

# Identifying the key microRNAs implicated in atrial fibrillation

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## ABSTRACT

**Objective:** This study investigated the potential microRNAs (miRNAs) having a diagnostic value in atrial fibrillation (AF).

**Methods:** The miRNA and mRNA expression profiles of atrial tissue from healthy individuals and patients with AF were downloaded from the Gene Expression Omnibus database. Differentially expressed miRNAs/mRNAs (DEMis/DEMs) were identified in patients with AF. Furthermore, an interaction network between DEMis and DMEs was constructed. The biological processes, molecular functions, and signaling pathways of DEMs were enriched. Then, the diagnostic values of candidate DECs among healthy individuals and patients with AF were preliminarily evaluated in the GSE101586, GSEE101684, and GSE112214 datasets.

**Results:** Twenty DEMis were identified in patients with AF, including seven upregulated and 13 downregulated DEMis. Furthermore, 2,307 DEMs were identified in patients with AF. In the DEMi-DEM interaction network, downregulated miR-193b and upregulated miR-16 interacted with the most targeted DEMs, which interacted with 72 and 65 targeted DEMs, respectively. The targeted DEMs were significantly enriched in biological functions including apoptosis and the PI3K-Akt, mTOR, Hippo, HIF-1, and ErbB signaling pathways. Four of the 20 DEMis (i.e., miR-490-3p, miR-630, miR-146b-5p, and miR-367) had a potential value to distinguish patients with AF from healthy individuals in the GSE68475, GSE70887, and GSE28954 datasets. The area under the curve values for those four DEMis were 0.751, 0.719, 0.709, and 0.7, respectively.

**Conclusion:** DEMis might play key roles in AF progression through the mTOR and Hippo signaling pathways. miR-409-3p, miR-630, miR-146b-5p, and miR-367 had a potential diagnostic value to discriminate patients with AF from healthy controls in this study.

**Keywords:** atrial fibrillation, microRNA, diagnosis

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## Introduction

Atrial fibrillation (AF) is one of the most common types of cardiac arrhythmias, which is characterized by irregular high-frequency excitation and contraction affecting circulation and oxygen supply (1). The Global Burden of Disease study has shown that the estimated number of individuals with AF was 34 million, and the estimated prevalence of AF globally is 2.5%–3.2% (2, 3). Given the morbidity and mortality from stroke, heart failure, and dementia, much focus has been directed toward AF prevention.

microRNAs (miRNA) are small endogenous noncoding RNAs with a length of 20–25 nt, which act to decrease the expression

of messenger RNAs containing stretches of sequence complementary to miRNAs (4, 5). They are crucial regulators of gene expression and promising candidates for biomarker development (4). Recent studies have shown that miRNAs may be involved in the pathophysiology of AF. The overexpression of miR-27b-3p targeting Wnt3a regulates the Wnt/ $\beta$ -Catenin signaling pathway and attenuates atrial fibrosis in rats with AF (6). Atrial-specific upregulation of miR-31 in human AF begets arrhythmia by depleting atrial dystrophin and neuronal nitric oxide synthase (7). Plasma miR-21 and miR-150 are lower in patients with AF than those in patients without AF, and these two miRNAs are lower in patients with paroxysmal AF than those in patients with persistent AF; in addition, those two miRNAs are

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**HIGHLIGHTS**

- Twenty DEMs were identified in AF patients compared with healthy individuals.
- mTOR and Hippo signaling pathways were significantly enriched in AF.
- miR-409-3p, miR-630, miR-146b-5p, and miR-367 had a potential diagnostic value to discriminate AF patients from healthy controls in this study.

significantly increased after AF catheter ablation (8). Studies have revealed that circulating miRNAs are potential biomarkers of AF (9-12). Liu et al. (13) have reported that reduced circulating miRNA-150 is significantly associated with AF; furthermore, miRNA-150 levels in patients with AF were substantially lower than those in healthy people.

Currently, no feasible biomarker exists for early diagnosis of AF. In this study, significantly dysregulated miRNAs in AF were investigated based on the miRNA expression profiles obtained from the Gene Expression Omnibus database; moreover, the diagnostic value of dysregulated miRNAs in AF was evaluated. This study provides valuable information for identifying a feasible biomarker for early diagnosis of AF in the future.

**Methods****Gene expression datasets of AF**

The expression profiles of human AF were searched from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo>). The keywords "Atrial fibrillation" and "Homo sapiens" [porgn] and "gse" [Filter] were searched in the GEO database. The microarray datasets generated from the heart tissue of patients with AF and healthy controls were incorporated into our studies. The inclusion criteria for the datasets were as follows: (1) mRNA/miRNA expression profile generated from a whole genome and (2) datasets including atrial tissue obtained from patients with AF and healthy controls. After manual inspection and filtration, three miRNA and six mRNA expression profiling datasets were incorporated into this study. The details of the datasets included in this study are shown in Tables S1 and S2.

**Differentially expressed mRNA/miRNA (DEM/DEMi) in AF**

The selected datasets were analyzed individually. To minimize heterogeneity among the datasets included in integrated analysis, normalization and log<sub>2</sub> transformation were performed for raw data. Then, the metaMA package in R language was used to calculate the *p* value of each dataset; *p* values were combined to identify DEM/DEMi in AF compared with controls. Benjamini-Hochberg's method was used to obtain the false discovery rate (FDR) of multiple comparisons correction for correcting *p* value. mRNA with FDR <0.05 and miRNA with *p*<0.01 were selected as DEM/DEMi.

**miRNA-mRNA network**

miRTarBase V7.0 (<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>) was used to predict the targeted genes of miRNA, and miRNA-mRNA interaction was constructed using Cytoscape (<http://cytoscape.org/>).

**Functional enrichment**

g:Profiler (<http://biit.cs.ut.ee/gprofiler/gost>), a web server for functional interpretation of gene lists, was used to enrich the Gene Ontology (GO) function of DEMs. KOBAS3.0 (<http://kobas.cbi.pku.edu.cn/index.php>), a web server for gene/protein functional annotation (Annotate module) and functional gene set enrichment (Enrichment module), was used to predict the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEMs. FDR <0.05 was the cut-off for selecting significant GO terms and KEGG pathway. The GOplot package in R language was used to describe GO and KEGG enrichment.

**Statistical analysis**

The expression level of mRNA/miRNA in patients with AF and healthy individuals was presented as mean ± standard deviation. The unpaired Student's *t*-test was used to calculate the statistical differences between patients with AF and healthy individuals in DEMs and DEMis.

Receiver operating characteristic (ROC) curve analysis is a tool used to describe the discrimination accuracy of a diagnostic test or prediction model. In this study, ROC curve analysis is used to evaluate the diagnostic value of DEMis to distinguish patients with AF from healthy individuals in three datasets (GSE68475, GSE70887, and GSE28954). The pROC package in R language (version R3.3.1, <https://www.r-project.org/>) was used for depicting ROC curves, and area under the curve (AUC) values of the ROC curves were calculated to assess the performance of DEMis. *P* values of <0.05 were used to denote statistical significance.

**Results****Identifying DEMis**

Three miRNA expression datasets of atrial tissue obtained from patients with AF and healthy individuals retrieved from the GEO database were used to identify DEMis in patients with AF (Table S1). Four hundred and thirty-two miRNAs were overlapped from the three miRNA datasets. Twenty DEMis were identified in patients with AF, including seven upregulated and 13 downregulated DEMis. miR-146b-5p and miR-99a were the most significantly upregulated and downregulated DEMis in AF, respectively (Table 1). Heat map analysis indicated that the 20 DEMis could distinguish patients with AF from healthy individuals (Fig. 1).

**Identifying differentially expressed mRNAs**

To investigate DEMs in patients with AF compared to healthy individuals, six mRNA expression datasets of atrial tissue from patients with AF and healthy individuals were downloaded from

**Table 1. The dysregulated miRNAs in AF**

Up/down-regulation	miRNA	AF	Control	P-value
Up-regulation	hsa-miR-146b-5p	0.783588	-0.66418	0.002747
	hsa-miR-630	0.900664	-0.4271	0.004166
	hsa-miR-184	-0.2852	-0.51578	0.004331
	hsa-miR-22	1.359494	0.954209	0.00568
	hsa-miR-16	1.350736	0.701235	0.005711
	hsa-miR-513b	0.038484	-0.1852	0.006205
	hsa-miR-17*	-0.13649	-0.41805	0.008079
Down-regulation	hsa-miR-99a	0.909484	1.225932	0.000384
	hsa-miR-100	0.730686	1.260344	0.000601
	hsa-miR-193b	0.279992	0.761553	0.001656
	hsa-miR-338-3p	0.241065	0.686402	0.002899
	hsa-miR-490-3p	-0.13122	0.789981	0.003523
	hsa-miR-367	-0.47095	-0.07467	0.004304
	hsa-miR-302d	-0.49751	-0.27813	0.004464
	hsa-miR-302a	-0.47395	-0.11171	0.00561
	hsa-miR-892b	-0.4223	-0.03796	0.006323
	hsa-miR-125b-2*	-0.16719	0.250087	0.006389
	hsa-let-7c	1.067001	1.296778	0.007283
	hsa-miR-143*	-0.28559	0.194946	0.007605
	hsa-miR-302c	-0.47047	-0.22427	0.00835

Atrial fibrillation and controls indicated the mean log2 expression level of certain miRNA in atrial fibrillation group and healthy individual group, respectively. miRNA - microRNA; AF - atrial fibrillation

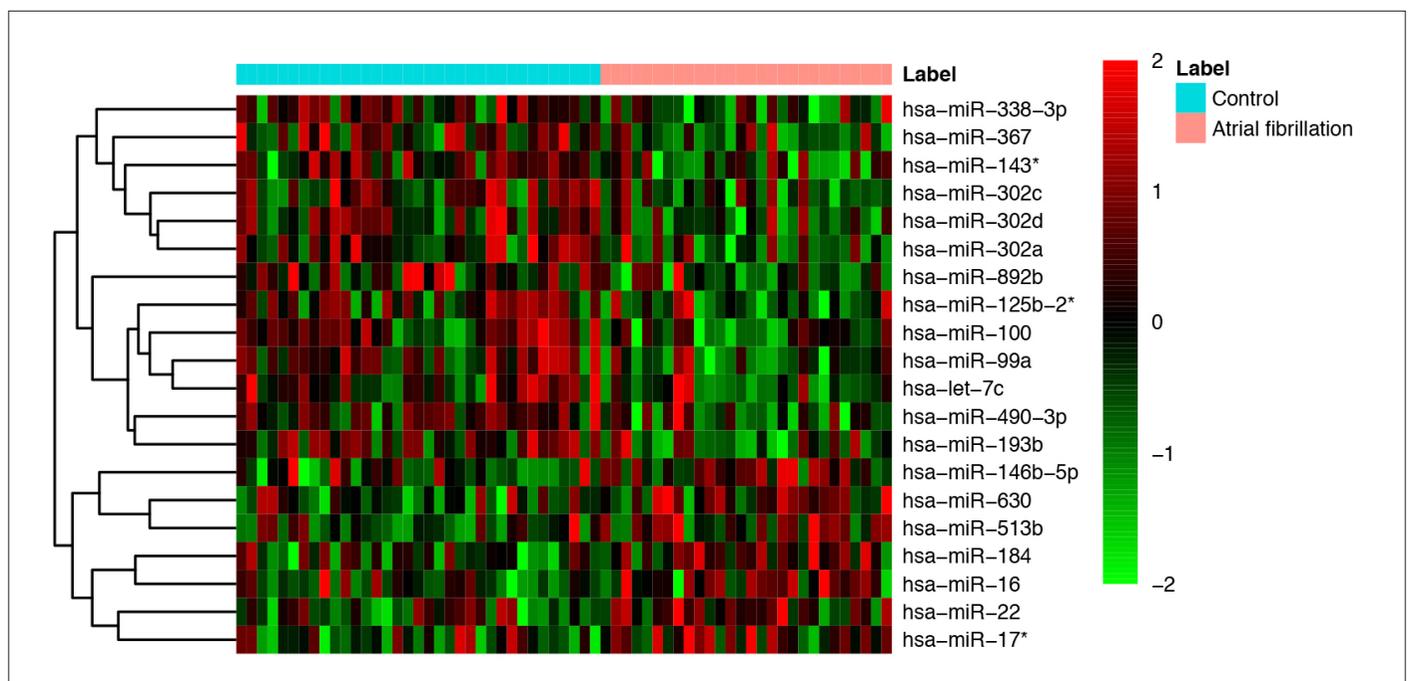
the GEO database for further analyses. In total, 19,545 genes were overlapped from the six mRNA datasets; furthermore, 2,307 DEMs were identified in AF, including 1,247 upregulated and 1,060 downregulated genes. *ANGPTL2* and *NECAP1* were the most significantly upregulated and downregulated genes, respectively (Table 2). In addition, heat map analysis indicated that those DEMs could distinguish patients with AF from healthy individuals (Fig. S1).

**Investigation of the miRNA-mRNA network**

To explore the associations between DEMs and DEMs, miR-TarBase V7.0 was used to predict the DEMs negatively targeted by DEMs. Four hundred and ninety-eight correlation pairs of DEMs and DEMs were eventually obtained, including 107 correlation pairs between upregulated DEMs and downregulated DEMs (Fig. S2) and 391 downregulated DEMs and upregulated DEMs (Fig. S3). In the upregulated DEMi/downregulated DEM network, miR-16, miR-17\*, miR-513b had targeted the most DEMs, which targeted 65, 11, and 10 DEMs, respectively (Fig. 2a-2c). In the downregulated DEMi/upregulated DEM network, six DEMs targeted more than 30 DEMs, namely, miR-193b, miR-302a, miR-302d, let-7c, miR-302c, and miR-143\*. In addition, miR-193b, miR-302a, and miR-302d targeted 72, 52, and 46 DEMs, respectively (Fig. 2d-2f).

**The enriched GO terms and pathways of DEMs targeted by DEMs**

To determine the biological functions of DMEs targeted by DEMs, the GO terms and KEGG pathway of the targeted DEMs were enriched. The targeted DEMs were significantly enriched in the biological processes of GO terms including apoptosis, cellular metabolic process, and intracellular transport (Fig. 3a).



**Figure 1.** The heatmap of DEMs in AF compared to healthy individuals

**Table 2. Top 20 up-regulated and down-regulated DEMs in AF**

Up-regulated			Down-regulated		
Gene ID	Gene symbol	FDR	Gene ID	Gene symbol	FDR
23452	<i>ANGPTL2</i>	3.54E-09	25977	<i>NECAP1</i>	0
54476	<i>RNF216</i>	1.17E-08	100507477	<i>C1orf105</i>	1.20E-08
10652	<i>YKT6</i>	1.20E-08	92346	<i>TLL2</i>	1.20E-08
3275	<i>PRMT2</i>	3.20E-08	7093	<i>TRDN</i>	1.20E-08
10577	<i>NPC2</i>	1.29E-07	10345	<i>FAM181B</i>	1.99E-08
10170	<i>DHRS9</i>	2.62E-07	220382	<i>RGS6</i>	5.14E-07
8668	<i>EIF3I</i>	4.49E-07	9628	<i>SMTNL2</i>	6.98E-07
51260	<i>PBDC1</i>	7.47E-07	342527	<i>ANKRD23</i>	9.84E-07
2487	<i>FRZB</i>	2.84E-06	200539	<i>OTOGL</i>	1.90E-06
81606	<i>LBH</i>	3.59E-06	283310	<i>HOOK1</i>	2.84E-06
10189	<i>ALYREF</i>	6.28E-06	51361	<i>KCNJ3</i>	2.84E-06
252969	<i>NEIL2</i>	7.06E-06	3760	<i>AQP4</i>	2.84E-06
3608	<i>ILF2</i>	7.08E-06	361	<i>SYT13</i>	2.98E-06
8048	<i>CSRP3</i>	7.67E-06	57586	<i>DEGS1</i>	3.32E-06
1114	<i>CHGB</i>	9.60E-06	8560	<i>SLC26A9</i>	3.59E-06
80851	<i>SH3BP5L</i>	1.30E-05	115019	<i>KIAA0895</i>	4.53E-06
55850	<i>USE1</i>	1.36E-05	23366	<i>SUSD4</i>	4.99E-06
11015	<i>KDEL3</i>	1.36E-05	55061	<i>NSDHL</i>	4.99E-06
10093	<i>ARPC4</i>	1.37E-05	50814	<i>LGR6</i>	5.06E-06
27338	<i>UBE2S</i>	1.60E-05	9097	<i>USP14</i>	5.59E-06

DEM - differentially expressed mRNA; FDR - false discovery rate; AF - atrial fibrillation

Furthermore, the targeted DMEs were significantly enriched in the following molecular functions of GO terms: protein binding, protein kinase binding, and actin binding (Fig. 3b). For the KEGG pathway, the targeted DMEs were significantly enriched in the PI3K–Akt, mTOR, Hippo, HIF-1, and ErbB signaling pathways (Table 3).

### The diagnostic value of DEMs in AF

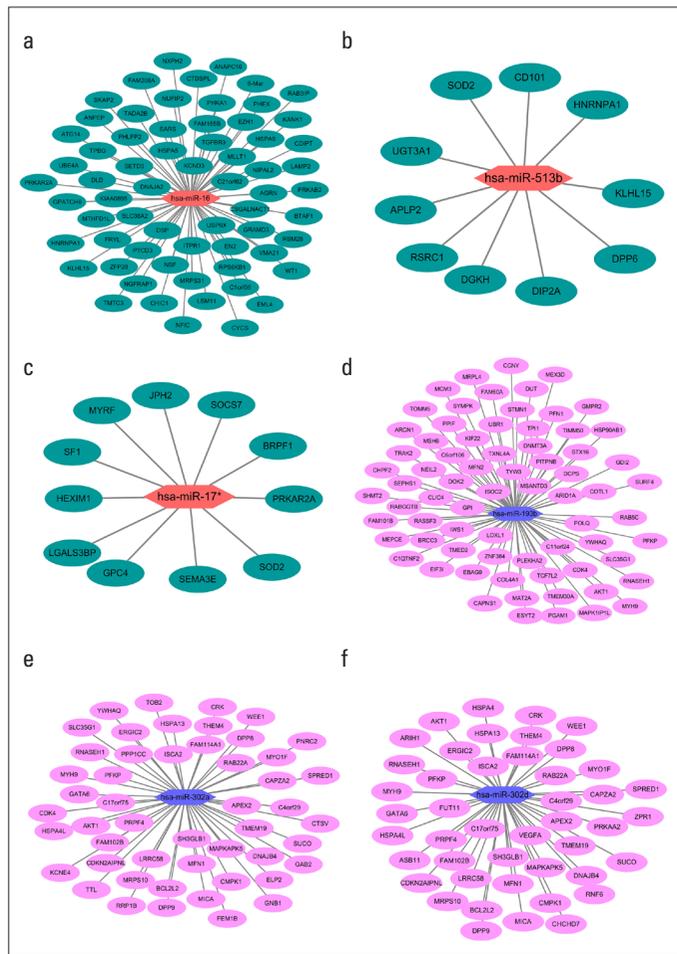
To evaluate the diagnostic value of DEMs in AF, ROC analyses of the GSE68475, GSE70887, and GSE28954 datasets including the miRNA expression data from patients with AF and healthy controls were performed. 2018 DEMs had diagnostic value in AF, except for miR-99a and let-7c; the AUC values of the 18 DEMs were more than 0.6 (Fig. S4 and Fig. 4). miR-490-3p, miR-630, miR-146b-5p, and miR-367 had a higher diagnostic value than other DEMs in distinguishing patients with AF from healthy controls; the AUC values of those 4 DEMs were 0.751, 0.719, 0.709, and 0.7, respectively (Fig. 4a-4d).

### Discussion

The GSE28954 and GSE70887 datasets involving data from patients with chronic AF and GSE68475 dataset involving data from patients with persistent AF were incorporated into this

study to identify DEMs that might be potential biomarkers for early detection of AF (14-16). This study revealed that 20 DEMs were dysregulated in atrial tissue of patients with AF compared with healthy controls. Four DEMs, namely, miR-490-3p, miR-146b-5p, miR-630, and miR-367, had a potential diagnostic value to distinguish patients with AF from healthy controls.

In a recent study, Zhang et al. (17), have used the GSE68475 dataset, which was also included in this study, and have shown that miR-204-5p, miR-31-5p, and miR-223-3p are key miRNAs in AF, which have more target genes based on the results of prediction of miRNA-target genes. However, these three miRNAs were not differentially expressed in AF compared with healthy individuals in this study. Furthermore, Larupa Santos et al. (18) have found that miR-130b-3p, miR-338-5p, and miR-208a-3p are differentially expressed in AF tissue samples. Although miR-130b-3p, miR-338-5p, and miR-208a-3p were not differentially expressed in AF, miR-338-3p as the homolog miRNA of miR-338-5p was significantly downregulated in AF in this study. Wang et al. (19) have identified 10 DEMs in AF tissue samples, including miR-146b-5p, miR-193b, and miR-155. In this study, both miR-146b-5p and miR-193b were differentially expressed in AF as well. Fan and Wei (20) have reported that miR-3123, miR-548g-3p, and miR-9-5p are most closely related to human AF. These miRNAs were not identified as DEMs in AF in this study. The



**Figure 2.** The subnetwork of up-regulated and down-regulated DEMs in AF. a: miR-16; b: miR-513b; c: miR-17\*; d: miR-193b; e: miR-302a; f: miR-302d. The red nodes indicate up-regulated DEMs, blue nodes indicated down-regulated DEMs, green nodes indicated down-regulated DEMs and pink nodes indicated up-regulated DEMs

discordant DEMs between this study and other studies might be attributed to several factors. First, different expression profiles of miRNAs between studies were used. Some studies have used only one dataset to identify DEMs in AF. Second, different criteria for screening DEMs were used. Third, some key miRNAs in AF were predicted based on the results retrieved from the GEO database, which were not identified by comparing the different expressions between patients with AF and healthy individuals.

miR-146b-5p was the most significantly upregulated DEMi in AF; moreover, it could discriminate patients with AF from healthy controls. This study is the first to report the dysregulation of miR-146-5p in AF. Published data have shown that microRNA-146a-5p levels are associated with circulating trimethylamine-N-oxide (TMAO) after atherogenic diet in animal models, such as liver-specific insulin receptor knockout mice fed a chow diet and African green monkeys fed a high-fat/high-cholesterol diet. TMAO is associated with increased atherosclerotic lesion formation and cardiovascular disease risk (21). miR-146b-5p is overexpressed in the atherosclerotic lesions of patients with AS

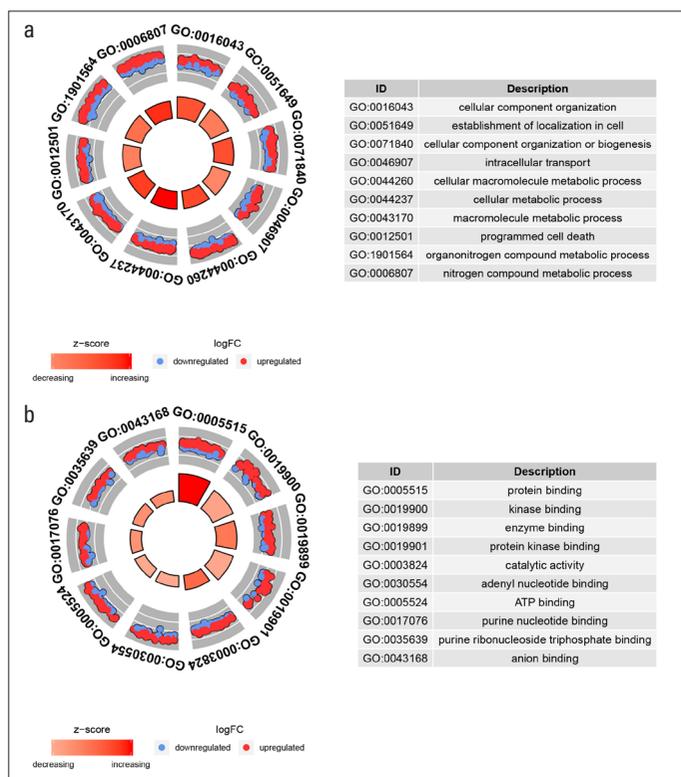
**Table 3. The enriched KEGG pathway of DEMs targeted by DEMis**

ID	Term	Number of DEMs	FDR
hsa04151	PI3K-Akt signaling pathway	16	1.78E-06
hsa04922	Glucagon signaling pathway	9	7.77E-06
hsa05200	Pathways in cancer	16	9.84E-06
hsa04910	Insulin signaling pathway	10	1.00E-05
hsa04150	mTOR signaling pathway	10	2.20E-05
hsa04390	Hippo signaling pathway	10	2.20E-05
hsa05169	Epstein-Barr virus infection	10	1.83E-04
hsa05205	Proteoglycans in cancer	10	1.89E-04
hsa04152	AMPK signaling pathway	8	2.09E-04
hsa04728	Dopaminergic synapse	8	2.62E-04
hsa04213	Longevity regulating pathway - multiple species	6	3.00E-04
hsa00010	Glycolysis / Gluconeogenesis	6	3.72E-04
hsa01100	Metabolic pathways	26	3.91E-04
hsa04066	HIF-1 signaling pathway	7	4.35E-04
hsa01521	EGFR tyrosine kinase inhibitor resistance	6	8.67E-04
hsa04141	Protein processing in endoplasmic reticulum	8	1.08E-03
hsa04022	cGMP-PKG signaling pathway	8	1.12E-03
hsa05222	Small cell lung cancer	6	1.13E-03
hsa04012	ErbB signaling pathway	6	1.26E-03
hsa04666	Fc gamma R-mediated phagocytosis	6	1.61E-03

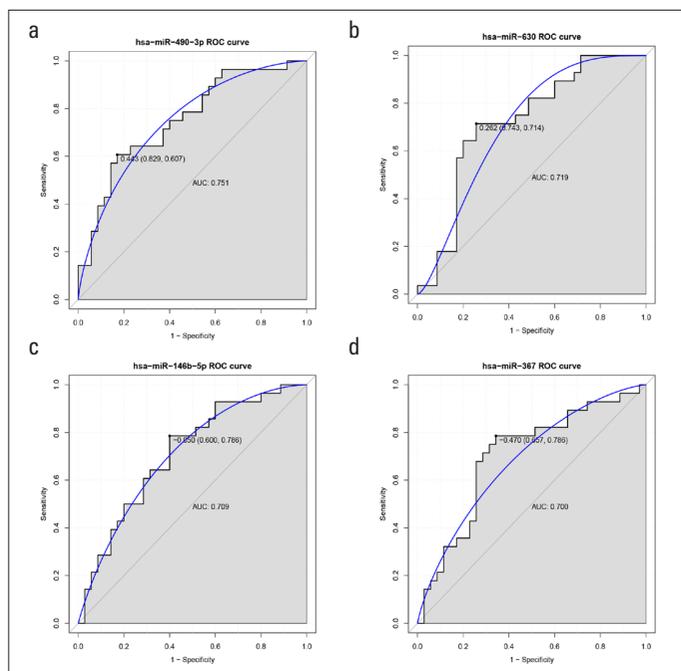
KEGG - Kyoto Encyclopedia of Genes and Genomes; DEM - differentially expressed mRNAs; DEMi - differentially expressed microRNAs

and is induced by oxidized low-density lipoprotein (oxLDL) in human macrophages; blockade of 146b-5p has a damaging role in AS-associated foam cell formation by targeting TRAF6 to promote chronic inflammation *in vitro* (22). In this study, among the four DEMs targeted by miR-146b-5p, only KDM6B, which was significantly downregulated in AF, implicated in cardiovascular disease has been reported. Bone marrow with myeloid *Kdm6b* deficiency (*Kdm6b*<sup>del</sup>) in mice results in more advanced AS (23). Moreover, KDM6B is implicated in the developing heart where co-expressed *Kdm6b* proteins promote cardiomyocyte proliferation coupled with the initial stages of cardiac trabeculation (24). However, the biological roles of miR-146b-5p and its targets are uncovered, and *in vitro* and *in vivo* studies should be performed to investigate the biological functions of miR-146-5p and its targets in the development of AF.

miR-630 is the top two significantly upregulated DEMi in AF, which targeted six DEMs, namely, IL6R, BTLA, MRO, LSAMP, DIP2A, and TMTC3, in this study. Studies have revealed that miRNA-630 participates in the regulation of epithelia-to-mesenchymal transition, tumorigenesis, metastasis, and radioresis-



**Figure 3.** The enrichment GO terms. a: the enrichment of biological processes of GO terms; b: the enrichment of molecular functions of GO terms



**Figure 4.** The ROC analysis of 4 DEMs in AF and healthy individuals in GSE68475, GSE70887 and GSE28954 datasets. a: miR-490-3p; b: miR-630; c: miR-146b-5p; d: miR-367

tance of cancers (25-27), but its biological roles in AF remain elusive. Among the six targets of miR-630 in this study, only IL6R involved in pathogenesis and prognosis of AF was reported. rs4845625 in the IL6R gene is associated with AF in Caucasians,

but not in African-Americans, and no association is observed between rs4845625 and stroke in Caucasians (28). Moreover, rs11265611, in intronic to IL6R, is significantly associated with AF (29). IL6R polymorphism rs4845625 is associated with the recurrence of AF after catheter ablation in a Han Chinese population (30). This and other studies indicated that miR-630 plays vital roles in AF pathogenesis, and further experiment needs to be performed to investigate its roles in AF.

miR-490-3p and miR-367 were upregulated in AF, and both DEMs could distinguish patients with AF from healthy controls. Those two DEMs involved in AF have not been reported. miR-490-3p is implicated in AS progression. A study has revealed that miR-490-3p modulates the proliferation of vascular smooth muscle cells (VSMCs) induced by oxLDL by targeting pregnancy-associated plasma protein-A (31). In AS, linc00299 acts as a sponge for miR-490-3p to upregulate aurora kinase A, increasing the proliferation and migration of VSMCs and human umbilical vein endothelial cells (32); in addition, HOTTIP knockdown suppresses cell proliferation and migration by regulating the miR-490-3p/HMGB1 axis and PI3K–Akt signaling pathway in oxLDL-induced VSMCs (33). The PI3k–AKT and Hippo signaling pathways were significantly enriched in the KEGG pathway in AF. Published data have shown that the PI3k–Akt signaling pathway is implicated in AF progression and a potential target of drugs. Ibrutinib increases the risk of AF, potentially by inhibiting cardiac PI3K–Akt signaling pathway (34). Telmisartan reduces the susceptibility to atrial arrhythmia and reverses imbalances in the RAS–ERK and PI3K–Akt–eNOS pathways in spontaneously hypertensive rats (35). Resveratrol reduces AF susceptibility in the failing heart by activating the PI3K/AKT/eNOS signaling pathway (36). In a study, weighted gene co-expression network analysis revealed that the Hippo signaling pathway is associated with AF (37). For miR-367, the downregulation of long noncoding RNA linc00961 promotes proliferation and inhibits apoptosis of VSMCs by sponging miR-367 in patients with coronary heart disease (38).

## Conclusion

In summary, in this study, we found that four miRNAs had a diagnostic value to distinguish patients with AF from healthy individuals, namely, miR-490-3p, miR-146b-5p, miR-630, and miR-367. The biological roles of those four miRNAs in AF progression have not been elucidated, which needed to be explored in further studies. In addition, the prognostic value of those four miRNAs is needed to be further validated in a prospective, large cohort of patients with AF and healthy individuals.

**Conflict of interest:** None declared.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept – Y.C., L.C.; Design – Y.C.; Supervision – Y.C., L.C.; Fundings – Y.C.; Materials – L.C.; Data collection &/or processing – Y.C.; Analysis &/or interpretation – Y.C., L.C.; Literature search – Y.C.; Writing – Y.C.; Critical review – Y.C., L.C.

## References

1. Harada M, Melka J, Sobue Y, Nattel S. Metabolic Considerations in Atrial Fibrillation-Mechanistic Insights and Therapeutic Opportunities. *Circ J* 2017; 81: 1749–57. [\[Crossref\]](#)
2. Storz MA, Helle P. Atrial fibrillation risk factor management with a plant-based diet: A review. *J Arrhythm* 2019; 35: 781–8. [\[Crossref\]](#)
3. Zulkifly H, Lip GYH, Lane DA. Epidemiology of atrial fibrillation. *Int J Clin Pract* 2018; 72: e13070. [\[Crossref\]](#)
4. Lu TX, Rothenberg ME. MicroRNA. *J Allergy Clin Immunol* 2018; 141: 1202–7. [\[Crossref\]](#)
5. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinformatics* 2009; 7: 147–54. [\[Crossref\]](#)
6. Lv X, Li J, Hu Y, Wang S, Yang C, Li C, et al. Overexpression of miR-27b-3p Targeting Wnt3a Regulates the Signaling Pathway of Wnt/ $\beta$ -Catenin and Attenuates Atrial Fibrosis in Rats with Atrial Fibrillation. *Oxid Med Cell Longev* 2019; 2019: 5703764. [\[Crossref\]](#)
7. Reilly SN, Liu X, Carnicer R, Recalde A, Muszkiewicz A, Jayaram R, et al. Up-regulation of miR-31 in human atrial fibrillation begets the arrhythmia by depleting dystrophin and neuronal nitric oxide synthase. *Sci Transl Med* 2016; 8: 340ra74. [\[Crossref\]](#)
8. McManus DD, Tanriverdi K, Lin H, Esa N, Kinno M, Mandapati D, et al. Plasma microRNAs are associated with atrial fibrillation and change after catheter ablation (the miRhythm study). *Heart Rhythm* 2015; 12: 3–10. [\[Crossref\]](#)
9. da Silva AM, de Araújo JN, de Freitas RC, Silbiger VN. Circulating MicroRNAs as Potential Biomarkers of Atrial Fibrillation. *Biomed Res Int* 2017; 2017: 7804763. [\[Crossref\]](#)
10. Gomes da Silva AM, Silbiger VN. miRNAs as biomarkers of atrial fibrillation. *Biomarkers* 2014; 19: 631–6. [\[Crossref\]](#)
11. Weckbach LT, Grabmaier U, Clauss S, Wakili R. MicroRNAs as a diagnostic tool for heart failure and atrial fibrillation. *Curr Opin Pharmacol* 2016; 27: 24–30. [\[Crossref\]](#)
12. Santulli G, Iaccarino G, De Luca N, Trimarco B, Condorelli G. Atrial fibrillation and microRNAs. *Front Physiol* 2014; 5: 15. [\[Crossref\]](#)
13. Liu Z, Zhou C, Liu Y, Wang S, Ye P, Miao X, et al. The expression levels of plasma microRNAs in atrial fibrillation patients. *PLoS One* 2012; 7: e44906. [\[Crossref\]](#)
14. Cooley N, Cowley MJ, Lin RC, Marasco S, Wong C, Kaye DM, et al. Influence of atrial fibrillation on microRNA expression profiles in left and right atria from patients with valvular heart disease. *Physiol Genomics* 2012; 44: 211–9. [\[Crossref\]](#)
15. Cañón S, Caballero R, Herraiz-Martínez A, Pérez-Hernández M, López B, Atienza F, et al. miR-208b upregulation interferes with calcium handling in HL-1 atrial myocytes: Implications in human chronic atrial fibrillation. *J Mol Cell Cardiol* 2016; 99: 162–73. [\[Crossref\]](#)
16. Morishima M, Iwata E, Nakada C, Tsukamoto Y, Takanari H, Miyamoto S, et al. Atrial Fibrillation-Mediated Upregulation of miR-30d Regulates Myocardial Electrical Remodeling of the G-Protein-Gated K(+) Channel, IK.ACh. *Circ J* 2016; 80: 1346–55. [\[Crossref\]](#)
17. Zhang H, Yang G, Zhong N, Shan J, Li X, Wu Y, et al. Possible key microRNAs and corresponding molecular mechanisms for atrial fibrillation. *Anatol J Cardiol* 2020; 23: 324–33. [\[Crossref\]](#)
18. Larupa Santos J, Rodríguez I, M SO, Hjorth Bentzen B, Schmitt N. Investigating gene-microRNA networks in atrial fibrillation patients with mitral valve regurgitation. *PLoS One* 2020; 15: e0232719. [\[Crossref\]](#)
19. Wang J, Wang Y, Han J, Li Y, Xie C, Xie L, et al. Integrated analysis of microRNA and mRNA expression profiles in the left atrium of patients with nonvalvular paroxysmal atrial fibrillation: Role of miR-146b-5p in atrial fibrosis. *Heart Rhythm* 2015; 12: 1018–26. [\[Crossref\]](#)
20. Fan G, Wei J. Identification of potential novel biomarkers and therapeutic targets involved in human atrial fibrillation based on bioinformatics analysis. *Kardiol Pol* 2020; 78: 694–702. [\[Crossref\]](#)
21. Coffey AR, Kanke M, Smallwood TL, Albright J, Pitman W, Gharaibeh RZ, et al. microRNA-146a-5p association with the cardiometabolic disease risk factor TMAO. *Physiol Genomics* 2019; 51: 59–71. [\[Crossref\]](#)
22. Lin N, An Y. Blockade of 146b-5p promotes inflammation in atherosclerosis-associated foam cell formation by targeting TRAF6. *Exp Ther Med* 2017; 14: 5087–92. [\[Crossref\]](#)
23. Neele AE, Gijbels MJJ, van der Velden S, Hoeksema MA, Boshuizen MCS, Prange KHM, et al. Myeloid Kdm6b deficiency results in advanced atherosclerosis. *Atherosclerosis* 2018; 275: 156–65. [\[Crossref\]](#)
24. Akerberg AA, Henner A, Stewart S, Stankunas K. Histone demethylases Kdm6ba and Kdm6bb redundantly promote cardiomyocyte proliferation during zebrafish heart ventricle maturation. *Dev Biol* 2017; 426: 84–96. [\[Crossref\]](#)
25. Wu DW, Wang YC, Wang L, Chen CY, Lee H. A low microRNA-630 expression confers resistance to tyrosine kinase inhibitors in EGFR-mutated lung adenocarcinomas via miR-630/YAP1/ERK feedback loop. *Theranostics* 2018; 8: 1256–69. [\[Crossref\]](#)
26. Zhou CX, Wang CL, Yu AL, Wang QY, Zhan MN, Tang J, et al. MiR-630 suppresses breast cancer progression by targeting metadherin. *Oncotarget* 2016; 7: 1288–99. [\[Crossref\]](#)
27. Zhang Y, Liu H, Li W, Yu J, Li J, Shen Z, et al. CircRNA\_100269 is downregulated in gastric cancer and suppresses tumor cell growth by targeting miR-630. *Aging (Albany NY)* 2017; 9: 1585–94. [\[Crossref\]](#)
28. Schnabel RB, Kerr KF, Lubitz SA, Alkylbekova EL, Marcus GM, Sinner MF, et al.; Candidate Gene Association Resource (CARE) Atrial Fibrillation/Electrocardiography Working Group. Large-scale candidate gene analysis in whites and African Americans identifies IL6R polymorphism in relation to atrial fibrillation: the National Heart, Lung, and Blood Institute's Candidate Gene Association Resource (CARE) project. *Circ Cardiovasc Genet* 2011; 4: 557–64. [\[Crossref\]](#)
29. Lin H, Sinner MF, Brody JA, Arking DE, Lunetta KL, Rienstra M, et al.; CHARGE Atrial Fibrillation Working Group. Targeted sequencing in candidate genes for atrial fibrillation: the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Targeted Sequencing Study. *Heart Rhythm* 2014; 11: 452–7. [\[Crossref\]](#)
30. Wu G, Cheng M, Huang H, Yang B, Jiang H, Huang C. A variant of IL6R is associated with the recurrence of atrial fibrillation after catheter ablation in a Chinese Han population. *PLoS One* 2014; 9: e99623. [\[Crossref\]](#)
31. Sun Y, Chen D, Cao L, Zhang R, Zhou J, Chen H, et al. MiR-490-3p modulates the proliferation of vascular smooth muscle cells induced by ox-LDL through targeting PAPP-A. *Cardiovasc Res* 2013; 100: 272–9. [\[Crossref\]](#)
32. Liu Y, Chen Y, Tan L, Zhao H, Xiao N. Linc00299/miR-490-3p/AURKA axis regulates cell growth and migration in atherosclerosis. *Heart Vessels* 2019; 34: 1370–80. [\[Crossref\]](#)

33. Guo X, Liu Y, Zheng X, Han Y, Cheng J. HOTTIP knockdown inhibits cell proliferation and migration via regulating miR-490-3p/HMGB1 axis and PI3K-AKT signaling pathway in ox-LDL-induced VSMCs. *Life Sci* 2020; 248: 117445. [\[Crossref\]](#)
34. McMullen JR, Boey EJ, Ooi JY, Seymour JF, Keating MJ, Tam CS. Ibrutinib increases the risk of atrial fibrillation, potentially through inhibition of cardiac PI3K-Akt signaling. *Blood* 2014; 124: 3829–30. [\[Crossref\]](#)
35. Wang WW, Zhang FL, Chen JH, Chen XH, Fu FY, Tang MR, et al. Telmisartan reduces atrial arrhythmia susceptibility through the regulation of RAS-ERK and PI3K-Akt-eNOS pathways in spontaneously hypertensive rats. *Can J Physiol Pharmacol* 2015; 93: 657–65. [\[Crossref\]](#)
36. Chong E, Chang SL, Hsiao YW, Singhal R, Liu SH, Leha T, et al. Resveratrol, a red wine antioxidant, reduces atrial fibrillation susceptibility in the failing heart by PI3K/AKT/eNOS signaling pathway activation. *Heart Rhythm* 2015; 12: 1046–56. [\[Crossref\]](#)
37. Li W, Wang L, Wu Y, Yuan Z, Zhou J. Weighted gene co-expression network analysis to identify key modules and hub genes associated with atrial fibrillation. *Int J Mol Med* 2020; 45: 401–16. [\[Crossref\]](#)
38. Wu CT, Liu S, Tang M. Downregulation of linc00961 contributes to promote proliferation and inhibit apoptosis of vascular smooth muscle cell by sponging miR-367 in patients with coronary heart disease. *Eur Rev Med Pharmacol Sci* 2019; 23: 8540–50.

Supplementary data

GEO ID	NC	AF	Platform	Country	Author	Year	PMID
GSE68475	20	14	GPL15018 Agilent-031181 Unrestricted_Human_miRNA_V16.0_Microarray 030840 (Feature Number version)	Japan	Masaki Morishima	2017	27180889
GSE70887	4	4	GPL19546 Agilent-021827 Human miRNA Microarray [miRBase release 17.0 miRNA ID version]	Spain	Susana Cañón	2015	-
GSE28954	11	10	GPL8227 Agilent-019118 Human miRNA Microarray 2.0 G4470B (miRNA ID version); GPL10850 Agilent-021827 Human miRNA Microarray (V3) (miRBase release 12.0 miRNA ID version)	Australia	Mark Cowley	2011	22147268

NC - normal controls; AF - atrial fibrillation

GEO ID	NC	AF	Platform	Country	Author	Year	PMID
GSE115574	31	28	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	Turkey	Gunseli Cubukcuoglu Deniz	2019	-
GSE31821	2	4	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	France	Emmanuelle Meugnier	2018	-
GSE62871	9	7	GPL17077 Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 (Probe Name version)	France	Lefebvre Philippe	2017	23644086
GSE79768	12	14	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	china taiwan	Yun-Shien Lee	2016	27494721
GSE41177	6	32	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	china taiwan	Yun-Shien Lee	2013	23183193
GSE14975	5	5	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	Germany	Sabine Ameling	2010	20117462

NC - normal controls; AF - atrial fibrillation

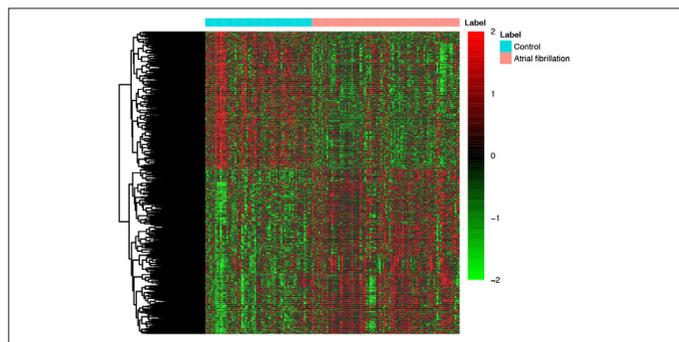


Figure S1. The heatmap of DEMs in AF compared to healthy individuals

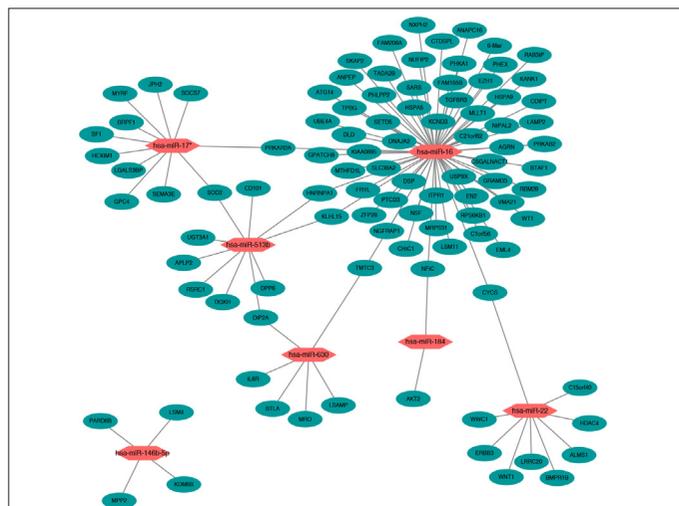


Figure S2. The interaction network between up-regulated DEMs and down-regulated DEMs in AF. The red nodes indicate up-regulated DEMs and green nodes indicated down-regulated DEMs

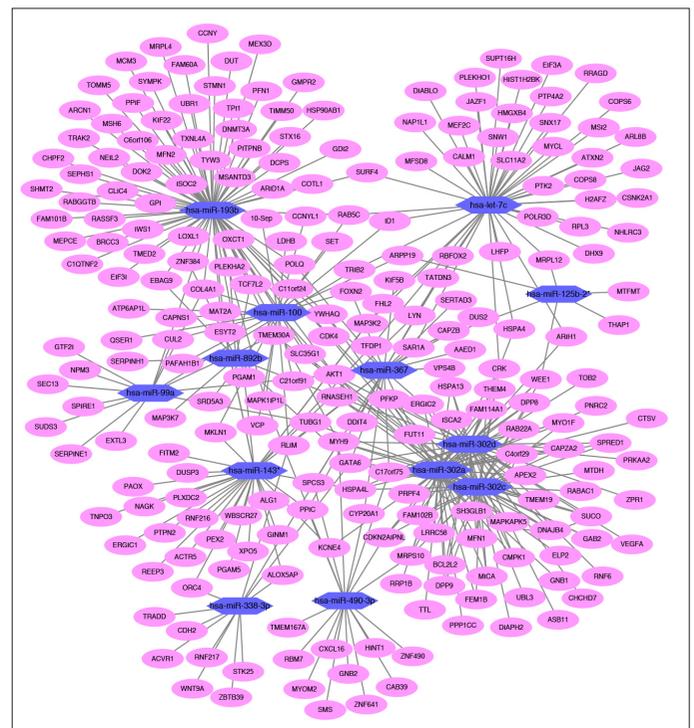
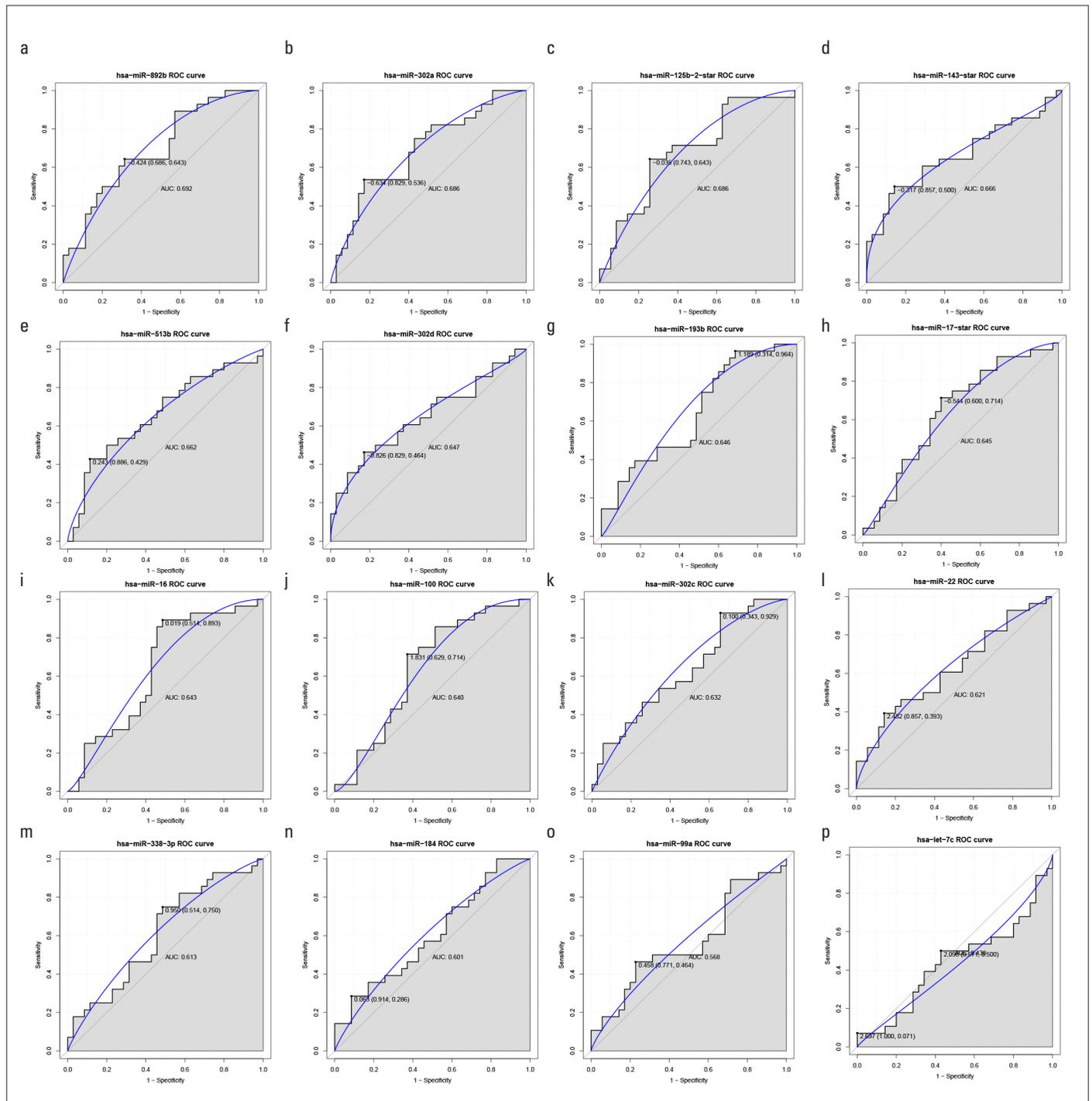


Figure S3. The interaction network between down-regulated DEMs and up-regulated DEMs in AF. The blue nodes indicated down-regulated DEMs and pink nodes indicated up-regulated DEMs



**Figure S4.** The ROC analysis of 16 DEMis in AF and healthy individuals in GSE68475, GSE70887 and GSE28954 datasets