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Bioinformatics Analysis for Potential Biomarkers and Therapeutic Targets of Obstructive Sleep Apnea-Related Atrial Fibrillation

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Abstract

Background: Epidemiological studies indicated that Obstructive Sleep Apnea (OSA) and Atrial Fibrillation (AF) could mutually affect the development and prognosis of diseases, but the underlying pathophysiological mechanisms are still unclear.

Methods: We downloaded two mRNA data sets GSE75097 and GSE128188 from Gene Expression Omnibus (GEO) data base, and the common Differentially Expressed Genes (co-DEGS) were identified. OSA-DEGs, LAA-AF-DEGs and RAA-AF-DEGs were functionally annotated by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and Protein-Protein Interaction (PPI) networks were constructed to screen out their respective hub-genes. Meanwhile, micro RNAs targeting co-DEGs were predicted and GO and KEGG analysis was performed for micro RNAs.

Results: We detected 343, 159, and 182 DEGs from OSA, LAA-AF, and RAA-AF patients, and screened out 4 co-DEGs, RASD1, PHLDA1, FOSL1 and ACPP. The GO and KEGG analysis suggested that Wnt signaling pathway might be a specific signaling pathway in the interaction mechanism between OSA and AF.

Conclusion: Our study suggested that RASD1, PHLDA1, FOSL1 and ACPP were the critical genes involved in the interaction between OSA and AF. Moreover, OSA may be involved in the process of AF through different mechanisms in LAA and RAA. These results provided potential new therapeutic targets for OSA and AF.

Keywords: Atrial fibrillation; Biomarkers; Gene analysis; Left atrial appendage; Obstructive sleep apnea; Right atrial appendage

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Introduction

Atrial Fibrillation (AF) is the most common persistent arrhythmia, and existing studies have shown that with economic growth, population aging, and an increase in atrial fibrillation risk factors such as obesity, hypertension, and diabetes, the global prevalence of atrial fibrillation have surged, leading to increased global disease burden. It is estimated that by 2050, 9 million people over 60 in China will suffer from atrial fibrillation [1]. Complications of AF mainly include stroke and thromboembolism, myocardial infarction, heart failure [2], dementia, chronic kidney disease and so on. Among them, thromboembolic events are the most severe complication of AF [3], which can increase the mortality rate [2]. Notably, there is evidence that the predisposition to AF differs between the Left Atrial Appendage (LAA) and Right Atrial Appendage (RAA). LAA plays an essential role in the maintenance of AF, and LAA has a significantly higher rate of AF induction and thrombosis than RAA [4]. Differences in gene expression [5], proteomics [6], electrophysiological activity [7,8], and even morphological histology [9] can be observed between the left and right atrial appendage. These differences may reflect different mechanisms by which LAA and RAA participate in AF.

Obstructive Sleep Apnea (OSA) is one of the most common clinical sleep-disordered breathing disorders. It is estimated that nearly 1 billion middle-aged and older people worldwide may suffer from OSA, of which almost 425 million have moderate to severe obstructive sleep apnea [10]. The main feature of OSA is the recurrent complete or partial collapse of the upper airway during sleep, resulting in hypopnea or apnea [11], which is typically characterized by frequent arousals and daytime sleepiness, causing circadian disturbances. Risk factors for OSA include obesity, smoking, estrogen, nocturnal nasal congestion of various causes [12], and structural abnormalities of the craniofacial and upper airways [13]. Available studies have shown that OSA is associated with the development of diabetes, hypertension, coronary artery disease, myocardial infarction, congestive heart failure and stroke [11,14,15].

Many studies have shown a strong correlation between OSA and AF. Epidemiological studies have shown that 76% to 85% of OSA patients also have atrial fibrillation [16]. Furthermore, in cohort studies and meta-analyses, patients with OSA have an increased risk of both developing AF and recurrence of AF after catheter ablation [17]. Intermittent hypoxia caused by OSA causes chemoreceptor excitation, sympathetic activation, changes in atrial electrophysiology and structure, affects atrial conduction and increases susceptibility to AF. At the same time, OSA can cause an increase in systemic inflammatory products, and these inflammatory products are related to the development and prognosis of AF [18]. However, the specific interaction mechanism between OSA and AF has not yet been fully elucidated, and few studies have demonstrated that OSA may have different mechanisms of action from LAA and RAA. Therefore, it is necessary to explore its underlying mechanism and provide new therapeutic targets based on the mechanism.

In this study, we screened out co-DEGs of OSA, LAA-AF and RAA-AF, and used online tools to predict the miRNAs of co-DEGs, explaining the upstream regulatory factors of co-DEGs expression. GO and KEGG analyses were used to analyze the role of miRNA pathways. At the same time, GO and KEGG analyses were performed on OSA, LAA-AF and RAA-AF, respectively. It was found that OSA and LAA-AF, OSA and RAA-AF have their own common pathways.

Materials and Methods

Data sources

GSE75097 and GSE128188 datasets were downloaded from the Gene Expression Omnibus database (GEO; www.ncbi.nlm.nih.gov/geo) [19], and expression profiling arrays were generated using GPL579 Illumina Human HT-12 V4.0 expression bead chips (Illumina Inc., San Diego, CA, USA) and GPL18573 Illumina Next Seq 500 (Illumina Inc.), respectively. The GSE75097 dataset included 48 peripheral blood samples collected from patients with primary snoring (n=6), moderate to serve OSA (n=16), very serve OSA (n=12), and very serve OSA patients treated by long-term continuous favorable airway pressure treatment (n=14). The samples from the primary snoring and moderate to help OSA patients were used for analysis in the present study. The GSE128188 dataset contained 20 left and right atrial appendages specimens from 10 patients with permanent AF or Sinus Rhythm (SR). It was used to identify differentially expressed genes between left and right atrial in AF.

Data preprocessing and DEGs analysis

R packages of "Lumi," "edgeR," and "limma" (www.bioconductor.org/packages/release/bioc/html/limma.html) provided by a Bioconductor project [20] were applied to assess GSE75097 and GSE128188 raw datasets. Background correction, quantile normalization, probe summarization, and log2-transformation were used to create a robust multi-array average, a log-transformed perfect match, and a mismatch probe. The Benjamini-Hochberg method was used to adjust original p-values, and the false discovery rate procedure was used to calculate fold changes. The fold-change of the gene expression ratio of >1.5 and adjusted P<0.05 were used to identify OSA-DEGs in the present study. The fold-change of the gene expression ratio of >2 and adjusted P<0.05 were used for filtering AF-DEGsin LAA and RAA. Venn diagrams for co-DEGs in OSA- and AF-DEGs (LA and RA) were also created using Venny software (version 2.1; https://bioinfogp.cnb.csic.es/tools/venny/index.html).

Meanwhile, the online prediction tools and software, including microRNA Data Integration Portal (mirDIP, version 4.1.11.1, http://ophid.utoronto.ca/mirDIP) [21], miRDB (http://mirdb.org/) [22], Target Scan (version 7.1; http://www.targetscan.org/vert_71/) [23], and DIANA Tools (http://diana.imis.athena-innovation.gr/DianaTools/) [24], were used to predict potential micro RNA targeting and predict which of selected miRNAs could target co-DEGs. We selected 5 top candidate miRNAs based on higher predicted scores for ≥ 3 prediction tools for each co-DEGs.

PPI construction

PPI networks of OSA-DEGS and AF-DEGs were analyzed by the search tool for the retrieval of interacting genes (STRING; https://string-db.org/) [25] database that predicted functional associations and protein-protein interactions. Firstly, we mapped the OSA-DEGS and AF-DEGs into the STRING database to identify significant protein pairs with a combined score of >0.4. Cytoscape software (version 3.6.1, www.cytoscape.org) was then used to visualize and analyze biological networks and node degrees [26]. Additionally, nodes with a higher degree of interaction were considered hub genes.

Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/) [27] and REACTOME (version 70, https://reactome.org/) [28] datasets were used to perform Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of OSA- and AF-DEGs. GO terms and KEGG maps of biological processes, molecular function, and cellular components associated with a P<0.05 were significantly enriched.

Additionally, we use the AmiGO database (v2.0; http://amigo.geneontology.org/amigo/) to analyze the GO consortium for selected co-DEGs and verify the accuracy and annotate bio functions of identified co-DEGs [29]. Online tools from Diana-miRPath (v3.0; http://www.microrna.gr/miRPathv3) [24] were applied to evaluate interactions between miRNA previously identified using prediction tools and co-DEGs involved in OSA and AF.

The comparative toxicogenomics database (http://ctdbase.org/) was used to find integrated chemical-gene, chemical-disease, and gene-disease interactions to generate expanded networks and predict novel associations [30]. We used the database to analyze relationships between gene products and respiratory or cardiovascular diseases. Here, relationships between co-DEGs and diseases and association or an implied association were identified.

Results

Identification of DEGs and co-DEGs

We identified 343 DEGs (157 down-regulated genes and 186 upregulated genes) in peripheral blood samples of OSA patients compared with primary snoring patients (Figure 1A). Meanwhile, a total of 159 DEGs (102 down-regulated genes and 57 upregulated genes) and 182 DEGs (121 down-regulated genes and 61 upregulated genes) were identified in LAA and RAA specimens of AF patients compared with SR patients, respectively (Figure 1B, 1C). Interestingly, four co-expressed DEGs, including Ras-Related Dexamethasone-Induced 1 (RASD1), Pleckstrin Homology Like Domain family A member 1 (PHLDA1), FOS Like 1 (FOSL1), and Acid Phosphatase (ACPP), were observed in OSA-DEGs and LAA-AF-DEGs. Additionally, RASD1 existed in OSA-DEGs and RAA-AF-DEGs (Figure 1D).

PPI network analysis

As shown in Figure 2, a total of 231 nodes and 454 protein pairs of OSA-DEGs, 102 nodes and 114 protein pairs of LAA-AF-DEGs, 102 nodes and 138 protein pairs of RAA-AF-DEGs, with a combined score of >0.4 based on the STRING database, were obtained. The results showed that Tumor Necrosis Factor (TNF; degree =40), Vascular Endothelial Growth Factor A (VEGFA; degree =20), Interleukin 1 beta (IL-1; degree =20), Activating Transcription Factor 3 (ATF3; degree =18), Hypoxia-Inducible Factor 1 Subunit Alpha (HIF1A; degree =14), TNF Alpha-Induced Protein 3 (TNFAIP3; degree =14), Aurora Kinase A (AURKA; degree =14) and Superoxide Dismutase 2 (SOD2; degree =14) were the top 8 hub genes in OSA-DEGs. Additionally, the top 6 hub genes, including WNT signaling pathway inhibitor 1 (DKK1; degree =8), Bone Morphogenetic Protein 7 (BMP7; degree =6), Aggrecan (ACAN; degree =6), Glutamate Ionotropic Receptor AMPA Type Subunit 1 (GRIA1; degree =6), Fibroblast Growth Factor Receptor 2 (FGFR2, degree =6) and Metallothionein 3 (MT3; degree =6), were identified in LAA-AF-DEGs. Meanwhile, the top 5 hub genes identified in RAA-AF-DEGs were Proopiomelanocortin (POMC; degree =13), Insulin-Like Growth Factor 1 (IGF1; degree =10), Endothelin 1 (EDN1; degree =8), GRIA1 (degree =8) and Glutamate Ionotropic Receptor NMDA Type Subunit 2A (GRIN2A, degree =8).

Functional GO terms and pathway enrichment analyses

Using the DAVID database, a total of 48 biological processes of OSA-DEGs, 47 biological processes of LAA-AF-DEGs, and 40 biological processes of RAA-AF-DEGs were enriched (Figure 3). The top five biological processes concerned OSA-DEGs, 'apoptotic process,' 'response to lipopolysaccharide', 'response to hypoxia', 'positive regulation of fever generation', and 'oxygen homeostasis'. As for LAA-AF-DEGs, the top 5 biological processes were 'SMAD protein signal transduction', 'response to retinoic acid', 'transition between fast and slow fiber', 'cartilage development', and 'protein kinase C-activating G-protein coupled receptor signaling pathway'. Meanwhile, the five top biological processes associated with 'bicarbonate transport', 'membrane depolarization during SA node cell action potential', 'positive regulation of cell growth involved in cardiac muscle cell development', 'cell-cell signaling' and 'protein

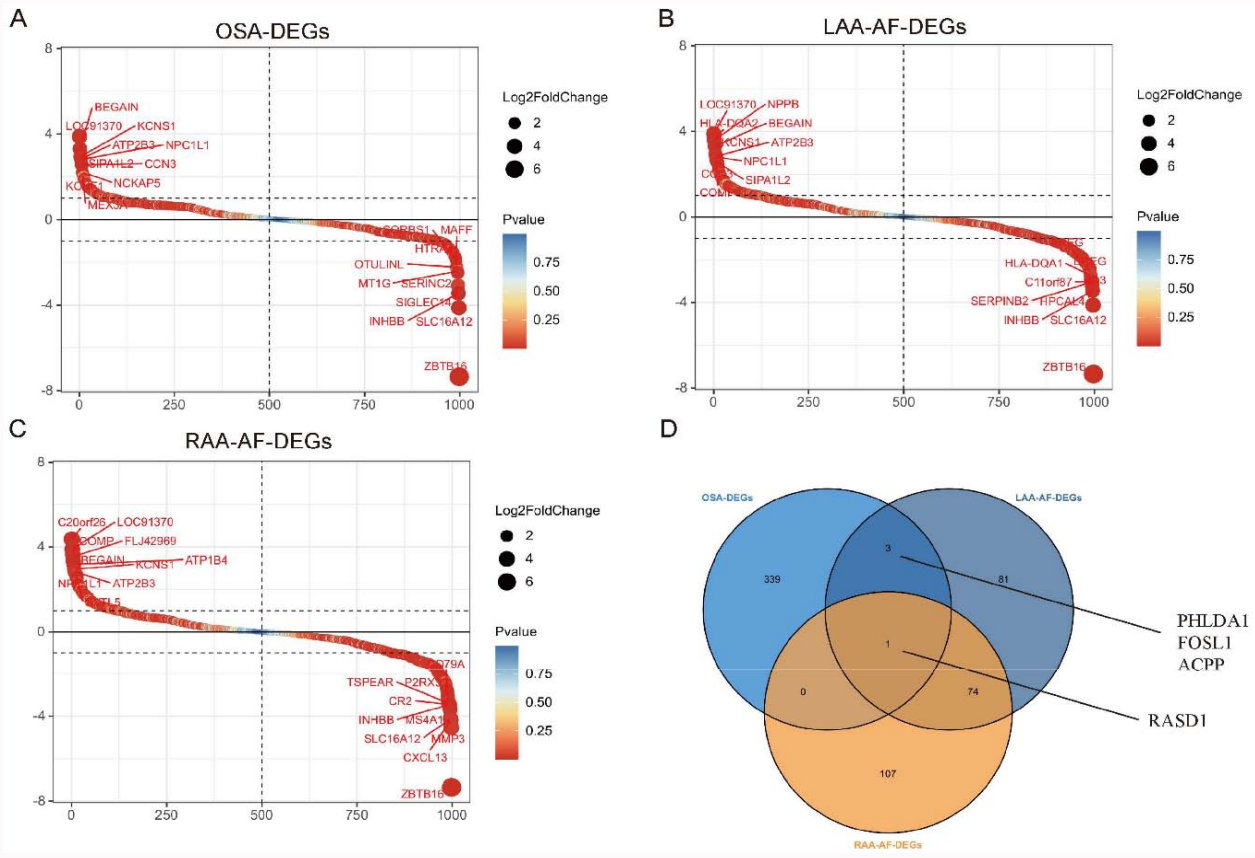

Figure 1: OSA and AF-related differentially expressed genes.
A-C: Differential gene sequence map of OSA, LAA-AF, and RAA-AF; D: Venn diagrams of DEGs. Dot sizes represent Log2 Fold Change of DEGs, and dot colors represent adjusted P<0.05.

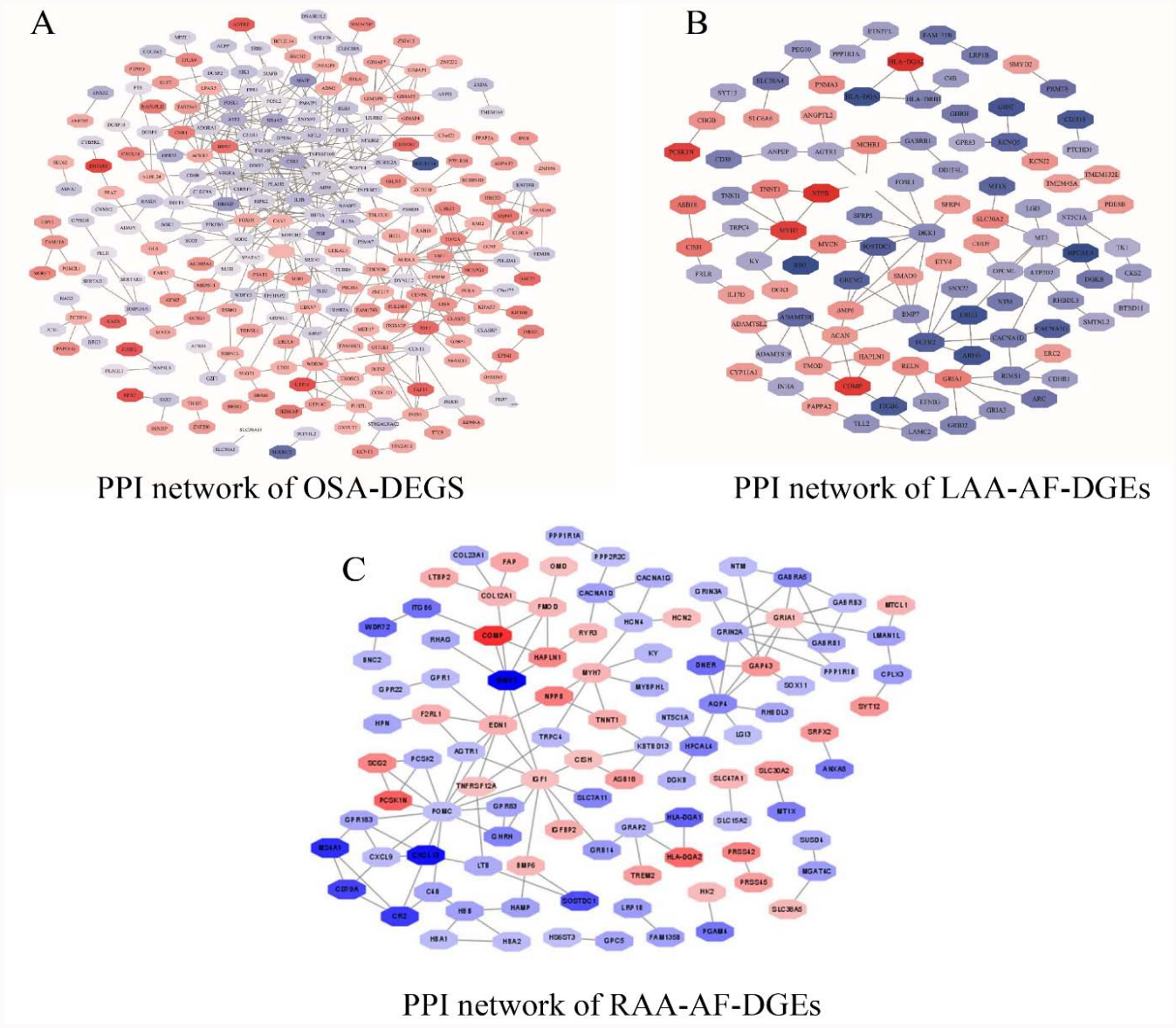

Figure 2: PPI networks of OSA-DEGS and AF-DEGs.
A-C: PPI network of OSA-DEGs, LAA-AF-DGEs and RAA-AF-related DEGs. Red, greater degree. Blue, lesser degree.

kinase C-activating G-protein coupled receptor signaling pathway' were observed in RAA-AF-DEGs. Notably, among all the biological processes, we found 'negative regulation of canonical Wnt signaling pathway' and 'cell-cell signaling' was enriched in OSA-DEGs and LA-AF-DEGs. Additionally, 'cell-cell signaling' and 'signal transduction' was observed in OSA-DEGs and RA-AF-DEGs.

KEGG pathway analysis data were presented in Figure 3. The results showed that the OSA-DEGs were mainly enriched in the 'NOD-like receptor signaling pathway'. KEGG pathway included 'Neuroactive ligand-receptor interaction', 'Circadian entrainment', '*Staphylococcus aureus* infection', 'Retrograde endocannabinoid signaling', 'Viral myocarditis', 'Long-term depression', 'Amphetamine addiction', 'Calcium signaling pathway', 'Asthma', 'Graft-versus-host disease' and 'Aldosterone synthesis and secretion' were enriched in LAA-AF-DEGs. Additionally, the RAA-AF-DEGs were mainly enriched in the pathway of 'Nicotine addiction', 'Neuroactive ligand-receptor interaction', 'Circadian entrainment', 'African trypanosomiasis', 'Amphetamine addiction', 'cAMP signaling pathway', 'GABAergic synapse', 'Malaria', 'Adrenergic signaling in cardiomyocytes', 'Retrograde endocannabinoid signaling' and 'Glutamatergic synapse' (Table 1).

Identification of functional and pathway enrichment among predicted miRNAs and co DEGs

Prediction analysis using mirDIP, miRDB, Target Scan, and DIANA bioinformatic tools identified the top 5 selected miRNAs targeting each Co-DEG involved in OSA-related AF, and these data appear in Table 2. These data enable us to understand how predicted miRNAs are related to OSA-related AF.

Discussion

OSA and AF are two high-morbidity diseases with many common risk factors. Intermittent hypoxia in OSA causes hypercapnia, sympathetic and parasympathetic imbalances [17], larger intrathoracic pressure fluctuations, and increased cardiac wall stress

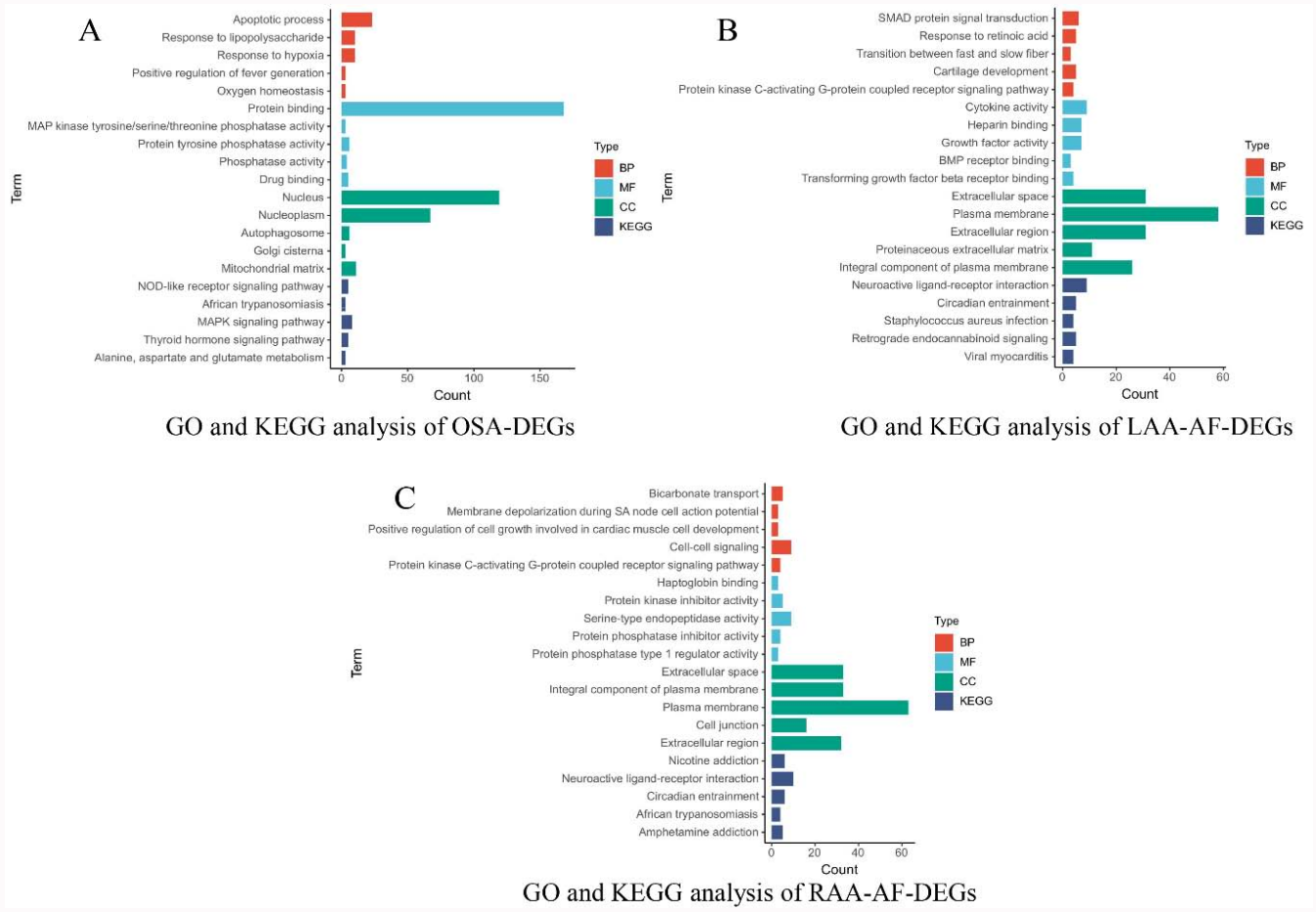

Figure 3: The top 5 GO terms and KEGG pathway enrichment for DEGs.
A-C: OSA, LAA-AF- and RAA-AF GO term and KEGG pathway enrichment for DEGs.

[31], and alters atrial electrophysiology and structure, resulting in increased susceptibility to AF. In atrial fibrillation, increased left atrial pressure and decreased positive flow can lead to unstable ventilatory control and worsen OSA [16]. OSA and AF mutually promote disease progression, thus affecting the efficacy of a single disease, but the causal relationship and underlying mechanisms of the two are not yet clear. Moreover, the mechanisms of LAA and RAA involved in AF may be different, and intervention strategies only targeting LAA or RAA cannot improve the efficacy of AF and prevent complications. Our study is the first to carry out bioinformatics analysis of LAA-AF and RAA-A with OSA, respectively, to find out their common pathophysiological mechanisms and molecular characteristics, so as to help discover new therapeutic targets.

We downloaded the mRNA datasets of peripheral blood mononuclear cells from OSA and left and right atrial appendages of AF patients from the GEO database, and identified co-DEGs, including RASD1, PHLDA1, FOSL1 and ACPP, using bioinformatics analysis.

RASD1, also known as dexRas, belongs to the Ras family and is essentially a small GTP-binding protein that is intensely, rapidly and transiently induced by dexamethasone treatment [32]. RASD1 plays a crucial role in regulating the circadian system and is a vital regulator of the circadian clock [33]. RASD1 was significantly associated with an Apnea-Hypopnea Index (AHI), the most widely used clinical measure of OSA in a genome-wide analysis in OSA patients, and RASD1 mutations or deletions were found can cause higher AHI in non-REM sleep in males [34], suggesting a higher incidence of OSA. In the volume overload rat model experiments of Monica Forero McGrath et al. [35], it was shown that atrial myocytes after RASD1 knockout secreted more Atrial Natriuretic Factor (ANF), which caused an increase in cardiac preload, leading to structural remodeling of the heart, resulting in heart failure, AF and other heart diseases. A study by Kotaro Shinone et al. [36] had demonstrated that in the Methamphetamine (MAP)-induced model, RASD1 can stimulate the secretion of inflammatory factors such as TNFα, IL-1, and IL-6 by activating the G protein signaling pathway, thereby causing myocardial injury and even suffering from sudden death. While elevated levels of TNF can increase the susceptibility of mice to atrial fibrillation [37].

PHLDA1 is a protein-coding gene involved in apoptosis, autophagy, cell proliferation and differentiation, inflammation and fat metabolism [38,39]. The pathophysiological characteristics of OSA are similar to those of asthma, and there is a high frequency of hypoxia-reoxygenation. In the Th2-mediated inflammatory asthma population, the expression of PHLDA1 is significantly up-regulated [40]. It is involved in the production of inflammatory cytokines IL-6, TNF-α, CCL2, etc. through the TLR2 signaling pathway. At the same time, the increase of TNF-α, IL-6 and IL-8 can also be observed in OSA patients [41], so it can be speculated that PHLDA1 may mediate the inflammatory activation of OSA. Meanwhile, Jia XU et al. [42] showed that PHLDA1 was significantly up-regulated after cell reoxygenation

Table 1: The top 5 GO terms enrichment for the co-expressed genes of the OSA-related AF.

Genes	GO class (direct)	Evidence	Evidence with	Reference
RASD1	GTPase activity	IEA	InterPro:IPR001806	GO_REF:0000002
	Protein binding	IPI	UniProtKB:O94972	PMID:25416956
	GTP binding	IEA	UniProtKB-KW:KW0342	GO_REF:0000037
	nucleus	IEA	UniProtKB-SubCell:SL-0191	GO_REF:0000039
	Plasma membrane	IEA	UniProtKB-SubCell:SL-0039	GO_REF:0000039
PHLDA1	G2/M transition of mitotic cell cycle	TAS		Reactome:R-HAS-69275
	Protein binding	IPI	UniProtKB:O15371	PMID:11369516
	nucleoplasm	IDA		GO_REF:0000052
	nucleolus	IDA		GO_REF:0000052
	cytosol	TAS		Reactome:R-HSA-8853429
FOSL1	nuclear chromatin	ISA	tfclass:1.1.2	GO_REF:0000113
	RNA polymerase II proximal promoter sequence-specific DNA binding	IDA		PMID:21673316
	DNA-binding transcription factor activity, RNA polymerase II-specific	ISA/ISM	UniProtKB:P10158	PMID:19274049
		/NAS/IEA		
	in utero embryonic development	IEA	UniProtKB:P48755	GO_REF:0000107
	DNA-binding transcription factor activity	TAS		PMID:10918580
ACPP	acid phosphatase activity	IMP		PMID:8132635
	protein binding	IPI	UniProtKB:P04626	PMID:9705354
	extracellular space	IDA		PMID:10639192
	extracellular space	IDA		PMID:15280042
	nucleus	HDA		PMID:21630459

IEA: The Evidence Used in Automatic Assertion; IPI: Physical Interaction Evidence Used in Manual Opinion; TAS: Author Statement Supported By Traceable Reference Used in Manual Opinion; IDA: Direct Assay Evidence Used in Manual Assertion; ISA: Sequence Alignment Evidence Used in the Manual Claim; ISM: Match To Sequence Model Evidence Used in Manual View; NAS: Author Statement Without Traceable Support Used in Manual Assertion; HAD: High Throughput Direct Assay Evidence Used in Manual Opinion; IMP: Mutant Phenotype Evidence Used in a Manual Report

and promoted vascular endothelial cell hypoxia/reoxygenation injury by binding to BCL2-Associated X protein (BAX). The experimental study by Guo et al [43]. found that PHLDA1 is highly expressed in cardiomyocytes in an ischemia-reperfusion model, and its mechanism may promote oxidative stress-induced cardiomyocyte damage. Wang et al., [44] by analyzing the gene expression dataset of Ischemic Cardiomyopathy (ICM), establishing a rat ICM model and culturing cardiomyocytes under ischemia, found that PHLDA 1 was significantly upregulated, increasing the expression of p53 and BAX proteins in cardiomyocytes, and inhibiting the expression of Bcl-2, thereby promoting cardiomyocyte apoptosis. In the past, many studies have shown that ischemic cardiomyopathy can promote the occurrence of AF, and they have the same risk factors such as obesity, smoking, hypertension, diabetes and so on. Therefore, we can speculate that the hypoxia-reoxygenation of OSA upregulates the expression of PHLDA1 and promotes cardiomyocyte injury, thereby increasing the susceptibility to AF.

FOSL 1 is a multifunctional transcription factor that binds to the JUN family to form the Activator Protein one complex (AP-1). The study by Mrinalini Singh et al. demonstrated that under prolonged hypoxia, FOSL1 increases the production of TNFα and IL6 in lung tissue through ERK, p38 and NFκB signaling pathways [45]. TNFα activated by FOSL1 can induce the expression of Matrix Metalloproteinase 9 (MMP9), and MMP9 can promote the degradation of vascular endothelial extracellular matrix and atrial fibrosis, resulting in impaired vascular integrity and atrial structural remodeling, leading to the occurrence of AF [46,47].

It can be seen from the above that co-DEGs are jointly involved in the progression of OSA and AF through various pathogenic mechanisms, providing potential new common therapeutic targets for OSA and OA.

At the same time, we performed GO and KEGG enrichment analysis on OSA-DEGs, LAA-AF-DEGs and RAA-AF-DEGs respectively, and found that OSA-DEGs and LAA-AF-DEGs were jointly enriched in negative regulation of canonical Wnt signaling pathway. The canonical Wnt signaling pathway functions by regulating the phosphorylation and degradation of β-catenin, which plays a crucial role in cardiac development, cell proliferation, cardiomyocyte gap junctions, pulmonary fibrosis and wound repair in many organs, including the respiratory system. At the same time, the study by Bingyan Guo et al [48]. suggested that the Wnt signaling pathway is closely related to the physiological disturbance of circadian rhythm. In human beings, the Wnt signaling pathway maintains lung homeostasis, and interference with this pathway may cause pathological responses such as pulmonary fibrosis [49] and asthmatic airway remodeling [50]. In severe asthma and COPD patients, it has been observed that post-injury repair of bronchial and alveolar epithelial cells was inhibited by reducing canonical Wnt signaling, then causing senescence [51,52]. Furthermore, down regulation of Wnt/β-catenin signaling pathway can promote an excessive inflammatory response in alveolar epithelial cells and disrupt lung homeostasis [53]. Meanwhile, studies by Zhaowei et al [54]. have shown that activation of the Wnt signaling pathway can induce the expression of Cx, which is the most abundant cardiovascular gap junction

Table 2: The GO terms and KEGG pathways enrichment among predicted miRNAs and co-DEGs.

Genes	Predicted miRNAs	Category		P-value
RASD1	hsa-miR-106b-5p	GO-BP terms	cellular nitrogen compound metabolic process	3.61E-56
	hsa-miR-20a-5p		the cellular protein modification process	2.25E-47
	hsa-miR-30d-5p		biosynthetic process	8.55E-31
	hsa-miR-578		gene expression	1.82E-25
	hsa-miR-3944-5p		neurotrophin TRK receptor signaling pathway	2.32E-23
		KEGG pathway	FoxO signaling pathway	4.61E-07
			TGF-beta signaling pathway	4.96E-06
			Axon guidance	1.85E-05
			Proteoglycans in cancer	1.85E-05
			Endocytosis	0.0001
PHLDA1	hsa-miR-3163	GO-BP terms	cellular nitrogen compound metabolic process	1.12E-113
	hsa-miR-875-3p		the cellular protein modification process	5.98E-73
	hsa-miR-548a-5p		biosynthetic process	1.66E-68
	hsa-miR-3148		neurotrophin TRK receptor signaling pathway	7.22E-33
	hsa-miR-5590-3p		gene expression	1.26E-31
		KEGG pathway	TGF-beta signaling pathway	6.26E-10
			Signaling pathways regulating pluripotency of stem cells	3.78E-09
			Proteoglycans in cancer	3.78E-09
			Mucin type O-Glycan biosynthesis	1.17E-08
			Pathways in cancer	2.45E-08
FOSL1	hsa-miR-454-3p	GO-BP terms	biosynthetic process	1.94E-20
	hsa-miR-34a-5p		cellular nitrogen compound metabolic process	1.83E-19
	hsa-miR-449a		the cellular protein modification process	2.00E-17
	hsa-miR-301a-3p		epidermal growth factor receptor signaling pathway	2.06E-10
	hsa-miR-4295		neurotrophin TRK receptor signaling pathway	2.32E-09
		KEGG pathway	Prion diseases	3.43E-24
			Glioma	8.18E-05
			MicroRNAs in cancer	0.0003
			Glycosphingolipid biosynthesis - Lacto and neolacto series	0.0004
			Phosphatidylinositol signaling system	0.0009
ACPP	hsa-miR-330-3p	GO-BP terms	cellular nitrogen compound metabolic process	3.81E-76
	hsa-miR-576-5p		biosynthetic process	2.55E-46
	hsa-miR-3926		the cellular protein modification process	5.94E-33
	hsa-miR-129-5p		gene expression	4.53E-24
	hsa-miR-4728-5p		neurotrophin TRK receptor signaling pathway	3.64E-20
		KEGG pathway	Long-term depression	3.79E-09
			Lysine degradation	3.72E-06
			Axon guidance	2.11E-05
			Glioma	2.20E-05
			ErbB signaling pathway	4.86E-05

channel protein and exists in the gap junctions of cardiomyocytes. Its reduced expression is associated with an increased predisposition to arrhythmias [55]. Similar to the mechanism of asthma and COPD, the obstructive ventilatory dysfunction in OSA can reduce the expression level of Cx43 by inhibiting the Wnt signaling pathway, resulting in an increased incidence of various arrhythmias including AF.

In addition, we also found that OSA-DEGs and LA-AF-DEGs were co-enriched in cell-cell signaling, and OSA-DEGs and RA-AF-DEGs were co-enriched in cell-cell signaling and signal transduction related to intercellular signaling. All of the above indicated specific or non-specific cell signal transduction pathways between OSA and AF in left and right atrial appendages that participated in the disease process.

MicroRNAs (miRNAs) control various biological processes by

fine-tuning gene expression and are increasingly involved in the pathogenesis of diseases, becoming emerging biomarkers. MiRNAs are directly or indirectly involved in the control of AF by mediating the regulation of cardiomyocyte ion channels, oxidative stress, and atrial structural remodeling [56]. In OSA, intermittent hypoxia modulates miRNA expression to affect cardiomyocyte injury, increasing the risk of cardiovascular disease [57,58]. We analyzed potential miRNAs regulating co-DEGs, and found that RASD1, PHLDA1, FOSL1 and ACPP were all controlled by their respective miRNAs. GO and KEGG analysis showed that these miRNAs were commonly enriched in the metabolic process of cellular nitrogen compounds, the modification process of cellular proteins and the biosynthesis process. In existing studies, these miRNAs are more or less involved in the processes of chronic obstructive pulmonary disease, ischemic cardiomyopathy, airway epithelial cell transformation, etc. From this, we can speculate that RASD1, PHLDA1, FOSL1 and ACPP have vital roles in the interaction of OSA and AF, and may be the potential biomarkers.

There are still some limitations of our study. First, this study is based on sequencing data or microarray datasets to perform microarray analysis on all results, focusing on the mRNA level, which cannot directly reflect protein expression. Second, we need experimental studies to confirm our conclusions.

Conclusion

More and more research has shown a strong correlation between AF and OSA, but the specific pathophysiological mechanism is still unclear. In this study, the co-DEGs of OSA and AF, including RASD1, PHLDA1, FOSL1 and ACPP, were screened by bioinformatics analysis. These genes can serve as targets for the interaction of OAS and AF. These genes mediate atrial structural remodeling and electrophysiological activity changes by regulating oxidative stress and altering cell signaling, thereby promoting the occurrence and development of AF. And we found that OSA-DEGs and LAA-AF-DEGs were co-enriched in the negative regulation of canonical Wnt signaling pathway, and OSA-DEGs and RA-AF-DEGs were co-enriched in cell-cell signaling and signal transduction, which are related to cell signaling. Inflammatory factors produced in AF also acted on pulmonary vascular endothelial cells and alveolar epithelial cells, aggravating the progression of OSA. These findings provide new directions for the interaction mechanism and treatment of OSA and AF.

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References

1. Rahman F, Kwan GF, Benjamin EJ. Global epidemiology of atrial fibrillation. Nat Rev Cardiol. 2014;11(11):639-54.
2. Stewart S, Hart CL, Hole DJ, McMurray JJV. A population-based study of the long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study. Am J Med. 2002;113(5):359-64.
3. Wang TJ, Massaro JM, Levy D, Vasan RS, Wolf PA, Agostino RBD, et al. A risk score for predicting stroke or death in individuals with new-onset atrial fibrillation in the community. The Framingham Heart Study. ACC Curr J Rev. 2003;12(6):54.
4. Ozer O, Sari I, Davutoglu V. Right atrial appendage: Forgotten part of the heart in atrial fibrillation. Clin Appl Thromb. 2010;16(2):218-20.
5. Zhu H, Zhang W, Zhong M, Zhang G, Zhang Y. Differential gene expression during atrial structural remodeling in human left and right atrial appendages in atrial fibrillation. Acta Biochim Biophys Sin. 2011;43(7):535-41.
6. Liu H, Chen G, Zheng H, Qin H, Liang M, Feng K, et al. Differences in atrial fibrillation-associated proteins between the left and right atrial appendages from patients with rheumatic mitral valve disease: A comparative proteomic analysis. Mol Med Rep. 2016;14(5):4232-42.
7. Tai CT, Lo LW, Lin YJ, Chen SA. Arrhythmogenic difference between the left and right atria in a canine ventricular pacing-induced heart failure model of atrial fibrillation. Pacing Clin Electrophysiol PACE. 2012;35(2):188-95.
8. Li D, Zhang L, Kneller J, Nattel S. Potential ionic mechanism for repolarization differences between canine right and left atrium. Circ Res. 2001;88(11):1168-75.
9. Park JH, Lee JS, Ko YG, Lee SH, Lee BS, Kang SM, et al. Histological and biochemical comparisons between right atrium and left atrium in patients with mitral valvular atrial fibrillation. Korean Circ J. 2014;44(4):233-42.
10. Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: A literature-based analysis. Lancet Respir Med. 2019;7(8):687-98.
11. Krieger J, McNicholas WT, Levy P, Backer WD, Douglas N, Marrone O, et al. Public health and medicolegal implications of sleep apnoea. Eur Respir J. 2002;20(6):1594-609.
12. Young T, Skatrud J, Peppard PE. Risk factors for obstructive sleep apnea in adults. JAMA. 2004;291(16):2013-6.
13. Dempsey JA, Skatrud JB, Jacques AJ, Ewanowski SJ, Woodson BT, Hanson PR, et al. Anatomic determinants of sleep-disordered breathing across the spectrum of clinical and nonclinical male subjects. Chest. 2002;122(3):840-51.
14. Shamsuzzaman ASM, Gersh BJ, Somers VK. Obstructive sleep apnea: Implications for cardiac and vascular disease. JAMA. 2003;290(14):1906-14.
15. Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: A population health perspective. Am J Respir Crit Care Med. 2002;165(9):1217-39.
16. The prevalence of obstructive sleep apnea in patients with atrial fibrillation.
17. Kwon Y, Koene RJ, Johnson AR, Lin GM, Ferguson JD. Sleep, sleep apnea and atrial fibrillation: Questions and answers. Sleep Med Rev. 2018;39:134-42.
18. Hu YF, Chen YJ, Lin YJ, Chen SA. Inflammation and the pathogenesis of atrial fibrillation. Nat Rev Cardiol. 2015;12(4):230-43.
19. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: Archive for functional genomics data sets—update. Nucleic Acids Res. 2013;41(Database issue):D991-5.
20. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
21. Tokar T, Pastrello C, Rossos AEM, Abovsky M, Hauschild AC, Tsay M, et al. mirDIP 4.1-integrative database of human microRNA target predictions. Nucleic Acids Res. 2018;46(D1):D360-70.
22. Liu W, Wang X. Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. Genome Biol. 2019;20(1):18.
23. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. eLife. 2015;4:e05005.
24. Paraskevopoulou MD, Georgakilas G, Kostoulas N, Vlachos IS, Vergoulis T, Reczko M, et al. DIANA-microT web server v5.0: Service integration into miRNA functional analysis workflows. Nucleic Acids Res. 2013;41(Web

Server issue):W169-73.

25. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013;41(Database issue):D808-15.

26. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.

27. Dennis G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol. 2003;4(5):P3.

28. Croft D, O'Kelly G, Wu G, Haw R, Gillespie M, Matthews L, et al. Reactome: A database of reactions, pathways and biological processes. Nucleic Acids Res. 2011;39(Database issue):D691-7.

29. Munoz-Torres M, Carbon S. Get GO! Retrieving GO Data Using AmiGO, QuickGO, API, Files, and Tools. Methods Mol Biol Clifton NJ. 2017;1446:149-60.

30. Davis AP, Grondin CJ, Johnson RJ, Sciaky D, McMorran R, Wiegers J, et al. The comparative toxicogenomics database: Update 2019. Nucleic Acids Res. 2019;47(D1):D948-54.

31. Schäfer H, Hasper E, Ewig S, Koehler U, Latzelsberger J, Tasci S, et al. Pulmonary haemodynamics in obstructive sleep apnoea: Time course and associated factors. Eur Respir J. 1998;12(3):679-84.

32. Colicelli J. Human RAS superfamily proteins and related GTPases. Sci STKE. 2004;2004(250):RE13.

33. Cheng HYM, Obrietan K, Cain SW, Lee BY, Agostino PV, Joza NA, et al. Dexras1 potentiates photic and suppresses nonphotic responses of the circadian clock. Neuron. 2004;43(5):715-28.

34. Chen H, Cade BE, Gleason KJ, Bjonnes AC, Stilp AM, Sofer T, et al. Multiethnic meta-analysis identifies RAI1 as a possible obstructive sleep apnea–related quantitative trait locus in men. Am J Respir Cell Mol Biol. 2018;58(3):391-401.

35. MF McGrath, Ogawa T, de Bold AJ. Ras dexamethasone-induced protein 1 is a modulator of hormone secretion in the volume overloaded heart. Am J Physiol Heart Circ Physiol. 2012;302(9):H1826-37.

36. Shinone K, Tomita M, Inoue H, Nakagawa Y, Ikemura M, Nata M. Molecular-biological analysis of the effect of methamphetamine on the heart in restrained mice. Leg Med. 2010;12(2):79-83.

37. Saba S, Janczewski AM, Baker LC, Shusterman V, Gursoy EC, Feldman AM, et al. Atrial contractile dysfunction, fibrosis, and arrhythmias in a mouse model of cardiomyopathy secondary to cardiac-specific overexpression of tumor necrosis factor-{alpha}. Am J Physiol Heart Circ Physiol. 2005;289(4):H1456-67.

38. Johnson EO, Chang K-H, de Pablo Y, Ghosh S, Mehta R, Badve S, PHLDA1 is a crucial negative regulator and effector of Aurora A kinase in breast cancer. J Cell Sci. 2011;124(Pt 16):2711-22.

39. Sellheyer K, Nelson P. Follicular stem cell marker PHLDA1 (TDAG51) is superior to cytokeratin-20 in differentiating between trichoepithelioma and basal cell carcinoma in small biopsy specimens. J Cutan Pathol. 2011;38(7):542-50.

40. Baos S, Calzada D, Cremades-Jimeno L, Sastre J, Picado C, Quiralte J. Nonallergic asthma and its severity: Biomarkers for its discrimination in peripheral samples. Front Immunol. 2018;9:1416.

41. Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. Circulation. 2005;112(17):2660-7.

42. Xu J, Jiang JK, Li XL, Yu XP, Xu YG, Lu YQ. Comparative transcriptomic analysis of vascular endothelial cells after hypoxia/re-oxygenation induction based on microarray technology. J Zhejiang Univ-Sci B. 2020;21(4):291-304.

43. Guo Y, Jia P, Chen Y, Yu H, Xin X, Yang H, et al. PHLDA1 is a new therapeutic target of oxidative stress and ischemia reperfusion-induced myocardial injury. Life Sci. 2020;245:117347.

44. Wang J. Transcriptome profiling reveals PHLDA1 as a novel molecular marker for ischemic cardiomyopathy. J Mol Neurosci. 2018;65(1):102-9.

45. Singh M, Yadav S, Kumar M, Saxena S, Saraswat D, Bansal A, et al. The MAPK-activator protein-1 signaling regulates changes in lung tissue of rat exposed to hypobaric hypoxia. J Cell Physiol. 2018;233(10):6851-65.

46. Sygitowicz G, Maciejak-Jastrzębska A, Sitkiewicz D. A review of the molecular mechanisms underlying cardiac fibrosis and atrial fibrillation. J Clin Med. 2021;10(19):4430.

47. Nakano Y, Niida S, Dote K, Takenaka S, Hirao H, Miura F, et al. Matrix metalloproteinase-9 contributes to human atrial remodeling during atrial fibrillation. J Am Coll Cardiol. 2004;43(5):818-25.

48. Guo B, Chatterjee S, Li L, Kim JM, Lee J, Yechoor VK, et al. The clock gene, brain and muscle Arnt-like 1, regulates adipogenesis *via* Wnt signaling pathway. FASEB J. 2012;26(8):3453-63.

49. Burgy O, Königshoff M. The WNT signaling pathways in wound healing and fibrosis. Matrix Biol. 2018;68–69:67-80.

50. Hussain M, Xu C, Lu M, Wu X, Tang L, Wu X. Wnt/β-catenin signaling links embryonic lung development and asthmatic airway remodeling. Biochim Biophys Acta BBA - Mol Basis Dis. 2017;1863(12):3226-42.

51. Hachim MY, Elemam NM, Ramakrishnan RK, Bajbouj K, Olivenstein R, Hachim IY, et al. Wnt signaling is deranged in asthmatic bronchial epithelium and fibroblasts. Front Cell Dev Biol. 2021;9:641404.

52. Wu X, van Dijk EM, Ng-Blichfeldt JP, Bos IST, Ciminieri C, Königshoff M, et al. Mesenchymal WNT-5A/5B signaling represses lung alveolar epithelial progenitors. Cells. 2019;8(10):1147.

53. Li Y, Shi J, Yang J, Ma Y, Cheng L, Zeng J, et al. A Wnt/β-catenin negative feedback loop represses TLR-triggered inflammatory responses in alveolar epithelial cells. Mol Immunol. 2014;59(2):128-35.

54. Ai Z, Fischer A, Spray DC, Brown AMC, Fishman GI. Wnt-1 regulation of connexin43 in cardiac myocytes. J Clin Invest. 2000;105(2):161-71.

55. Zeevi-Levin N, Barac YD, Reisner Y, Reiter I, Yaniv G, Meiry G, et al. Gap junctional remodeling by hypoxia in cultured neonatal rat ventricular myocytes. Cardiovasc Res. 2005;66(1):64-73.

56. Wang Z, Lu Y, Yang B. MicroRNAs and atrial fibrillation: New fundamentals. Cardiovasc Res. 2011;89(4):710-21.

57. Lin G, Huang J, Chen Q, Chen L, Feng D, Zhang S, et al. miR-146a-5p mediates intermittent hypoxia-induced injury in H9c2 cells by targeting XIAP. Oxid Med Cell Longev. 2019;2019:6581217.

58. Chen Q, Lin G, Chen Y, Li C, Wu L, Hu X, et al. miR-3574 ameliorates intermittent hypoxia-induced cardiomyocyte injury through inhibiting Axin1. Aging. 2021;13(6):8068-77.