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Diagnostic and prognostic significance of miRNA-15a-5p, 16-5p, and 92a-3p in arrhythmogenic right ventricular cardiomyopathy

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ABSTRACT

BACKGROUND Arrhythmogenic right ventricular cardiomyopathy (ARVC) presents diagnostic challenges and significant clinical burden because of life-threatening ventricular arrhythmias, compounded by the limited ability to predict patient prognosis using current clinical parameters. MicroRNAs (miRNAs) offer potential as markers in cardiac diseases, including ARVC, providing insights into disease pathogenesis, identification, and prognosis. However, current diagnostic criteria lack sensitivity and specificity, highlighting the need for novel markers such as miRNAs to better understand ARVC's complex pathophysiologic mechanisms.

OBJECTIVE This multisite study assessed circulating miRNA expression in ARVC patients, stratified by 5-year event-free survival risk, to explore their potential as a marker for improving ARVC diagnosis and prognosis.

METHODS Blood samples from 102 ARVC patients, 24 Brugada syndrome (BrS) patients, and 22 healthy controls were analyzed for the expression of 20 miRNAs using TaqMan quantitative real-time polymerase chain reaction (PCR), ARVC patients were stratified by 5-year event-free survival risk. Six candidate miRNAs were selected for further analysis, and machine learning algorithms were applied for classification and risk stratification based on miRNA profiles. Additionally, genotyping and functional annotation of miRNA targets were performed.

RESULTS Six miRNAs exhibited differential expression between high- and low-risk ARVC patients. MiR-15a-5p, miR-16-5p, and miR-92a-3p demonstrated the best performance in risk stratification. MiR-15a-5p also displayed higher expression in patients with adverse cardiac events. Comparative analysis with BrS patients and healthy controls consistently demonstrated increased expression of these miRNAs in ARVC.

CONCLUSION This study highlights miRNAs' potential to enhance the diagnosis, disease progression, and clinical outcomes of ARVC, supporting further research to improve patient care.

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KEYWORDS Arrhythmogenic right ventricular cardiomyopathy; ARVC risk calculator; miRNA expression; Biomarker; Ventricular arrythmia

Introduction

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a genetic heart muscle disorder predominantly affecting with the right ventricular free wall, characterized by fibrofatty replacement, life-threatening ventricular arrhythmias, and disruptions in cell–cell junctions that impair intercellular conduction and signaling.[1] Although the classic form of ARVC primarily involves the right ventricle, recent updates to the disease spectrum have recognized forms with isolated left ventricular ("left-dominant") or biventricular involvement. ARVC occurs in approximately 1 in 2000 individuals and presents significant clinical and diagnostic challenges, contributing to up to 11% of sudden cardiac deaths in adults younger than 65 years and 25% in children and adolescents.[2,3] The ARVC Task Force Criteria (TFC) were initially established in 1994 and subsequently updated in 2010 to reflect advancement in the understanding and diagnosis of the condition.[4] The criteria encompass a thorough evaluation of imaging, pathology, electrocardiography, family history, and genetics, categorizing patients into definite, borderline, possible, or no ARVC groups based on the total number of fulfilled criteria. However, the TFC demonstrates only modest sensitivity and specificity, often not exceeding 70%,[5] and their application can be both time-consuming and laborious, presenting challenges for clinicians and patients alike. Despite the identification of various risk factors, predicting the progression and severity of the condition remains highly complex and difficult.[6] Risk stratification in ARVC remains a challenge because of uncertainties in predicting disease progression, assessing arrhythmic risk, determining the need for implantable cardioverter-defibrillators and personalizing treatment strategies. Various clinical predictors have been proposed, but their validation is often based on small and heterogeneous cohorts. The revised 2022 ARVC risk model is a significant step in providing a validated tool for estimating individual risk of sustained ventricular arrhythmias (VA) in definite ARVC patients with no prior sustained VA.[7] However, external validation studies have reported varying accuracy, with some demonstrating good performance and others, including the current study, observing risk overestimation.[8–10] Discrepancies may stem from differences in patient selection biases, center focus, and genotype composition between cohorts.

Abbreviations

ARVC: arrhythmogenic right ventricular cardiomyopathy

AUC: area under the curve

BrS: Brugada syndrome

CI: 95% confidence interval

HC: healthy controls

HR: hazard ratio

MACE: major adverse cardiovascular events

miRNA: microRNA

mRNA: messenger RNA

PCR: polymerase chain reaction

ROC: receiver operating curve

susVA: sustained ventricular arrhythmia

TFC: Task Force Criteria

VA: ventricular arrhythmias

The limited understanding of ARVC's pathophysiology has hindered the development of highly sensitive diagnostic markers and risk predictors, highlighting the need for novel approaches. In recent years, emerging research on microRNAs (miRNA) has shown some promise as a supplementary disease marker, offering insights into the intricate mechanisms of ARVC, thereby improving diagnostic accuracy and enhancing patient management.[11]

MiRNAs are small noncoding RNAs that bind to messenger RNA (mRNA) transcripts to regulate their stability and translation.[12] Their tissue- and disease-specific expression, stability in circulation and ease of detection position them as promising diagnostic markers and therapeutic targets in cardiac disorders.[13–18] Some evidence suggests that the overexpression of specific miRNAs is associated with increased risks of VA.[19] However, only a few studies have assessed circulating miRNA expression in ARVC patients, isolating miRNAs from samples such as tissue, plasma, pericardial fluid, and blood samples.[11,20,21] Despite their potential as biomarkers, variability in patient cohorts, miRNA expression patterns, and methodologies have limited this clinical use. Additionally, there is a paucity of research on their role in disease progression, clinical outcomes, and prognosis.

This study aims to assess circulating miRNA expression in ARVC, stratified by estimated 5-year event-free survival risk. It seeks to identify miRNAs with significant changes in expression. Additionally, the study will explore their association with future cardiac events and assess the potential of miRNA-based classification to improve ARVC diagnosis and prognostic assessment.

Materials and methods

Study population

This multi-site study was conducted at the National Institute of Cardiology, Poland; the University Hospital Zurich, Switzerland; the Heart Institute (Institute do Coração), Brazil; and The Hospital for Sick Children (SickKids), Canada. The study protocol adhered to the Declaration of Helsinki and was approved by the Research Ethics Boards of participating centers. All participants provided written informed consent.

A total of 83 ARVC patients from the National Institute of Cardiology in Poland and 19 ARVC patients from the University Hospital Zurich in Switzerland were included in the study. All patients met the minimum criteria for definite ARVC according to the 2010 revised Task Force Criteria (TFC),[4] requiring at least 2 major criteria or 1 major plus 2 minor criteria in the clinical, imaging, and genetic categories Genotyping was performed through sequencing to analyze variants

located in the coding or splicing regions of genes associated with ARVC, as outlined in the supplementary section (detailed methodology in the supplementary section).

The final cohort of 102 ARVC patients was stratified into 3 subgroups based on the estimated 5-year event-free survival risk, using the ARVC Risk Calculator version 3.0 (www.arvcrisk.com).[22] These subgroups represented the low-, intermediate-, and high-risk tertiles (inferior, medium, and superior, respectively).

Eighty-three Polish patients were clinically monitored for major adverse cardiovascular events (MACE) over a follow-up period of 6 months to 6.2 years from admission. MACE included cardiac death, heart transplantation, sudden cardiac death, sustained ventricular arrhythmia (susVA), ventricular fibrillation, and arrhythmogenic syncope. The presence of MACE at admission was also recorded in this study.

Additionally, 24 patients diagnosed with definite Brugada syndrome (BrS) were included based on a Shanghai Score >3.5 points (Supplemental Table 1). Among these, 10 BrS patients were from the University Hospital Zurich in Switzerland, and 14 were from the Heart Institute (Instituto do Coração) in Brazil. Furthermore, 22 healthy control serum samples from healthy volunteers were acquired from the Precision for Medicine company (HC, healthy control group).

miRNA extraction, complementary DNA synthesis, and real-time polymerase chain reaction

The miRNAs were selected based on an in-silico analysis using 2 ARVC heart tissue datasets in the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/gds). Myocardial long-noncoding RNA-seq data (access code GSE107475)[23] was first reanalyzed using a specific pipeline for miARmaseq[24] to determine miRNAs of interest. Next, TargetScanHuman 8.0 software was used to predict target miRNA in a second dataset (access code GSE29819)[25] based on mRNAs differentially expressed by microarray technology. The 20 miRNAs identified by both approaches and known to be expressed in plasma and serum samples were selected as final targets for the current study (Supplemental Table 2).

Peripheral blood samples were collected on admission from patients with ARVC in the study. Serum was isolated by centrifugation (1500*g*) from 10 mL entire blood and stored at –80 °C until use. Total RNA, including miRNA, was extracted using the miRNeasy Serum/Plasma kit and spike-in control (Qiagen, RedwoodCity, CA), following the manufacturer's protocols. All steps involved in RNA extraction were performed using RNase-free materials, and RNA samples were stored at −80 °C.

The reverse transcription of miRNA samples was performed using the TaqMan™ Advanced miRNA complementary DNA Synthesis Kit (Applied Biosystems®, Foster City, CA) according to the manufacturer's instructions. miRNA expressions were assessed using custom TaqMan™ Advanced miRNA Plates, TaqMan™ Advanced miRNA assays, TaqMan™ Fast Advanced Master Mix Kit (Applied Biosystems®), and OneStep™ Real-Time polymerase chain reaction (PCR) System, 96-well. miRNAs with cycle threshold values higher than 35 were excluded from the analysis. Relative miRNA levels were measured using an adapted protocol developed by Schmittgen and Livak,[26] using exogenous cel-mir-39-3p as a reference.

Data analysis

The expression profiles of 20 miRNAs were compared between the top 10 individuals with the highest and the top 10 individuals with the lowest ARVC risk, as calculated by the ARVC Risk Calculator.[7,22] Only miRNAs showing differential expressions were selected for further analysis. This included differentiating between ARVC patients classified into low-, intermediate-, and high-risk tertiles, as well as between ARVC, BrS, and HC. Furthermore, ARVC patients were stratified by genotype, to compare gene-elusive, gene-positive (pathogenic or likely pathogenic genotypes according to ACMG guidelines, Supplemental Table 3), as well as the presence of MACE on admission (Supplemental Table 4). The Kaplan-Meier curve was employed to analyze time-to-MACE event data, considering only Polish cases that showed any MACE on admission (60 patients among 83 Polish cases).

Continuous variables were analyzed using the Shapiro-Wilk test. Variables with non-skewed distribution are shown as mean ± standard deviation and were compared by 2-sample *t*-test for independent variables. Those with skewed distributions are shown as median and interquartile range and compared using the Mann-Whitney *U*-test or Kruskal-Wallis test, expressing the results as mean ± standard deviation. Categorical variables were assessed via Fisher's exact and χ^2 tests with results depicted as relative (in parenthesis) and absolute frequencies.

The area under the receiver operating curve (ROC), with a 95% confidence interval (CI), was used to determine the discriminatory power of the selected miRNA in ARVC identification and risk stratification. Optimal cutoff points for miRNA were identified based on an excellent combination of sensitivity and specificity (area under the curve [AUC] $\geq$ 0.7).

Statistical analysis was performed using SPSS Statistics V23.0 software (IBM, Chicago, IL), or R program (v4.1), using packages ggplot2 (v3.4.0), and $P < .05$ was considered statistically significant.

Machine learning for patient prediction and classification

Random forest algorithm was used to classify patients based on a 5-year event-free risk score and ARVC diagnosis. The mean decrease accuracy and Gini coefficient were obtained for each miRNA relative expression, and the model was tested considering 95% CI. Principal Component Analysis was used to stratify patients into low- and high-risk and differentiate between ARVC, BrS, and HC.

Two generalized linear models were used to analyze the relationship between miRNA relative expressions and ARVC identification. Univariate analysis categorized patients into low- and high-miRNA expression. Stepwise multivariate

logistic regression was used to define low- and high-miRNA expression based on the cutoff value of miRNA expression obtained from sensitivity and 1-specificity ROC analysis.

Results

Patient characteristics

The description of the clinical characteristics of the ARVC cohort is shown in Table 1. ARVC risk stratification and MACE subset are shown in Supplemental Tables 3 and 4. Most patients enrolled in the study were women, with 71 (70%) showing potential genetic markers for ARVC diagnosis. However, no differences were observed between the sexes when analyzing risk groups for ARVC (Supplemental Table 5). Notably, patients classified as high-risk were younger (37 ± 12, $P < .001$). Among the ARVC patients with up to 6 years of clinical follow-up, 40 (58%) experienced 1 or more MACE, with 28 (70%) of those cases having sustained ventricular arrythmias as the primary event.

miRNAs differently expressed in ARVC

Among the 20 analyzed miRNAs, 6 (miR-15a-5p, miR-16-5p, miR-19a-3p, miR-29a-3p, miR-92a-3p, and miR-145-5p) were significantly upregulated in the top 10 individuals with the highest ARVC risk compared with the bottom 10 individuals with the lowest ARVC risk (Supplemental Table 6). These miRNAs demonstrated the same results when we reanalyzed ARVC cases comparing patients classified into high- vs low-risk tertiles subgroups based on the estimated 5-year event-free survival risk (low, intermediate, and high tertiles) using the ARVC Risk Calculator. However, miR-15a-5p, miR-19a-3p, and miR-92a-3p were overexpressed in higher-risk individuals compared with intermediate-risk ones (Figure 1). Univariate analysis showed hazard ratios (HR) for miR-15a-5p (HR, 12.70; 95% CI, 3.40–77.00; $P = .005$), miR-16-5p (HR, 3.90; 95% CI, 1.80–12.20; $P = .003$), and miR-92a-3p (HR, 3.50; 95% CI, 1.90–8.30, $P = .002$). In a subsequent multivariate stepwise analysis, miR-16-5p was excluded from the model, and the significant influence of miR-15a-5p was emphasized (Figure 2A).

Random forest analysis confirmed the effectiveness of these 6 miRNAs in differentiating between patients classified into high- vs low-risk tertiles based on their estimated 5-year event-free survival risk, achieving an accuracy of 71% (95% CI, 0.42–0.92). Notably, 3 specific miRNAs (miR-15a-5p, 92a-3p, and miR-16-5p) increased the accuracy to 85% (95% CI, 0.57–0.98, Figure 2B), explaining 79.9% of the variability among ARVC risk patients (Figure 2C–D). Finally, the ROC curve for low-risk and high-risk showed an AUC of 80% and 81% (95% CI) for miR-15a-5p and miR-92a-3p, respectively (Figure 2E).

These 6 miRNAs consistently exhibited overexpression in ARVC compared with both BrS and HC (Figure 3). Notably, random forest analysis confirmed that these 6 miRNAs were sufficient to distinguish ARVC patients from HC with 94% accuracy (CI: 81%–99%) with a highlight on miR-16-5p, miR-92a-3p, and miR-19a-3p (Supplemental Figure S1A–C). These miRNAs were also identified as significant in the univariate analysis (miR-92a-3p HR, 7.30; 95% CI, 3.50–20.90; $P = 8.33e^{-06}$; miR-16-5p HR, 6.90; 95% CI, 3.40–21.10; $P = 1.8e^{-05}$; and miR-19a-3p HR, 4.60; 95% CI, 2.60–10.10; $P = 8.23e^{-06}$). However, in multivariate analysis, miR-16-5p remained significant (Supplemental Figure S1D), with the ROC curve demonstrating an AUC exceeding 98% in differentiating ARVC from HC (Supplemental Figure 1E).

ARVC patients with a positive gene demonstrated overexpression of miR-15a-5p with borderline significance (Figure 4). This miRNA also exhibited upregulation in 24 ARVC patients who experienced any MACE events, as described at admission (Figure 5) and postadmission clinical follow-up recruitment, compared with the 6 cases who did not experience MACE ($P = .002$, Figure 6B). Results from Kaplan-Meier curve analysis indicated that the patient group exhibiting initially high expression of miRNA-15a-3p had a significant probability of developing MACE in the subsequent years ($P = .002$, Figure 6A). Finally, generalized linear models indicated that ARVC with elevated expression of mir-15a-5p showed a 6-fold increased risk of experiencing any MACE compared with those with low expression (*P*-value,.002; 95% CI, 1.9–19.0).

Pathway enrichment analysis of miRNA targets

Enrichment analysis indicated that shared genes between miR-15a-3p and miR-16-5p were involved in the bone morphogenetic protein signaling pathway, cell–cell migration

Table 1 Clinical information about the ARVC cohort

Clinical variables	All ARVC (n = 102)
Age, y, mean ± SD	48 ± 15
Sex, male n (%)	35 (34)
ARVC Risk Calculator parameters	
RVEF, mean ± SD	37 ± 10
PVC count, mean ± SD	2551 ± 3319
Cardiac syncope (<6 mo), n (%)	16 (16)
No. of inv. T-W, mean ± SD	5 ± 2
Nonsustained VT, n (%)	61 (73)
susVA (np)5, mean ± SD	34 ± 20
Genetics	
DES, n (%)	3 (3)
DSC2, n (%)	5 (5)
DSG2, n (%)	9 (9)
DSP, n (%)	4 (4)
FLNC, n (%)	1 (1)
LMNA, n (%)	3 (3)
PKP2, n (%)	44 (43)
More than 1 gene, n (%)	7 (7)
Gene elusive, n (%)	26 (25)
Genetic variants classification	
LP, n (%)	5 (5)
P, n (%)	55 (54)
VUS, n (%)	11 (11)

LVEF = left ventricular ejection fraction; PVC = premature ventricular contraction; RVEF = right ventricular ejection fraction; susVA = No. of inv. T-w; number of inverted T-waves observed on an electrocardiogram; susVA (np)5 = risk calculator estimating the 5-year risk of sustained ventricular arrhythmias (VA) in patients with definite ARVC diagnosis (www.arvcrisk.com).

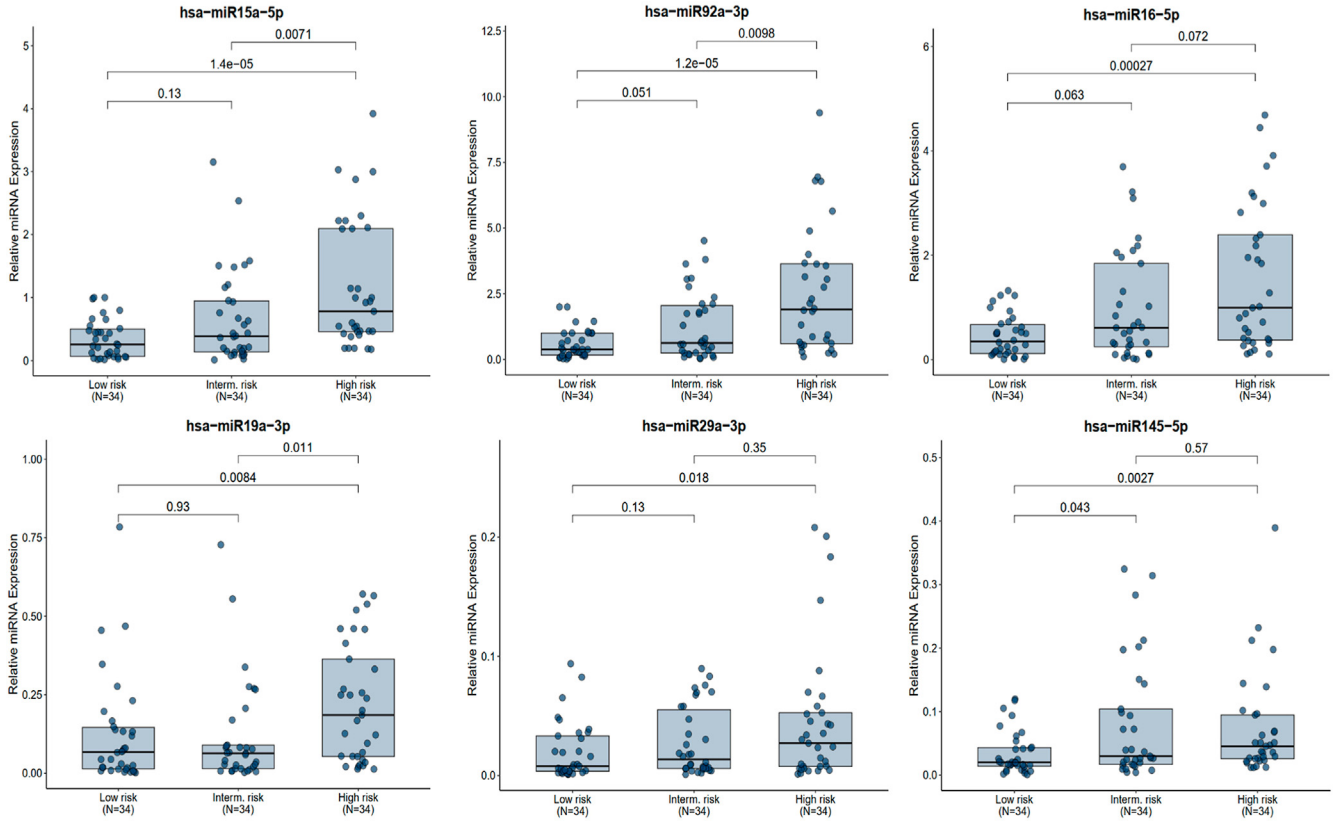

Figure 1

miRNAs stratify ARVC patients based on 5 years of event-free survival. Differentially expressed miRNAs in serum between Low (n = 34), Intermediate (n = 34), and high-risk (n = 34). Data are shown as the median of relative expression ($2-\delta^{CT} \times 10^4$) and were compared using *U*-Mann Whitney; $^*P < .05$ was significant. The definition of low, intermediate, and high risk is determined by arranging the ARVC risk calculated values from lowest to highest and dividing them into terciles (inferior, medium, and superior), with an equal number of patients in each subgroup.

associated with sprouting angiogenesis, and phosphatidylinositol dephosphorylation (Figure 7A). The latter 2 biological processes were also associated with miR-19a-3p and miR-92a-3p. Furthermore, through examining targets of each miRNA separately, we identified potential targets for miR-15a-5p (177), miR-16-5p (208), miR-92a-3p (193), and miR-19a-3p (191) (Supplemental Tables 7 and 8). Notably, miR-19a-3p was significantly associated with positive regulation of vasculogenesis and atrioventricular valve morphogenesis. Moreover, miR-92a-3p displayed enrichment in biological processes associated with vesicle cytoskeletal trafficking (Figure 7B).

Discussion

The current study explores the diagnostic and prognostic value of circulating miRNA expression in ARVC, and the potential associated regulatory pathways identified through functional enrichment analysis. Six miRNAs (miR-15a-5p, miR-16-5p, miR-19a-3p, miR-29a-3p, miR-92a-3p, and miR-145-5p) were significantly upregulated in ARVC patients with a higher estimated 5-year risk of experiencing sustained ventricular arrythmias. Among these, 3 miRNAs (15a-5p, miR-16-5p, and miR-92a-3p) showed greater accuracy in distinguishing between ARVC patients with high and low 5-year event-free risk, with miR-15a-5p notably associated with adverse clinical events, suggesting a link to disease severity or increased vulnerability. Although several MACE parameters (eg, cardiac death, heart transplant, arrhythmic syncope) did not correlate with miRNA expression, elevated miR-15a-5p was strongly associated with a higher risk of MACE, particularly sustained ventricular arrhythmias. This suggests that upregulation of specific miRNAs, such as miR-15a-5p, may be more closely related to arrhythmic events than other cardiac outcomes. Comparative analysis with BrS patients and HC consistently revealed elevated expression of all identified miRNAs in ARVC, supporting their diagnostic potential. Machine learning analysis further validated the accuracy of miRNA-based classification, emphasizing their diagnostic utility. These findings highlight the promising role of circulating miRNAs in ARVC diagnosis and prognosis, emphasizing the need for further exploration into their application in other cardiac disorders.

In miRNA research, it is increasingly evident that a single miRNA is unlikely to be disease specific. Rather, patterns of specific miRNAs may serve as unique signatures that reflect the nuanced aspects of disease pathologies. The complexity and heterogeneity of cardiac diseases, particularly in their symptomology and pathology, often lead to overlapping clinical manifestations among different conditions.[27] Given that miRNAs regulate multiple genes and pathways, their

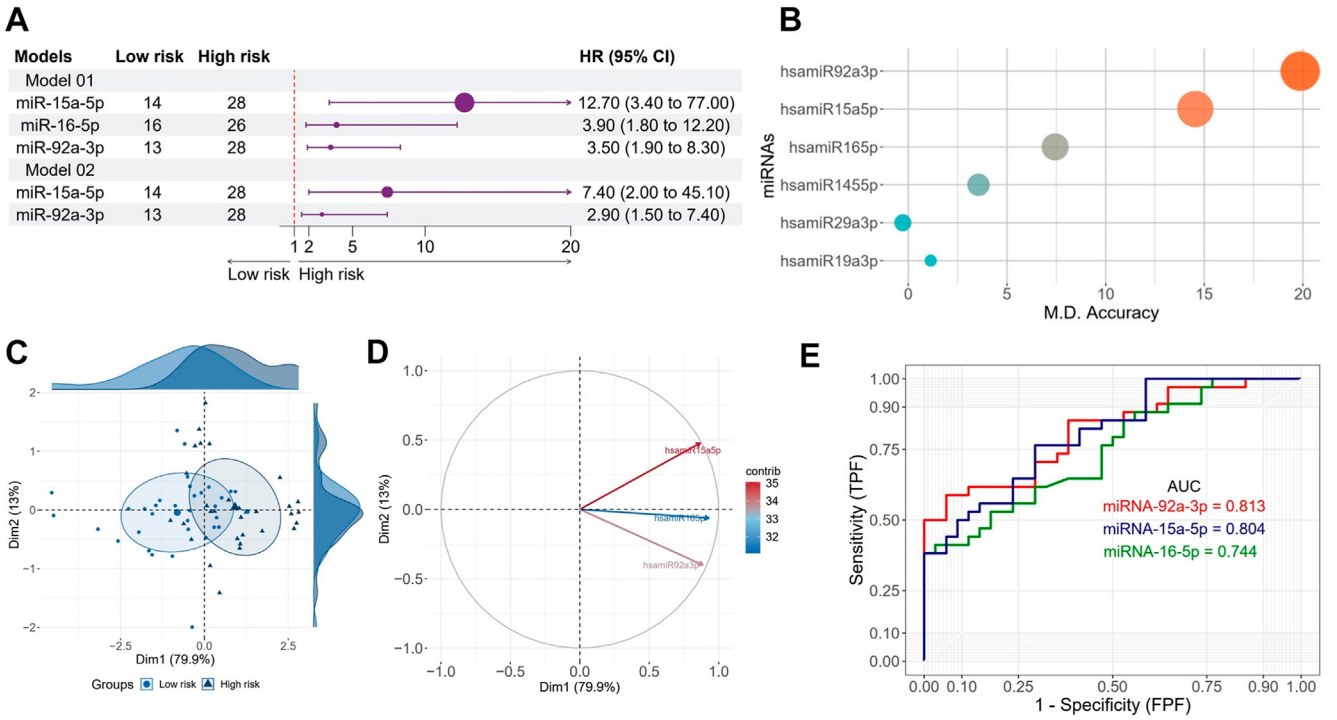

Figure 2

A Generalized linear model results. The first model was the univariate analysis, and the second was multivariate analysis following the stepwise approach. Dependent variables were low- and high-risk, and the miRNA expressions were added as covariables. **B:** Machine learning analysis by Random Forest analysis of the 6-miRNA model in ARVC score risk (low and high-risk). The mean decrease in accuracy is shown in the x-axis, and the mean decrease Gini value is represented by color and size dots. **C:** Principal component analysis with spectral decomposition based on 3 different miRNA (miR-15a-5p, 92a-3p, and miRNA-16-5p) expressions shows the stratification of low- and high-risk patients. *Circular blue* and *triangular dark blue dots* represent the low- and high-risk-score groups. **D:** miRNAs with positive correlation *arrows* in the same direction as the plot. **E:** Receiver operating characteristic (ROC) curve based on miR-15a-5p, 92a-3p, and miRNA-16-5p expression by sensitivity and inverse specificity.

dysregulation may result in similar clinical findings across various cardiac pathologic conditions. Specifically, miR-15a-5p and miR-92a-3p are linked to cardiac tissue remodeling, fibrotic processes, and disease progression in conditions such as hypertrophic cardiomyopathy and heart failure.[28] We observed substantial overexpression of miR-15a-5p in ARVC patients, particularly those with gene-positive or high-risk profiles, suggesting a possible association with diffuse myocardial fibrosis, similar to findings in hypertrophic cardiomyopathy.[28] Tijsen and collaborators[29] demonstrated that the miR-15 family, including miR-15b, modulates the transforming growth factor beta pathway by targeting transforming growth factor BR1, SMAD3, and related genes, ultimately regulating fibrosis and hypertrophy in hypertrophic cardiomyopathy.[29] Although the study emphasized the protective role of miR-15 inhibition in limiting excessive fibrosis, the dysregulation of miR-15a-5p observed in ARVC suggests a disease-specific role in pathologic remodeling. This dysregulation may compromise structural integrity and functional capacity, contributing to the fibrotic processes central to ARVC pathogenesis. Elevated levels of miR-15a-5p in high-risk ARVC patients may exacerbate the fibrotic response, further impairing cardiac function. These findings suggest that miR-15a-5p could serve as a prognostic marker for poorer outcomes in ARVC.

Additionally, miRNA-92a, critical in cardiovascular diseases and arrhythmias, influences endothelial function, angiogenesis, apoptosis, and cardiac hypertrophy.[30,31] Its dysregulation disrupts ion channel expression, cardiac fibrosis, and electrical conduction, contributing to arrhythmogenic events. In our study, we observed overexpression of miR-92a in ARVC, and its ability to differentiate between low and high risk underscores its prognostic significance. Similarly, miRNA-16-5p, is associated with reduced cardiac fibrosis,[11] likely through regulatory effects on extracellular matrix-related processes, apoptosis and inflammation, further highlighting its potential relevance in disease progression.[32] Pathway enrichment analysis revealed that miR-16-5p was linked to cell migration and tissue remodeling processes, which are important in fibrosis and tissue repair in ARVC.

Other miRNAs such as miR-21-5p and miR-135b are associated with key signaling pathways, including Wnt and Hippo, which play essential roles in maintaining myocardial homeostasis and regulating cardiac remodeling.[11] Dysregulation of these pathways may drive fibrofatty substitution, a hallmark of ARVC, by promoting aberrant cell proliferation, differentiation, and apoptosis, thereby impacting disease severity and frequency of VAs. Specifically, downregulation of desmoplakin expression releases plakoglobin from the desmosomes, allowing plakoglobin to translocate to the nucleus and inhibit the Wnt/β-catenin pathway. This suppression encourages adipogenesis via transcription factors such as PPARγ and C/EBP-α. Furthermore, crosstalk between Wnt/β-catenin and Hippo/YAP pathways enhances adipogenesis and disease

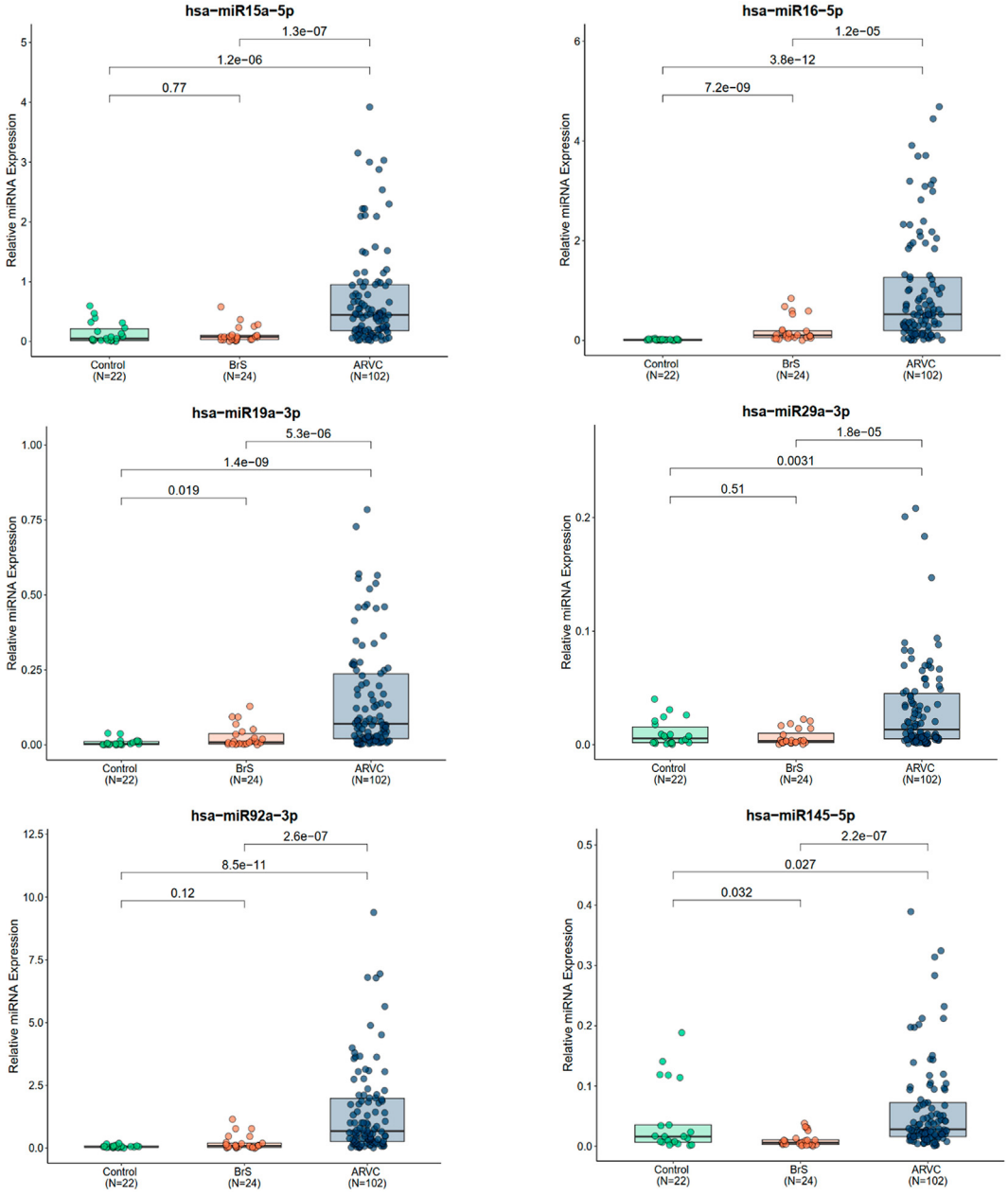

Figure 3

Differentially expressed miRNAs in serum between health control (n = 22), Brugada syndrome (BrS, n = 24), and ARVC patients (n = 102). Data are shown as the median of relative expression ($2-\delta^{CT} \times 10^4$) and were compared using *U*-Mann Whitney; *$P < .05$ was considered significant.

progression, with desmosome disruption activating Hippo/YAP to suppress Wnt signaling and worsen fibrofatty replacement.[11] Our pathway analysis suggests that miR-92a-3p also may play a role in these processes, because it was enriched in vesicle trafficking pathways involved in cellular functions such as migration and differentiation, processes that are crucial for tissue remodeling in ARVC. Collectively, these findings emphasize the capacity of miRNAs to offer enhanced clinical support and effective surveillance mechanisms during the progression of this cardiac disorder.

Our study revealed elevated levels of miRNA-16-5p and miRNA-92a-3p to be predictive of 5-year risk for ventricular arrhythmias. Despite limited direct research linking these miRNAs to ARVC, their involvement in key biological processes, such as fibrosis inhibition and inflammation regulation, underscores their relevance in ARVC pathophysiology.[32] Their roles in fibrosis, hypertrophy, and arrhythmia further highlight their broader impact on cardiac health, offering insights into the mechanisms underlying ARVC and related pathologic conditions.

We also observed elevated levels of miR-19a-3p, miR-92a-3p, and miR-145-5p n both ARVC and BrS patients compared with their healthy counterparts (Supplemental Figure 1), suggesting shared pathologic features such as genetic overlaps in desmosomal and sodium channel genes. The ARVC-BrS crossover phenotype, characterized by the presence of features from both conditions, highlights the interplay between electrical disturbances (ie,

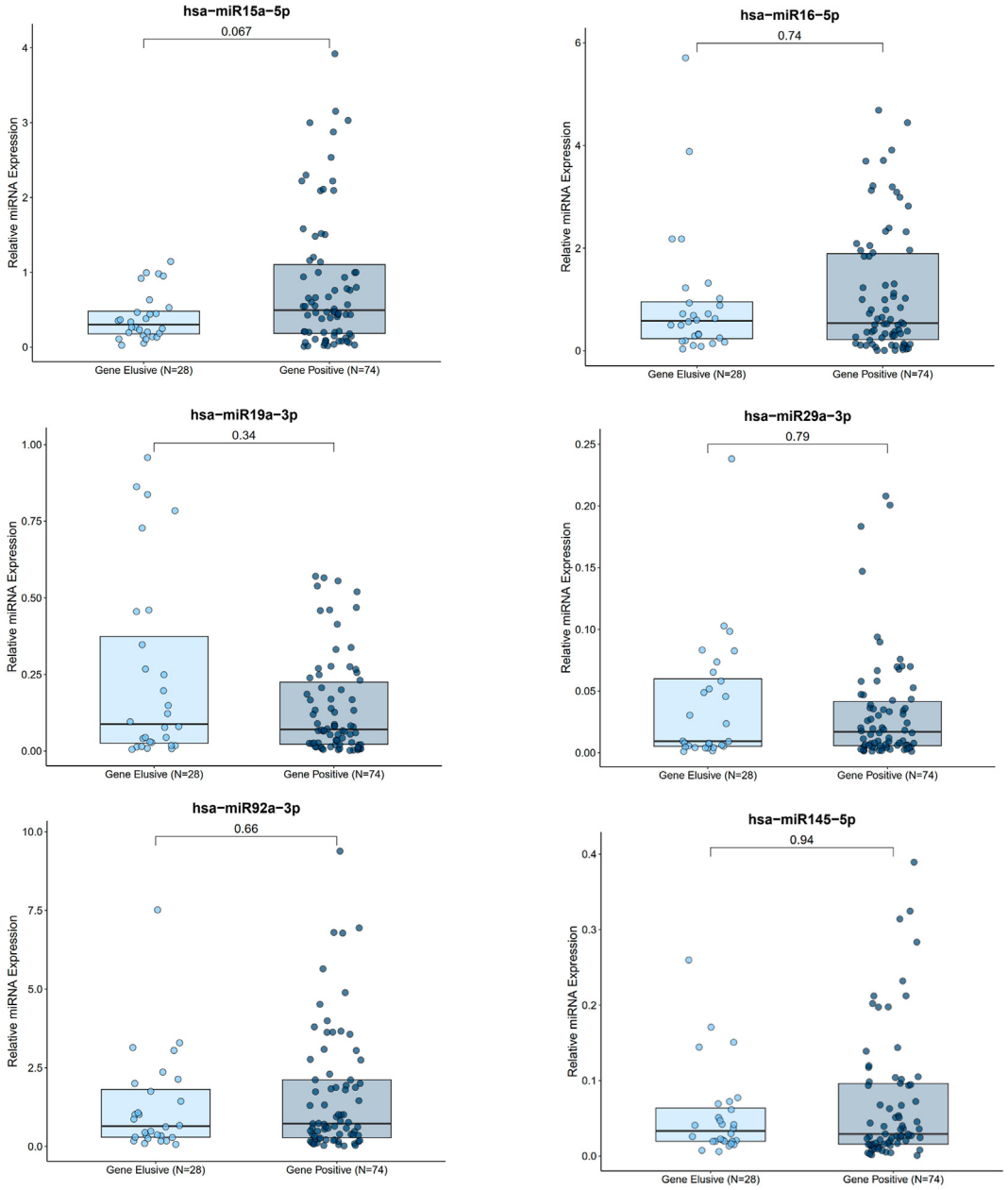

Figure 4
miRNAs stratify ARVC patients based on genotype. Differentially expressed miRNAs in serum between gene elusive (n = 28) and gene positive (n = 74) patients. Data are shown as the median of relative expression ($2-\delta^{CT} \times 10^4$) and were compared using *U*-Mann Whitney; **P* < .05 was considered significant.

arrythmias) and structural changes, suggesting the mechanisms driving ARVC extend beyond structural defects.[33] The differential miRNA expression between ARVC and BrS suggests that, despite sharing arrhythmic features, their miRNA profiles reflect distinct pathogenic mechanisms. ARVC is associated with structural remodeling and fibrosis, whereas BrS primarily involves electrical disturbances, highlighting the complex interplay between structural and electrical abnormalities in arrhythmia-related diseases. Similar elevations in miR-145-5p have been reported in ARVC,[21] heart failure, hypertrophic cardiomyopathy,[34,35] and dilated cardiomyopathy patients. Additionally, varying levels of these miRNAs have been observed in cardiac fibrosis and myocardial infarction.[11,20,21,36] These findings suggest a shared pool of dysregulated miRNAs across cardiac pathologic conditions, emphasizing the need to identify disease-specific miRNA signatures for accurate diagnosis and prognosis.

These miRNAs also may play a compensatory role in response to structural disruptions caused by mutations in desmosomal proteins such as DSG2, crucial for cardiac cell adhesion in ARVC. By regulating gene expression, they may stabilize disrupted cellular interaction and promote tissue remodeling and repair through processes such as cell migration and vasculogenesis.[37] Mutations in DSG2 can impair cell–cell adhesion, triggering inflammation and compensatory miRNA

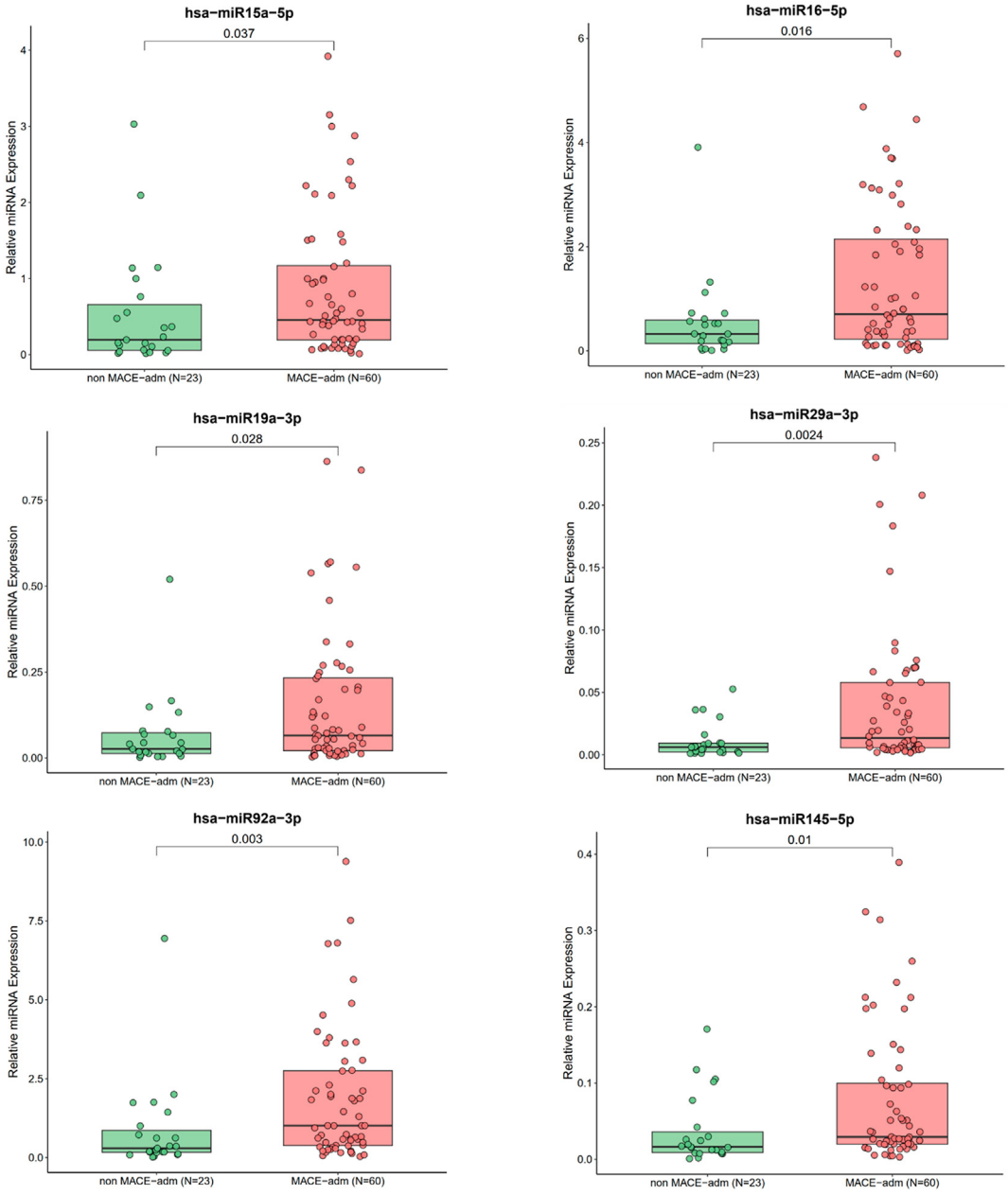

Figure 5
miRNAs stratify ARVC patients based on presence of MACE at admission. Differentially expressed miRNAs in serum between patients with non-MACE at admission (n = 23) and MACE (n = 60) by Polish cohort. Data are shown as the median of relative expression ($2-\delta^{CT} \times 10^4$) and were compared using *U*-Mann Whitney; $*P < .05$ was considered significant.

expression changes.[11] Elevated miRNA levels in ARVC patients likely reflect this adaptive response, underscoring the intricate relationship between inflammation and miRNA regulation in managing structural and functional disruptions.

Despite its valuable insights into miRNA expression in ARVC, this study has some limitations. The small cohort size and limited geographic variability may affect generalizability. The inclusion of a Brazilian cohort may introduce ethnic variation, although no significant differences have been reported between South American and European populations for Brugada syndrome. Single-timepoint miRNA measurements restrict the ability to track dynamic changes during disease progression, and overlapping miRNA profiles with other cardiac conditions, such as hypertrophic and dilated cardiomyopathy, challenge diagnostic specificity. Samples were primarily collected during clinical stability; however, miRNA levels may fluctuate during arrhythmic flares or disease exacerbations. Additionally, since the analysis was limited to microRNAs (miRNAs) derived from peripheral blood mononuclear cells, their direct involvement in cardiac structural changes remains uncertain. Although functional enrichment analysis suggests potential pathways, it does not establish direct links to ARVC's defining features, such as fibrofatty replacement or desmosomal defects, highlighting

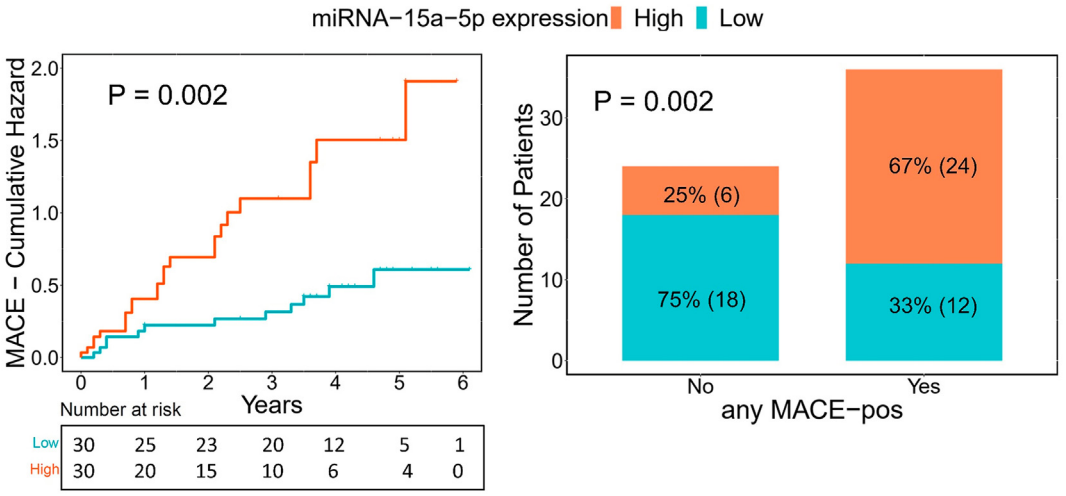

Figure 6
A: Kaplan-Meier survival curve depicting the cumulative incidence of major adverse cardiovascular events (MACE) over time, stratified by high and low miRNA expression groups in ARVC patients from the Polish cohort (n = 60). The y-axis represents the probability of remaining free from MACE, and the x-axis denotes time in years. B: Barblot distribution of patients based on low and high miRNA expression. groups (miR-15a-5p) and their distribution by major cardiovascular events (MACE) observed (non-MACE N=24, any-MACE N=36).

the need for external validation and mechanistic studies. Future research should focus on identifying ARVC-specific miRNA signatures through larger disease-specific cohorts and functional studies to improve differentiation from other cardiac conditions.

Our findings emphasize the potential of miRNAs as valuable diagnostic and prognostic tools in ARVC. By integrating miRNA profiling with established markers, diagnostic accuracy and risk stratification can be improved, to allow for improved disease management and patient care. Although miRNA-based diagnostics remain in the early stages of development, significant progress has been made toward their clinical application. To fully translate miRNA profiling into practice, future research should focus on expanding cohort sizes, incorporating diverse patient populations, and conducting longitudinal studies with multi-timepoint sampling

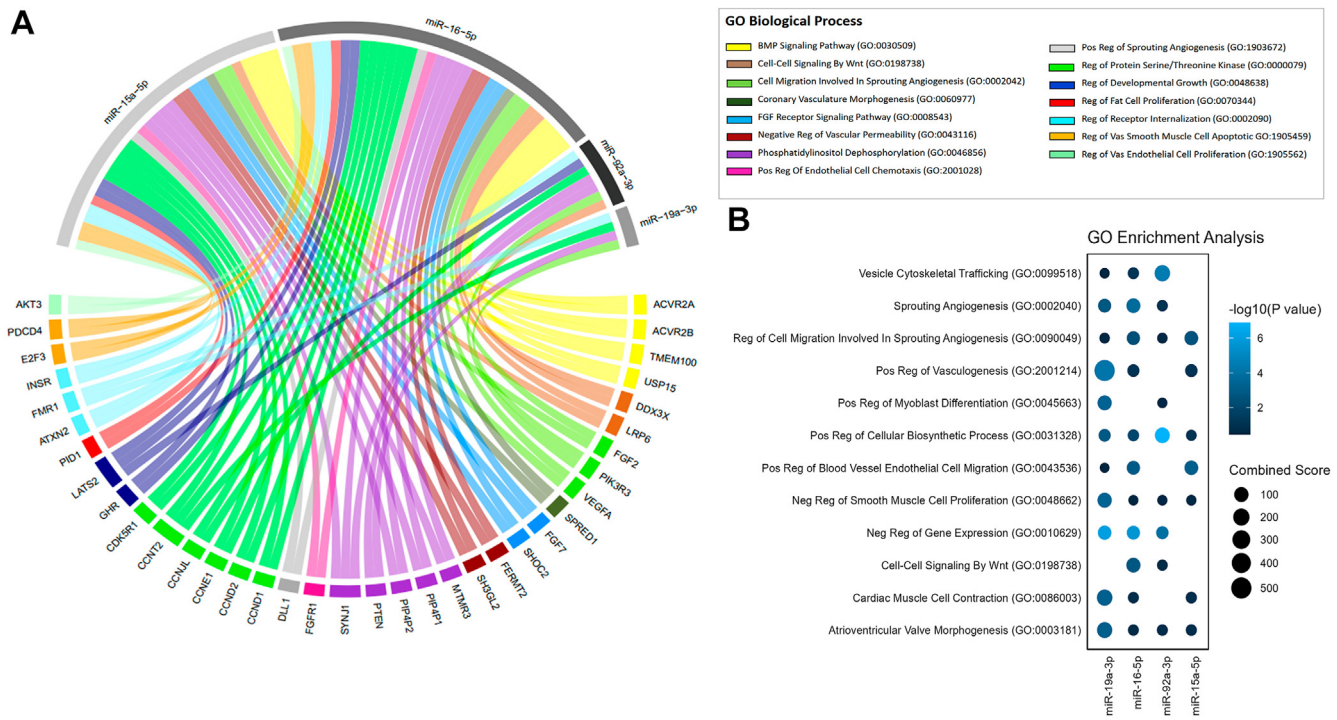

Figure 7
A: Prediction of potential miRNA targets and gene ontology (GO). Circular network illustrating the interactions between miRNAs and target genes, with different colors representing GO-enriched genes. B: Enrichment analysis relevant to arrhythmogenic right ventricular cardiomyopathy (ARVC). GO biological process of the targets of the miRNAs was displayed in a bubble plot. Size is related to the combined score (number of genes in each process identified about all total genes, and color black to blue increase –log10 of the *P*-value).

to capture miRNA expression dynamics throughout disease progression. Additionally, addressing challenges such as variability in miRNA expression across populations, standardization of quantification methods, and integration into routine diagnostics will be crucial. Despite these challenges, ongoing advancements in miRNA research hold promising potential for improving ARVC diagnosis, prognosis, and targeted therapeutic interventions.

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Authorship: All authors attest they meet the current ICMJE criteria for authorship.

Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrthm.2025.04.014.

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