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Development of a prognostic gene model for myocardial infarction

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ABSTRACT

Background: Ischemic cardiomyopathy due to myocardial infarction frequently induces adverse events. However, the lack of predictive models impedes clinical decisions.

Objective: We aimed to construct a gene-based model to predict post–myocardial infarction adverse cardiac events by integrating gene expression profiles and clinical factors.

Methods: This multicenter prospective cohort study was conducted between 2021 and 2024 and included patients from Jiangsu Provincial People’s Hospital and Hangzhou First People’s Hospital admitted between June 2021 and January 2024. All participants underwent follow-up until 36 months post-discharge. They were divided into model-construction (n=291) and validation (n=100) cohorts. The primary endpoints were major adverse cardiovascular events, including cardiac death, recurrent myocardial infarction, stroke, heart failure, or rehospitalization for further intervention. We used Cox regression analysis to identify risk factors. Model specificity and sensitivity were assessed via the concordance index, calibration curves, and receiver operating characteristic curves. Model performance was evaluated against previous models using integrated discrimination improvement and continuous net reclassification improvement. Kaplan-Meier curves were used for visualization and log-rank tests for between-group comparisons.

Results: Patients in the construction cohort (mean age: 63±13 years) were predominantly male (77.7%). Among 55 patients who reached an endpoint event, 13 experienced cardiovascular death, 20 were readmitted for heart failure, and 22 underwent further intervention for chest pain. In the validation cohort, 25 patients experienced major adverse cardiovascular events. Biometric analysis and machine learning identified four candidate genes—*FADS2*, *FMN1*, *TMEM176A*, and *RPS4Y1*. A prognostic model combining polymerase chain reaction results and clinical factors achieved a concordance index of 0.83, increasing integrated discrimination improvement and continuous net reclassification improvement by at least 0.239 and 0.69, respectively, compared to

previous models. The internal and external validation concordance indices were 0.864 and 0.723, respectively.

Conclusions: The developed prognostic model for myocardial infarction demonstrated robust sensitivity and specificity and favorable validation in external cohorts, offering substantial clinical guidance. Future studies should investigate the function and mechanism of action of identified targets.

Keywords: prognostic gene model, myocardial infarction, cardiac events, clinical factors

INTRODUCTION

According to the World Health Organization,¹ cardiovascular-related diseases caused approximately 18 million deaths in 2019, representing 32% of global mortality. Particularly, acute myocardial infarction (AMI) arising from coronary heart disease is a major international health concern. It accounts for approximately 75% of sudden cardiac deaths and over 2 million deaths in the United States, over 4 million in Europe and North Asia, and over one-third of all deaths in developed nations.² In China, 11.39 million of approximately 330 million patients with cardiovascular disease are affected by coronary heart disease. Although evidence-based treatments and lifestyle modifications have decreased mortality rates, many patients still experience cardiac insufficiency.³ The economic impact is substantial, with total hospitalization costs for cardiovascular disease reaching \$165,222 million in 2020.⁴ In 2021, mainland China registered 1,164,117 cases involving coronary intervention.

An effective therapeutic approach to inhibit cardiomyocyte apoptosis, promote local angiogenesis, and attenuate cardiac remodeling is urgently needed to halt irreversible myocardial damage. Bioinformatics-guided gene therapy is a promising approach for diagnosing and treating AMI. Initially applied in protein self-sequencing analysis, genealogy studies, and amino acid framework design, bioinformatics has expanded to DNA identification, evolving alongside sequencing technologies such as first-generation and second-generation sequencing, leading to precision-targeted therapy at the forefront of medical science.⁵ Bioinformatics in precision medicine is used to integrate histological biomarkers identified in individuals or patient cohorts (single-nucleotide polymorphisms, structural variants, circulating DNA, methylated DNA, mRNA, miRNA, proteins, and metabolites). These are used for decision-making in cancer treatment,⁶ pharmacogenomic design,^{7,8} biomarker screening for disease diagnosis and treatment,⁹ and guidance for basic research.¹⁰

The fusion of genomics, advanced bioinformatic analyses, mathematical modeling, and

fundamental experiments offers insights into gene expression changes during disease progression and treatment, aiding patient subgroup classification and tailored medical interventions.¹¹ Precision medicine is evolving to customize care for small groups or individual patients, factoring in genetics, environment, and lifestyle. However, research is needed to develop a multifaceted approach. Therefore, we aimed to investigate the potential of precision therapy for enhancing clinical treatment.

METHODS

Research design and study population

This multicenter prospective cohort study adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committees of The First Affiliated Hospital of Nanjing Medical University and Hangzhou First People's Hospital (No. 2021-SR-066). Between June 2021 and January 2024, 391 consecutive patients with AMI were enrolled from Jiangsu Provincial People's Hospital and Hangzhou First People's Hospital and underwent follow-up. After excluding 9 patients owing to missing basic data, 382 patients were included in the analysis.

The inclusion criteria were patients with AMI diagnosed based on the criteria outlined in the Guidelines for the Diagnosis and Treatment of Non-ST-Segment Elevation Acute Coronary Syndromes and the Guidelines for the Diagnosis and Treatment of Acute ST-Segment Elevation Myocardial Infarction^{12,13} and those voluntarily agreeing to participate and provide written informed consent. The exclusion criteria were history of AMI; AMI associated with coronary artery bypass grafting; diagnosis of malignant neoplasm, severe hepatic or renal insufficiency, or other uncontrolled systemic disease; cognitive dysfunction or dementia; and declining to provide written informed consent.

Consent

Written informed consent was obtained from all participants, and data were analyzed anonymously.

Baseline data collection

Baseline participant characteristics, including demographic information, current and past medical history, laboratory test results, diagnostic investigations, and medication usage, were collected within 24 h of admission. Standardized blood sample analyses were conducted at the central laboratory of Nanjing Medical University.

Primary endpoints and follow-up

The primary endpoints were major adverse cardiovascular events (MACE), including cardiac death, recurrent AMI, stroke, heart failure, or rehospitalization for further intervention. Patient data were documented according to the study protocol, and participants underwent follow-up (in person or by telephone) at 3, 6, 12, 24, and 36 months post-discharge.

Bioinformatics analysis

Microarray datasets GSE59867 and GSE123342, relevant to AMI prognosis, were retrieved from the Gene Expression Omnibus database. To mitigate bias and variability in the data, the “sva” package was used. Subsequently, differential gene expression analysis between the experimental and control groups was conducted using the “limma” package. Genes exhibiting significant expression disparities in dataset GSE59867 were validated in dataset GSE123342. Finally, least absolute shrinkage and selection operator regression analysis (LASSO) was used to select the most informative candidate genes.

RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction (PCR)

1. RNA extraction was performed using TRIzol reagent.
2. Chloroform was added and vigorously mixed for 30–40 s, then centrifuged at 12,000 rpm at 4 °C for 15–20 min.
3. Isopropanol was added to the upper layer of the liquid and centrifuged for 10 min, allowing the RNA to precipitate at the bottom of the tube.
4. The RNA samples were washed with ethanol, dried at room temperature, and dissolved in an appropriate amount of enzyme-free water.
5. The total RNA concentration of the sample was determined, followed by reverse transcription to obtain cDNA. Specific primers were then added to conduct PCR to verify the expression levels. The PCR procedure included initial incubation at 95 °C for 5 min, followed by 40 cycles of incubation at 95 °C for 10 s, and final incubation at 60 °C for 20 s. The primer sequence is provided in **Supplemental Table 1**.

Enzyme-linked immunosorbent assay (ELISA)

1. In blank wells, add the standard while adding test samples to the other wells.
2. Wash the plate thoroughly by adding wash solution to each well.
3. Add biotinylated antibody dilution solution to the blank wells and biotinylated antibody working solution to the remaining wells.
4. After washing, add streptavidin-biotin complex working solution to each well.
5. Following another wash, add the tetramethylbenzidine mixture.
6. Add termination solution to each well and measure the absorbance using an enzyme lab.

Statistical analyses

Continuous variables are presented as the mean $\pm$ standard deviation, and categorical variables as numbers and percentages. Normality and chi-square tests were conducted prior to data analysis. Between-group comparisons were made using Welch's t-test, Student's t-test, and the Mann–Whitney U test based on data distribution. Chi-square tests were used to compare rates between groups. Serum N-terminal pro b-type natriuretic peptide (NT-proBNP) and C-reactive protein were log-transformed to achieve normal distribution consistency. Multiple imputations addressed missing data when missing values were <2%.

Three Cox regression models were used to investigate the association between gene expression and adverse events. Variables were screened based on clinical significance and statistical criteria; those with a significance level of $p < 0.1$ in Cox regression were considered for inclusion. Model 1 was unadjusted; Model 2 was adjusted for age, sex, hypertension, hyperlipidemia, diabetes mellitus, and smoking; and Model 3 was further adjusted for blood creatinine, hemoglobin, high-density lipoprotein, low-density lipoprotein, troponin, BNP, medication use, cardiac systolic function, and cardiac short-axis contractility. Additionally, quartiles of gene expression levels were compared to assess the relationship with MACE. Kaplan-Meier analysis was used to plot MACE survival curves and log-rank tests to compare survival probabilities between groups.

Variables selected via the LASSO method were used to assess linearity between gene expression and endpoints using a restricted cubic spline function. Optimal cutoff values for gene expression were determined. PCR results were used to construct a nomograph. Sensitivity was evaluated using the C-index and receiver operating characteristic curve analysis, whereas specificity was assessed with calibration plots. Decision curve analysis was used to determine the benefit ratio. Performance was compared with previous models using integrated discrimination improvement (IDI) and continuous net reclassification improvement (c-NRI). For internal validation, we used 1000 bootstrap iterations, whereas we used participants from the First People's Hospital of Hangzhou for the external validation set. A two-sided p-value of < 0.05

indicated statistical significance. Analyses were conducted using SPSS (version 23.0, IBM, Chicago) and R (version 4.2.1, R Development Core Team).

RESULTS

Baseline patients characteristics

In total, 391 participants were enrolled in this study; 291 were recruited from Jiangsu Provincial People's Hospital for model construction, and 100 were recruited from Hangzhou First People's Hospital for external validation. Eight patients were excluded from the construction set and one from the validation set owing to missing data. Clinical baseline characteristics are summarized in Tables 1 and 2, with the study process illustrated in Figure. 1. In the construction cohort, 55 experienced MACE, and the affected tended to be older (66 ± 12 vs. 62 ± 13 , $p=0.0076$). They also exhibited higher troponin T (1248 ± 1655 vs. 850 ± 1396 , $p=0.049$), NT-proBNP (6256 ± 9787 vs. 3515 ± 6647 , $p=0.014$), blood urea nitrogen (BUN) (8.5 ± 5.6 vs. 6.9 ± 3.9 , $p=0.013$), and fasting glucose (7 ± 3.6 vs. 6.1 ± 2.5 , $p=0.035$) levels, alongside lower hemoglobin (122 ± 23 vs. 135 ± 20 , $p=0.0001$) and red blood cell counts (4 ± 0.8 vs. 4.4 ± 0.7 , $p=0.001$). Cardiac ultrasound and medication use data indicated the MACE group exhibited a smaller ventricular short-axis systolic rate (28 ± 7 vs. 30 ± 6 , $p=0.006$), worse cardiac function (53 ± 11 vs. 56 ± 10 , $p=0.012$), and lower β -blocker levels (48 [87.3%] vs. 166 [72.8%], $p=0.016$). *FADS2* and *RPS4Y1* exhibited significantly higher expression in the MACE group than in the non-MACE group, whereas *TMEM176A* exhibited the opposite. No significant difference was observed for *FMN1*. In the external validation cohort, basic clinical information and laboratory test results were similar (except for BUN and alkaline phosphatase levels). These differences in gene expression were consistent with those observed in the construction cohort.

Screening and validation of AMI and prognosis-related hub genes

From the integrated normalized data, 18,837 genes were identified after adjusting for batch effects. In the GSE59867 dataset, 13 differentially expressed genes (DEGs) associated with poor post-infarction prognosis were identified, with two upregulated and 11 downregulated genes. Heatmaps and volcano plots illustrating the DEGs are shown in Supplemental Figure. 2A–B. The pivotal genes included *FADS2*, *LRRN3*, *FMN1*, *GPR15*, *TAS2R43*, *TMEM176A*, *USP9Y*, *TXLNGY*, *UTY*, *DDX3Y*, *RPS4Y1*, and *KDM5D*. Following LASSO regression, *TMEM176A*, *FADS2*, *RPS4Y1*, and *FMN1* were identified and used to construct the model Figure. 2D–F. The area under the curve of the receiver operating characteristic curve for the GSE59867 dataset was 0.922, corresponding to a C-index of 0.935. Validation of expression was performed using the GSE123342 dataset, shown in Figure. 2C.

Construction of gene models

In the model construction cohort, optimal cutoff values of 7, 6.63, 6.45, and 12.7 were selected for *FADS2*, *FMN1*, *TMEM176A*, and *RPS4Y1*, respectively, based on the results of the restricted cubic spline (Figure. 3). Gene expression was categorized as low or high using these values. In the construction cohort, high expression of *TMEM176A*, *RPS4Y1*, *FADS2*, and *FMN1* was associated with MACE in 50%, 50.4%, 51.1%, and 50.4% of cases, respectively. In the validation cohort, the corresponding percentages were 53.5%, 46.5%, 50.5%, and 46.5%. Four subgroups were created based on quartiles of *FADS2*, *FMN1*, *TMEM176A*, and *RPS4Y1* expression levels. Sixteen risk factors were identified via univariate Cox regression analysis, with six attributed to basic clinical information, six to laboratory test results, two to laboratory examination results, and two to medication use (**Supplemental Table 2**). To demonstrate model efficacy and clinical usability, variables such as age, sex, and ejection fraction were combined with gene expression to develop a visual column chart (**Supplemental Table 2**). Line graphs are shown in Figure. 4.

Effectiveness of the nomogram model

The new model exhibited strong discriminatory power with consistency indices of 0.76, 0.83, and 0.84 (Table 3). The calibration plot (Figure. 5) indicates a close alignment between predicted and actual post-AMI MACE occurrence. The overlap between the predicted and actual line segments illustrates this. When expressions were transformed into categories based on thresholds/quartiles, the models maintained a high fit. Models using raw data, thresholds, and quartiles achieved high net benefits within thresholds and did not intersect with the no-intervention group in decision curve analysis.

Internal and external model validation and model comparison

Internal validation with 1000 replicates yielded a C-index of 0.864, whereas external validation using a similar method yielded 0.723. To assess the superiority of the model, we evaluated the degree of model improvement using the area under the curve, c-NRI, and IDI. The cutoff for the assessment was 12 months. The prognostic base model included age, sex, blood creatinine level, ejection fraction, New York Heart Association (NYHA) cardiac function class, BNP level, and troponin level, yielding a C-index of 0.645. NRI and IDI results are presented in Table 3 and Supplemental Figure 1. The genetic model demonstrated stronger predictive capability despite incorporating fewer clinical variables than the older model.

Prognostic value of the AMI hub genes

Table 4 summarizes the association between each hub gene and MACE. After full covariate adjustment, PCR results indicated positive associations between *FADS2* (hazard ratio [HR]: 1.517, 95% confidence interval [CI]: 1.257–1.83, $p < 0.0001$) and *RPS4Y1* expression (HR: 1.268, 95% CI: 1.099–1.464, $p = 0.001$) and MACE. Conversely, *TMEM176A* was negatively correlated

with MACE (HR: 0.612, 95% CI: 0.505–0.74, $p<0.0001$). *FMNI* had no significant association (HR: 0.93, 95% CI: 0.73–1.185, $p=0.559$). ELISA results revealed negative correlations between *FADS2* (HR: 0.54, 95% CI: 0.416–0.7, $p<0.0001$), *RPS4Y1* (HR: 0.168, 95% CI: 0.066–0.432, $p<0.0001$), and *FMNI* (HR: 0.38, 95% CI: 0.18–0.803, $p=0.011$) expression and MACE, whereas *TMEM176A* (HR: 2.776, 95% CI: 1.853–4.158, $p<0.0001$) showed a positive correlation. Furthermore, log-rank tests revealed significant differences among quartile groups for *FADS2*, *TMEM176A*, and *RPS4Y1* (Figure. 6).

Table 5 summarizes the associations between different expression levels of each hub gene and MACE. After full covariate adjustment, significant differences were observed between PCR and ELISA results for *TMEM176A*, *FADS2*, and *RPS4Y1*. PCR results indicated increasing expression levels of *FADS2* and *RPS4Y1* were associated with higher MACE risk. For *FADS2*, the odds ratios (ORs) and 95% CIs for models I, II, and III were 4.52 (1.255–16.275), 3.173 (0.811–12.414), and 13.95 (3.903–49.856), respectively. For *RPS4Y1*, the OR values and 95% CIs were 1.684 (0.625–4.54), 0.497 (0.157–1.575), and 4.627 (1.777–12.05). Conversely, lower expression of *TMEM176A* was associated with higher MACE occurrence, with ORs and 95% CIs of 0.229 (0.109–0.48), 0.13 (0.053–0.318), and 0.057 (0.017–0.193). No significant differences were observed for *FMNI* in models I, II, or III ($p>0.05$). ELISA results showed a similar trend. Detailed PCR and ELISA results are shown in **Supplemental Table 3**.

Subgroup analyses

The study population was stratified according to sex, diabetes mellitus, hypertension, hyperlipidemia, and smoking. As depicted in the forest plot in Supplemental Figure. 2, higher *TMEM176A* expression was correlated with decreased MACE risk in men, corresponding to an OR and 95% CI of 0.524 (0.413–0.665, $p<0.0001$). However, no significant difference was observed in women (OR: 0.822, 95% CI: 0.67–1.009, $p=0.061$). Compared to individuals with

dyslipidemia, those with normolipidemia exhibited lower MACE risk, with increasing *FADS2* and *RPS4YI* values, corresponding to ORs and 95% CIs of 1.449 (1.22–1.719, $p<0.0001$) and 1.31 (1.169–1.469, $p<0.0001$), respectively. Conversely, increased *TMEM176A* expression was associated with decreased MACE risk in individuals with normolipidemia (OR: 0.652, 95% CI: 0.558–0.762, $p<0.0001$). Non-smokers showed increased MACE risk with increasing *RPS4YI* values (OR: 1.3, 95% CI: 1.148–1.473, $p<0.0001$), whereas no significant difference was observed in smokers (OR: 1.178, 95% CI: 0.894–1.552, $p=0.245$). In the remaining subgroups, changes in gene expression leading to changes in MACE risk remained consistent across populations.

DISCUSSION

In this study, we used high-throughput sequencing to identify candidate hub genes associated with AMI prognosis. We observed that elevated *FADS2* and *RPS4YI* expression and decreased *TMEM176A* expression were linked to increased MACE risk. Using spline function analysis, we determined optimal cutoff values for gene expression, facilitating the establishment of a prognostic gene prediction model. Internal and external validation cohorts confirmed the model's sensitivity and specificity. This approach holds promise for enhancing early prognosis prediction and guiding targeted interventions for patients who experience AMI.

This study identified four hub genes, confirming the association of *FADS2*, *TMEM176A*, and *RPS4YI* with adverse events. *FADS2*, a member of the fatty acid desaturase gene family (FADS), regulates fatty acid unsaturation by introducing double bonds between specific carbons in the fatty acyl chain. These desaturases are implicated in various metabolic disorders and are widely expressed in different biological contexts.^{14–16} *FADS* has been linked to reduced inflammation, lower total and low-density lipoprotein cholesterol levels, and decreased risk of coronary artery disease.^{17,18} A Chinese study suggested *FADS* polymorphisms may influence plasma fatty acid

concentrations and desaturase activity. Saturated fatty acids are associated with an increased risk of coronary artery disease, whereas unsaturated fatty acids decrease this risk. This association may partially explain the effect of *FADS2* on prognosis. A post-hoc analysis of the OMEGA-REMODEL trial revealed that *FADS2* polymorphisms may modify the therapeutic response to high-dose omega-3 fatty acids in patients with AMI.¹⁹ Reducing left ventricular end-systolic volume and minimizing noninfarct myocardial fibrosis improve left ventricular remodeling. However, the precise mechanisms by which *FADS2* influences the prognosis of these patients require further investigation.

TMEM176A regulates dendritic cell differentiation and influences apoptosis, cell proliferation, migration, and invasion. The role of *TMEM176A* in apoptosis suggests it could substantially impact cardiac remodeling by affecting cardiomyocytes within the cardiovascular system.²⁰ *TMEM176A* expression is susceptible to methylation, with regulation occurring via the methylation of promoter regions.²¹ This highlights the potential of *TMEM176A* as an intervention target.

RPS4Y1, a member of the ribosomal protein S4E family, encodes ribosomal protein S4, a component of the 40S subunit. *RPS4Y1* expression is often upregulated in human endothelial cells and associated with endothelial dysfunction, crucial in the pathogenesis of coronary artery disease.²² *RPS4Y1* overexpression disrupts normal endothelial cell functions, including migration and neovascularization, whereas inhibition enhances endothelial cell performance. Additionally, *RPS4Y1* may induce mitochondria-dependent apoptosis, reducing cell viability via increased mitochondrial depolarization. Conversely, *RPS4Y1* may also induce cell death by modulating proinflammatory cytokines such as interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , and IL-8.²³ Further validation and exploration of its functions and mechanisms in appropriate cardiac models are warranted.

Previous studies have attempted to predict patient outcomes following AMI based on clinical

data.²⁴⁻²⁷ However, these studies exhibited several deficiencies. First, they lacked consideration for genomics, which represents the future of medical decision-making. Second, their predictive efficacy diminishes outside the model development phase, with most lacking external validation; they were often constructed retrospectively, potentially limiting real-world application. Finally, they typically failed to compare other models to evaluate prediction robustness. Despite incorporating only three clinical factors, our model outperformed previous models in C-index, IDI, and INR values, affirming its enhanced stability and predictive accuracy.

Our study had some limitations. First, RNA detection may degrade, particularly with repeated freeze-thaw cycles. Second, RNA detection is not a one-time process and may be subject to batch effects. Third, our study had a relatively small number of patients, especially in the external validation cohort. Finally, data were collected from only two centers, warranting further evaluation of our predictive model with additional cases from different centers.

CONCLUSION

Our study introduced a novel gene-based model using qPCR data that effectively predicted patient prognosis following AMI. Notably, the feasibility and reproducibility of our model were validated across multiple centers. The prognostic capability of our model holds promise for optimizing early management strategies for patients with AMI. This model provides a foundation for future investigations into drug target identification, biomarker discovery, and mechanistic exploration in AMI.

ABBREVIATIONS

acute myocardial infarction (AMI)
continuous net reclassification improvement (c-NRI)
differentially expressed genes (DEGs)

352 Enzyme-linked immunosorbent assay (ELISA)
353 fatty acid desaturase gene family (FADS)
354 least absolute shrinkage and selection operator regression analysis (LASSO)
355 integrated discrimination improvement (IDI)
356 major adverse cardiovascular events (MACE)
357 N-terminal pro b-type natriuretic peptide (NT-proBNP)
358 New York Heart Association (NYHA)
359

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University and Hangzhou First People's Hospital (No. 2021-SR-066). Written informed consent was obtained from all participants prior to their enrollment in the study.

Consent for publication

All participants were fully informed about the study. All authors have reviewed and approved the final manuscript and consent to its publication.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy restrictions but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Contributions

QG contributed to the conception and design of the study. QG and QQ performed the statistical analysis. QG wrote the first draft of the manuscript. All authors contributed to the manuscript revisions and read and approved the submitted version.

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Figure Legends

Figure 1: Flowchart depicting the study methodology. LASSO: least absolute shrinkage and selection operator; AUC: area under the curve of the receiver operating characteristic curve.

Figure 2: A–C: Bioinformatics analysis and validation. A: Heatmap illustrating the results of the differential analysis of GSE59867; B: Volcano plot displaying the results of GSE59867; C: Validation of the expression of pivotal genes from GSE59867 using the GSE123342 dataset. D–E: Results of machine learning analysis on GSE59867 dataset, depicting the process of selecting hub genes using LASSO regression; F: Area under the ROC curve corresponding to the model. LASSO: least absolute shrinkage and selection operator; ROC: receiver operating characteristic.

Figure 3: Restricted cubic spline selection of cutoff values for gene expression. A: Selection of cutoff values for *TMEM176A*; B: Selection of cutoff values for *RPS4Y1*; C: Selection of cutoff values for *FADS2*; D: Selection of cutoff values for *FMN1*.

Figure 4: Visualization of the model. A: Nomograph constructed using cutoff values of the three genes; B: Nomograph constructed using quartiles of the three genes; C: Nomograph constructed using raw data.

Figure 5: Visual evaluation of the three models. A: Calibration plot for cutoff value modeling; B: Calibration plot for quartile modeling; C: Calibration plot for raw data modeling; D: Net benefit plot for cutoff value modeling; E: Net benefit plot for quartile modeling; F: Net benefit plot for raw data modeling.

489 Figure 6: Log-rank results of gene expression. A: Intergroup comparison results of *TMEM176A*;
490 B: Intergroup comparison results of *RPS4Y1*; C: Intergroup comparison results of *FADS2*; D:
491 Intergroup comparison results of *FMNI*.

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493 Supplemental Figure 1: A: NRI results for cutoff value modeling; B: NRI results for quartile
494 modeling; C: NRI results for raw data modeling; D: IDI results for cutoff value modeling; E: IDI
495 results for quartile modeling; F: IDI results for raw data modeling. NRI: net reclassification
496 improvement; IDI: integrated discrimination improvement

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498 Supplemental Figure 2: Forest plots of subgroup analyses. A: Forest plot of subgroup analyses of
499 *TMEM176A*; B: Forest plot of subgroup analyses of *FADS2*; C: Forest plot of subgroup analyses
500 of *RPS4Y1*.

Table 1

Model training set	Total	Non-MACE	MACE	p-value
	283	228	55	
Baseline characteristics				
Sex (n, %)	226(77.7)	191(83.8)	35(65.5)	0.113
Age (n, %)	63±13	62±13	66±12	0.0076
Hypertension (n, %)	187(66.1)	146(64)	41(74.5)	0.092
Diabetes (n, %)	101(35.7)	78(34.3)	23(41.8)	0.184
Smoke (n, %)	109(38.5)	94(41.2)	15(27.3)	0.038
Alcohol (n, %)	63(22.3)	56(24.6)	7(12.7)	0.039
Hyperlipemia (n, %)	19(93.3)	13(5.7)	6(10.9)	0.478
PCI (n, %)	208(73.5)	163(71.5)	45(81.8)	0.293
BMI (kg/m ²)	37±12.2	37±14.7	36±3.5	0.521
SBP (mmHg)	130±42	131±46	126±18	0.456
DBP (mmHg)	76±12	77±12	73±11	0.009
NY				0.251
I	80(28.3)	69(30.3)	11(20)	
II	128(45.2)	96(42.1)	32(58.2)	
III	31(11)	26(11.4)	5(9.1)	
IV	3(1.1)	2(0.9)	1(1.8)	
Laboratory examination				
HbA1c (%)	6.54±1.52	6.51±1.54	6.69±1.44	0.492
TNT (ng/mL)	927±1455	850±1396	1248±1655	0.023
Myoglobin	83±136	78±133	101±150	0.273
CKMB (ng/mL)	36±63	37±68	31±51	0.483
NT-proBNP (ng/L)	4047±7421	3515±6647	6256±9787	0.014
ALT (U/L)	37±58	37±59	38±51	0.978
AST (U/L)	68±105	70±113	59±61	0.47
ALP (IU/L)	89±48	87±41	99±71	0.091
LDH (U/mg)	375±249	369±253	400±234	0.413
CK (ng/mL)	504±758	513±780	467±661	0.686
TBIL (μmol/L)	16.4±13.8	16.3±14	16.5±13.4	0.939
DBIL (μmol/L)	5.8±3.3	5.8±3.4	5.4±2.8	0.412
IBIL (μmol/L)	9.4±4.3	9.6±4.4	8.9±4.1	0.319
TCHOL (mmol/L)	4.1±4.28	4.1±1.22	4.1±1.16	0.626
TG (mmol/L)	1.7±1.2	1.7±1.3	1.5±0.8	0.184
HDL (mmol/L)	1±0.2	0.98±0.24	1.05±0.27	0.072
LDL (mmol/L)	2.5±0.8	2.6±0.86	2.4±0.73	0.244
LPa (mmol/L)	280±277	266±272	340±288	0.074

Glu (mmol/L)	6.3±2.8	6.1±2.5	7±3.6	0.035
BUN (mmol/L)	7.2±4.3	6.9±3.9	8.5±5.6	0.013
SCR (mmol/L)	106±122	101±114	127±155	0.169
UA (mmol/L)	360±118	359±106	363±163	0.819
Ca (mmol/L)	2.2±0.18	2.2±0.19	2.2±0.13	0.669
P (mmol/L)	1.1±0.29	1.1±0.27	1.2±0.33	0.141
Mg (mmol/L)	0.87±0.1	0.87±0.1	0.88±0.1	0.711
K (mmol/L)	3.7±0.4	3.7±0.38	3.8±0.46	0.363
Na (mmol/L)	140±3	140±3	141±4	0.202
Cl (mmol/L)	104±3.6	104±3.4	104±4.2	0.818
PT (s)	12±1.6	12±1.6	13±1.6	0.009
PTINR	1.3±2.3	1.3±2.5	1.3±1.4	0.816
APTT (s)	29±11	30±12	28±5	0.459
FIB (g/L)	6.7±31.8	7.3±35.4	4.4±3.2	0.552
TT (s)	18.8±14.6	19.3±16	17.3±5	0.368
DD2 (mg/L)	0.9±1.9	0.7±1.5	1.7±3	0.001
WBC (×10 ⁹ /L)	8.2±2.9	8.3±2.78	8.14±3.33	0.772
N (×10 ⁹ /L)	5.6±2.8	5.6±2.6	5.6±3.5	0.923
N (%)	66±15	66±14	67±20	0.973
RBC (×10 ¹² /L)	4.3±0.7	4.4±0.7	4±0.8	0.001
Hb (g/L)	132±21	135±20	122±23	<0.0001
PLT (×10 ⁹ /L)	202±77	206±79	189±68	0.156
Echocardiography				
EF (%)	56±10	56±10	53±11	0.012
FS (%)	30±6	30±6	28±7	0.006
LVDs (mm)	36±7.6	36±7	37±9	0.18
LVDd (mm)	59±59	58±59	65±59	0.442
Drug				
Aspirin	259(91.5)	210(92.1)	49(89.1)	0.313
Clopidogrel	129(45.6)	98(43)	31(56.4)	0.051
Tigrillo	175(61.8)	143(62.7)	32(58.2)	0.318
ARNI	113(39.9)	87(38.2)	26(47.3)	0.139
ARB/ACEI	30(10.6)	24(10.5)	6(10.9)	0.548
β-blockers	214(75.6)	166(72.8)	48(87.3)	0.016
Tirofiban	21(7.4)	16(7)	5(9.1)	0.387
Heparin	154(54.4)	120(52.6)	34(61.8)	0.141
Oral anticoagulant	27(9.5)	19(8.3)	8(14.5)	0.126
Statin	278(98.2)	223(97.8)	52(100)	0.337

PCSK9 inhibitors	46(16.3)	39(17.1)	7(12.7)	0.286
HbA1c, glycated hemoglobin; TNI, troponin I; TNT, troponin T; Mb, myoglobin; CKMB, creatine kinase isoenzyme; NT-proBNP, B-type natriuretic peptide precursor; ALT, glutamate aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CK, creatine kinase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TCHOL, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LP(a), lipoprotein a; GLU, glucose; BUN, urea nitrogen; SCR, blood creatinine; UA, uric acid; Ca, calcium; P, phosphorus; Mg, magnesium; K, potassium; Na, sodium; Cl, chloride; PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; FIB, fibrinogen; TT, prothrombin time; DD2, D-dimer; WBC, white blood cell; N, neutrophil; RBC, red blood cell; Hb, hemoglobin; Plt, platelet; EF, ejection fraction; FS, short-axis systolic rate; LVDs, left ventricular systolic internal diameter; LVDd, left ventricular diastolic internal diameter.				

Table 2

Model validation set	Total	Non-MACE	MACE	p-value
	99	74	25	
Baseline characteristics				
Sex (n, %)	99(77.8)	191(83.8)	35(65.5)	0.092
Age (n, %)	65±17	65±16	66±19	0.884
Hypertension (n, %)	66(66.7)	146(64)	41(74.5)	0.185
Diabetes (n, %)	32(32.3)	78(34.3)	23(41.8)	0.219
Smoke (n, %)	41(41.4)	94(41.2)	15(27.3)	0.666
Alcohol (n, %)	16(16.2)	56(24.6)	7(12.7)	0.714
Hyperlipemia (n, %)	29(29.3)	13(5.7)	6(10.9)	0.344
PCI (n, %)	74(74.7)	163(71.5)	45(81.8)	0.256
BMI (kg/m ²)	24.7±2.5	24.6±2.3	25.1±3	0.316
SBP (mmHg)	130±42	131±46	126±18	0.456
DBP (mmHg)	76±12	77±12	75±11	0.534
NY				0.289
I	67(67.7)	69(30.3)	11(20)	
II	14(14.1)	96(42.1)	32(58.2)	
III	10(10.1)	26(11.4)	5(9.1)	
IV	8(8.1)	2(0.9)	1(1.8)	
Laboratory examination				
HbA1c (%)	6.21±1.25	6.22±1.16	6.18±1.5	0.867
TNT (ng/mL)	12.9±22.2	850±1396	1248±1655	0.546
Myoglobin	83±136	78±133	101±150	0.273
CKMB (ng/mL)	63±104	73±116	32±49	0.09
NT-proBNP (ng/L)	3273±6375	3100±6235	3783±6878	0.646
CRP (mg/L)	21±37	23±40	17±27	0.531
ALT(U/L)	48±84	51±96	38±29	0.5
AST(U/L)	141±245	158±274	90±115	0.231
ALP (IU/L)	72±22	75±23	64±16	0.035
LDH (U/mg)	529±539	547±574	478±426	0.58
CK (ng/mL)	919±1573	1046±1743	543±819	0.169
TBIL (μmol/L)	14.6±7.9	14.8±7.6	14±8.6	0.673
DBIL (μmol/L)	4.9±2.5	5±2.5	4.7±2.6	0.662
IBIL (μmol/L)	9.7±5.7	9.8±5.5	9.2±6.2	0.679
TCHOL (mmol/L)	4.3±1.1	4.3±1.15	4.1±1	0.311
TG (mmol/L)	1.9±1.8	1.9±2	1.7±0.8	0.577
HDL (mmol/L)	0.99±0.2	0.99±0.25	0.97±0.22	0.707
LDL (mmol/L)	0.98±0.25	2.7±1.5	2.6±1.7	0.749

LPa (mmol/L)	31±25	30±22	33±31	0.631
Glu (mmol/L)	6.6±2.8	6.1±2.5	7±3.6	0.335
BUN (mmol/L)		6.9±3.9	6.2±2.4	0.013
SCR (mmol/L)	118±90	116±88	125±98	0.662
UA (mmol/L)	375±133	372±119	385±171	0.684
Ca (mmol/L)	2.2±0.14	2.2±0.14	2.2±0.14	0.489
P (mmol/L)	1.1±0.28	1.1±0.27	1.1±0.29	0.53
Mg (mmol/L)	0.86±0.1	0.87±0.1	0.84±0.1	0.23
K (mmol/L)	4.05±0.6	3.7±0.38	3.8±0.46	0.714
Na (mmol/L)	151±100	141±3	141±2	0.969
Cl (mmol/L)	105±3.7	105±3.6	105±4.3	0.816
PT (s)	13±9.7	13±11	12±1	0.606
PTINR	1.1±0.1	1.1±0.1	1.1±0.1	0.527
APTT (s)	32±16	33±18	28±3	0.197
FIB (g/L)	4±1.5	4.1±1.6	3.8±1.1	0.421
TT (s)	17±6.2	17±7	16±1	0.473
DD2 (mg/L)	1010±1656	891±1177	1362±2610	0.22
WBC (×10 ⁹ /L)	8.8±3.4	9.1±3.6	7.9±2.4	0.136
N (×10 ⁹ /L)	6.4±3.1	6.7±3.3	5.7±2.2	0.194
N (%)	71±12	71±12	71±11	0.99
RBC (×10 ¹² /L)	4.1±0.9	4.2±0.9	3.8±0.9	0.089
Hb (g/L)	124±29	126±29	119±29	0.273
PLT (×10 ⁹ /L)	224±89	219±68	239±133	0.348
Echocardiography				
EF (%)	58±12	58±12	59±10	0.718
FS (%)	32±7	32±7	33±7	0.644
LVDs (mm)	35±69	35±69	33±68	0.307
LVDd (mm)	51±62	51±59	49±68	0.178
Drug				
Aspirin	93(93.9)	210(92.1)	49(89.1)	0.525
Clopidogrel	53(53.5)	98(43)	31(56.4)	0.837
Tigrallo	43(43.4)	143(62.7)	32(58.2)	0.436
ARNI	31(31.3)	87(38.2)	26(47.3)	0.257
ARB/ACEI	39(39.4)	24(10.5)	6(10.9)	0.562
β-blockers	81(81.8)	166(72.8)	48(87.3)	0.106
Tirofiban	24(24.2)	16(7)	5(9.1)	0.602
Heparin	93(93.9)	120(52.6)	34(61.8)	0.475
Oral anticoagulant	90(90.9)	19(8.3)	8(14.5)	0.593

Statin	97(98)	223(97.8)	52(100)	0.062
PCSK9 inhibitors	21(21.2)	39(17.1)	7(12.7)	0.555

HbA1c, glycated hemoglobin; TNI, troponin I; TNT, troponin T; Mb, myoglobin; CKMB, creatine kinase isoenzyme; NT-proBNP, B-type natriuretic peptide precursor; ALT, glutamate aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CK, creatine kinase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TCHOL, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LP(a), lipoprotein a; GLU, glucose; BUN, urea nitrogen; SCR, blood creatinine; UA, uric acid; Ca, calcium; P, phosphorus; Mg, magnesium; K, potassium; Na, sodium; Cl, chloride; PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; FIB, fibrinogen; TT, prothrombin time; DD2, D-dimer; WBC, white blood cell; N, neutrophil; RBC, red blood cell; Hb, hemoglobin; Plt, platelet; EF, ejection fraction; FS, short-axis systolic rate; LVDs, left ventricular systolic internal diameter; LVDd, left ventricular diastolic internal diameter.

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Table 3

Model	C-statistic	c-NRI (95% CI)	IDI (95% CI)	p-value
Cutoff value model	0.76	0.42 (0.095–0.654)	0.165 (0.041–0.272)	0.008
Quartile model	0.83	0.69 (0.35–0.86)	0.239 (0.16–0.382)	<0.001
Raw data model	0.84	0.724 (0.2–0.88)	0.257 (0.117–0.35)	<0.001

CI, confidence interval; IDI, integrated discrimination improvement; NRI, continuous net reclassification improvement.

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Table 4

	Model 1	p-value	Model 2	p-value	Model 3	p-value
ELISA						
<i>FADS2</i>	0.519(0.411–0.654)	<0.0001	0.514(0.232–1.4)	<0.0001	0.54(0.416–0.7)	<0.0001
<i>FMN1</i>	0.44(0.227–0.856)	0.016	0.408(0.208–0.802)	0.009	0.38(0.18–0.803)	0.011
<i>TMEM176A</i>	2.513(1.823–3.465)	<0.0001	2.718(1.886–3.918)	<0.0001	2.776(1.853–4.158)	<0.0001
<i>RPS4Y1</i>	0.25(0.112–0.558)	0.001	0.26(0.113–0.596)	0.01	0.168(0.066–0.432)	<0.0001
PCR						
<i>FADS2</i>	1.458(1.239–1.716)	<0.0001	1.508(1.27–1.79)	<0.0001	1.517(1.257–1.83)	<0.0001
<i>FMN1</i>	0.929(0.755–1.141)	0.482	0.987(0.796–1.223)	0.904	0.93(0.73–1.185)	0.559
<i>TMEM176A</i>	0.646(0.556–0.749)	<0.0001	0.681(0.588–0.789)	<0.0001	0.612(0.505–0.74)	<0.0001
<i>RPS4Y1</i>	1.29(1.158–1.437)	<0.0001	1.259(1.046–1.441)	0.001	1.268(1.099–1.464)	0.001
Model 1 was unadjusted; model 2 was adjusted for age, sex, hypertension, hyperlipidemia, hyperglycemia, and smoking based on model 1; model 3 was also adjusted for blood creatinine, hemoglobin, HDL, LDL, troponin, BNP, medication use, cardiac systolic function, and cardiac short-axis contractility based on model 2						

Table 5

	Model 1	p-value	Model 2	p-value	Model 3	p-value
ELISA						
<i>FADS2</i>		0.005		0.001		0.001
Q1	1		1		1	
Q2	0.188(0.082–0.432)	<0.0001	0.189(0.081–0.443)	<0.0001	0.182(0.074–0.447)	<0.0001
Q3	0.165(0.071–0.382)	<0.0001	0.151(0.064–0.356)	<0.0001	0.175(0.071–0.431)	<0.0001
Q4	0.326(0.163–0.653)	0.002	0.282(0.14–0.568)	<0.0001	0.234(0.108–0.51)	<0.0001
<i>FMN1</i>		0.133		0.092		0.119
Q1	1		1		1	
Q2	0.391(0.185–0.825)	0.014	0.311(0.144–0.673)	0.003	0.321(0.144–0.716)	0.005
Q3	0.461(0.219–0.971)	0.041	0.4(0.188–0.847)	0.017	0.319(0.14–0.722)	0.006
Q4	0.536(0.254–1.129)	0.101	0.501(0.231–1.084)	0.079	0.647(0.279–1.499)	0.31
<i>TMEM176A</i>		<0.0001		<0.0001		0.001
Q1	1		1		1	
Q2	1.295(0.484–3.467)	0.607	1.228(0.456–3.305)	0.685	0.811(0.29–2.274)	0.691
Q3	1.35(0.516–3.529)	0.541	1.338(0.496–3.612)	0.565	0.943(0.344–2.581)	0.909
Q4	5.086(2.284–11.328)	<0.0001	4.505(1.977–10.264)	<0.0001	3.384(1.434–7.982)	0.005
<i>RPS4Y1</i>		0.001		0.002		<0.0001
Q1	1		1		1	
Q2	0.826(0.445–1.531)	0.543	0.892(0.468–1.699)	0.727	0.754(0.381–1.49)	0.416
Q3	0.549(0.259–1.161)	0.117	0.555(0.258–1.194)	0.132	0.52(0.235–1.152)	0.107
Q4	0.158(0.047–0.53)	0.003	0.182(0.054–0.615)	0.006	0.102(0.027–0.388)	0.001
PCR						
<i>FADS2</i>		<0.0001		<0.0001		<0.0001
Q1	1		1		1	
Q2	5.183(1.489–18.041)	0.01	5.304(1.513–18.593)	0.009	4.52(1.255–16.275)	0.021
Q3	3.697(1.107–13.437)	0.047	4.149(1.121–15.36)	0.033	3.173(0.811–12.414)	0.097
Q4	12.296(3.729–40.547)	<0.0001	14.812(4.398–49.889)	<0.0001	13.95(3.903–49.856)	<0.0001
<i>FMN1</i>		0.884		0.751		0.958
Q1	1		1		1	
Q2	1.127(0.527–2.409)	0.758	1.096(0.511–2.354)	0.814	1.049(0.468–2.354)	0.907
Q3	0.997(0.46–2.158)	0.993	0.975(0.444–2.143)	0.95	0.817(0.36–1.854)	0.629
Q4	0.989(0.456–2.142)	0.977	1.197(0.542–2.643)	0.656	1.073(0.475–2.424)	0.865

<i>TMEM176A</i>		<0.0001		<0.0001		<0.0001
Q1	1		1		1	
Q2	0.271(0.139–0.529)	<0.0001	0.286(0.144–0.567)	<0.0001	0.229(0.109–0.48)	<0.0001
Q3	0.15(0.066–3.434)	<0.0001	0.153(0.066–0.35)	<0.0001	0.13(0.053–0.318)	<0.0001
Q4	0.056(0.017–0.184)	<0.0001	0.056(0.017–0.185)	0.027	0.057(0.017–0.193)	<0.0001
<i>RPS4Y1</i>		<0.0001		0.005		0.009
Q1	1		1		1	
Q2	1.476(0.571–3.811)	0.421	1.44(0.547–3.79)	0.46	1.684(0.625–4.54)	0.303
Q3	0.777(0.271–2.222)	0.637	0.743(0.254–2.168)	0.586	0.497(0.157–1.575)	0.235
Q4	4.061(1.78–9.26)	0.001	3.62(1.519–8.632)	0.004	4.627(1.777–12.05)	0.002
Model 1 was unadjusted; Model 2 was adjusted for age, sex, hypertension, hyperlipidemia, hyperglycemia and smoking based on model 1; Model 3 was also adjusted for blood creatinine, hemoglobin, HDL, LDL, troponin, BNP, medication use, cardiac systolic function, and cardiac short-axis contractility based on model 2.						

Supplementary Table 1

Gene	3'sequence	5'sequence
<i>GAPDH</i>	CAACGAATTTGGCTACAGCA	AGGGGAGATTCAAGTGTGGTG
<i>TMEM176A</i>	CACCTCCTCGTCACCTC	CCAGCGTTAGCAGAGTCCT
<i>FADS2</i>	AGCCTCCTTTTCCTCAAC	GGCCTCCTGGTCAATCTC
<i>FMN1</i>	CGTGGGTTCTGGGCTAC	GCTGTGGTCAGGATGGAG
<i>RPS4Y1</i>	GCACCGGAAAAGAACAGA	TGAGGAAGACGATCAGAGG

Supplementary Table 2

Variable	Crude Model	p-value
Baseline characteristics		
Sex (n, %)	0.424(0.243–0.74)	0.003
Age (n, %)	1.02(0.999–1.042)	0.063
Hypertension (n, %)	1.717(0.934–3.155)	0.082
Diabetes (n, %)	1.316(0.77–2.249)	0.315
Smoke (n, %)	0.58(0.32–1.051)	0.072
Alcohol (n, %)	0.525(0.237–1.163)	0.112
Hyperlipemia (n, %)	2.45(1.042–5.763)	0.04
PCI (n, %)	1.597(0.781–3.267)	0.2
BMI (kg/m ²)		
SBP (mmHg)		
DBP (mmHg)		
NY	1.289(0.869–1.913)	0.207
Laboratory examination		
HbA1c (%)		
TNT (ng/mL)	1.483(1.046–2.101)	0.027

Myoglobin	1.001(0.999–1.002)	0.262
CKMB (ng/mL)	0.998(0.993–1.003)	0.394
NT-proBNP (ng/L)	2.132(1.421–3.22)	<0.0001
ALT (U/L)	1.001(0.997–1.005)	0.653
AST (U/L)	0.999(0.995–1.002)	0.452
ALP (IU/L)	1.006(1.002–1.01)	0.003
LDH (U/mg)	1(0.999–1.001)	0.489
CK (ng/mL)	1(1–1.001)	0.86
TBIL (μmol/L)	1.006(0.989–1.024)	0.489
DBIL (μmol/L)	0.986(0.905–1.074)	0.744
IBIL (μmol/L)	0.975(0.913–1.04)	0.439
TCHOL (mmol/L)	0.949(0.753–1.195)	0.656
TG (mmol/L)	0.817(0.59–1.132)	0.224
HDL (mmol/L)	2.558(0.944–6.934)	0.065
LDL (mmol/L)	0.817(0.58–1.15)	0.246
LPa (mmol/L)	1.001(1–1.002)	0.135
Glu (mmol/L)	1.084(1.004–1.171)	0.039
BUN (mmol/L)	1.078(1.06–1.123)	<0.0001
SCR (mmol/L)	1.002(1–1.003)	0.024
UA (mmol/L)	1.001(0.999–1.003)	0.416
Ca (mmol/L)	0.767(0.234–2.51)	0.661
P (mmol/L)	2.582(1.118–5.964)	0.026
Mg (mmol/L)	2.798(0.195–40.142)	0.449
K (mmol/L)	1.372(0.686–2.747)	0.371
Na (mmol/L)	1.053(0.96–1.155)	0.271
Cl (mmol/L)	1.005(0.932–1.084)	0.887
PT (s)	1.237(1.083–1.414)	0.002
PTINR	0.977(0.867–1.103)	0.711
APTT (s)	0.989(0.951–1.028)	0.577
FIB (g/L)	0.996(0.981–1.012)	0.635
TT (s)	0.986(0.949–1.025)	0.485
DD2 (mg/L)	1.156(1.072–1.246)	<0.0001
WBC (×10 ⁹ /L)	0.99(0.901–1.088)	0.84
N (×10 ⁹ /L)	1.004(0.986–1.023)	0.651
N (%)	1.013(0.922–1.112)	0.793
RBC(×10 ¹² /L)	0.486(0.341–0.694)	<0.0001
Hb (g/L)	0.973(0.962–0.985)	<0.0001
PLT (×10 ⁹ /L)	0.997(0.993–1.001)	0.152
Echocardiography		
EF (%)	0.969(0.947–0.991)	0.007
FS (%)	0.946(0.912–0.982)	0.004
LVDs (mm)	1.027(0.995–1.06)	0.098
LVDd (mm)	1.001(0.998–1.004)	0.471
Drug		

Aspirin	0.698(0.299–1.631)	0.406
Clopidogrel	1.562(0.917–2.662)	0.101
Tigrallo	0.902(0.527–1.541)	0.705
ARNI	1.381(0.811–2.351)	0.234
ARB/ACEI	0.773(0.328–1.823)	0.556
β-blockers	2.717(1.226–6.021)	0.014
Tirofiban	1.1(0.436–2.777)	0.84
Heparin	1.35(0.782–2.329)	0.281
Oral anticoagulant	2.16(1.012–4.61)	0.046
Statin	20.835(0.008–56224)	0.451
PCSK9 inhibitors	0.955(0.43–2.121)	0.909

HbA1c, glycated hemoglobin; TNI, troponin I; TNT, troponin T; Mb, myoglobin; CKMB, creatine kinase isoenzyme; NT-proBNP, B-type natriuretic peptide precursor; ALT, glutamate aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CK, creatine kinase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TCHOL, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LP(a), lipoprotein a; GLU, glucose; BUN, urea nitrogen; SCR, blood creatinine; UA, uric acid; Ca, calcium; P, phosphorus; Mg, magnesium; K, potassium; Na, sodium; Cl, chloride; PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; FIB, fibrinogen; TT, prothrombin time; DD2, D-dimer; WBC, white blood cell; N, neutrophil; RBC, red blood cell; Hb, hemoglobin; Plt, platelet; EF, ejection fraction; FS, short-axis systolic rate; LVDs, left ventricular systolic internal diameter; LVDd, left ventricular diastolic internal diameter.

Supplementary Table 3

	Model training set	Model validation set	p-value
ELISA			
<i>TMEM176A</i>	633±303	1134±780	<0.0001
<i>RPS4Y1</i>	25±81	8±7	<0.0001
<i>FADS2</i>	65016±43727	80740±85229	<0.0001
<i>FMN1</i>	169360±716263	81050±9339	0.165
qPCR			
<i>TMEM176A</i>	6.9±1.7	6.3±1.6	0.051
<i>RPS4Y1</i>	12.2±3.5	12.2±3.2	0.903
<i>FADS2</i>	6.6±1.9	7.1±1.9	0.823
<i>FMN1</i>	6.3±1.2	6.4±1.3	0.714
ELISA, enzyme-linked immunosorbent assay; qPCR: quantitative polymerase chain reaction.			

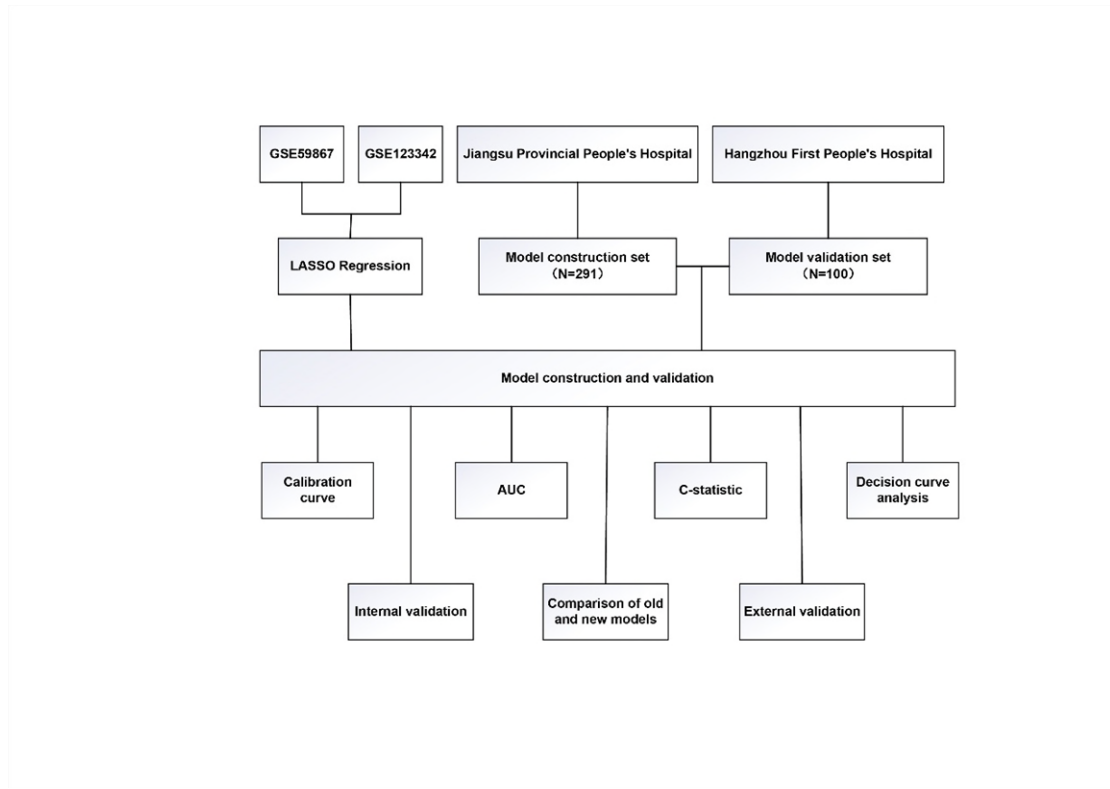


Figure 1: Flowchart depicting the study methodology. LASSO: least absolute shrinkage and selection operator; AUC: area under the curve of the receiver operating characteristic curve.

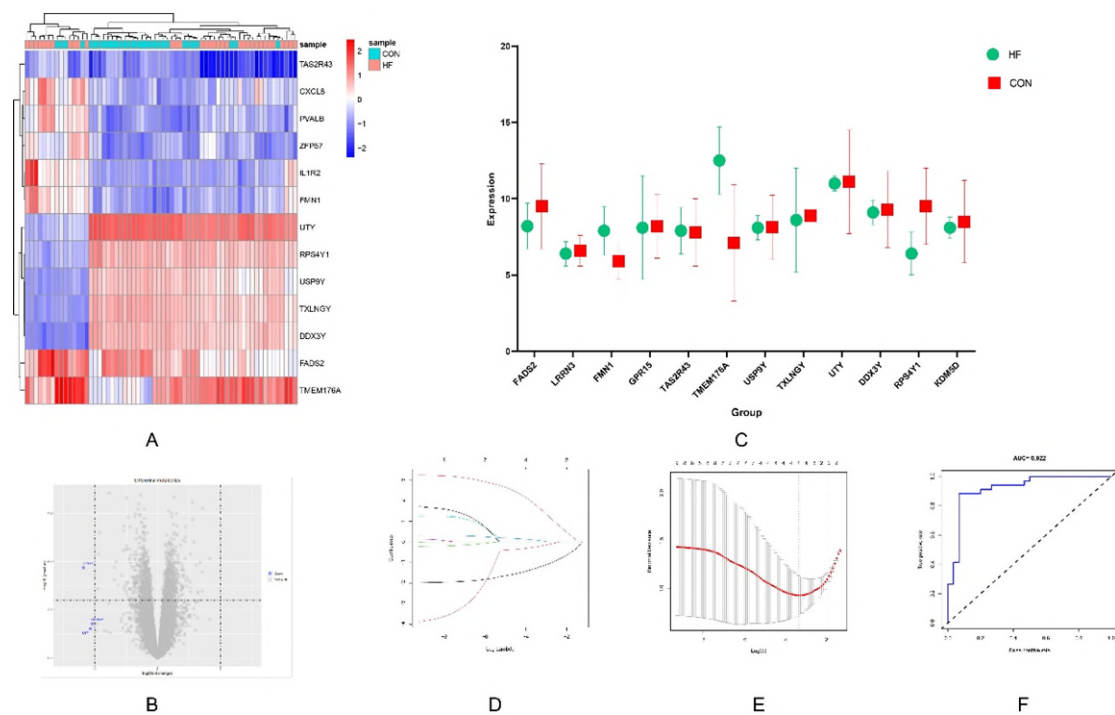


Figure 2: A–C: Bioinformatics analysis and validation. A: Heatmap illustrating the results of the differential analysis of GSE59867; B: Volcano plot displaying the results of GSE59867; C: Validation of the expression of pivotal genes from GSE59867 using the GSE123342 dataset. D–E: Results of machine learning analysis on GSE59867 dataset, depicting the process of selecting hub genes using LASSO regression; F: Area under the ROC curve corresponding to the model. LASSO: least absolute shrinkage and selection operator; ROC: receiver operating characteristic.

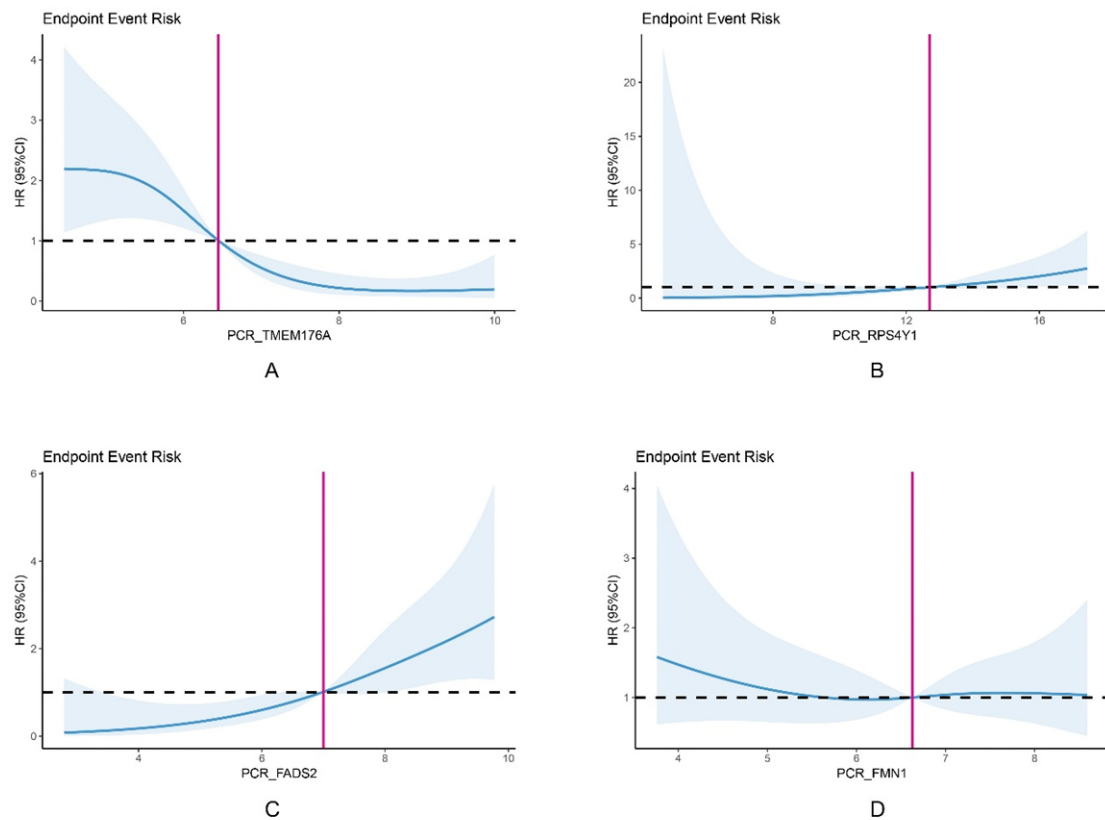


Figure 3: Restricted cubic spline selection of cutoff values for gene expression. A: Selection of cutoff values for *TMEM176A*; B: Selection of cutoff values for *RPS4Y1*; C: Selection of cutoff values for *FADS2*; D: Selection of cutoff values for *FMN1*.

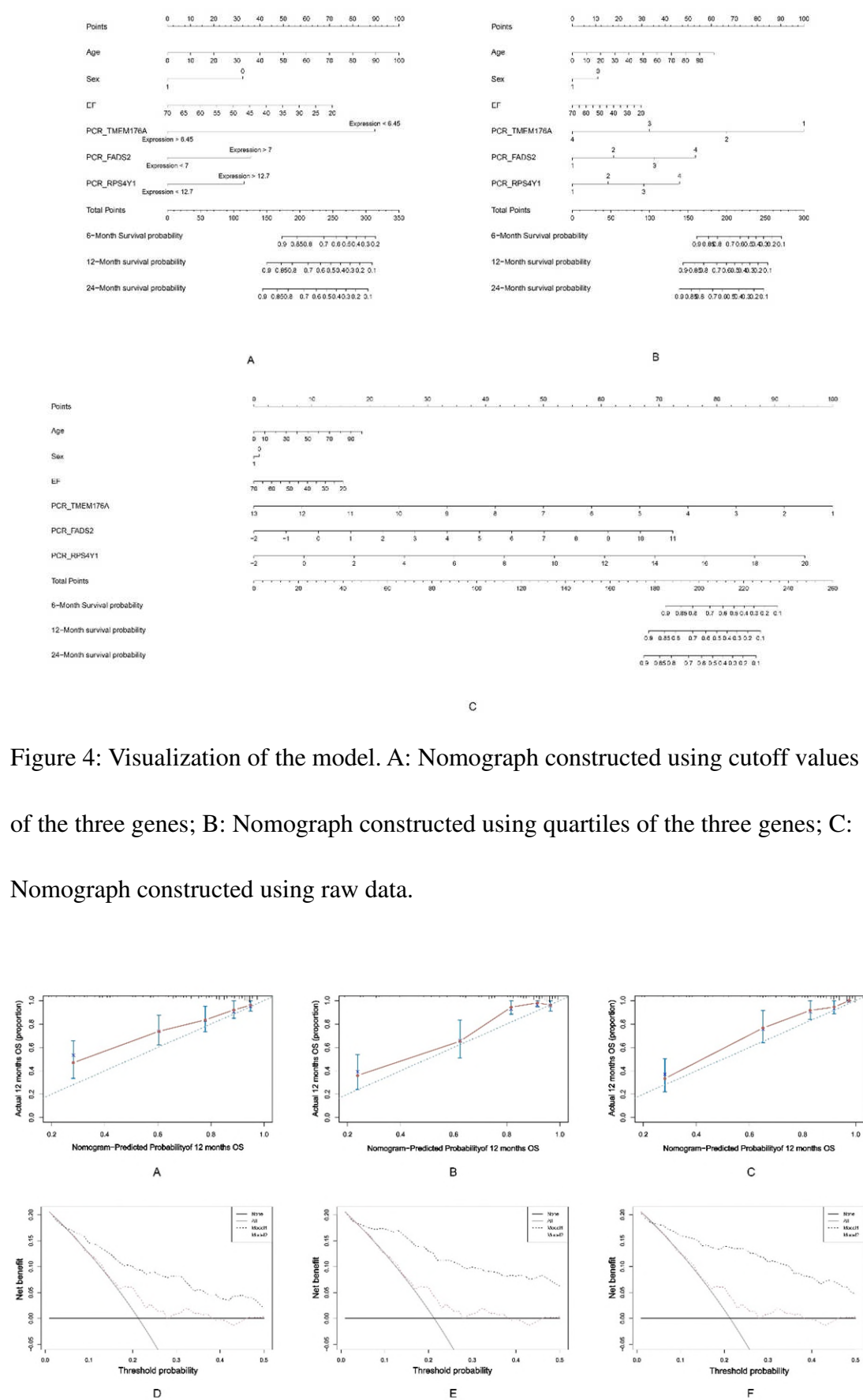


Figure 5: Visual evaluation of the three models. A: Calibration plot for cutoff value modeling; B: Calibration plot for quartile modeling; C: Calibration plot for raw data modeling; D: Net benefit plot for cutoff value modeling; E: Net benefit plot for quartile modeling; F: Net benefit plot for raw data modeling.

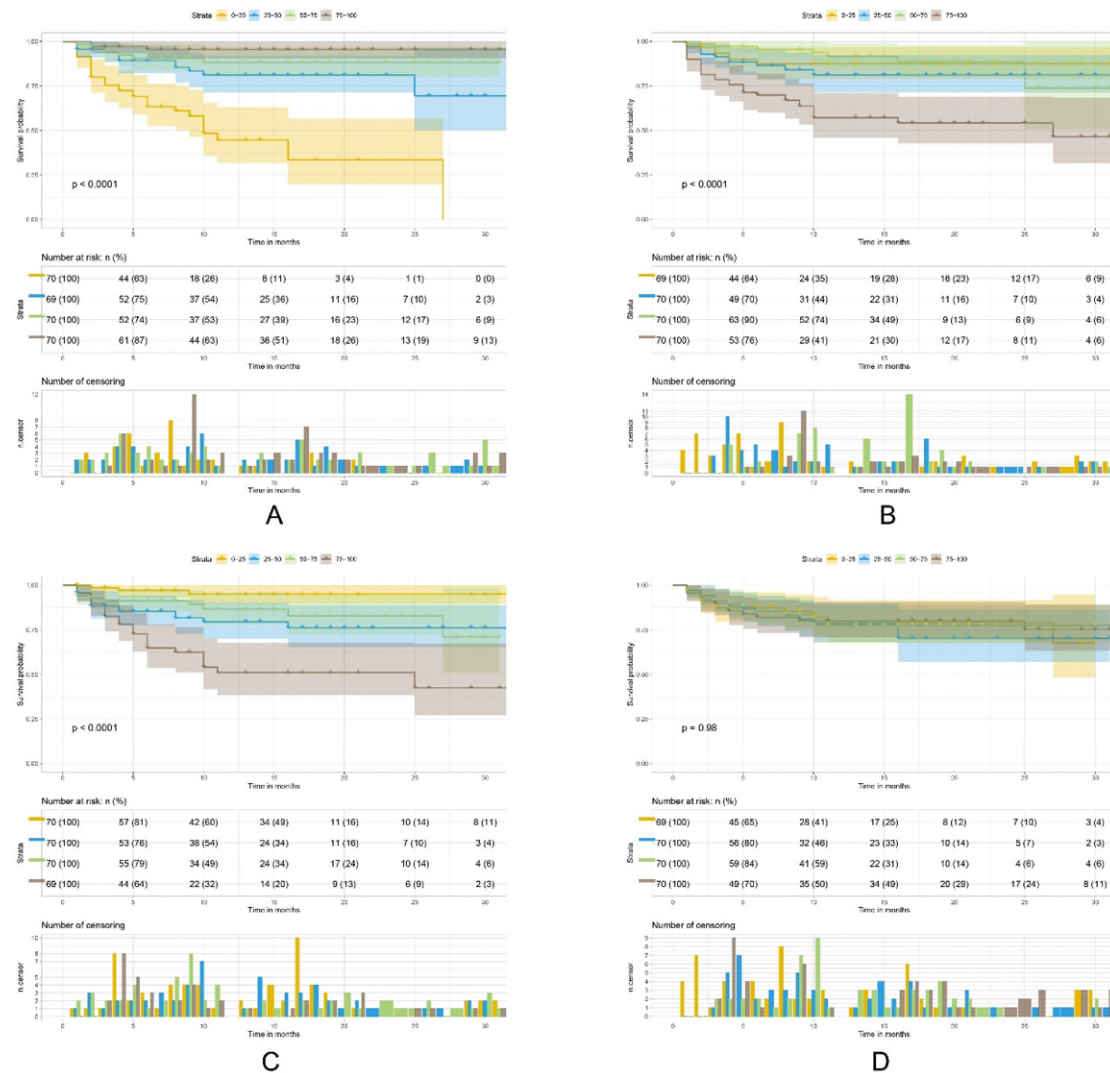
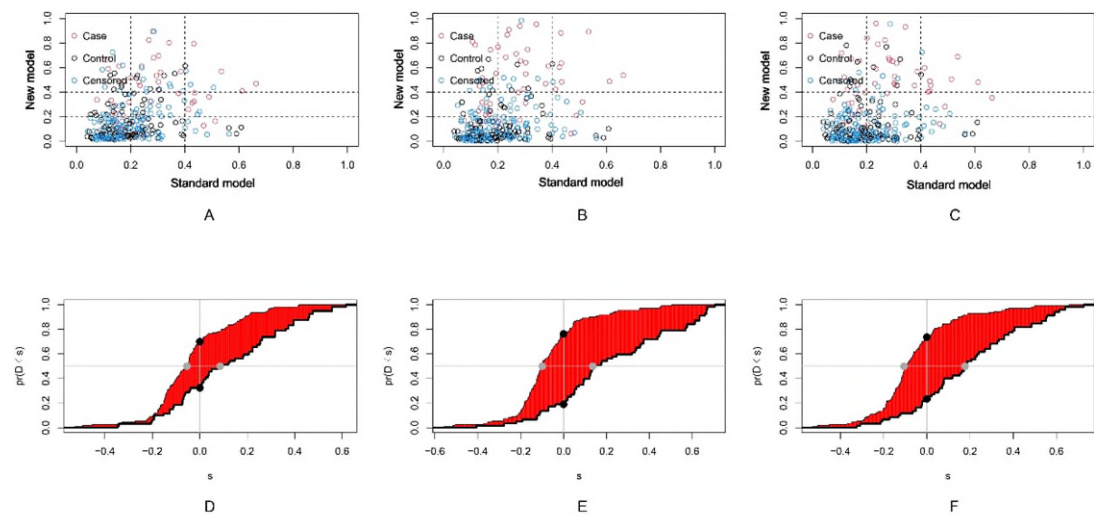
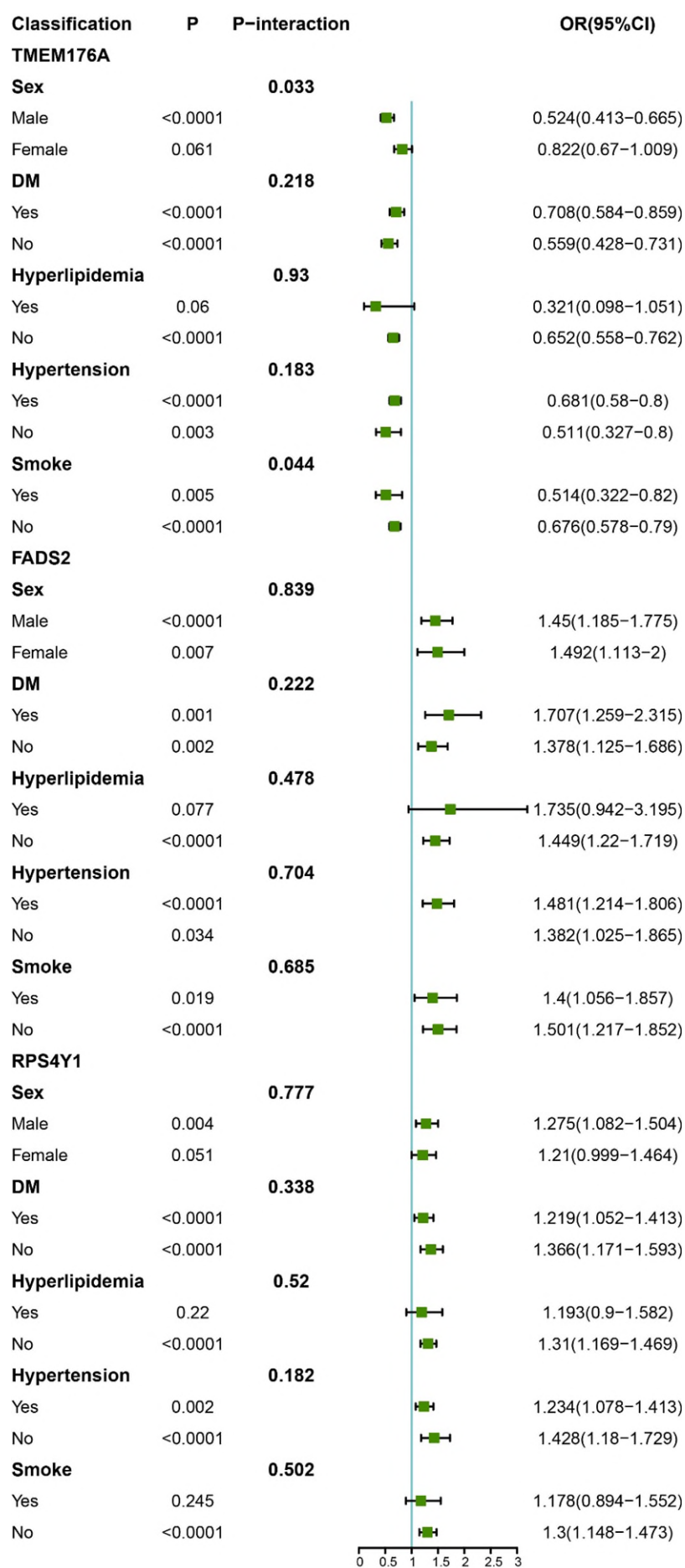


Figure 6: Log-rank results of gene expression. A: Intergroup comparison results of *TMEM176A*; B: Intergroup comparison results of *RPS4Y1*; C: Intergroup comparison results of *FADS2*; D: Intergroup comparison results of *FMN1*.



Supplemental Figure 1: A: NRI results for cutoff value modeling; B: NRI results for quartile modeling; C: NRI results for raw data modeling; D: IDI results for cutoff value modeling; E: IDI results for quartile modeling; F: IDI results for raw data modeling. NRI: net reclassification improvement; IDI: integrated discrimination improvement



Supplemental Figure 2: Forest plots of subgroup analyses. A: Forest plot of subgroup analyses of *TMEM176A*; B: Forest plot of subgroup analyses of *FADS2*; C: Forest plot of subgroup analyses of *RPS4Y1*.