

# Integrated Analysis Identifies Key lncRNA-mRNA Network in Atrial Fibrillation

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## Research Article

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# Abstract

*Background:* Atrial fibrillation (AF), the most common cardiac arrhythmias, is associated with the risk of stroke and pronounced morbidity and mortality. The application of biomarkers in the management of AF has been grown as an interesting topic. Long non-coding RNAs (lncRNAs) have been reported to participate in the pathogenesis of cardiovascular diseases by regulating mRNA networks.

*Results:* In this study, we firstly used two AF cohorts to identify circulating lncRNAs and mRNAs with potential diagnostic prediction value. The expression of 8,164 lncRNAs and 14,508 mRNAs were quantified in these two cohorts with lncRNA microarray. By using a stringent threshold ( $P < 0.01$ , fold change  $> 2.0$  or  $< 0.5$ ), we identified 10 lncRNAs and 7 mRNAs were significantly differentially expressed in AF in both cohorts. To further explore the function of these significantly dysregulated lncRNA and mRNA, we performed co-expression analysis by using RNA-seq data of 429 atrial appendage tissue samples. Interestingly, we found a significant lncRNA-mRNA network for 5 lncRNAs (AC109460.1, AL031123.1, MIAT, PTPRG-AS1 and ZNF815P) and 4 mRNAs (D2HGDH, SPNS1, KCND2 and TNFAIP8L3) with Pearson correlation  $r > 0.3$  (all  $P < 10^{-8}$ ). Moreover, in silico analysis showed that the lncRNA MIAT and PTPRG-AS1 may serve as miRNA sponges to regulate D2HGDH, SPNS1, KCND2 and TNFAIP8L3.

*Conclusions:* Our study suggested that the circulating lncRNA-mRNA network of MIAT and PTPRG-AS1 play an important role in AF and may be considerable diagnostic biomarkers. These results may contribute to the precise diagnosis and early detection of this disease.

## Introduction

Atrial fibrillation (AF) is a common cardiac arrhythmia in clinical practice and is known as a major risk factor of ischemic stroke, with an estimate incidence of 1–2% in the general population, rising up to 8–10% in the elderly [1]. The available data demonstrate that AF significantly increases morbidity and mortality [2] and provokes prominent socioeconomic burden [3]. Unfortunately, we often identify AF after a complication, e.g. a stroke, which can be prevented by initiation of oral anticoagulation [4]. Therefore, systematic AF screening is necessary for at risk populations [5]. However, electrocardiograph (ECG) screening is both bothersome and resource-intensive [6]. Therefore, serum diagnostic biomarkers of AF will be highly valuable. Biomarkers have been widely utilized in the management of cardiovascular disorders, such as acute myocardial infarction and heart failure, and have contributed to risk predictors and prognostic values [7]. On the contrary, the application of biomarkers in AF management has not yet well established in the major guidelines until the 2016 European Society of Cardiology guideline for management of atrial fibrillation developed in collaboration with EACTS [8]. Novel biomarkers may offer the possibility of earlier diagnosis with the opportunity to alleviate disease progression. Therefore, identification of new biomarkers targeting the early diagnosis for AF is pivotal.

long non-coding RNAs (lncRNAs) are transcripts generated from transcriptional units which resemble protein coding genes but lacking coding potential and characterized by large than 200 nt [9]. The

estimated amount of lncRNAs in the human genome is ~ 58 000 [10]. Various lncRNAs have been reported to serve as potential biomarkers for diagnosis and prognosis in cardiovascular diseases [4]. For example, the lncRNA, UCA1, was identified to initially decrease and then augment in patients with acute myocardial infarction, indicating a possible diagnostic value [11]. The lncRNA, LIPCAR, was reported as a valuable biomarker for cardiac remodeling and a predictor of the mortality of heart failure [12]. However, the evidence for the involvement of lncRNAs in AF is quite finite [13].

In this study, to systematically search for key lncRNA-mRNA networks in AF, we identified differentially expressed lncRNAs and mRNAs in patients with AF and performed co-expression analysis and bioinformatic predictions. The identified lncRNA-mRNA network may serve as potential diagnostic biomarkers for this disease.

## Materials And Methods

### Study participants and lncRNA microarray

In this study, we included two AF cohorts from the Gene Expression Omnibus (GEO) database (GSE64904 [14] and GSE75092 [15]) with RNA microarray data from the blood of AF patients and healthy controls. The first cohort (GSE64904) contains 3 normal blood samples and 3 AF blood samples. The expression patterns of lncRNAs and mRNAs was determined by using the Agilent Human lncRNA array V4.0 (4 × 180 K), which include 78,243 human lncRNAs and 30,215 coding transcripts. The second cohort (GSE75092) also included peripheral blood samples from 3 AF patients and 3 healthy controls. The lncRNAs and mRNAs profiling in leukocytes were conducted by using the Arraystar human lncRNA microarray 3.0 which contained probes for 30,586 lncRNAs and 26,109 protein coding transcripts. The probe annotation of these two microarrays were conducted using the idmap3 package in R.

### Co-expression analysis and miRNA target prediction

The gene TPM data of GTEx v8 [16] (GTEx\_Analysis\_2017-06-05\_v8\_RNASeQCv1.1.9\_gene\_tpm.gct.gz) were downloaded and 429 Heart - Atrial Appendage tissue samples were used to analyze the co-expression of lncRNAs and mRNAs. The co-expressions were calculated by using Pearson correlation test with the  $P$  value  $< 0.05$  and  $r > 0.3$  being considered significant. The cytoscape software [17] was used to build lncRNA-mRNA network. The miRcode [18] was used to predict miRNA target for these identified lncRNAs and mRNAs.

### Statistical analysis

The expression of lncRNAs and mRNAs in different groups were compared by using unpaired  $t$ -test with two-sided.  $P$  values below 0.01 and fold change  $> 2.0$  or  $< 0.5$  were considered statistically significant for the identification of differentially expressed lncRNAs and mRNAs. All statistical analysis was performed

by using R 3.3.0. The volcano plots were plotted by using the ggplot2 package in R. The venn plots were plotted by using the venn.diagram package in R. The heatmap plots were plotted by using heatmap.2 function from the gplots package in R.

## Results

### Identification of differentially expressed lncRNAs in AF patients

We obtained the circulating lncRNA profiling in AF patients and healthy controls from two cohorts. Both of the two cohorts consisted of blood samples from 3 AF patients and 3 healthy controls and the lncRNAs expression was profiled by using the Arraystar Human lncRNA Array. A total of 8,164 lncRNAs were annotated and analyzed in these two cohorts in this study. Based on the cut-off criteria of  $P$  value  $< 0.01$  and fold change  $> 2.0$  or  $< 0.5$ , we identified 116 and 639 differentially expressed circulating lncRNAs in AF patients, respectively (Figure 1A and 1B). Among these differentially expressed lncRNAs, 49 and 251 were up-regulated in these two cohorts, respectively (Figure 1C, top). Meanwhile, 67 and 388 lncRNAs were down-regulated in these two cohorts (Figure 1C, bottom). Two (LINC00174 and AP001360.3) and eight (AC109460.1, AC109460.3, AL031123.1, AL358113.1, MIAT, MIR4713HG, PTPRG-AS1 and ZNF815P) lncRNAs were significantly up-regulated and down-regulated in both the two cohorts, respectively (Figure 1C). Through unsupervised clustering analysis, the identified lncRNAs can categorize the blood samples into AF patients and healthy controls groups, showing good diagnostic prediction ability (Figure 1D and 1E).

### Identification of differentially expressed mRNAs in AF patients

We then systematically searched for differentially expressed mRNAs in AF patients among 14,508 mRNAs in the two cohorts. Based on the same criteria as significant dysregulation lncRNA identification, we identified 143 and 869 differentially expressed circulating mRNAs in AF patients from the two cohorts, respectively (Figure 2A and 2B). Among these differentially expressed mRNAs, only one (TEAD2) was significantly up-regulated in both the two cohorts with  $P$  value being 0.0078 (fold change = 2.23) and 0.0005 (fold change = 2.81) in GSE64904 and GSE75092, respectively (Figure 2C, top). Meanwhile, six mRNAs (D2HGDH, KCND2, PTPRG, SOCS3, SPNS1 and TNFAIP8L3) were significantly down-regulated in both the two cohorts (Figure 2C, bottom). Similarly, these mRNAs also showed great diagnostic prediction potential that can categorize the blood samples into AF patients and healthy controls by using unsupervised clustering analysis (Figure 2D and 2E).

### Identification of lncRNA-mRNA network

To further investigate the potential function of these identified dysregulated lncRNAs and mRNAs in AF patients, we conducted co-expression analysis by using the RNA-seq data of 429 normal Atrial Appendage tissue samples from the Genotype-Tissue Expression (GTEx) project database. Significant correlation (Pearson  $P$  value  $< 0.05$  and  $r > 0.3$  or  $< -0.3$ ) between the differentially expressed lncRNAs and mRNAs were shown in the Table 1. The most significant co-expression was PTPRG-AS1 and D2HGDH with Pearson  $P$  value being  $3.69 \times 10^{-36}$  and correlation  $r$  being 0.56. By using these significant lncRNA and mRNA co-expression, we construct a potential lncRNA-mRNA network (Figure 3A). As all the significant lncRNA-mRNA co-expressions were positive correlation (Table 1), we hypothesises a miRNA sponge mechanism for these lncRNAs. Therefore, we performed miRNA target prediction for these lncRNAs and mRNAs by using a transcriptome-wide miRNA target prediction tool including lncRNAs (miRcode) [18], which was based on Targetscan 6. The results showed that the lncRNA MIAT may regulate SPNS1 and D2HGDH by competitive binding of miR-15, miR-24, miR-27 et al (Figure 3B, left) and the PTPRG-AS1 also serve as a miRNA sponge to regulate D2HGDH, SPNS1, TNFAIP8L3 and KCND2 (Figure 3B, right). Collectively, these results indicate a potential dysregulated lncRNA-miRNA-mRNA network may function in both the blood and the Atrial tissues of AF patients.

Table 1  
Significant co-expression of identified dysregulation lncRNAs and mRNAs in AF

| LncRNAs    | LncRNA id         | mRNAs     | Pearson $P$            | $r$  |
|------------|-------------------|-----------|------------------------|------|
| AC109460.1 | ENSG00000260367.2 | SPNS1     | $2.00 \times 10^{-26}$ | 0.48 |
|            |                   | D2HGDH    | $1.54 \times 10^{-14}$ | 0.36 |
| AL031123.1 | ENSG00000226281.2 | SPNS1     | $6.01 \times 10^{-12}$ | 0.32 |
|            |                   | D2HGDH    | $2.95 \times 10^{-11}$ | 0.31 |
| MIAT       | ENSG00000225783.6 | SPNS1     | $2.00 \times 10^{-26}$ | 0.48 |
|            |                   | D2HGDH    | $1.54 \times 10^{-14}$ | 0.36 |
| PTPRG-AS1  | ENSG00000241472.6 | D2HGDH    | $3.69 \times 10^{-36}$ | 0.56 |
|            |                   | SPNS1     | $6.25 \times 10^{-22}$ | 0.44 |
|            |                   | TNFAIP8L3 | $1.84 \times 10^{-11}$ | 0.32 |
|            |                   | KCND2     | $1.27 \times 10^{-10}$ | 0.30 |
| ZNF815P    | ENSG00000235944.7 | SPNS1     | $1.17 \times 10^{-39}$ | 0.58 |
|            |                   | D2HGDH    | $2.92 \times 10^{-37}$ | 0.56 |

## Discussion

In this study, by systematically searching for key lncRNA-mRNA network in AF patients, we used two different cohorts with lncRNA microarray data and identified 10 lncRNAs and 7 mRNAs that were significantly differentially expressed in AF in both two cohorts. Through further co-expression and bioinformatics analysis, we constructed a lncRNA-miRNA-mRNA network including 5 lncRNAs, 4 mRNAs and a series of potential miRNAs. The identified key lncRNA-mRNA network may serve as diagnostic prediction biomarkers for the AF.

AF is associated with increasing morbidity and mortality worldwide. Even intermittent asymptomatic AF episodes could increase the risk for the ischemic stroke and systemic embolism [19]. Currently, AF can be detected by ECG and prolonged monitoring. Since the proportion of asymptomatic AF within all AF episodes is nearly 69.0% [20], the diagnosis of asymptomatic AF is surly challenging [21]. The sensitivity of standard ECG for the detection of AF is low especially in patients with paroxysmal AF [22]. Prolonged ECG monitoring has higher sensitivity but is much more expensive, time-consuming and hard to generalize to a routine scenario. Therefore, including biomarker measurements in clinical practice could better identify patients with undiagnosed prevalent AF.

Serum biomarkers have the potential to be incorporated into point-of-care tests thus encouraging screening for AF in community [23]. Previous studies have investigated some candidate biomarkers for detection of AF, such as N-terminal prohormone of brain natriuretic peptide (NT-proBNP) [24], brain natriuretic peptide (BNP) [25], C-reactive protein [26], Galectin-3 [5] and fibroblast growth factor-23 (FGF-23) [23]. A series of lncRNAs we identified in the present study may also be advantageous to screen and diagnose patients with AF in many settings, particularly in milieux without prompt input from medically cultivated personnel.

There are some limitations for this study. First, the identified dysregulated lncRNAs and mRNAs still need to be replicated in more cohorts with larger sample size. Second, the function of these identified lncRNAs in the development of AF need to be investigated. Importantly, the biological mechanism caused the differentially expressed of these lncRNAs in the serum of AF patients need to be explored. Third, the miRNA sponge mechanism for the identified lncRNAs need further validation.

In summary, this study identified a key lncRNA-mRNA network in AF that may serve as potential diagnostic biomarkers for this disease. The findings may contribute to the early detection and precise diagnosis of AF.

## Declarations

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

## Competing Interests

The authors declare that they have no competing interests.

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## Authors' contribution

RMH and XC conceived and designed the study. TS and ZL developed the methodology. ZL and XY collected the sample. RMH and TS analyzed and interpreted the data. RMH wrote the main manuscript text. All authors reviewed the manuscript.

## Acknowledgements

Not applicable.

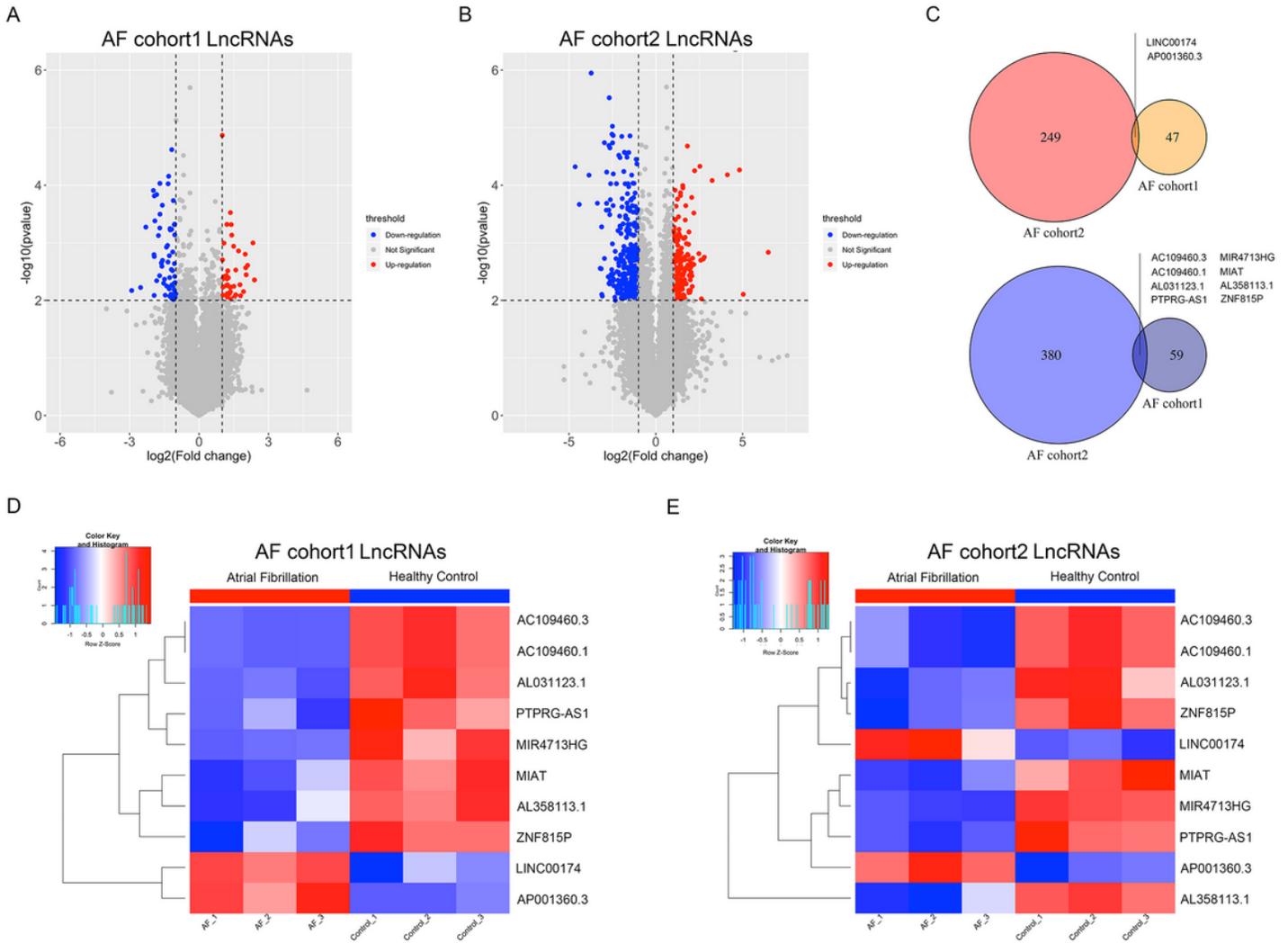
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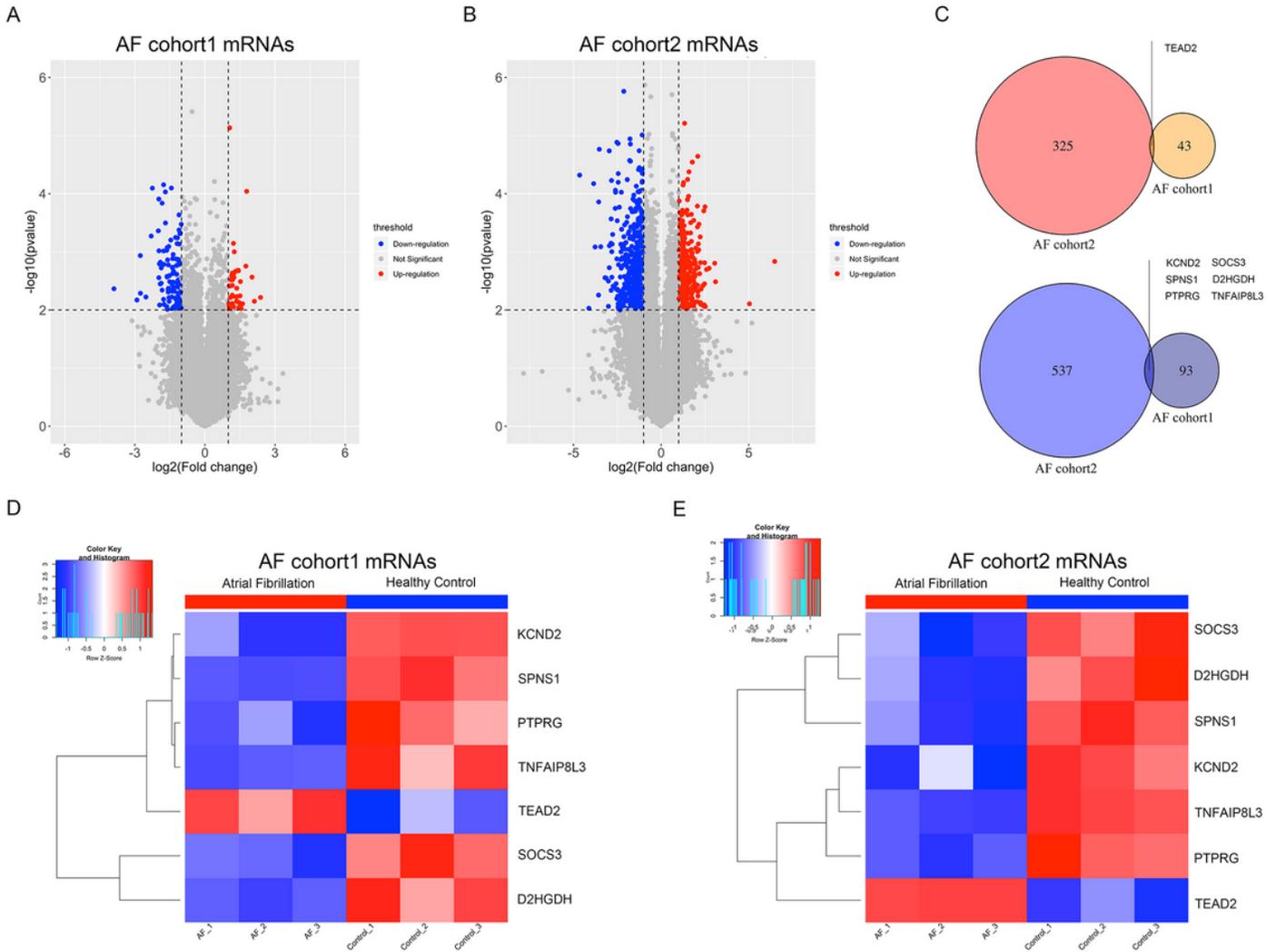
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## Figures



**Figure 1**

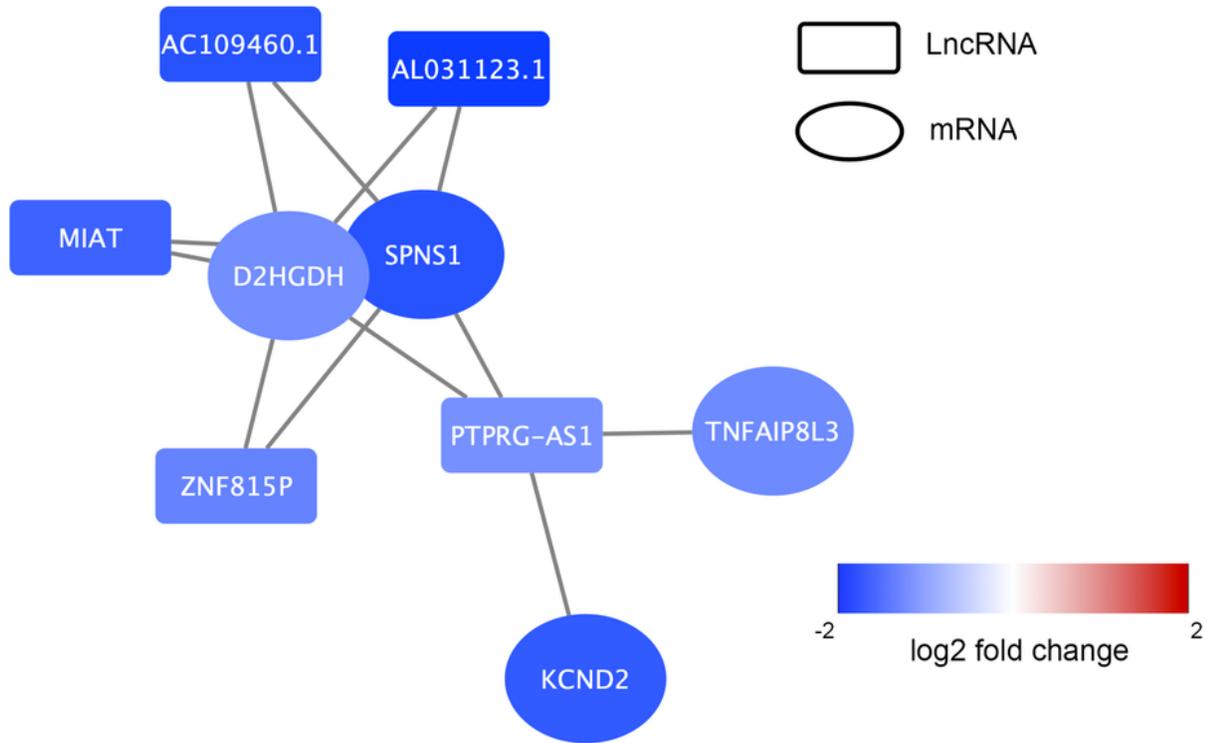
The dysregulated lncRNAs identified by using two AF cohorts. (A) Volcano plot of corresponding P values and fold change for circulating lncRNAs in the AF cohort1. (B) Volcano plot of corresponding P values and fold change for circulating lncRNAs in the AF cohort2. (C) Significantly up-regulated or down-regulated lncRNA in both cohorts. (D) Heatmap based on the expression of circulating lncRNAs in the AF cohort1. (E) Heatmap based on the expression of circulating lncRNAs in the AF cohort2.



**Figure 2**

The dysregulated mRNAs identified by using two AF cohorts. (A) Volcano plot of corresponding P values and fold change for circulating mRNA in the AF cohort1. (B) Volcano plot of corresponding P values and fold change for circulating mRNA in the AF cohort2. (C) Significantly up-regulated or down-regulated mRNAs in both cohorts. (D) Heatmap based on the expression of circulating mRNAs in the AF and control groups in the AF cohort1. (E) Heatmap based on the expression of circulating mRNAs in the AF cohort2.

A



B

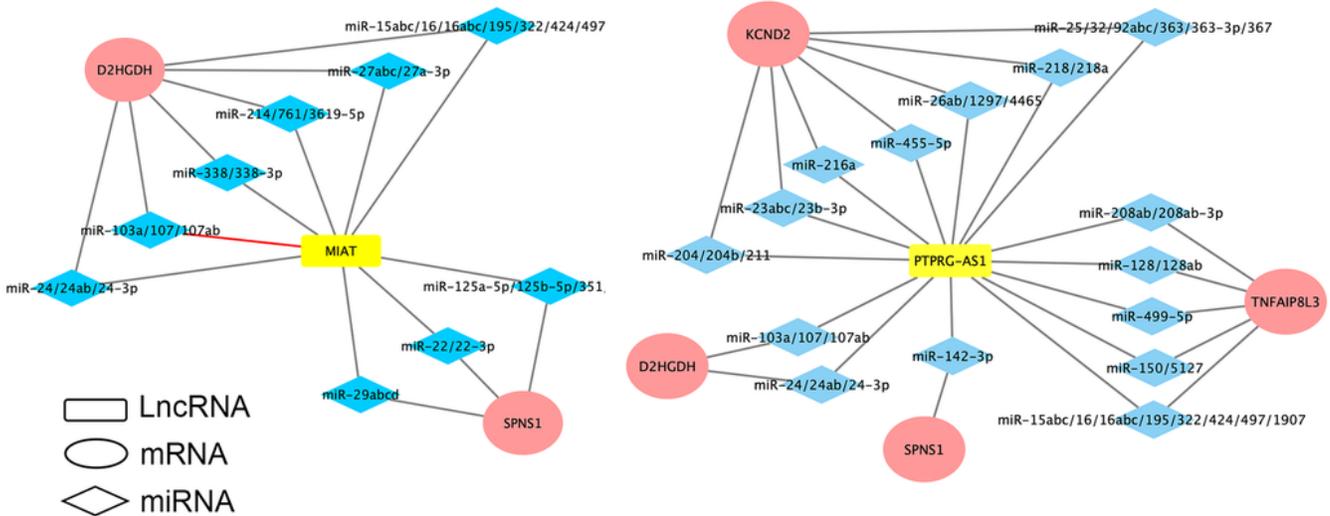


Figure 3

The dysregulated circulating lncRNA-miRNA-mRNA network. (A) The co-expression analysis of lncRNAs and mRNAs were calculated by using the RNA-seq data of 429 atrial appendage tissue samples. (B) The lncRNA-miRNA and mRNA-miRNA binding were predicted by using the miRcode. The rectangles in yellow indicate lncRNAs, ellipses in light red represent mRNAs and diamonds in blue represent miRNAs.