EXPERIMENTAL STUDY

**BIRC5, GAJ5, and IncRNA NPHP3-AS1 Are Correlated with the Development of Atrial Fibrillation-Valvular Heart Disease**

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**Summary**

The aim of this study was to explore the pivotal genes or IncRNAs involved in the progression of atrial fibrillation (AF)-valvular heart disease (VHD). The mRNA profiling GSE113013 was obtained from the Gene Expression Omnibus database. The identification of differentially expressed genes (DEGs) and differentially expressed long non-coding RNAs (DElncRNAs) was performed. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were carried out for DEGs. Then, the construction of the protein-protein interaction (PPI) network was conducted. An IncRNA-miRNA-target ceRNA network was constructed after obtaining microRNAs (miRNA) related to DElncRNAs. Ultimately, key disease-related genes were screened. A total of 399 DEGs and 145 DElncRNAs were obtained. There were 283 nodes and 588 interaction pairs in the PPI network, and synaptosome-associated protein 25 (SNAP25) had higher degrees (degree = 22) in the PPI network. There were 65 interaction pairs in the ceRNA network. Here, Bacculoviral IAP Repeat Containing 5 (BIRC5) was regulated by hsa-miR-1285-3p, which was regulated by IncRNA NPHP3-AS1. Gap Junction Protein Alpha 5 (GAJ5) was regulated by hsa-miR-4505, hsa-miR-1972, and hsa-miR-1199-5p. In particular, GAJ5 was enriched in the function of ion transmembrane transport regulation, whereas BIRC5 was enriched in the function of apoptosis-multiple species pathway. Similarly, Potassium Inwardly Rectifying Channel Subfamily J Member 6 (KCNJ6) was enriched in the function of an ion channel complex. VENN analysis identified BIRC5 and GAJ5 as key AF-related genes. KCNJ6, SNAP25, GAJ5, BIRC5, hsa-miR-1285-3p, and IncRNA NPHP3-AS1 were likely to be associated with AF-VHD development.

**Key words:** Protein-protein interaction network, Competing endogenous RNAs network, Differentially expressed genes, Ion channel complex, Differentially expressed IncRNAs

Atrial fibrillation (AF) is considered to be the most common heart arrhythmia of clinical significance that can cause irregular and rapid beating of the heart’s upper chambers. An estimated, 2% to 3% of the population was affected by AF in 2014 in Europe and North America. Approximately 0.6% of men and 0.4% of women also suffer from AF in developing countries. AF and ventricular fibrillation caused approximately 29,000 deaths in 1990 and increased to 112,000 in 2013. The increased morbidity and mortality leads to a higher burden for patients and countries. The most common risk factors correlated with AF are hypertension and valvular heart disease (VHD). Because of elevated dilatation and pressure of the right and left atria and pulmonary veins, patients with VHD frequently have AF. Additionally, heart failure, coronary artery disease, and congenital heart disease increase the risk of AF.

To date, microRNAs (miRNAs) have been identified as being involved in various pathological processes such as AF. For example, exosomal miR-320d is related to AF cardiomyocyte apoptosis and cell viability, and the miR-320d effect on cardiomyocytes is STAT3 dependent. Abnormal expressions of miR-483-5p, miR-223-3p, and miR-142-5p are regarded as associated with AF development. MiR-27b-3p has a crucial role in the development of AF through regulating the Wnt/β-Catenin signaling pathway. The increasing evidences suggest that several long non-coding RNAs (IncRNAs) are involved in the progression of cardiovascular disorders. For instance, IncRNA PVT1 has been considered to be playing a key role in promoting atrial fibrosis via the miR-128-3p-SP1-TGF-β1-Smad axis in AF. The IncRNA KCNQ1OT1 up-regulation regulates angiotensin II-induced AF by regulating the miR-384/CACNA1C axis. Besides, IncRNA...
GAS5 overexpression could inhibit cell proliferation of AF by suppressing ALK5 expression.\textsuperscript{13}

The greatest risk of AF is stroke, and stroke caused by VHD with AF is more serious. The risk for stroke and incidence of embolism in patients who have AF and VHD is higher than those in people with a normal sinus rhythm (SR).\textsuperscript{11,14} Wang, et al. analyzed the miRNA regulation in AF-VHD occurrence and identified AF-VHD-specific miRNAs.\textsuperscript{10} GSE113013 was analyzed by Rao, et al. to explore the lncRNA and mRNA expressions of atrial appendage tissues from SR-VHD and AF-VHD patients. However, the molecular mechanisms underlying the development of VHD to AF-VHD have not yet been understood. In the present study, non-coding RNA profiling GSE113013 was re-analyzed to profoundly search the specific mechanisms of AF-VHD development. We identified differentially expressed genes (DEGs) and differentially expressed long non-coding RNAs (DELncRNAs). Afterwards, enrichment analyses were performed. Then, we conducted protein-protein interaction (PPI) network and ceRNA network analyses. Moreover, key disease-related genes were further screened out. We expected to reveal the miRNAs, mRNAs, and lncRNAs associated with the AF-VHD development and to provide novel therapeutic targets for AF-VHD.

**Methods**

**Data source:** The non-coding RNA profiling GSE113013 (Species: Homo sapiens) was downloaded from NCBI Gene Expression Omnibus ( GEO)\textsuperscript{15} database (https://www.ncbi.nlm.nih.gov/gds/?term=). A total of 10 atrial appendage tissue samples containing 5 AF-VHD and 5 SR-VHD were included in this study. All samples were detected through the GPL16956 Agilent-045997 Arraystar human lncRNA microarray V3 (Probe Name Version) platform.

**Data preprocessing, DEG, and DELncRNA identification:** The limma\textsuperscript{23} package was applied for preprocessing the obtained data by conducting normalization, background correction, and concentration prediction. Probes were annotated by matrix data combined with a chip platform annotation file. The average values of diverse probes would be considered as the eventual expression level of a gene if they were corresponded with those of an identical mRNA.

For lncRNA annotation, genes whose information was “antisense,” “lncRNA,” “sense_intronic,” “sense_overlapping,” and “processed_transcript” were selected as lncRNA according to the GENCODE for human gene annotation files (Release 29).

The classical Bayesian test was utilized to analyze the differential expression of the samples. The significant differentially expressed cut-off was set as log: fold change (FC) > 1 and P value < 0.05. Finally, principal component analysis (PCA) and volcano maps for DEGs and DELncRNAs were generated using ggplot2\textsuperscript{17} (Version 3.0.0) in the R package.

**Enrichment analyses:** Metascape\textsuperscript{18} was used to calculate the up-regulated and down-regulated Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and the Kappa similarity of all the enriched items was then calculated. The items were clustered into the tree, and the sub-tree was then transformed into a cluster of similar items. The functional enrichment analysis can identify overlapping or related items, and functional network clustering was then conducted.

**PPI network analysis:** The interactions between protein and protein encoded by DEGs were analyzed utilizing the Search Tool for the Retrieval of Interacting Genes (STRING)\textsuperscript{24} (Version 10.0, http://www.string-db.org/) database. The PPI score was set at 0.4 (referred to as median confidence). Afterwards, the Cytoscape\textsuperscript{20} (Version 3.6.0, http://www.cytoscape.org/) software was used for constructing the PPI network.

The R package clusterProfiler\textsuperscript{21} (Version 2.4.3, http://bioconductor.org/packages/3.2/bioc/html/clusterProfiler.htm) was applied to perform the GO-biological process (GO-BP) and KEGG\textsuperscript{22} pathway enrichment analyses for the top 20 nodes in the PPI network. The cut-off criteria for significant results were set as the significance threshold $P$ value $< 0.05$ and the numbers of enriched genes count $\geq 2$.

**ceRNA network analysis:** The Pearson correlation coefficient of lncRNA and mRNA was calculated according to the matrix data of DELncRNAs and DEGs, and the correlation test was then conducted. LncRNA-mRNA was further screened under the threshold of $r > 0.95$ and $P < 0.05$, and these co-expressions of lncRNA-mRNA were considered to be significantly positively correlated.

The FASTA format sequence files of lnRNAs in the co-expression relationship and all the FASTA sequence files of mature miRNA in miRBase were further sorted out. The miRNA-lncRNA combination was predicted by miRanda (Version 3.3a, https://omictools.com/miranda-tool),\textsuperscript{25} and the regulatory pairs in miRNA-lncRNA were then obtained under the cut-off of score $> 160$ and energy $< -30$.

The target of miRNAs that regulated more than five lncRNAs in the LncRNA-miRNA interaction pairs was predicted. DIANA-microT web server\textsuperscript{26} (Version 5.0) was used to collate the regulation data of miRNA-target, and the regulatory pairs of DEGS in target genes were further screened. Finally, the regulatory pairs of miRNA-DEGs were obtained. The LncRNA-miRNA-target ceRNA network was constructed by Cytoscape.

We implemented the GO-BP and KEGG pathway enrichment analyses for the mRNA in the ceRNA network. The threshold was set as follows: the significance threshold $P < 0.05$ and the number of enriched genes count $\geq 2$.

**Screening of key disease-related genes:** Genes associated with disease were screened under the threshold of inference score $\geq 20$ according to the Comparative Toxicogenomics Database (http://ctd.mdibl.org)\textsuperscript{27} database, which included genes related to atrial fibrillation. Subsequently, a VENN analysis was performed for disease-related genes and mRNA in ceRNA.

**Results**

Identification of crucial DEGs and DELncRNAs: A total of 399 DEGs were obtained, including 135 up-regulated
[e.g., Gap Junction Protein Alpha 5 (GJA5)] and 264 down-regulated DEGs [e.g., Baculoviral IAP Repeat Containing 5 (BIRC5) and Potassium Inwardly Rectifying Channel Subfamily J Member 6 (KCNJ6)]. Analogously, a total of 145 DElncRNAs were screened, including 51 up-regulated and 94 down-regulated DElncRNAs (e.g., lncRNA NPHP3-AS1). The PCA results were illustrated in Figure 1. The two groups of samples can be significantly separated, indicating that the difference analysis results were reliable.

Analysis of DEG enrichment: We performed the KEGG pathway and GO function enrichment analyses for DEGs utilizing MetaScape. The network diagram of DEG enrichment results are illustrated in Figure 2A. The obtained DEGs were enriched in a variety of functions, such as a retinol metabolic process, cell chemotaxis and cell killing (Figure 2B).

Analysis of the PPI network: There were 588 interaction pairs and 283 nodes in the PPI network, and synaptosome-associated protein 25 (SNAP25) had higher degrees (degree = 22) in the PPI network (Figure 3). The top 20 DEGs (Table I) were enriched in 36 KEGG pathways and 172 GO-BP functions. Here, we present the top 10 enriched GO-BP functions and KEGG pathways for the top 20 DEGs (Table II).

Analysis of the ceRNA network: There were 65 interaction pairs in the ceRNA network, including 22 miRNA-lncRNAs, 32 miRNA-miRNAs, and 11 mRNA-lncRNAs. Meanwhile, 33 nodes were identified in the ceRNA network, including 10 mRNAs (e.g., GJA5, BIRC5 and KCNJ6), 3 IncRNAs (e.g., NPHP3-AS1) and 20 miRNAs (e.g., hsa-miR-4505, hsa-miR-1972, hsa-miR-1199-5p and hsa-miR-1285-3p) (Figure 4). Here, BIRC5 was regulated by hsa-miR-1285-3p, and hsa-miR-1285-3p was regulated by lncRNA NPHP3-AS1. GJA5 was regulated by hsa-miR-1285-3p and hsa-miR-1285-3p was regulated by IncRNA NPHP3-AS1. GAJ5 was significantly enriched in 16 GO-BP, 12 GO-cellular component (GO-CC), 14 GO-molecular function (GO-MF), and 6 KEGG pathways (Table III). In particular, GAJ5 was enriched in the function of ion transmembrane transport regulation, while BIRC5 was enriched in the function of an apoptosis-multiple species pathway. Similarly, KCNJ6 was enriched in the function of the ion channel complex.

**Identification of key AF-related genes:** A total of 1171 key disease-related genes were obtained, and a VENN analysis of these key AF-related genes and 10 genes contained in the ceRNA network was then conducted. As illustrated in Figure 5, five shared genes (BIRC5, GJA5, etc.) were screened out.

**Discussion**

Currently, AF-VHD has become a growing public health problem in developing countries. Hence, it is necessary to explore the progression of AF-VHD. Totally, 399 DEGs and 145 DElncRNAs were obtained in this study. Meanwhile, 33 nodes were identified in the ceRNA network, including 10 mRNAs, 3 IncRNAs, and 20 miRNAs. Here, BIRC5 was regulated by hsa-miR-1285-3p, and hsa-miR-1285-3p was regulated by lncRNA NPHP3-AS1. GAJ5 was regulated by hsa-miR-4505, hsa-miR-1972, and hsa-miR-1199-5p. In particular, GJA5 was enriched in the function of ion transmembrane transport regulation, whereas BIRC5 was enriched in the function of apoptosis-multiple species pathway. Similarly, KCNJ6 was enriched in the function of the ion channel complex. The VENN analysis identified BIRC5 and GJA5 as key AF-related genes.

It has been suggested that ion-channel remodeling has a key role in the pathophysiology of AF, promoting its initiation, and perpetuation. Moreover, AF results in the ion-channel changes differentiating AF-VHD from SR-VHD patients. The abnormal intracellular Ca\(^2+\) is the crucial contributor to atrial remodeling and focal firing during AF. These findings indicated that genes correlated with the ion channel complex, and regulation of ion transmembrane transport might be related to the development of VHD to AF-VHD. It has been widely accepted that KCNJ6 modulates many physiological processes, such as heart rate in cardiac cells. It has been found that KCNJ6 is involved in altered cardiac regulation in down...
syndrome patients. KCNJ6 was down-regulated in AF-VHD samples and enriched in the function of the ion channel complex. Besides, SNAP25 had higher degrees in the PPI network. However, SNAP25 and KCNJ6 roles in AF-VHD have not been elucidated. Hence, SNAP25 and KCNJ6 might be associated with the AF-VHD development through regulation of the ion channel function.

A previous study suggested that functional alterations in GJs have a crucial role in the AF pathogenesis in the mutant carriers. Recently, mutation of GJA5 has been identified to correlate with AF development. For instance, GJA5 mutations have been found in early onset lone AF patients in several families over generations. Because of either impaired gap junction channels or abnormal connexin localization, GJA5 mutation impairs the gap junction function, which may play a role in contributing to AF. These findings pointed that GJA5 is related to AF development. Here, we found that GJA5 was up-regulated and regulated by hsa-miR-4505, hsa-miR-1972, and hsa-miR-1199-5p. In this case, hsa-miR-4505, hsa-miR-1972,
Figure 3. PPI network for DEGs. Pink rounds and the green rhombus represent up-regulated and down-regulated DEGs, respectively. The size of nodes presents the value of degree. The larger nodes indicate a larger degree value. DEGs indicates differentially expressed genes, and PPI, protein–protein interaction.

and hsa-miR-1199-5p might participate in the AF-VHD development by modulating GJA5 expression.

Several studies have demonstrated that atrial apoptosis has a pivotal role in the human AF progression. The increased atrial apoptosis has been observed in both tachypacing-induced AF animal models and patients with AF. BIRC5 (also known as apoptosis inhibitor survivin) encodes negative regulatory proteins. It has been identified that survivin is considered to be a candidate biomarker reflecting the operability of congenital heart disease-pulmonary arterial hypertension patients. The up-regulation of survivin plays a vital role in a nicotinic agonist suppressing the apoptosis of cardiomyocyte in CVB3-induced myocarditis. Besides, simvastatin is regarded as being involved in the cardiomyocyte protection and survivin overexpression. These results indicated that
survivin is involved in the progression of heart-related diseases. Here, BIRC5 down-regulation was found in AF-VHD samples. We also found that BIRC5 was enriched in the apoptosis-multiple species pathway. Hence, BIRC5 might be related to the progression of AF-VHD via the apoptosis-multiple species pathway. Currently, several miRNAs have been identified to be related to AF-VHD, such as hsa-miR-101, hsa-miR-133a, and hsa-miR-29b. However, hsa-miR-1285-3p’s effect on AF development has not been searched. Interestingly, it has been uncovered that hsa-miR-1285-3p was dramatically up-regulated in heart and plasma during heart failure. Therefore, hsa-miR-1285-3p might also have been up-regulated during AF-VHD development. BIRC5 was down-regulated in AF-VHD samples and interacted with hsa-miR-1285-3p, and BIRC5 expression was reduced in the heart and plasma during heart failure. Therefore, hsa-miR-1285-3p might have been up-regulated during AF-VHD development.

### Table I. The Top 20 DEGs in the PPI Network

| Gene       | Degree | Regulate |
|------------|--------|----------|
| SNAP25     | 22     | Up       |
| GNG8       | 20     | Down     |
| KNG1       | 20     | Down     |
| GCG        | 17     | Up       |
| POLR2A     | 16     | Down     |
| DLG4       | 15     | Up       |
| PDYN       | 15     | Up       |
| ADCY2      | 15     | Down     |
| PPBP       | 14     | Up       |
| CD3D       | 14     | Up       |
| REEP2      | 14     | Down     |
| MAGEA10    | 13     | Down     |
| ISG15      | 12     | Down     |
| GNAO1      | 12     | Down     |
| NEK2       | 12     | Down     |
| CXCL13     | 12     | Down     |
| SLC6A1     | 12     | Down     |
| IFNG       | 12     | Up       |
| SYT1       | 11     | Down     |
| OAS1       | 11     | Down     |

DEGs indicates differentially expressed genes; and PPI, protein–protein interaction.

### Table II. Enrichment Analyses for the Top 20 DEGs in the PPI Network

| Category | Term Description                                      | P value         | Genes                                      |
|----------|-------------------------------------------------------|-----------------|--------------------------------------------|
| BP       | GO: 0071377 Cellar response to glucagon stimulus      | 9.43 × 10⁻⁶     | GNG8, GCG, ADCY2                           |
| BP       | GO: 0033762 Response to glucagon                      | 2.13 × 10⁻⁵     | GNG8, GCG, ADCY2                           |
| BP       | GO: 0015800 Acidic amino acid transport               | 3.48 × 10⁻⁵     | SNAP25, SLC6A1, SYT1                       |
| BP       | GO: 0046717 Acid secretion                            | 2.42 × 10⁻⁴     | SNAP25, SLC6A1, SYT1                       |
| BP       | GO: 0099072 Regulation of postsynaptic membrane neurotransmitter receptor levels | 2.50 × 10⁻⁴ | SNAP25, DLG4 |
| BP       | GO: 0051668 Localization within membrane              | 2.61 × 10⁻⁴     | SNAP25, DLG4, REEP2                        |
| BP       | GO: 0050804 Modulation of chemical synaptic transmission | 3.94 × 10⁻⁴ | SNAP25, DLG4, SLC6A1, etc.                |
| BP       | GO: 0099177 Regulation of trans-synaptic signaling     | 3.94 × 10⁻⁴     | SNAP25, DLG4, SLC6A1, etc.                |
| BP       | GO: 0007612 Learning                                   | 3.96 × 10⁻⁴     | SNAP25, DLG4, SLC6A1                       |
| BP       | GO: 0006865 Amino acid transport                      | 4.04 × 10⁻⁴     | SNAP25, SLC6A1, SYT1                       |
| KEGG     | hsa04727 GABAergic synapse                             | 3.13 × 10⁻⁵     | GNG8, ADCY2, SLC6A1, etc.                 |
| KEGG     | hsa05142 Chagas disease (American trypanosomiasis)    | 5.36 × 10⁻⁵     | GNG8, ADCY2, GNAO1, SLC6A1, etc.           |
| KEGG     | hsa04724 Glutamateric synapse                           | 8.28 × 10⁻⁵     | GNG8, DLG4, ADCY2, GNAO1                  |
| KEGG     | hsa04721 Synaptic vesicle cycle                        | 5.60 × 10⁻⁴     | SNAP25, SLC6A1, SYT1                      |
| KEGG     | hsa04062 Chemokine signaling pathway                  | 5.79 × 10⁻⁴     | GNG8, ADCY2, PPBP, CXCL13                 |
| KEGG     | hsa04911 Insulin secretion                             | 7.46 × 10⁻⁴     | SNAP25, GCG, ADCY2                        |
| KEGG     | hsa05032 Morphine addiction                           | 8.80 × 10⁻⁴     | GNG8, ADCY2, GNAO1                        |
| KEGG     | hsa04713 Circadian entrainment                        | 1.06 × 10⁻³     | GNG8, ADCY2, GNAO1                        |
| KEGG     | hsa04725 Cholinergic synapse                           | 1.61 × 10⁻³     | GNG8, ADCY2, GNAO1                        |
| KEGG     | hsa04926 Relaxin signaling pathway                    | 2.41 × 10⁻³     | GNG8, ADCY2, GNAO1                        |

DEGs indicates differentially expressed genes; GO, gene ontology; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; and PPI, protein–protein interaction.
**Figure 4.** The ceRNA regulatory network for DEGs and DElncRNAs. Pink rounds and the green rhombus represent up-regulated and down-regulated DEGs, respectively. Orange rounds and the blue rhombus represent up-IncRNAs and down-IncRNAs, respectively. Red triangles represent miRNAs. The size of nodes stands for the nodes' degrees, where the larger nodes have higher degrees. DEGs indicates differentially expressed genes, and DElncRNAs, differentially expressed long non-coding RNAs.

**Table III.** Enrichment Analyses for the DEGs in the ceRNA Network

| Category | Term | Description | P value | Genes |
|----------|------|-------------|---------|-------|
| BP       | GO: 0034765 | Regulation of ion transmembrane transport | 4.58 x 10^{-5} | DLG4, GJA5, KCNJ6, etc. |
| BP       | GO: 0086010 | Membrane depolarization during action potential | 2.68 x 10^{-4} | GJA5, CACNA1E |
| BP       | GO: 0051899 | Membrane depolarization | 1.14 x 10^{-3} | GJA5, CACNA1E |
| BP       | GO: 0042391 | Regulation of membrane potential | 1.14 x 10^{-3} | DLG4, GJA5, CACNA1E |
| BP       | GO: 0001508 | Action potential | 2.20 x 10^{-3} | GJA5, CACNA1E |
| CC       | GO: 0034703 | Cation channel complex | 1.74 x 10^{-4} | DLG4, KCNJ6, CACNA1E |
| CC       | GO: 0034702 | Ion channel complex | 4.34 x 10^{-4} | DLG4, KCNJ6, CACNA1E |
| CC       | GO: 1902495 | Transmembrane transporter complex | 5.50 x 10^{-4} | DLG4, KCNJ6, CACNA1E |
| CC       | GO: 1990351 | Transporter complex | 5.91 x 10^{-4} | DLG4, KCNJ6, CACNA1E |
| CC       | GO: 0033267 | Axon part | 7.25 x 10^{-4} | DLG4, PDYN, DYSPL3 |
| MF       | GO: 0015267 | Channel activity | 8.85 x 10^{-4} | DLG4, GJA5, KCNJ6, etc. |
| MF       | GO: 0022839 | Ion gated channel activity | 7.49 x 10^{-4} | DLG4, KCNJ6, CACNA1E |
| MF       | GO: 0022836 | Gated channel activity | 7.69 x 10^{-4} | DLG4, KCNJ6, CACNA1E |
| MF       | GO: 0005216 | Ion channel activity | 1.48 x 10^{-3} | DLG4, KCNJ6, CACNA1E |
| KEGG     | hasa05030 | Cocaine addiction | 5.50 x 10^{-4} | DLG4, PDYN |
| KEGG     | hasa04915 | Estrogen signaling pathway | 4.29 x 10^{-3} | KRT23, KCNJ6 |
| KEGG     | hasa04390 | Hippo signaling pathway | 5.32 x 10^{-3} | DLG4, BIRC5 |
| KEGG     | hasa04215 | Apoptosis—multiple species | 2.40 x 10^{-2} | BIRC5 |
| KEGG     | hasa04930 | Type II diabetes mellitus | 3.42 x 10^{-2} | CACNA1E |
| KEGG     | hasa04929 | GnRH secretion | 4.74 x 10^{-2} | KCNJ6 |

DEGs indicates differentially expressed genes; GO, gene ontology; BP, biological process; and KEGG, Kyoto Encyclopedia of Genes and Genomes.
with lncRNA NPHP3-AS1. In particular, lncRNA NPHP3-AS1 was also down-regulated in AF-VHD samples, and it later interacted with BIRC5. Therefore, lncRNA NPHP3-AS1 might function as a ceRNA by sponging with hsa-miR-1285-3p to up-regulated BIRC5.

However, there are some limitations in this study. Firstly, a small sample size limited the study. Given that a complicated secondary analysis was likely to weaken the accuracy of the aforementioned results, lncRNAs' effect should be validated in a large-scale study in the future. Besides, further studies are also essential to investigate the roles of specific lncRNAs, miRNAs, and their relationships with related mRNAs.

Conclusion

In summary, KCNJ6, SNAP25, GJA5, BIRC5, hsa-miR-1285-3p, and lncRNA NPHP3-AS1 were likely to be related to the progression of AF-VHD. These molecular markers might be used as the therapeutic target for AF-VHD.

Disclosure

Conflicts of interest: The authors declare that they have no conflicts of interest.

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