Integrative analysis of the circRNA–miRNA regulatory network in atrial fibrillation

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We aimed to investigate the circRNA–miRNA regulatory network in atrial fibrillation (AF) by using Cytoscape and HMDD v3.0. Finally, 120 differentially expressed circRNAs in peripheral blood monocytes of 4 AF patients were preliminarily screened by circRNA microarray. circRNA_4648, circRNA_4631, and circRNA_2875 were the first four circRNAs with the most binding nodes in the circRNA–miRNA network. The top three most frequent miRNAs for up-regulated circRNAs were hsa-miR-328 that interacted with 5 up-regulated circRNAs, hsa-miR-4685-5p with 4 up-regulated circRNAs, hsa-miR-3150a-3p, hsa-miR-4669-5p, hsa-miR-383-3p, and hsa-miR-8073 with 3 up-regulated circRNAs, while the top three most frequent miRNAs for down-regulated circRNAs were hsa-miR-328 that interacted with 14 down-regulated circRNAs, hsa-miR-4685-5p with 11 down-regulated circRNAs and hsa-miR-661 with 9 down-regulated circRNAs. According to HMDD v3.0, five up-regulated and eleven down-regulated circRNAs were found to interact with AF related miRNAs. These results indicated the possible regulatory network between circRNAs and miRNAs in the pathogenesis of AF.

Atrial fibrillation (AF), one of the most common arrhythmias in clinical practice, with a prevalence about 1–2% in the general population, is characterized with high relative risk of heart failure and embolic stroke. AF is also considered as a potential factor for high mortality and morbidity, especially in elderly individuals1,2. Recent growing reports indicate that structural remodeling and electrical remodeling are important pathophysiological contributors to onset and maintenance of AF 3,4. However, exact mechanism of how AF occurs is still unknown.

To our knowledge, non-coding RNAs (ncRNAs), include a class of RNAs, such as long non-coding RNAs (lncRNAs), micro-RNAs (miRNAs) and circular RNAs (circRNAs), play crucial roles in regulating gene expression under pathological and physiological conditions5–7. circRNAs, a novel type of endogenous ncRNAs , have been reported to play key ncRNAs in gene regulation and the pathophysiology of cardiovascular diseases8,9. It has been well-known that dysregulated miRNAs can contribute to the prevalence of AF by deregulating transcription factors, regulating atrial excitability and increasing atrial arrhythmogenicity10,11. Accumulating studies indicate that circRNAs may interact with miRNAs by a sequence-driven sponging effect and the circRNA–miRNA-network has emerging roles in physiological and pathological processes of cardiovascular diseases12,13. However, to our knowledge, there are few studies pointing to the expression of circRNAs in AF, and circRNA–miRNA network in AF remains unclear.

In the present study, we analyzed and predicted the differentially expressed circRNAs in human monocytes from patients with AF and healthy controls using microarray, the potential regulatory network between circRNAs and miRNAs were explored by using Cytoscape and HMDD v3.0. We hypothesized that there were differentially expressed circRNAs in human monocytes and highly possible interaction between circRNAs and miRNAs, which would provide an important landmark for investigating the mechanism of AF.

Materials and methods

Study population and specimen collection. 10 patients with AF (AF group) and 10 matched healthy subjects (Control group) who excluded AF were enrolled (Table 1). 10 ml of peripheral blood was collected, monocytes were purified from peripheral blood and frozen for analysis. The diagnosis of AF was consistent with the criteria listed in the 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS14. The Ethics Committee of Taizhou People’s Hospital approved the study, which was conducted according to the principles of the Declaration of Helsinki and the International Conference on Harmonisation

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Good Clinical Practice guidelines. All the enrolled subjects provided written informed consent before entering this experiment.

The differentially expressed circRNAs of AF detected by microarray analysis. The total RNA in monocytes was extracted using Trizol reagent (Ambion, USA) and purified by QIAGEN RNaseasy Mini Kit (QIGEN, Germany). Sample labeling and microarray hybridization were conducted by Outdo Bio-tech (Shanghai, P.R. China) with the same method as previously described. Simply, the circRNAs were measured with the Agilent One-Color Microarray Based Gene Expression Analysis Low. The arrays were scanned by Axon microarray 4000B microarray scanner and extracted using Agilent Feature Extraction software (version 11.0.1.1). Quantile normalization and data processing were conducted by the Gene Spring GXv11.5.1 software package (Agilent, USA). The fold-change between AF patients and healthy controls was calculated. The statistical significance was calculated by t test and further filtered with fold change. circRNAs with foldchange $>2$ and $p < 0.05$ were regarded as significant differential expression.

qRT-PCR validation of differentially expressed circRNAs. In order to confirm the results of microarray analysis, four upregulated circRNAs (circRNA_0031, circRNA_1837,circRNA_5901 and circRNA_7571) and four downregulated circRNAs (circRNA_2773, circRNA_5801, circRNA_7386 and circRNA_7577) were selected randomly for validation by qRT-PCR in all study population. Simply, 1 μl of cDNAs was added to 12.5 μl of SYBR Green Gene Expression Master Mix (Applied Biosystems, Inc.), 10.5 μl of DEPC-treated water, and 0.5 μl of reverse and forward primers. The gene expression level of target circRNAs was normalized to the housekeeping gene GAPDH (Sangon Biotech, Shanghai, China) and calculated using the $(2^{−ΔΔCt})$ method. The primer sequences for RT-PCR were shown in Table 2.

Construction of circRNA–miRNA regulatory networks. Acting as competing miRNA sponge, the sponging activity of differentially expressed circRNAs over corresponding miRNAs was calculated by the prediction of miRNA target binding sites using the miRanda software. Enrichment results of total differentially expressed circRNAs were sorted by $p$ value, and the potential connections between circRNAs and miRNAs were further explored by using Cytoscape 3.4.0 (http://cytoscape.org/). Finally, the regulatory networks of circRNA–microRNA in AF patients were constructed.

Analyze the AF related circRNAs according to HMDD v3.0. In order to further explore the AF related circRNAs, we used the website of HMDD v3.0. HMDD v3.0, a database for experimentally supported human microRNA–disease associations, integrated many past publications about microRNA–disease associations, and offered evidence-stratified miRNA–disease data based on six categories of 20 evidence codes. We used the keywords ‘atrial fibrillation’ to obtain AF related miRNAs from HMDD v3.0. If the differentially expressed circRNAs identified by microarray interacted with these reported AF related miRNAs, they were considered to be AF associated circRNAs.

Results

The differentially expressed circRNAs between AF patients and healthy controls. A total of 120 circRNAs was calculated as differentially expressed between AF patients and healthy controls (fold change $>2$, and $p < 0.05$) (Fig. 1). In which, 65 circRNAs were upregulated (Table 3) and 55 circRNAs were downregulated (Table 4).

### Table 1. Baseline characteristics of the subjects.

| Variable                        | AF group      | Control group | $P$ value |
|--------------------------------|---------------|---------------|-----------|
| Age                            | 52.10 ± 8.19  | 49.60 ± 10.92 | $> 0.05$  |
| Gender (%)                     |               |               |           |
| Female                         | 6             | 5             | $> 0.05$  |
| Male                           | 4             | 5             | $> 0.05$  |
| Complicated diseases           |               |               |           |
| Rheumatic heart disease        | 0             | 0             | $> 0.05$  |
| Hypertension                   | 1             | 0             | $> 0.05$  |
| Hyperlipidemia                 | 1             | 0             | $> 0.05$  |
| Diabetes mellitus              | 0             | 0             | $> 0.05$  |
| Coronary heart disease         | 0             | 0             | $> 0.05$  |
| Infectious disease             | 0             | 0             | $> 0.05$  |
| Connective tissue disease      | 0             | 0             | $> 0.05$  |
| Other autoimmune diseases      | 0             | 0             | $> 0.05$  |
| Other cardiovascular diseases  | 0             | 0             | $> 0.05$  |
| Left atrial diameter (mm)      | 43.20 ± 4.02  | 31.3 ± 3.59   | $< 0.05$  |
| Ejection fraction              | 48.10 ± 8.26  | 53.20 ± 8.43  | $> 0.05$  |
Table 2. Primer sequences for reverse transcription polymerase chain reaction.

| Gene name | circbase_id | Primer sequences | Fragment (bp) |
|-----------|-------------|------------------|---------------|
| GAPDH     |             | 5'-TCTCTGCTCCTCCCTCTGTTCTA-3' | 177 |
|           |             | 5'-ATGAAAGGGCTTGATTGGG-3' |   |
| circRNA_0031 | hsa_circ_0008737 | 5'-ACUGCCCCAGAGGGCAAUCUCGG-3' | 179 |
|           |             | 5'-AGAGAGGGGCGCTGAGGCGAGG-3' |   |
| circRNA_1837 |             | 5'-GCUGGGCGACAGGGAUGGCCC-3' | 192 |
|           |             | 5'-GGCTCAAGCTGTAATCCCGAGG-3' |   |
| circRNA_5901 | hsa_circ_0001240 | 5'-CAGUGGCCAGACCCUGAGCAG-3' | 159 |
|           |             | 5'-TGCTCGGCGAGCATCGGACCAGTCG-3' |   |
| circRNA_7571 |             | 5'-GGUGAGGGG CGCTGTAATCCCGAGG-3' | 165 |
|           |             | 5'-ATCCCCGTTCCATGTCGTGACC-3' |   |
| circRNA_2773 |             | 5'-GGGGUUGGCCAGGCAUGGAUUU-3' | 163 |
|           |             | 5'-TCAAAAGGACCCCTAGGAAACCC-3' |   |
| circRNA_5801 | hsa_circ_0062426 | 5'-UGGUGAGAGAGGACGCAGAGG-3' | 181 |
|           |             | 5'-CTTCTGCAACCCTTGTACACCC-3' |   |
| circRNA_7386 |             | 5'-UGAGGCCCUGUGGGCCACAGUGG-3' | 168 |
|           |             | 5'-ACACTTAGCTTACAGGGGGCCTCA-3' |   |
| circRNA_7577 | hsa_circ_0006109 | 5'-UGCCACACGUCCAGCCACCCCU-3' | 166 |
|           |             | 5'-CCCCGGTGCCGGCTTGGGGCTG-3' |   |

Figure 1. Differentially expressed circRNAs between AF group and control group. (A) Volcano plots are displayed for visualizing the differential expression of circRNAs. The red and green points in the plot represent the differentially expressed circRNAs with statistical significance. (B) Hierarchical cluster analysis of all the deregulated circRNAs.
| circRNA_id   | circbase_id     | circRNA_Chr | Type               | Gene    | Fold change | P value |
|-------------|-----------------|-------------|--------------------|---------|-------------|---------|
| circRNA_0031 | hsa_circ_0008737 | Chr1        | Sense-overlapping  | CAMTA1  | 3.34        | 0.031   |
| circRNA_0095 | –                | Chr1        | Intrinsic          | CAPZB   | 8.01        | 0.011   |
| circRNA_0161 | –                | Chr1        | Antisense          | THEMIS2 | 4.14        | 0.001   |
| circRNA_0312 | hsa_circ_0004877 | Chr1        | Sense-overlapping  | EPS15   | 4.06        | 0.011   |
| circRNA_0544 | –                | Chr1        | Intergenic         | SUCO    | 2.49        | 0.014   |
| circRNA_0685 | hsa_circ_000160 |Chr1        | Sense-overlapping  |            |             |         |
| circRNA_1166 | –                | Chr10       | Intrinsic          | IMJD1C  | 8.73        | 0.042   |
| circRNA_1402 | –                | Chr1        | Sense-overlapping  | HTTMT2  | 5.78        | 0.049   |
| circRNA_1415 | hsa_circ_000274 | Chr11       | Sense-overlapping  | NUP98   | 5.24        | 0.047   |
| circRNA_1417 | –                | Chr11       | Intrinsic          | NUP98   | 3.84        | 0.015   |
| circRNA_1513 | hsa_circ_000302 | Chr11       | Sense-overlapping  | SP1     | 3.06        | 0.010   |
| circRNA_1741 | hsa_circ_0005589 | Chr11       | Sense-overlapping  | ARCN1   | 4.21        | 0.008   |
| circRNA_1837 | –                | Chr12       | Sense-overlapping  | KLRC2   | 9.3         | 0.025   |
| circRNA_2116 | hsa_circ_0004901 | Chr12       | Sense-overlapping  | APAF1   | 3.88        | 0.037   |
| circRNA_2294 | hsa_circ_0007547 | Chr13       | Sense-overlapping  | SKA3    | 4.18        | 0.011   |
| circRNA_2371 | –                | Chr13       | Sense-overlapping  | ELF1    | 4.3         | 0.009   |
| circRNA_2482 | –                | Chr13       | Sense-overlapping  | SLAIN1  | 3.86        | 0.020   |
| circRNA_2551 | –                | Chr14       | Intergenic         |         | 3.8         | 0.029   |
| circRNA_2616 | hsa_circ_0008002 | Chr14       | Sense-overlapping  | POBE2   | 3.2         | 0.030   |
| circRNA_2681 | hsa_circ_0032109 | Chr14       | Sense-overlapping  | PAI1A   | 3.54        | 0.020   |
| circRNA_3140 | –                | Chr15       | Sense-overlapping  | PDCD1   | 5.52        | 0.002   |
| circRNA_3337 | hsa_circ_0000672 | Chr16       | Sense-overlapping  | SLC16A  | 3.08        | 0.040   |
| circRNA_3359 | hsa_circ_0002771 | Chr16       | Sense-overlapping  | JARID1N | 3.64        | 0.024   |
| circRNA_3421 | hsa_circ_0008223 | Chr16       | Sense-overlapping  | XPO6    | 2.91        | 0.048   |
| circRNA_3448 | hsa_circ_0031961 | Chr16       | Sense-overlapping  | IGTA7   | 8.18        | 0.000   |
| circRNA_4003 | hsa_circ_0005347 | Chr17       | Sense-overlapping  | RPTF    | 5.73        | 0.034   |
| circRNA_4284 | hsa_circ_0008099 | Chr17       | Intrinsic          | ZNF516  | 5.63        | 0.008   |
| circRNA_4314 | hsa_circ_0004891 | Chr19       | Sense-overlapping  | CNN2    | 4.06        | 0.040   |
| circRNA_4656 | hsa_circ_0008847 | Chr2        | Sense-overlapping  | MBOAT2  | 3.76        | 0.015   |
| circRNA_4657 | hsa_circ_0008752 | Chr2        | Sense-overlapping  | MBOAT2  | 2.45        | 0.010   |
| circRNA_4661 | –                | Chr2        | Sense-overlapping  | MBOAT2  | 5.89        | 0.022   |
| circRNA_4864 | hsa_circ_0001756 | Chr2        | Sense-overlapping  | RTN4    | 3.43        | 0.029   |
| circRNA_4959 | –                | Chr2        | Sense-overlapping  | DYSF    | 3.69        | 0.026   |
| circRNA_5325 | –                | Chr3        | Antisense          | NOP58   | 3.21        | 0.045   |
| circRNA_5335 | hsa_circ_0003493 | Chr2        | Sense-overlapping  | CARF    | 3.55        | 0.026   |
| circRNA_5399 | hsa_circ_00058514 | Chr2        | Sense-overlapping  | AGFG1   | 3.89        | 0.014   |
| circRNA_5401 | –                | Chr3        | Intrinsic          | CTSZ    | 6.47        | 0.024   |
| circRNA_5439 | hsa_circ_0061286 | Chr3        | Sense-overlapping  | USP25   | 3.08        | 0.045   |
| circRNA_5531 | hsa_circ_0008021 |Chr3        | Sense-overlapping  | PDZK1   | 13.23       | 0.004   |
| circRNA_5637 | hsa_circ_0000806 | Chr3        | Sense-overlapping  | CCDC134 | 5.19        | 0.022   |
| circRNA_5901 | hsa_circ_0001240 | Chr3        | Sense-overlapping  | NFIAM1  | 6.34        | 0.033   |
| circRNA_5988 | hsa_circ_0001274 | Chr3        | Sense-overlapping  | PLCL2   | 8.66        | 0.046   |
| circRNA_6087 | hsa_circ_0001289 | Chr3        | Sense-overlapping  | SET2D2  | 3.18        | 0.032   |
| circRNA_6264 | hsa_circ_0006959 | Chr3        | Sense-overlapping  | HCLS1   | 3.62        | 0.028   |
| circRNA_6360 | –                | Chr3        | Sense-overlapping  | PLOD2   | 3.69        | 0.015   |
| circRNA_6574 | hsa_circ_0001394 | Chr4        | Exonic             | TBC1D14 | 4.04        | 0.004   |
| circRNA_6624 | –                | Chr4        | Exonic             | TLR6    | 3.43        | 0.033   |
| circRNA_6644 | –                | Chr4        | Sense-overlapping  | RBM47   | 3.13        | 0.050   |
| circRNA_6903 | hsa_circ_00071174 | Chr4        | Sense-overlapping  | LRBA    | 3.18        | 0.032   |
| circRNA_6955 | hsa_circ_0001460 |Chr4        | Sense-overlapping  | NEIL3   | 3.25        | 0.044   |
| circRNA_6991 | –                | Chr5        | Intergenic         |         | 5.86        | 0.002   |
| circRNA_7097 | hsa_circ_0072697 | Chr5        | Sense-overlapping  | PPWD1   | 6.69        | 0.008   |
| circRNA_7571 | –                | Chr6        | Sense-overlapping  | HLA-A   | 28.22       | 0.005   |
| circRNA_7672 | hsa_circ_0003700 |Chr6        | Sense-overlapping  | FBXO9   | 6.12        | 0.030   |
| circRNA_7952 | hsa_circ_0004662 |Chr6        | Sense-overlapping  | SOD2    | 5.68        | 0.011   |
| circRNA_7964 | hsa_circ_0078665 |Chr6        | Sense-overlapping  | RNASET2 | 3.43        | 0.033   |

Continued
Within the eleven down-regulated circRNAs, five of them (circRNA_4624, hsa_circRNA_1496, hsa_circRNA_3138, hsa_circRNA_3138, hsa_circRNA_6086, hsa_circRNA_2773) were found to interact with AF related miRNAs. Figures 5 and 6 showed the expression pattern of these dysregulated circRNAs, respectively. We predicted that these miRNAs may be more relevant with the differentially expressed circRNAs in AF.

**Construction of circRNA–miRNA networks.** We calculated the terms of miRNAs that targeted these dysregulated circRNAs by using Cytoscape 3.4.0 (http://cytoscape.org/) and conducted the circRNA–miRNA networks (shown in Fig. 3). Results showed that circRNA_7571, circRNA_4648, circRNA_4631, and circRNA_2875 were the first four circRNAs with the most binding nodes in the co-expression network, interacted with 34 miRNAs, 26 miRNAs, 24 miRNAs and 24 miRNAs, respectively (Fig. 4). The top three most frequent miRNAs for up-regulated circRNAs were hsa-miR-328 that interacted with 4 up-regulated circRNAs, hsa-miR-4685-5p that interacted with 3 up-regulated circRNAs, and hsa-miR-8073 that interacted with 3 up-regulated circRNAs, whereas the three most frequent miRNAs for down-regulated circRNAs were hsa-miR-328 that interacted with 4 down-regulated circRNAs, and hsa-miR-661 that interacted with 9 down-regulated circRNAs. We predicted that these miRNAs may be more relevant with the differentially expressed circRNAs in AF.

**Analyze the AF related circRNAs according to HMDD v3.0.** We confirmed 100 AF related miRNAs from HMDD v3.0 by using the keywords 'atrial fibrillation'. If the differentially expressed circRNAs identified by microarray interacted with these reported AF related miRNAs, they were considered to be AF associated circRNAs. Finally, five up-regulated (hsa_circRNA_7571, hsa_circRNA_3448, hsa_circRNA_1402, hsa_circRNA_4284 and hsa_circRNA_1415) and eleven down-regulated circRNAs (hsa_circRNA_2527, hsa_circRNA_4624, hsa_circRNA_1496, hsa_circRNA_3138, hsa_circRNA_3138, hsa_circRNA_6086, hsa_circRNA_2875, hsa_circRNA_3807, hsa_circRNA_4402, hsa_circRNA_4631 and hsa_circRNA_2773) were found to interact with AF related miRNAs and considered as the AF associated circRNAs by the construction of circRNA–miRNA network and the analysis using HMDD v3.0.

| circRNA_id | circbase_id | circRNA_Chr | Type          | Gene          | Fold change | P value |
|------------|-------------|-------------|---------------|---------------|-------------|---------|
| circRNA_8132 | hsa_circ_0001707 | Chr7        | Intron         | ABCA13        | 15.44       | 0.010   |
| circRNA_8233 |                   | Chr7        | Sense-overlapping | ANKIB1        | 3.43       | 0.037   |
| circRNA_8255 | hsa_circ_0007940 | Chr7        | Sense-overlapping | ARPC1B        | 3.62       | 0.028   |
| circRNA_8317 | hsa_circ_0082096 | Chr7        | Sense-overlapping | ZNF800        | 4.88       | 0.031   |
| circRNA_8548 | hsa_circ_0006376 | Chr8        | Sense-overlapping | HOOK3         | 3.31       | 0.043   |
| circRNA_8895 | hsa_circ_0003945 | Chr9        | Sense-overlapping | UBA5P2        | 3.37       | 0.015   |
| circRNA_9098 | hsa_circ_0081192 | Chr9        | Sense-overlapping | PTPRF         | 4.22       | 0.014   |
| circRNA_9396 | hsa_circ_0001947 | ChrX        | Exonic         | AFF2          | 7.79       | 0.001   |
| circRNA_9422 | hsa_circ_0008297 | ChrY        | Sense-overlapping | DDX3Y         | 5.27       | 0.037   |

Table 3. Upregulation circular RNA.

**qRT-PCR validation of differentially expressed circRNAs.** Four upregulated circRNAs (circRNA_0031, circRNA_1837, circRNA_5901 and circRNA_7571) and four downregulated circRNAs (circRNA_2773, circRNA_7386 and circRNA_7577) were selected randomly by Random Number Generator Pro V1.79 software for qRT-PCR validation to confirm the microarray results. As a result, all of 4 upregulated circRNAs (p < 0.05 or p < 0.01 for circRNA_0031, circRNA_1837, circRNA_5901 and circRNA_7571, respectively) and 3 out of 4 downregulated circRNAs (p < 0.05 or p < 0.01 for circRNA_5901, circRNA_7577, circRNA_7386 and circRNA_7577, respectively) showed a significantly different expression (Fig. 2), which was consistent with microarray results.

**Ethical approval.** No treatment was tested in patients by the authors for this article. Informed consent was obtained from all individual participants included in the study.

**Discussion** In the present study, we provide two experimental findings on circRNAs involved in AF. On the one hand, there was significantly different expression profiles of circRNAs between AF patients and normal controls. On the other hand, five up-regulated (hsa_circRNA_7571, hsa_circRNA_3448, hsa_circRNA_1402, hsa_circRNA_4284 and hsa_circRNA_1415) and eleven down-regulated circRNAs (hsa_circRNA_2527, hsa_circRNA_4624, hsa_circRNA_1496, hsa_circRNA_3138, hsa_circRNA_3138, hsa_circRNA_6086, hsa_circRNA_2875, hsa_circRNA_3807, hsa_circRNA_4402, hsa_circRNA_4631 and hsa_circRNA_2773) were found to interact with AF related miRNAs and considered as the AF associated circRNAs by the construction of circRNA–miRNA network and the analysis using HMDD v3.0.
| circRNA_id      | circbase_id      | circRNA_Chr | Type              | Gene     | FoldChange | pValue |
|-----------------|------------------|-------------|-------------------|----------|------------|--------|
| circRNA_0259    | hsa_circ_0009142 | Chr1        | Sense-overlapping | CAP1     | 3.41       | 0.029  |
| circRNA_0323    | hsa_circ_0012553 | Chr1        | Sense-overlapping | ZCCHC11  | 2.88       | 0.014  |
| circRNA_0831    | –                 | Chr1        | Sense-overlapping | LYPLA1   | 4.38       | 0.024  |
| circRNA_0835    | hsa_circ_0004417 | Chr1        | Sense-overlapping | LYPLA1   | 9.69       | 0.023  |
| circRNA_0947    | hsa_circ_0002802 | Chr1        | Sense-overlapping | ZNF124   | 6.37       | 0.042  |
| circRNA_0995    | hsa_circ_0000211 | Chr10       | Sense-overlapping | SFMBT2   | 4.55       | 0.024  |
| circRNA_1111    | –                 | Chr10       | Sense-overlapping | CCDC7    | 2.94       | 0.028  |
| circRNA_1292    | –                 | Chr10       | Sense-overlapping | EXOSC5C1 | 3.23       | 0.015  |
| circRNA_1335    | hsa_circ_0000260 | Chr10       | Sense-overlapping | SMC3     | 4.44       | 0.037  |
| circRNA_1450    | –                 | Chr11       | Sense-overlapping | SERGEF   | 3.47       | 0.010  |
| circRNA_1496    | –                 | Chr11       | Sense-overlapping | PRR5L    | 3.79       | 0.011  |
| circRNA_1693    | hsa_circ_0006208 | Chr11       | Sense-overlapping | NPAT     | 7.11       | 0.003  |
| circRNA_1786    | hsa_circ_0002881 | Chr12       | Sense-overlapping | KDM5A    | 3.04       | 0.000  |
| circRNA_1787    | hsa_circ_0024946 | Chr12       | Sense-overlapping | KDM5A    | 3.52       | 0.002  |
| circRNA_1800    | –                 | Chr12       | Antisense         | CACNA1C  | 5.73       | 0.005  |
| circRNA_1834    | –                 | Chr12       | Sense-overlapping | KLRC4-KLR | 2.69      | 0.000  |
| circRNA_2370    | –                 | Chr13       | Exonic            | ELF1     | 2.97       | 0.021  |
| circRNA_2527    | hsa_circ_0004096 | Chr13       | Sense-overlapping | RASG5C8  | 4.38       | 0.001  |
| circRNA_2683    | hsa_circ_0032116 | Chr14       | Sense-overlapping | MNT1     | 3.67       | 0.007  |
| circRNA_2773    | –                 | Chr14       | Intergenic        | 12.02     | 0.043     |
| circRNA_2875    | –                 | Chr14       | Intergenic        | 3.06      | 0.030     |
| circRNA_3138    | –                 | Chr15       | Intergenic        | 4.33      | 0.036     |
| circRNA_3307    | hsa_circ_0007788 | Chr16       | Sense-overlapping | SNT4R1L  | 10.03      | 0.023  |
| circRNA_3807    | –                 | Chr17       | Sense-overlapping | CCL3L3   | 7.42       | 0.016  |
| circRNA_3830    | –                 | Chr17       | Sense-overlapping | ERBB2    | 3.01       | 0.004  |
| circRNA_4184    | –                 | Chr18       | Sense-overlapping | RNF138   | 6.13       | 0.000  |
| circRNA_4402    | –                 | Chr19       | Sense-overlapping | ZNF564   | 3.51       | 0.014  |
| circRNA_4581    | hsa_circ_0003912 | Chr19       | Exonic            | DBP      | 4.63       | 0.005  |
| circRNA_4624    | –                 | Chr19       | Sense-overlapping | LILRA1   | 7.92       | 0.002  |
| circRNA_4631    | –                 | Chr19       | Sense-overlapping | KIR2DL1  | 8.77       | 0.009  |
| circRNA_4648    | –                 | Chr19       | Intergenic        | 4.41      | 0.007     |
| circRNA_4737    | –                 | Chr2        | Exonic            | GTF3C2   | 4.23       | 0.011  |
| circRNA_5440    | hsa_circ_0001119 | Chr2        | Sense-overlapping | DGKD     | 2.13       | 0.050  |
| circRNA_5625    | hsa_circ_0003998 | Chr20       | Sense-overlapping | ARFGF2   | 6.95       | 0.037  |
| circRNA_5801    | hsa_circ_000426  | Chr22       | Sense-overlapping | PPIL2    | 4.82       | 0.043  |
| circRNA_5996    | –                 | Chr3        | Intergenic        | 4.12      | 0.021     |
| circRNA_6103    | –                 | Chr3        | Sense-overlapping | SETD2    | 4.63       | 0.005  |
| circRNA_6910    | hsa_circ_0006937 | Chr4        | Sense-overlapping | ARAP2    | 7.28       | 0.043  |
| circRNA_6775    | hsa_circ_0002782 | Chr4        | Sense-overlapping | SLC39A8  | 5.38       | 0.019  |
| circRNA_7610    | hsa_circ_0007477 | Chr4        | Sense-overlapping | PPA2     | 5.64       | 0.030  |
| circRNA_7032    | hsa_circ_0072380 | Chr5        | Exonic            | ZNF131   | 4.18       | 0.009  |
| circRNA_7335    | hsa_circ_0006716 | Chr5        | Sense-overlapping | UBE2D2   | 3.66       | 0.032  |
| circRNA_7386    | –                 | Chr5        | Sense-overlapping | SGCD     | 4.37       | 0.007  |
| circRNA_7777    | hsa_circ_0006109 | Chr6        | Sense-overlapping | C6orf136 | 2.29       | 0.028  |
| circRNA_7599    | –                 | Chr6        | Sense-overlapping | HLA-DRB1 | 3.16       | 0.042  |
| circRNA_7797    | hsa_circ_0001638 | Chr6        | Sense-overlapping | MFSD4B   | 3.21       | 0.031  |
| circRNA_8031    | hsa_circ_0005519 | Chr7        | Sense-overlapping | SNX13    | 8.57       | 0.045  |
| circRNA_8108    | –                 | Chr7        | Sense-overlapping | TARP     | 6.28       | 0.001  |
| circRNA_8280    | hsa_circ_0007395 | Chr7        | Sense-overlapping | KMT2E    | 12.57      | 0.033  |
| circRNA_8455    | –                 | Chr8        | Intrinsic         | ER11     | 9.61       | 0.023  |
| circRNA_8731    | hsa_circ_0085438 | Chr8        | Sense-overlapping | TBC1D31  | 5.03       | 0.002  |
| circRNA_8841    | –                 | Chr9        | Sense-overlapping | KIAA2026 | 3.34       | 0.025  |
| circRNA_8857    | hsa_circ_0008732 | Chr9        | Sense-overlapping | BNC2     | 3.62       | 0.022  |
| circRNA_9064    | –                 | Chr9        | Sense-overlapping | NIPSNAP3A| 7.75       | 0.000  |
| circRNA_9326    | hsa_circ_0091775 | ChrX        | Sense-overlapping | BRWD3    | 3.69       | 0.020  |

Table 4. Downregulation circRNA.
Figure 2. Quantitative reverse transcription polymerase chain reaction analysis for validation of differentially expressed circRNAs. Compared with control group, *P < 0.05 and **P < 0.01.

Figure 3. circRNA–miRNA coexpression network explored by using Cytoscape. The size of each node represents functional connectivity of each circRNA. The network consists of 37 circRNAs and 90 miRNAs. The red node represents circRNA and the green node represents miRNA. circRNA_7571, circRNA_4648, circRNA_4631 and circRNA_2875 were the four largest nodes in the network. hsa-miR-328 was the highest positive correlated miRNA in the networks.
Atrial electric remodeling associated with profound reduction of L-type Ca\(^{2+}\) current and shortening of the action potential duration was the characteristic of both clinical and experimental AF. It was reported that miR-328, diminished L-type calcium current, shortened the atrial action potential duration, and increased AF vulnerability, would contribute to the atrial electric remodeling in AF and can be used as a diagnosis biomarker of AF.\(^{17,18}\) Our findings indicated that hsa-miR-328 interacted with both up-regulated and downregulated circRNAs, which was consistent with the reports and indicated that circRNA_7571, circRNA_3448, circRNA_1415, circRNA_4648, circRNA_4624, circRNA_4402, circRNA_2527 and circRNA_1496 could be regarded as the diagnosis biomarkers of circRNAs for AF.

miR-486 was related to the accumulation of superoxide anion, induction of DNA damage, reduction of cell proliferation and senescence phenotype in human fibroblasts.\(^{19}\) Slagsvold et al. reported that hsa-miR-486 was upregulated in AF with left atria. Another report from Wang et al. showed that hsa-miR-486 was found to be up-regulated in left atrial appendage in patients with AF.\(^{20}\) Thus, hsa-miR-486 was considered as a AF related miRNA. At the same time, circRNA_1402, interacted with hsa-miR-486 in our findings could be considered as one of the AF related circRNAs.

A large number of studies have reported the relationships between the miRNAs (hsa-miR-133a, hsa-miR-574, hsa-miR-92a, hsa-miR-26b and hsa-miR-199a) and AF. For example, miR-133 has a cardioprotective role dependent on AKT serine/threonine kinase (AKT) signaling in control situation, inducing apoptosis in AF patients due to its down-regulation.\(^{22}\) hsa-miR-26b increases IK1 current and membrane resting potential, the downregulation of hsa-miR-26b may reduce AF vulnerability.\(^{23}\) hsa-miR-574 may promote electrical remodeling via Cav1.2 and contribute to cardiac arrhythmia pathogenesis of AF.\(^{24}\) hsa-miR-92a can attenuate cardiomyocyte apoptosis in AF patients induced by hypoxia/reoxygenation via the up-regulation of SMAD7 and down-regulation of nuclear NF-xB p65.\(^{25}\) MiR-26b directly targeted KCNJ2. Both in vivo and in vitro inhibition of miR-26b increased IK1 and AF vulnerability, whereas overexpression of lammed AF vulnerability.\(^{26}\) miR-199a down-regulation induces Sirtuin 1, a cardio-protective protein, as a compensatory mechanism to inhibit the process of oxidative stress which contributes to the pathogenesis of AF.\(^{27}\) These miRNAs were considered as the potential biomarkers and therapeutic targets related to AF. Therefore, the differentially expressed circRNAs of circRNA_4284, circRNA_6086, circRNA_3138, circRNA_2773, circRNA_2875, circRNA_3807 and circRNA_4631 in the current study were more likely to be AF associated circRNAs.

**Study limitations.** First, the small sample size does not provide sufficient power for such an analysis. Second, we just preliminarily investigated the circRNA–miRNA regulatory network in AF, the target gene or pathway analysis and functional assays of circRNA–miRNA regulatory network in the AF process should be further explored.

**Conclusions**

Our study showed that there were differentially expressed circRNAs in AF patients, five up-regulated and eleven down-regulated circRNAs were considered as the AF related circRNAs. The differentially expressed circRNAs had a possible regulatory network with miRNAs, which indicated the possible regulatory network between circRNAs and miRNAs in the pathogenesis of AF.
Figure 5. The expression pattern of the five up-regulated circRNAs that interact with AF related miRNAs. (A) The expression pattern of hsa_circRNA_7571 that interact with has-miR-133a. (B) The expression pattern of hsa_circRNA_3448 that interact with has-miR-328. (C) The expression pattern of hsa_circRNA_1402 that interact with has-miR-486. (D) The expression pattern of hsa_circRNA_4284 that interact with has-miR-328. (E) The expression pattern of hsa_circRNA_1415 that interact with has-miR-328.
Figure 6. The expression pattern of the eleven down-regulated circRNAs that interact with atrial fibrillation related miRNAs. (A) The expression pattern of hsa_circRNA_2527 that interact with has-miR-328. (B) The expression pattern of hsa_circRNA_4648 that interact with has-miR-328. (C) The expression pattern of hsa_circRNA_4624 that interact with has-miR-328. (D) The expression pattern of hsa_circRNA_3138 that interact with has-miR-328. (E) The expression pattern of hsa_circRNA_3138 that interact with has-miR-574. (F) The expression pattern of hsa_circRNA_2875 that interact with has-miR-574. (G) The expression pattern of hsa_circRNA_6086 that interact with has-miR-574. (H) The expression pattern of hsa_circRNA_3807 that interact with has-miR-92a. (I) The expression pattern of hsa_circRNA_4402 that interact with has-miR-199a. (J) The expression pattern of hsa_circRNA_4631 that interact with has-miR-199a. (K) The expression pattern of hsa_circRNA_2773 that interact with has-miR-574.
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Author contributions

Z.R. and L.Z. conceived the idea and designed the project. Z.R., Q.Y. and G.C. helped in experimentation and data acquisition. F.W. contributed to clinical evaluation and sample provision. Z.R. and G.C. contributed to data analysis and the interpretation of the results. Z.R. took the lead in writing the manuscript along F.W., Q.Y., L.Z. supervised the research. All authors read and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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