP, The x-axis plot shows their effect sizes on miR-4286 obtained from the GWAS results in the present study, and the y-axis shows the effect on TG or HDL-C as estimated in 3240 samples and 3238 samples using linear regression model adjusted for age and sex, respectively. Error bars represent the 95% confidence intervals of each SNP. The slope of different colored

lines represent the causal effects estimated by the IVW test, MR-Egger test, weighted median test, or MR-PRESSO test. IVW, inverse variance-weighted; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

| MR method | β (95% CI) | Pleiotropy |
|---|---|---|
| **TG** | | |
| IVW | 0.01 (0.00, 0.02) | |
| MR-Egger | 0.02 (-0.01, 0.04) | 0.32 |
| Weighted Median | 0.01 (-0.01, 0.02) | |
| MR-PRESSO | 0.01 (0.00, 0.02) | 0.90 |
| **HDL-C** | | |
| IVW | 0.00 (-0.01, 0.00) | |
| MR-Egger | -0.02 (-0.03, 0.00) | 0.08 |
| Weighted Median | 0.00 (-0.01, 0.01) | |
| MR-PRESSO | 0.00 (-0.01, 0.00) | 0.40 |

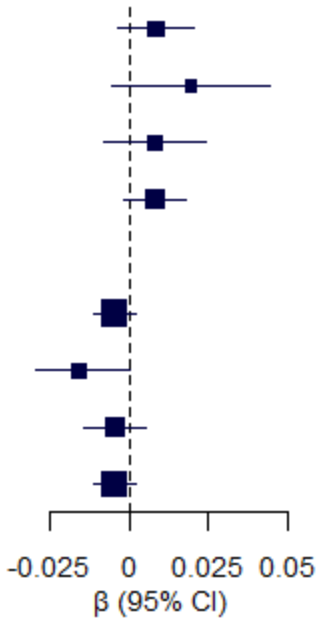


**Figure S11. Causal effects of plasma miR-4286 on TG and HDL-C.**

The causal effect (95% CI) of miR-4286 on TG used 24 SNPs of miR-4286, and the causal effect (95% CI) of miR-4286 on HDL-C used 23 SNPs of miR-4286, after removing 1 pleiotropic SNP (rs544792245) that associated with HDL-C. Pleiotropy *P* value derived from the intercept of MR-Egger test or MR-PRESSO Global test, a small *P* value indicates an unbalanced pleiotropy. CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; IVW, inverse variance-weighted; TG, triglyceride.

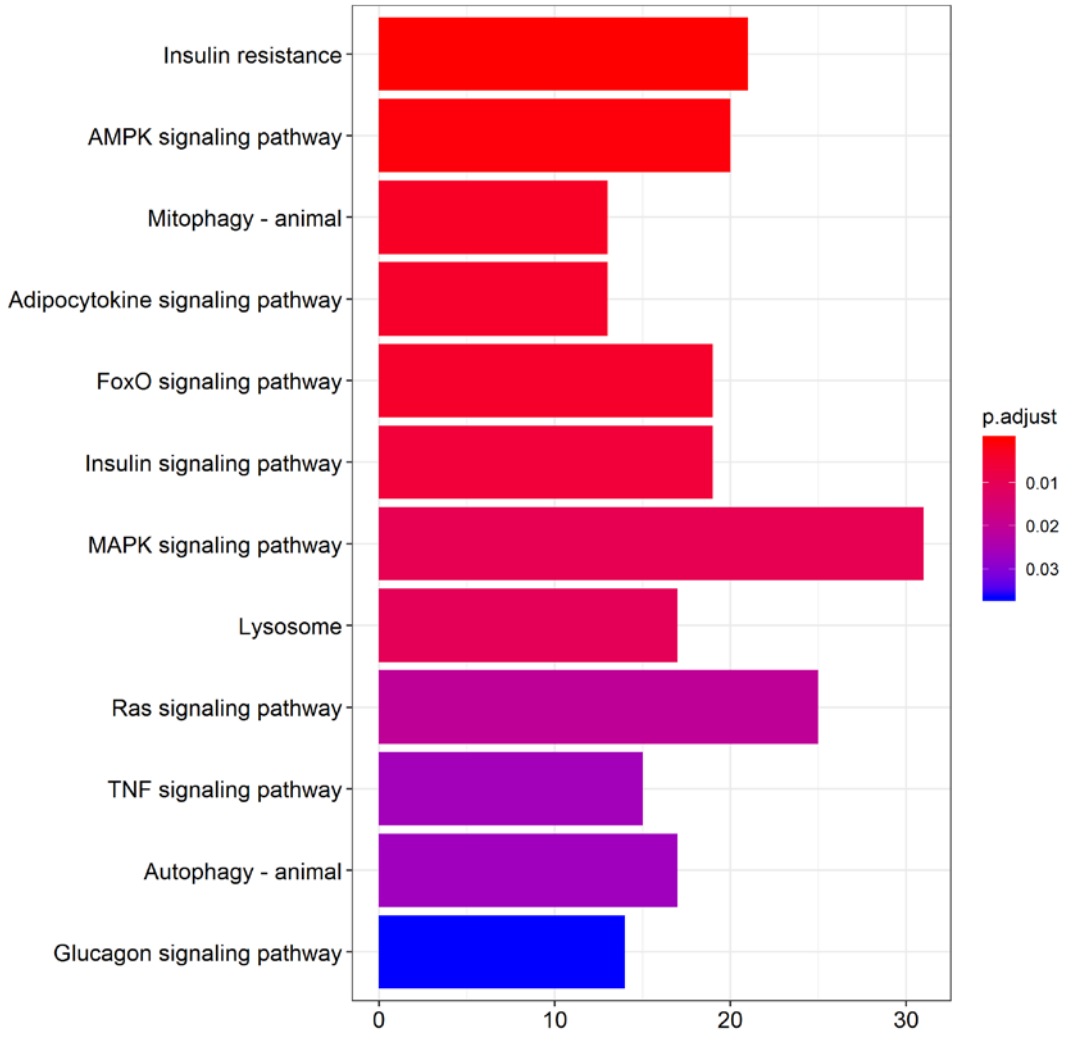


**Figure S12. Enriched KEGG pathways for target genes of miR-4286.**

Bar chart shows the significant KEGG pathways (Benjamini-Hochberg adjusted $P<0.05$) for miR-4286, using highly expressed target genes in the cardiovascular system. The x-axis represents the counts of target genes enriched in each pathway, and the y-axis represents the pathways in order by increased adjusted *P* value. KEGG, Kyoto Encyclopedia of Genes and Genomes.