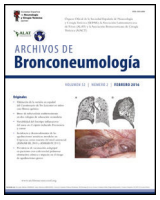
Archivos de Bronconeumología xxx (xxxx) xxx–xxx

Sociedad Española de Neumología y Cirugía Torácica SEPAR

ARCHIVOS DE
Bronconeumología

www.archbronconeumol.org



Original Article

Synergic Integration of the miRNome, Machine Learning and Bioinformatics for the Identification of Potential Disease-Modifying Agents in Obstructive Sleep Apnea

Q1 Thalia Belmonte[a,b], Iván D. Benitez[a,b,c], María C. García-Hidalgo[a,b], Marta Molinero[a,b], Lucía Pinilla[b,d], Olga Mínguez[a], Rafaela Vaca[a], Maria Aguilà[a], Anna Moncusí-Moix[a], Gerard Torres[b,d], Olga Mediano[e], Juan F. Masa[f], Maria J. Masdeu[g], Blanca Montero-San-Martín[h], Mercè Ibarz[h], Pablo Martinez-Camblor[i,j], Alberto Gómez-Carballa[b,k,l], Antonio Salas[b,k,l], Federico Martinón-Torres[b,k,m], Ferran Barbé[a,b], Manuel Sánchez-de-la-Torre[b,d,n,o,*,1], David de Gonzalo-Calvo[a,b,*,1]

[a] *Translational Research in Respiratory Medicine, University Hospital Arnau de Vilanova and Santa Maria, IRBLleida, Lleida, Spain*
[b] *CIBER of Respiratory Diseases (CIBERES), Institute of Health Carlos III, Madrid, Spain*
[c] *Department of Basic Medical Sciences, University of Lleida, Lleida, Spain*
[d] *Precision Medicine in Chronic Diseases, University Hospital Arnau de Vilanova and Santa Maria, IRB Lleida, Department of Nursing and Physiotherapy, Faculty of Nursing and Physiotherapy, University of Lleida, Lleida, Spain*
[e] *Pneumology Department, University Hospital of Guadalajara, Guadalajara, Spain*
[f] *San Pedro de Alcantara Hospital, Instituto Universitario de Investigación Biosanitaria de Extremadura, Cáceres, Spain*
[g] *Respiratory and Sleep Department, Parc Taulí University Hospital, Parc Taulí Research and Innovation Institute, Autonomous University of Barcelona, Sabadell, Spain*
[h] *Department of Clinical Laboratory, University Hospital Arnau de Vilanova, IRBLleida, Lleida, Spain*
[i] *Anesthesiology Department, Geisel School of Medicine at Dartmouth, Hanover, NH, USA*
[j] *Faculty of Health Sciences, Universidad Autonoma de Chile, Providencia, Chile*
[k] *Genetics, Vaccines and Infections Research Group (GenViP), Instituto de Investigación Sanitaria de Santiago, Universidade de Santiago de Compostela (USC), 15706 Santiago de Compostela, Galicia, Spain*
[l] *Unidade de Xenética, Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela (USC) and Genetica de Poblaciones en Biomedicina (GenPoB) Research Group, Instituto de Investigación Sanitaria (IDIS), Hospital Clínico Universitario de Santiago (SERGAS), Santiago de Compostela, Galicia, Spain*
[m] *Translational Pediatrics and Infectious Diseases, Department of Pediatrics, Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Galicia, Spain*
[n] *Group of Precision Medicine in Chronic Diseases, Hospital Nacional de Parapléjicos, IDISCAM, Spain*
[o] *Department of Nursing, Physiotherapy and Occupational Therapy, Faculty of Physiotherapy and Nursing, University of Castilla-La Mancha, Toledo, Spain*

ARTICLE INFO

Article history:
Received 30 July 2024
Accepted 22 November 2024
Available online xxx

Keywords:
Disease-modifying interventions
Machine learning
microRNAs
Molecular mechanisms
Obstructive sleep apnea
Therapeutic targets

ABSTRACT

Introduction: Understanding the diverse pathogenetic pathways in obstructive sleep apnea (OSA) is crucial for improving outcomes. microRNA (miRNA) profiling is a promising strategy for elucidating these mechanisms.
Objective: To characterize the pathogenetic pathways linked to OSA through the integration of miRNA profiles, machine learning (ML) and bioinformatics.
Methods: This multicenter study involved 525 patients with suspected OSA who underwent polysomnography. Plasma miRNAs were quantified via RNA sequencing in the discovery phase, with validation in two subsequent phases using RT-qPCR. Supervised ML feature selection methods and comprehensive bioinformatic analyses were employed. The associations among miRNA targets, OSA and OSA treatment were further explored using publicly available external datasets.
Results: Following the discovery and technical validation phases in a subset of patients with and without confirmed OSA ($n = 53$), eleven miRNAs were identified as candidates for the subsequent feature selection

* Corresponding authors.
E-mail addresses: sanchezdelatorre@gmail.com (M. Sánchez-de-la-Torre), dgonzalo@irblleida.cat (D. de Gonzalo-Calvo).
[1] Equally contributed.

https://doi.org/10.1016/j.arbres.2024.11.011

Please cite this article as: T. Belmonte, I.D. Benitez, M.C. García-Hidalgo et al., Synergic Integration of the miRNome, Machine Learning and Bioinformatics for the Identification of Potential Disease-Modifying Agents in Obstructive Sleep Apnea, Archivos de Bronconeumología, https://doi.org/10.1016/j.arbres.2024.11.011

process. These miRNAs were then quantified in the remaining population ($n = 472$). Feature selection methods revealed that the miRNAs let-7d-5p, miR-15a-5p and miR-107 were the most informative of OSA. The predominant mechanisms linked to these miRNAs were closely related to cellular events such as cell death, cell differentiation, extracellular remodeling, autophagy and metabolism. One target of let-7d-5p and miR-15a-5p, the *TFDP2* gene, exhibited significant differences in gene expression between subjects with and without OSA across three independent databases.

Conclusion: Our study identified three plasma miRNAs that, in conjunction with their target genes, provide new insights into OSA pathogenesis and reveal novel regulators and potential drug targets.

Introduction

Obstructive sleep apnea (OSA) is a prevalent sleep disorder characterized by the recurrent occurrence of obstructive apnea and hypopnea, leading to repeated decreases in oxygen saturation and disrupted sleep patterns. OSA is an important contributor to poor health outcomes, including a spectrum of morbidities and an elevated risk of major causes of mortality.[1,2] OSA is a complex condition with a heterogeneous profile of clinical and physiological phenotypes,[3,4] presenting a challenge in tailoring effective therapeutic strategies. The identification of the pathogenetic pathways is a pivotal step in improving outcomes in OSA patients. Continuous positive airway pressure (CPAP) represents the cornerstone therapeutic intervention. Despite its well-established efficacy in reducing daytime somnolence and enhancing quality of life, a proportion of individuals treated with CPAP continues to experience residual symptoms and associated comorbidities.[5] This scenario suggests the necessity for adjuvant therapies capable of complementing CPAP therapy and addressing the multifactorial nature of OSA.

microRNAs (miRNAs) are small noncoding RNAs (ncRNAs) composed of 19–25 nucleotides that play a key role in the post-transcriptional regulation of gene expression. miRNAs typically inhibit gene expression by binding to target mRNAs, thereby inducing their degradation or translational repression. However, in specific instances, miRNAs can also activate translation.[6] It has been predicted that a significant proportion of the human transcriptome is regulated by miRNAs.[7] As such, miRNAs are mediators of numerous fundamental biological processes and play significant roles in various diseases, including respiratory conditions.[8] Previous studies have shown that miRNAs play a role in various pathobiological mechanisms associated with OSA. For example, the SREBP2/miR-210 axis and its impact on mitochondrial dysfunction represent a mechanistic link between OSA and endothelial cell dysfunction.[9] The downregulation of miR-15b-5p and miR-92b-3p in OSA patients may contribute to intermittent hypoxia/reoxygenation-induced oxidative stress by influencing the eicosanoid inflammatory pathway.[10]

Over the past decade, miRNAs have emerged as crucial tools for developing next-generation therapeutics. Numerous well-designed studies have demonstrated the efficacy and safety of miRNA-based treatments.[11,12] A notable example is miravirsen, a locked nucleic acid–modified DNA phosphorothioate antisense oligonucleotide that sequesters mature miR-122, resulting in a dose-dependent and sustained reduction in hepatitis C virus (HCV) RNA levels in patients with chronic HCV genotype 1 infection.[13] In addition, second-line therapies using miRNA mimics, such as miR-34a and let-7g, are being investigated to reduce tumor burden and promote tumor suppression.[14,15] Beyond their intracellular location, miRNAs serve as mediators of intercellular communication through their secretion into the extracellular milieu and regulation of gene expression in recipient cells.[16–18] Although the hormone-like effect of miRNAs has been a topic of debate,[19] a multitude of studies have provided evidence supporting their role as endocrine genetic signals.[20–22] Overall, the circulating miRNome has emerged as a promising tool for improving the mechanistic understanding of disease and identifying novel therapeutic treatments.

The analysis of ncRNAs, including miRNAs, constitutes a valuable strategy in the field of network medicine, as it facilitates the discovery of novel mechanistic insights that were previously elusive.[23,24] Investigating these transcripts enhances the ability to identify molecular mechanisms associated with a given disease. Notably, the integration of extracellular ncRNA profiling with machine learning (ML) methods has been previously employed to identify molecular drivers of disease.[25] The distinct advantage of ML is its ability to decipher previously unknown multidimensional interactions between predictors and outcomes and therefore to identify novel biological features. This is particularly relevant for miRNAs, given their involvement in regulating gene expression through dynamic, complex and coordinated networks.[26]

In the current investigation, we synergistically employed circulating cell-free miRNome profiling with ML feature selection techniques and bioinformatic analyses. This integrative approach aimed to characterize the mechanistic pathways associated with OSA and to propose innovative disease-modifying agents.

Methods

Methods for blood collection, miRNA sequencing and external dataset are provided in Supplemental Material.

Study Population

The study included 525 subjects referred to the sleep unit due to suspected OSA (ClinicalTrials.gov identifier: NCT03513926). The recruitment was conducted at University Hospital Arnau de Vilanova-Santa María of Lleida and Hospital San Pedro Alcántara of Cáceres in Spain. Patients aged older than 18 years were recruited for the present study. The exclusion criteria were the presence of a previously diagnosed sleep disorder, a history of CPAP treatment, inability to complete the questionnaires and any medical, social or geographic factors that could compromise the eligibility of the subject, i.e., pregnancy, substance abuse, cancer, renal insufficiency, severe chronic obstructive pulmonary disease, chronic depression and other very limiting chronic diseases or life expectancy less than 1 year. General physical and anthropometric parameters were documented, and information on sociodemographic characteristics, medical history, medication use and lifestyle habits was collected by trained clinicians.

Sleep Evaluation

All eligible participants underwent a comprehensive polysomnographic (PSG) sleep study (Sleepware G3, Philips, Amsterdam, Netherlands). All procedures were conducted according to national guidelines and regulations governing clinical practice.[27] Trained personnel who adhered to standard criteria[28] analyzed the results of the sleep studies. Apnea was characterized

as an interruption or reduction in oronasal airflow by ≥90% for at least 10 s, while hypopnea was defined as a 30–90% reduction in oronasal airflow for at least 10 s, associated with either oxygen desaturation by at least 3% or an arousal on the electroencephalogram. The apnea–hypopnea index (AHI), which is indicative of OSA severity, was calculated based on the average number of apnea and hypopnea events per hour of sleep. Following the PSG study, subjects were categorized according to the International Consensus Document on Obstructive Sleep Apnea[29] into non-OSA (AHI < 15 events/h) and OSA (AHI ≥ 15 events/h) groups.

Ethics

All subjects provided written informed consent for study participation prior to the collection of blood samples. This study was revised and approved by the Clinical Research Ethics Committee of the University Hospital Arnau de Vilanova-Santa María of Lleida (no. 1153/1411). The study was performed according to the Declaration of Helsinki.

Study Design

The study design is presented in Fig. 1. The experimental approach was composed of three clearly defined phases: (i) Discovery phase: from the initial population of 525 participants with suspected OSA, 27 subjects with confirmed OSA were randomly selected. Subsequently, 26 controls without OSA from the same study population were selected using nearest neighbor propensity score matching by age, sex and body mass index (BMI). miRNA sequencing was conducted on these 53 subjects. The number of samples aligns with the recommendations for transcriptomic profiling studies.[30] (ii) Technical validation phase: to confirm the reliability of the miRNA sequencing findings, a rigorous assessment of miRNA candidates was conducted using RT-qPCR, which is acknowledged as the gold standard for circulating miRNA quantification. This technical validation was performed on the same set of samples employed in the discovery phase ($n = 53$). (iii) Comprehensive evaluation: miRNA candidates that successfully passed the technical validation phase were further evaluated in the entire study population, excluding samples utilized in the previous steps ($n = 472$).

Plasma microRNA Profiling

Total RNA was extracted from 200 μL of frozen plasma utilizing the miRNeasy Serum/Plasma Advanced Kit (Qiagen, Hilden, Germany). To establish a normalization strategy, synthetic *Caenorhabditis elegans* miR-39-3p (cel-miR-39-3p) (Qiagen) was introduced as an external reference miRNA at a concentration of 1.6×10^8 copies/μL. Additionally, 1 μg of MS2 carrier RNA (Roche Diagnostics, Mannheim, Germany) was added to the mixture to increase the yield of extracellular miRNAs. Quality control for RNA isolation was assured by the inclusion of the RNA Spike-In Kit (synthetic UniSp2, UniSp4, and UniSp5) (Qiagen). All reagents were spiked into samples during RNA isolation after incubation with the denaturing solution. The isolated RNA was then eluted in 20 μL of RNAse-free water and stored at −80 °C.

For miRNA quantification, the miRCURY LNA Universal RT microRNA PCR System (Qiagen) protocol was followed. Reverse transcription (RT) to synthesize complementary DNA (cDNA) was performed using a miRCURY LNA RT Kit (Qiagen) in a total reaction volume of 10 μL. The spike-in UniSp6 (Qiagen) was added to monitor the RT reaction. The RT conditions included incubation at 42 °C for 60 min, inactivation at 95 °C for 5 min and immediate cooling at 4 °C. Subsequently, the cDNA was stored at −20 °C. We used the miRCURY LNA miRNA Custom Panels (384-well plates) (Qiagen), which contained the selected miRNAs and spike-in primers (Qiagen). A QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) was used for qPCR in a 10 μL reaction volume. The RT-qPCR conditions included an initial incubation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 56 °C for 1 min and a final melting curve analysis. Synthetic UniSp3 served as an interplate calibrator and qPCR control. Amplification curves were assessed by melting curve analysis using QuantStudio Software v1.3 (Thermo Fisher Scientific, MA, USA), ensuring the presence of single products and the absence of primer dimers.

The quantification cycle (Cq) was determined as the fractional cycle number at which the fluorescence surpassed a defined threshold. Cq values of spike-in RNA templates were initially scrutinized to monitor the homogenous efficiencies of RNA extraction procedures, the robustness of RT and the absence of PCR inhibitors. The ΔCq ratio (miR-23a-3p–miR-451a), as proposed by Blondal et al.,[31] was used to exclude hemolyzed samples. Cq values exceeding 35 cycles were considered undetectable and censored at the minimum level observed for each miRNA. miRNAs detected below the limit of detection in more than 80% of the samples were categorized as nonexpressed. Samples that did not pass the quality control test were excluded from subsequent statistical analysis. Relative quantification was accomplished using the 2^{-Cq} method ($\Delta Cq = Cq_{miRNA} - Cq_{cel\text{-}miR\text{-}39\text{-}3p}$). The expression levels were log-transformed for subsequent statistical analysis.

Functional Assessment

We retained the predicted miRNA–target interactions utilizing the web-based TargetScan (Release 8.0, https://www.targetscan.org/vert_80/).[32] The search settings were set to all predicted targets, regardless of conservation. To elucidate the biological relevance and pathways associated with the identified miRNA targets, we conducted a functional enrichment analysis using the R package *ClusterProfiler* 4.6.2.[33] *ClusterProfiler* is released through the Bioconductor project and can be accessed via https://bioconductor.org/packages/clusterProfiler/. The Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO) and Reactome were used as reference databases. To further explore the expression patterns of selected miRNA targets, tissue patterns from the Genotype-Tissue Expression (GTEx) Portal (https://www.gtexportal.org/home/) project v.8 were used. To explore potential therapeutic interventions, we investigated the repositioning potential of upregulated miRNA targets using the Drug–Gene Interaction database (DGIdb v.5.0.5) (https://dgidb.org).[34]

Statistical Analysis

All the statistical analyses were performed using R version 4.2.2 (R Foundation for Statistical Computing). Descriptive statistics were employed to explore the characteristics of the study population. Continuous variables were compared between groups using the Mann–Whitney *U* test, whereas categorical variables were compared using Fisher's exact test. The data are presented as frequencies (percentages) for categorical variables and as medians (25th and 75th percentiles) for continuous variables. Spearman correlation was utilized to estimate the correlation between continuous variables. A correlation coefficient (rho) greater than 0.3 was considered as biologically relevant.[35] The *p*-value threshold defining statistical significance was set at <0.05.

Three distinct ML-based feature selection methods were implemented simultaneously: Random forest with Boruta,[36] variable selection using random forests (VSURF)[37] and sparse partial least squares (sPLS).[38] To ensure the robustness and consistency of our selection process, we repeated each method 50 times. In each itera-

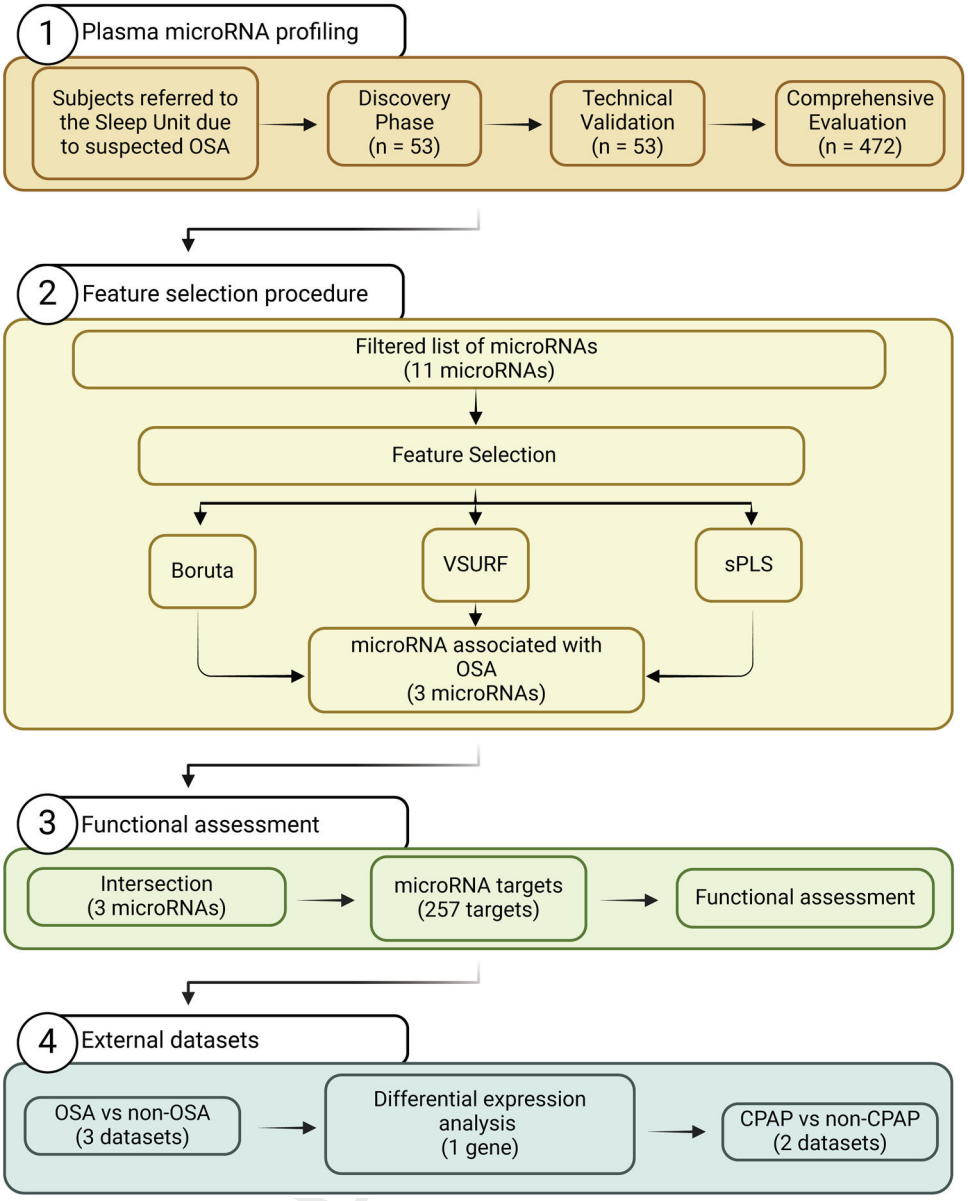


Fig. 1. Study workflow. Figure created with BioRender.com (https://app.biorender.com/), license number: TH26UP0M73. *Abbreviations*: CPAP: continuous positive airway pressure; OSA: obstructive sleep apnea.

tion, miRNAs selected in at least one run were considered important variables. Finally, miRNAs consistently identified across all three methods were designated as candidates for further investigation through bioinformatics analysis, as previously described.[39]

For the external datasets, we obtained the preprocessed gene expression data and normalized them using quantile normalization. The raw data were processed and normalized using methods implemented in the *Limma* R package[40] for the Illumina and Agilent microarrays and the *oligo* R package[41] for the Affymetrix chips. Subsequently, we applied a base 2 logarithmic scale transformation. Following data normalization, we averaged the expression data from multiple probes associated with the same gene. An extensive outlier analysis was performed to assess the plausibility of extreme values. After thorough evaluation, outliers were excluded from further analysis. Differential expression analysis was performed with processed data between groups of each microarray dataset using the *Limma* R package. For the longitudinal analysis, we used a paired-samples design to account for interindividual differences with respect to the pretreatment state. For each comparison, we utilized the *Limma* moderated *t*-test (implemented with the "lmFit" and "eBayes" functions) to calculate differential expression for each gene.

Results

Selection of microRNA Candidates

miRNA sequencing was conducted in specific matched subgroups of subjects with and without confirmed OSA ($n = 53$). The main characteristics of the study groups are detailed in Table 1. miRNA candidates were chosen based on the following criteria: over the 90th decile of expression (to ensure their detection in the following phases), $\geq$1.3-fold differential expression and p-value ≤ 0.2 (to identify a broader range of potential biomarkers). A total of 38 miRNAs fulfilled these criteria, as depicted in Fig. 2A.

To validate the miRNA sequencing results, the selected candidates were further examined through RT-qPCR using the same sample set ($n = 53$). Eight samples were excluded from further anal-

Table 1
Clinical Characteristics of the Study Population at Baseline (Discovery and Technical Validation Phases).

	All $n = 53$	Non-OSA $n = 26$	OSA $n = 27$	*p*-Value	*n*
Clinical data					
Demographic/anthropometric					
Age, years	59.0 [52.0;65.0]	57.0 [52.5;60.0]	61.0 [53.0;67.0]	0.200	53
Sex, female	22 (41.5%)	11 (42.3%)	11 (40.7%)	1.000	53
BMI, kg/m^2	24.0 [12.0;37.0]	20.0 [10.2;36.2]	27.0 [15.5;38.0]	0.262	53
Polysomnographic data					
Respiratory disturbances					
AHI, events/h	15.1 [10.1;39.9]	9.75 [3.01;12.6]	39.9 [33.1;56.3]	**<0.001**	53

Data are presented as the median [25th;75th percentile] for continuous variables and as frequencies (percentage) for categorical variables. *p*-Values < 0.05 are presented in bold. AHI = apnea–hypopnea index; BMI = body mass index; OSA = obstructive sleep apnea.

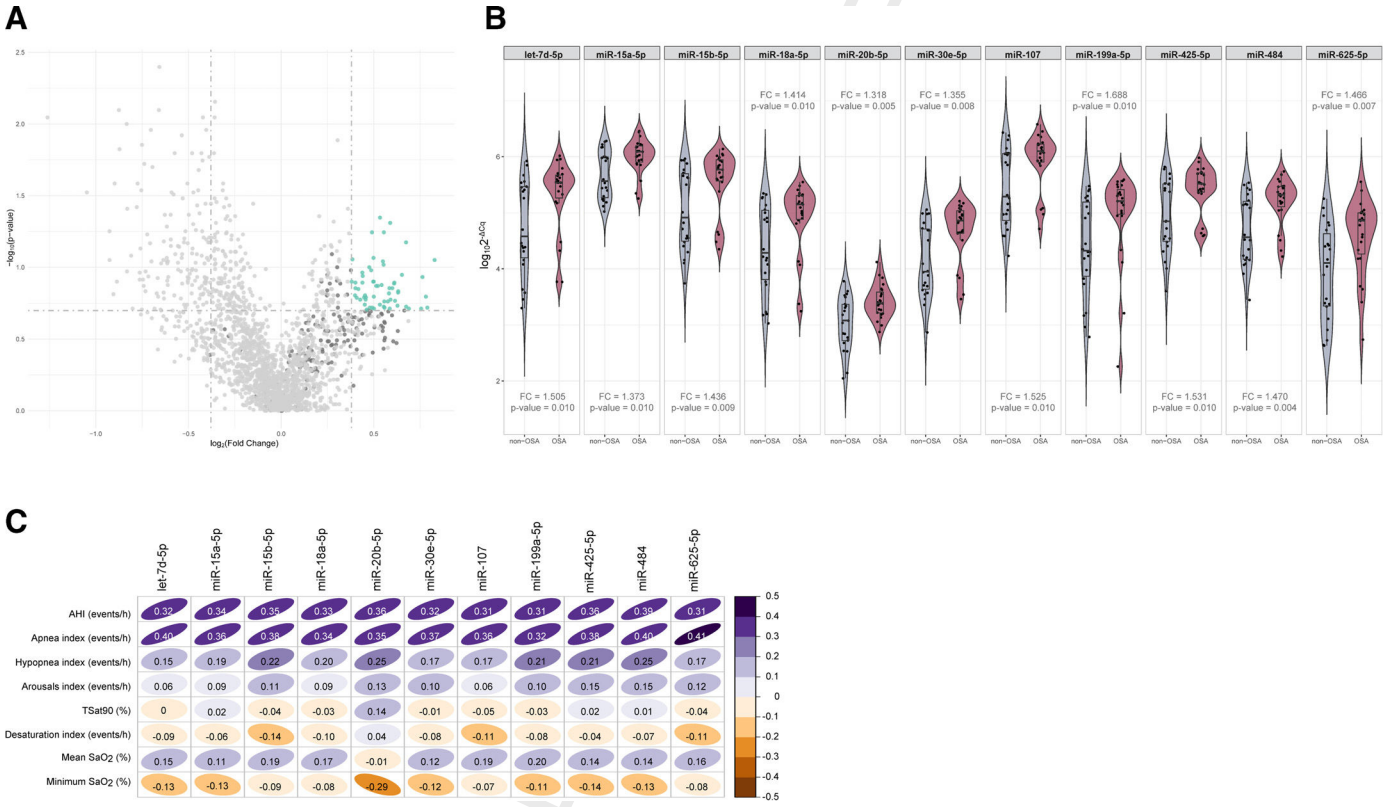


Fig. 2. Identification of microRNAs related to obstructive sleep apnea. (A) Volcano plot showing the *p*-value versus the fold change for the microRNAs analyzed in the discovery phase. microRNAs over the 90th decile of expression are depicted in dark gray. The green dots indicate microRNA candidates. (B) Violin plots including microRNAs that fulfilled the selection criteria in the technical validation phase. Plots depict $\log_{10}2^{-\Delta Cq}$ as the microRNA expression unit. The gray color indicates the nonobstructive sleep apnea group, and the red color indicates the obstructive sleep apnea group. The plot presents the median (25th and 75th percentiles) estimator of the density (as density curves) and individual values (black dots). Fold changes and *p*-values are displayed. (C) Correlogram showing spearman correlation coefficients between polysomnographic parameters and the microRNAs that fulfilled the selection criteria in the technical validation phase. The color scale illustrates the degree of correlation and ranges, indicating the negative to positive correlations. *Abbreviations*: AHI: apnea–hypopnea index; OSA: obstructive sleep apnea; SaO_2: oxygen saturation; TSat90: time during the night with SaO_2 below 90%.

ysis due to failing the quality control test, which was attributed to the presence of hemolysis and/or low quality of the spike-ins. miR-6877-5p was expressed at low levels (Cq ≥ 35 in more than 80% of the samples) and was therefore excluded from further analysis. Among the remaining 37 miRNA candidates, those with a ≥1.3-fold difference between study groups, with a *p*-value ≤ 0.01 and that correlated with the AHI (rho > 0.3) were retained (Fig. 2B, C and Supplemental table* S1). Ultimately, 11 miRNAs—let-7d-5p, miR-15a-5p, miR-15b-5p, miR-18a-5p, miR-20b-5p, miR-30e-5p, miR-107, miR-199a-5p, miR-425-5p, miR-484 and miR-625-5p—were identified as candidates for the subsequent feature selection process. With the exception of the AHI and the apnea index, weak correlations were observed between the miRNA panel and the parameters obtained from PSG (Fig. 2C).

Feature Selection Procedure and Bioinformatics

Following the identification of miRNA candidates associated with OSA, quantification was performed using RT-qPCR in the entire study population. A total of 9.5% of the samples did not pass quality control due to hemolysis or variability in spike-ins and were discarded from further analysis. The detailed characteristics of the study population are presented in Table 2. As anticipated, patients with OSA were older, predominantly men, had a higher BMI and

Table 2
Characteristics of the Study Population (Comprehensive Evaluation).

	All $n = 427$	Non-OSA $n = 108$	OSA $n = 319$	p-Value	n
Clinical data					
Demographic/anthropometric					
Age, years	51.0 [45.0;57.0]	45.5 [39.0;53.2]	53.0 [47.0;58.5]	**<0.001**	427
Female	129 (30.2%)	51 (47.2%)	78 (24.5%)	**<0.001**	427
BMI, kg/m^2	30.9 [27.2;35.2]	27.8 [25.1;32.0]	32.0 [28.4;35.8]	**<0.001**	427
Smoking status				0.863	423
Never smoker	164 (38.8%)	44 (40.7%)	120 (38.1%)		
Current smoker	114 (27.0%)	29 (26.9%)	85 (27.0%)		
Former smoker	145 (34.3%)	35 (32.4%)	110 (34.9%)		
Comorbidities					
Hypertension	178 (41.7%)	26 (24.1%)	152 (47.6%)	**<0.001**	427
Cardiovascular disease	83 (19.7%)	13 (12.1%)	70 (22.2%)	**0.034**	422
Diabetes	58 (13.6%)	6 (5.56%)	52 (16.3%)	**0.008**	427
Dyslipidemia	135 (32.1%)	20 (18.7%)	115 (36.6%)	**0.001**	421
Medications use					
ACE inhibitors	92 (21.5%)	13 (12.0%)	79 (24.8%)	**0.008**	427
Beta-blockers	63 (14.8%)	7 (6.48%)	56 (17.6%)	**0.008**	427
Diuretic agents	65 (15.3%)	12 (11.2%)	53 (16.7%)	0.230	425
Calcium-channel blockers	35 (8.22%)	7 (6.48%)	28 (8.81%)	0.578	426
Angiotensin II receptor blockers	42 (9.86%)	9 (8.33%)	33 (10.4%)	0.668	426
Anticoagulants	14 (3.28%)	1 (0.93%)	13 (4.08%)	0.206	427
Insulin	22 (5.15%)	1 (0.93%)	21 (6.58%)	**0.041**	427
Lipid-lowering drugs	95 (22.3%)	15 (13.9%)	80 (25.2%)	**0.022**	426
Polysomnography data					
Respiratory disturbances					
AHI, events/h	31.6 [14.7;57.5]	8.34 [4.30;11.6]	43.5 [27.4;65.4]	**<0.001**	427
Hypopneas, events/h	17.4 [9.66;28.9]	6.96 [3.61;10.2]	22.4 [15.3;33.2]	**<0.001**	422
Nocturnal hypoxemia					
Mean SaO_2, %	93.0 [91.0;94.0]	94.0 [93.0;95.0]	92.0 [91.0;94.0]	**<0.001**	427
Minimum SaO_2, %	82.0 [73.0;87.0]	88.5 [85.0;91.0]	79.0 [71.0;84.0]	**<0.001**	421
Percentage of time spent with O_2 saturation below 90% (CT90), %	2.97 [0.30;14.1]	0.09 [0.00;0.66]	5.60 [1.60;21.1]	**<0.001**	425
Sleep fragmentation					
Arousal index, events/h	36.7 [24.6;58.8]	19.5 [13.9;26.4]	46.1 [32.6;65.5]	**<0.001**	422
Movement arousal index, events/h	2.80 [0.84;6.35]	4.18 [2.00;7.18]	2.30 [0.55;5.94]	**0.001**	420
Respiratory arousal index, events/h	19.5 [7.27;39.1]	4.88 [1.96;7.93]	25.7 [15.7;48.4]	**<0.001**	416
Sleep quality					
ESS	10.0 [7.00;14.0]	11.0 [7.00;14.0]	10.0 [6.00;14.2]	0.618	413

Data are presented as the median [25th;75th percentile] for quantitative variables and as frequencies (percentage) for qualitative variables. p-Values < 0.05 are presented in bold. Definitions of abbreviations: ACE = angiotensin-converting enzyme; AHI = apnea–hypopnea index; BMI = body mass index; ESS = Epworth sleepiness scale; OSA = obstructive sleep apnea; SaO_2 = oxygen saturation.

had a more unfavorable profile of comorbidities and disease-related sleep parameters.

Due to the intricate dynamics of miRNA networks, the circulating levels of miRNAs were entered into a supervised feature selection protocol that integrates three distinct ML-based methods (Fig. 3A). The outcome of each method resulted in a unique feature subset: (i) VSURF selected eight candidates: let-7d-5p, miR-15a-5p, miR-18a-5p, miR-30e-5p, miR-107, miR-199a-5p, miR-425-5p and miR-484; (ii) sPLS selected six candidates: let-7d-5p, miR-15a-5p, miR-15b-5p, miR-20b-5p, miR-107 and miR-625-5p; and (iii) Boruta selected all candidates. Notably, let-7d-5p, miR-15a-5p and miR-107 demonstrated robust and consistent selection across the three methods.

Target prediction for let-7d-5p, miR-15a-5p and miR-107 utilizing TargetScan v8.0 revealed a total of 3436 transcripts as potential targets (1993 targets for let-7d-5p, 1605 targets for miR-15a-5p and 101 targets for miR-107), with 257 targets shared by at least two of the selected miRNAs. To elucidate the underlying molecular pathways and biological processes associated with these identified target transcripts, an exhaustive enrichment analysis was conducted employing the KEGG, GO and Reactome databases. The analyses identified 9 KEGG pathways, 220 GO processes and 15 Reactome pathways (Fig. 3B–D and Supplemental Tables S2–S4). The predominant significant pathways and processes were closely linked to cellular events such as cell death, cell differentiation, extracellular remodeling, autophagy and metabolism.

External Datasets

To assess the robustness of our findings, we systematically validated the results by analyzing the expression of the target transcripts in external datasets obtained from GEO. This process was divided into two steps. Initially, we conducted a comparative examination of miRNA targets between individuals with and without OSA. Subsequently, we analyzed the influence of CPAP therapy on the identified miRNA targets.

First, three distinct external datasets, including OSA patients and non-OSA subjects, were evaluated (GSE226379, GSE135917 and GSE75097). Among the targetomes of let-7d-5p, miR-15a-5p and miR-107, one gene, the transcription factor Dp-2 (*TFDP2*), which is a target of let-7d-5p and miR-15a-5p, exhibited significant differential expression in the three comparisons (fold change ≥ 1.5 and p-value ≤ 0.05) (Fig. 4A–C). To further elucidate the expression profile of the miRNA targets, we retrieved data from the GTEx project, which revealed that *TFDP2* is expressed in most human tissues (Fig. 4D).

To explore the impact of the current therapeutic strategy, the gene expression levels of *TFDP2* were evaluated in two independent external datasets (GSE133601 and GSE49800), including samples from patients with OSA collected before and after CPAP therapy. No alterations in the transcript levels were observed following therapy (fold change ≥ 1.5 and p-value ≤ 0.05) (Fig. 4E and F). Next, we evaluated the repositioning potential of the

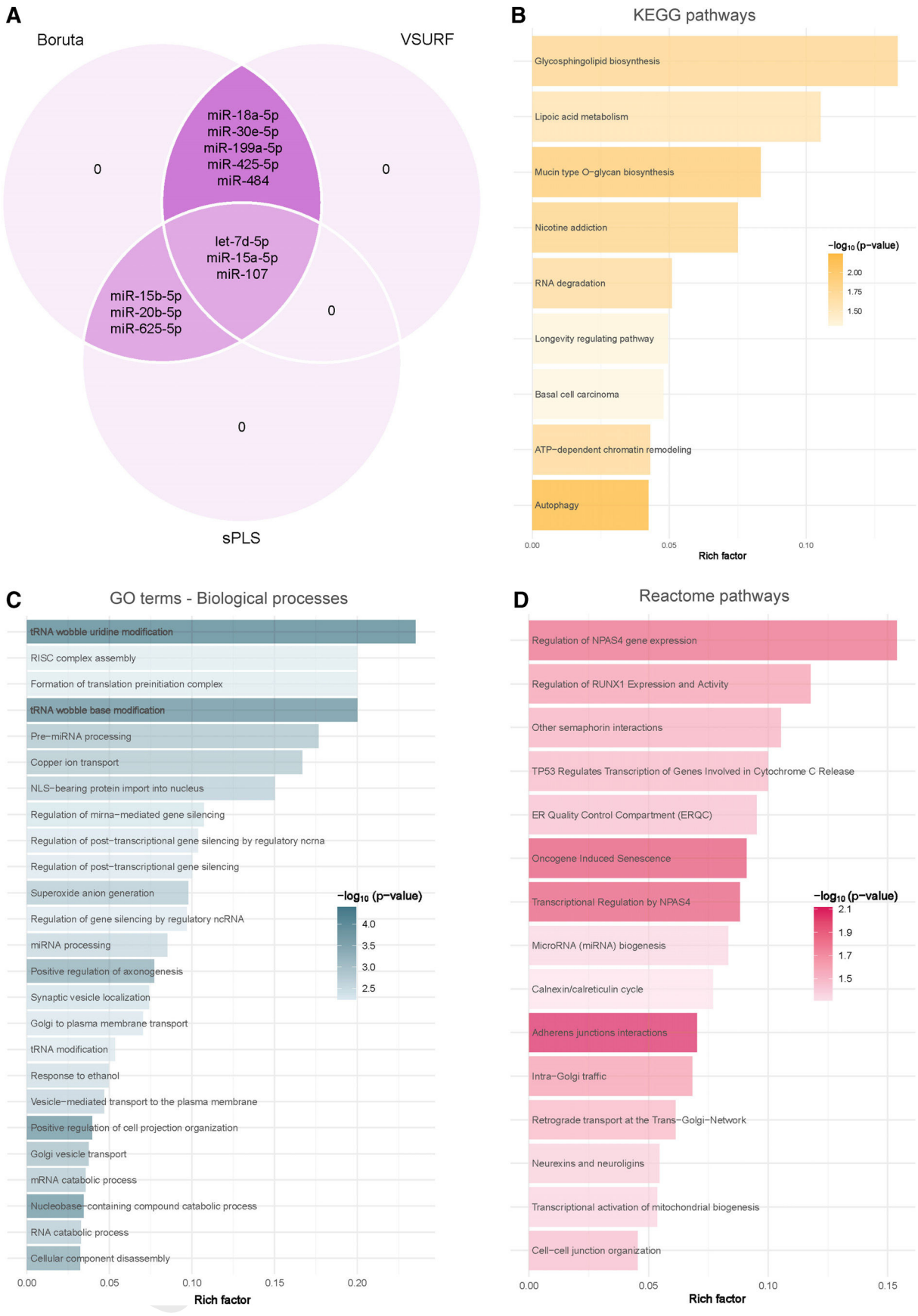


Fig. 3. Identification of microRNA-mediated molecular mechanisms associated with obstructive sleep apnea. (A) Identification of microRNAs associated with obstructive sleep apnea using a consensus of three supervised machine learning feature selection algorithms (Boruta, VSURF and sPLS) with the intersected microRNAs among the methods selected as candidates. (B–D) Functional enrichment analysis of the predicted downstream target genes using KEGG (B), GO (C) and Reactome (D) in the R package ClusterProfileR 4.0. The plots present the rich factors of the pathways with the most significant differences, considering their *p*-values. The intensity of the colors of the bars denotes the *p*-values. The rich factor consists of a ratio of target genes annotated in the molecular processes to all genes annotated in the processes.

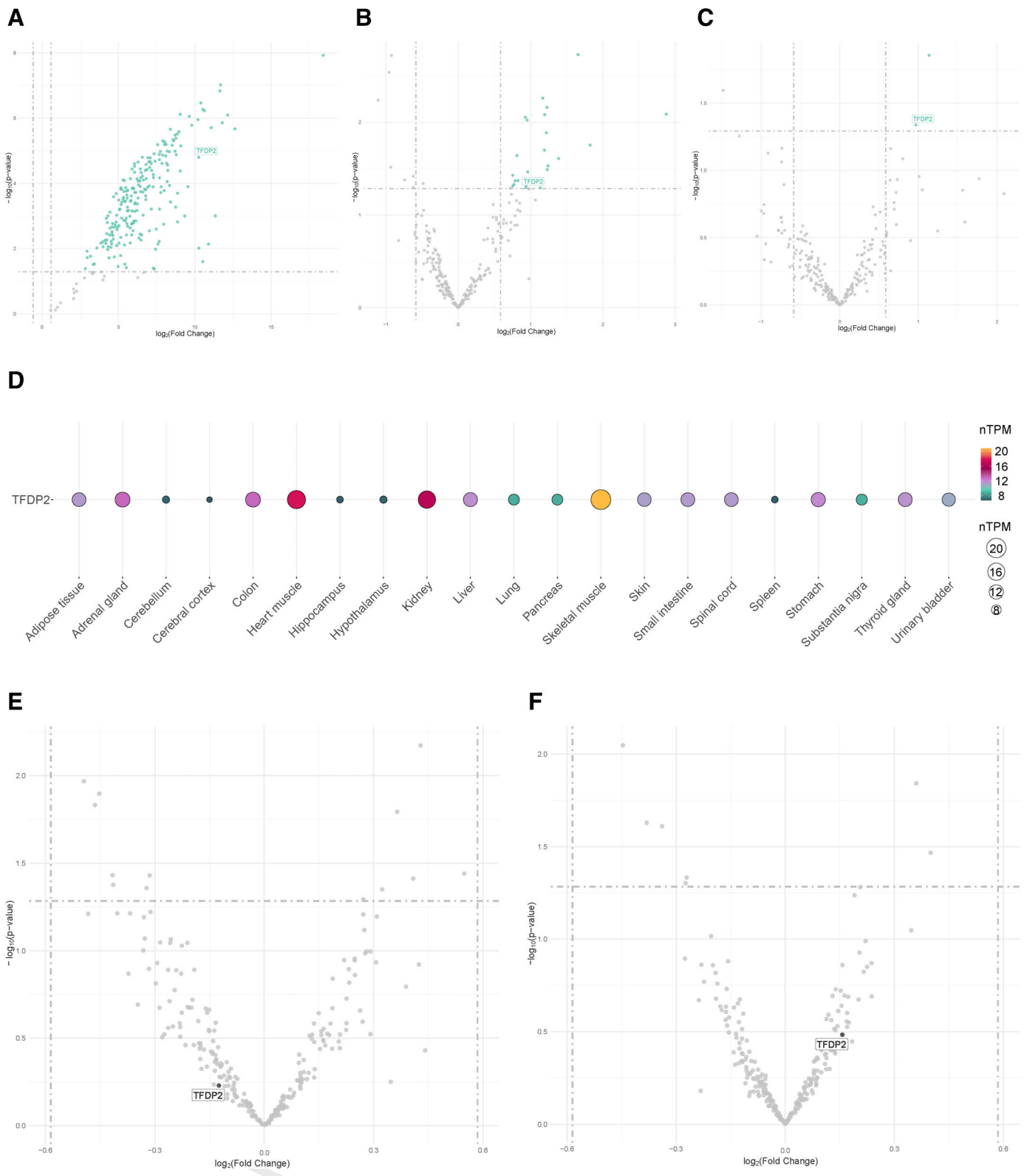


Fig. 4. External datasets. (A–C) Volcano plots showing the *p*-value versus the fold change for the predicted targetome in three external RNA sequencing datasets obtained from the GEO database from patients with obstructive sleep apnea. Significantly differentially expressed genes are presented in green (fold change > 1.5 and *p*-value ≤ 0.05). Intersected genes among the datasets are labeled. (A) Plasma, GSE226379; (B) subcutaneous fat, GSE135917; and (C) peripheral blood mononuclear cells (PBMCs), GSE75097. (D) Tissue enrichment analysis using GTEx (https://www.gtexportal.org/home/). Each column denotes a tissue type, and the row represents the intersected genes among the external datasets. The size and color of the points reflect the expression level of the gene, represented as normalized transcripts per million (nTPM) values. The GTEx Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health and by the NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. (E, F) Volcano plots showing the *p*-value versus the fold change for the predicted targetome in two external RNA sequencing datasets, including obstructive sleep apnea patients allocated to CPAP treatment, obtained from the GEO database. Intersected genes among the A–C datasets are labeled. (E) PBMCs, GSE133601; and (F) peripheral blood leukocytes, GSE49800.

miRNA target using DGIdb. No FDA-approved drugs were identified.

Discussion

Recognizing and characterizing the diverse pathogenetic pathways of OSA are pivotal steps toward improving patient outcomes. In this study, we implemented a complete approach that synergistically integrates plasma miRNA profiling with ML feature selection techniques and bioinformatic analyses to elucidate the mechanistic pathways associated with OSA and to propose innovative disease-modifying agents.

Through the combination of a high-throughput technique, i.e., sequencing, and the gold-standard methodology, i.e., RT-qPCR, we identified a set of 11 plasma miRNAs potentially linked to OSA. miRNAs play a significant role in finely regulating gene expression through their multifaceted interactions. A comprehensive understanding of the dynamics of miRNA-mediated regulation is crucial for deciphering their impact on cellular function and molecular pathways. ML methodologies capture complex and nonlinear relationships that may remain elusive using conventional univariable analyses.[42] Consequently, the candidates quantified in the whole population were subjected to a ML feature selection process to define the most informative miRNAs. A combination of three distinct feature selection methods was employed to limit the number of selected miRNAs, thereby enhancing confidence and reproducibility. Our approach revealed three relevant miRNAs: let-7d-5p, miR-15a-5p and miR-107. The use of ML-based feature selection algorithms represents an innovative approach in OSA research, limiting direct comparisons with previous findings obtained using classical statistical approaches.[43] Nevertheless, our candidates have been previously associated with OSA. Serum levels of miR-107 have been shown to be significantly altered in OSA patients compared with non-OSA controls.[44] More recently, a study including individuals referred to the otorhinolaryngology service due to snoring or apnea revealed that both let-7d-5p and miR-107 were differentially expressed in nonhypertensive OSA and hypertensive OSA patients compared with controls, respectively.[45] Furthermore, Santamaria-Martos et al.[46] demonstrated the association of miR-107 with OSA severity parameters such as the AHI and arousal index. Consequently, earlier investigations provide evidence that the miRNAs identified here may be associated with OSA, further validating our ML approach.

In the next step, we used predicted miRNA interactions and enrichment analyses to provide novel insights into OSA pathobiology. Our analysis defined an array of specific mechanisms associated with the disease. Using three independent public datasets generated from different tissues, i.e., plasma, peripheral blood mononuclear cells (PBMCs) and subcutaneous fat, our approach identified *TFDP2* as a potential contributor to OSA pathology. *TFDP2*, a member of the DP transcription factor family, forms heterodimers with E2F transcription factors, facilitating the transcriptional activation of genes critical for cell proliferation, differentiation and programmed cell death. For example, loss of *TFDP2* disrupts the normal downregulation of E2F2 target genes, which are essential for regulating the cell cycle. This disruption leads to an accumulation of cells in the S phase and an increase in erythrocyte size.[47] Importantly, prior research has established *TFDP2* as a target of specific miRNAs. Bone marrow mesenchymal stem cells alleviate renal injury and fibrosis by modulating the miR-146a-5p/*TFDP2* axis in mouse renal tubular epithelial cells.[48] Furthermore, E2F transcription factors have been recognized as critical mediators of apoptosis across multiple experimental models and conditions.[49,50] Although no existing literature directly associates *TFDP2* with pathogenic mechanisms in OSA, the biological role of this mediator may explain our findings. Apoptosis, a cellular process essential for maintaining cellular homeostasis and survival, is a well-described pathogenetic mechanism in the context of OSA. Intermittent hypoxia, a hallmark of OSA, has been implicated in the generation of reactive oxygen species (ROS), which trigger endoplasmic reticulum stress and inhibit the synthesis of functional proteins, ultimately promoting apoptotic responses.[51] In individuals diagnosed with OSA, the degree of impairment in endothelial-dependent vasodilation correlates with the extent of endothelial cell apoptosis.[52] Additionally, reduced apoptosis and increased expression of selectin adhesion molecules have been observed in the polymorphonuclear leukocytes of patients with OSA, suggesting potential effects of the condition on inflammatory and immunological changes in blood leukocytes.[53]

More accurate pathway detection, achieved through the integration of miRNAs, may facilitate the discovery and development of novel drug targets aligned with pathogenetic mechanisms. Understanding the interplay between the mechanistic pathways and OSA could offer crucial insights for designing adjuvant therapeutic strategies to address the cellular responses to the physiological challenges associated with this medical condition. Nevertheless, our analyses of drug-gene interactions did not reveal any FDA-approved drugs for *TFDP2*. Furthermore, examination of external datasets suggested that CPAP treatment had no observable impact on the levels of miRNA targets. Therefore, the potential of miRNA-based therapeutics as novel candidates for treatment is particularly relevant considering not only the limited repositioning potential identified in our study but also the promising advances in the use of this technology.[13] The regulatory effects of miRNAs on multiple genes of underlying pathways may be advantageous in addressing the multifactorial nature of conditions such as OSA.[54] Additional investigations based on current data are therefore warranted.

Strengths and Limitations

The current investigation has several strengths, including the adoption of a "real clinical context", multicentric design, comprehensive clinical assessments, the analysis of the plasma miRNome and the employment of diverse feature selection algorithms. However, it is imperative to acknowledge the inherent limitations of this study. Although the discovery phase included a subset of 53 subjects, which may appear limited, this sample size is relatively large compared to typical RNA sequencing studies. Nevertheless, we acknowledge that this limitation may affect the robustness of our findings. To further investigate the relevance of our results, we utilized different external datasets. However, validation in additional cohorts with more diverse demographic and clinical characteristics and an appropriate sample size to ensure adequate statistical power is essential. A significant limitation of our study is the lack of mechanistic investigations to confirm the biological roles of the identified miRNAs and their target genes in the pathogenesis of OSA. While we have established associations between specific miRNAs and OSA using various datasets, experimental studies are vital to elucidate the causal relationships and underlying signaling pathways influenced by these miRNAs. Finally, the criteria for selecting candidate miRNAs in the discovery and technical validation phases were intentionally permissive. This approach was chosen for two reasons. First, the inherent biology of miRNAs allows them to regulate biological responses even with minor fluctuations in their levels,[26] which justifies the use of lower fold-change thresholds. Second, the primary goal of these phases was to "feed" the feature selection analysis rather than to identify individual miRNAs specifically associated with OSA.

Conclusions

Our ML feature selection approach identified three plasma miRNAs linked to OSA that, along with their target genes, offer new insights into the underlying pathogenesis of OSA and reveal novel regulatory elements of disease and potential drug targets. These findings hold promise for advancing the development of targeted disease-modifying agents aimed at improving outcomes.

Authors' Contribution

All authors were involved in the conception and design of the work, the acquisition, analysis and interpretation of the data, as well as in the drafting and critical revision of the work. All authors approved the final version of the manuscript.

Artificial Intelligence Involvement

The authors declare non artificial intelligence involvement.

Funding Statement

This study was funded by the Instituto de Salud Carlos III (ISCIII) through the projects "PI20/00577" and "PI22/00636" and cofunded by the European Union. DdGC has received financial support from Instituto de Salud Carlos III (Miguel Servet 2020: CP20/00041) cofunded by European Union. FB is supported by the ICREA Academia Program. MCGH held a predoctoral fellowship, "Ayudas al Personal Investigador en Formación", from IRBLleida/Diputación de Lleida. MM is the recipient of a predoctoral fellowship (PFIS 2021: FI21/00187) from Instituto de Salud Carlos III and cofunded by the European Union. MSdlT has received financial support from the "Ramón y Cajal" grant (RYC2019-027831-I) from the "Ministerio de Ciencia e Innovación—Agencia Estatal de Investigación" cofunded by the European Social Fund (ESF)/"Investing in your future". CIBERES (CB07/06/2008) is an initiative of the Instituto de Salud Carlos III.

Conflicts of Interests

The authors declare no competing interests.

Data Availability

HTG EdgeSeq miRNA Whole Transcriptome Assay data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE229362 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE229362).

Acknowledgments

We thank the individuals who participated in this study, their families, and the clinical and research team in the sleep unit. Human sample manipulation was performed at the Cell Culture Facility, Universitat de Lleida (Lleida, Catalonia, Spain). Work supported by IRBLleida Biobank (B.000682) and Biobank and Biomodels Platform ISCIII PT23/00032.

Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.arbres.2024.11.011.

References

1. Sánchez-de-la-Torre M, Campos-Rodriguez F, Barbé F. Obstructive sleep apnoea and cardiovascular disease. Lancet Respir Med. 2013;1:61–72.
2. Hirotsu C, Haba-Rubio J, Togeiro SM, Marques-Vidal P, Drager LF, Vollenweider P, et al. Obstructive sleep apnoea as a risk factor for incident metabolic syndrome: a joined Episono and HypnoLaus prospective cohorts study. Eur Respir J. 2018;52:1801150.
3. Zinchuk AV, Jeon S, Koo BB, Yan X, Bravata DM, Qin L, et al. Polysomnographic phenotypes and their cardiovascular implications in obstructive sleep apnoea. Thorax. 2018;73:472–80.
4. Zinchuk AV, Gentry MJ, Concato J, Yaggi HK. Phenotypes in obstructive sleep apnea: a definition, examples and evolution of approaches. Sleep Med Rev. 2017;35:113–23.
5. Gasa M, Tamisier R, Launois SH, Sapene M, Martin F, Stach B, et al. Residual sleepiness in sleep apnea patients treated by continuous positive airway pressure. J Sleep Res. 2013;22:389–97.
6. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science. 2007;318:1931–4.
7. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120:15–20.
8. Benincasa G, DeMeo DL, Glass K, Silverman EK, Napoli C. Epigenetics and pulmonary diseases in the horizon of precision medicine: a review. Eur Respir J. 2021;57, 2003406.
9. Shang F, Wang SC, Gongol B, Han SY, Cho Y, Schiavon CR, et al. Obstructive sleep apnea-induced endothelial dysfunction is mediated by miR-210. Am J Respir Crit Care Med. 2023;207:323–35.
10. Chen YC, Hsu PY, Su MC, Chen TW, Hsiao CC, Chin CH, et al. Microrna sequencing analysis in obstructive sleep apnea and depression: anti-oxidant and maoa-inhibiting effects of mir-15b-5p and mir-92b-3p through targeting ptgs1-nf-κb-sp1 signaling. Antioxidants. 2021;10:1854.
11. Foinquinos A, Batkai S, Genschel C, Viereck J, Rump S, Gyöngyösi M, et al. Preclinical development of a miR-132 inhibitor for heart failure treatment. Nat Commun. 2020;11:633.
12. Dragomir MP, Fuentes-Mattei E, Winkle M, Okubo K, Bayraktar R, Knutsen E, et al. Anti-miR-93-5p therapy prolongs sepsis survival by restoring the peripheral immune response. J Clin Invest. 2023;133, e158348.
13. Janssen HLA, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med. 2013;368:1685–94.
14. Trang P, Medina PP, Wiggins JF, Ruffino L, Kelnar K, Omotola M, et al. Regression of murine lung tumors by the let-7 microRNA. Oncogene. 2010;29:1580.
15. Wiggins JF, Ruffino L, Kelnar K, Omotola M, Patrawala L, Brown D, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer Res. 2010;70:5923–30.
16. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654–9.
17. Anfossi S, Babayan A, Pantel K, Calin GA. Clinical utility of circulating non-coding RNAs – an update. Nat Rev Clin Oncol. 2018;15:541–63.
18. Rossi-Herring G, Belmonte T, Rivas-Urbina A, Benítez S, Rotllan N, Crespo J, et al. Circulating lipoprotein-carried miRNome analysis reveals novel VLDL-enriched microRNAs that strongly correlate with the HDL-microRNA profile. Biomed Pharmacother. 2023:162.
19. Bär C, Thum T, de Gonzalo-Calvo D. Circulating miRNAs as mediators in cell-to-cell communication. Epigenomics. 2019;11:111–3.
20. Castaño C, Mirasierra M, Vallejo M, Novials A, Párrizas M. Delivery of muscle-derived exosomal miRNAs induced by HIIT improves insulin sensitivity through down-regulation of hepatic FoxO1 in mice. Proc Natl Acad Sci USA. 2020;117:30335–43.
21. Sánchez-Ceinos J, Rangel-Zuñiga OA, Clemente-Postigo M, Podadera-Herreros A, Camargo A, Alcalá-Diaz JF, et al. miR-223-3p as a potential biomarker and player for adipose tissue dysfunction preceding type 2 diabetes onset. Mol Ther Nucleic Acids. 2021;23:1035–52.
22. Ying W, Gao H, Dos Reis FCG, Bandyopadhyay G, Ofrecio JM, Luo Z, et al. MiR-690, an exosomal-derived miRNA from M2-polarized macrophages, improves insulin sensitivity in obese mice. Cell Metab. 2021;33:781–90, e5.
23. Gysi DM, Barabási AL. Noncoding RNAs improve the predictive power of network medicine. Proc Natl Acad Sci USA. 2023;120, e2301342120.
24. García-Hidalgo MC, González J, Benítez ID, Carmona P, Santisteve S, Pérez-Pons M, et al. Identification of circulating microRNA profiles associated with pulmonary function and radiologic features in survivors of SARS-CoV-2-induced ARDS. Emerg Microbes Infect. 2022;11:1537–49.
25. de Gonzalo-Calvo D, Karaduzovic-Hadziabdic K, Dalgaard LT, Dieterich C, Perez-Pons M, Hatzigeorgiou A, et al. Machine learning for catalysing the integration of noncoding RNA in research and clinical practice. EBioMedicine. 2024;106. Q2
26. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature. 2011;469:336–42.
27. Lloberes P, Durán-Cantolla J, Martínez-García MÁ, Marín JM, Ferrer A, Corral J, et al. Diagnosis and treatment of sleep apnea–hypopnea syndrome. Spanish Society of Pulmonology and Thoracic Surgery. Arch Bronconeumol. 2011;47:143–56.
28. Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea:

an American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med. 2017;13:479–504.
29. Mediano O, González Mangado N, Montserrat JM, Alonso-Álvarez ML, Almendros I, Alonso-Fernández A, et al. International consensus document on obstructive sleep apnea. Arch Bronconeumol. 2022;58:52–68.
30. Schurch NJ, Schofield P, Gierliński M, Cole C, Sherstnev A, Singh V, et al. How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? RNA. 2016;22:839–51.
31. Blondal T, Jensby Nielsen S, Baker A, Andreasen D, Mouritzen P, Wrang Teilum M, et al. Assessing sample and miRNA profile quality in serum and plasma or other biofluids. Methods. 2013;59:S1–6.
32. McGeary SE, Lin KS, Shi CY, Pham TM, Bisaria N, Kelley GM, et al. The biochemical basis of microRNA targeting efficacy. Science. 2019;366:eaav1741.
33. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation (Cambridge (Mass)). 2021;2:100141.
34. Cannon M, Stevenson J, Stahl K, Basu R, Coffman A, Kiwala S, et al. DGIdb 5.0: rebuilding the drug-gene interaction database for precision medicine and drug discovery platforms. Nucleic Acids Res. 2024;52:D1227–35.
35. Mukaka MM. A guide to appropriate use of Correlation coefficient in medical research. Malawi Med J. 2012;24:69.
36. Kursa MB, Rudnicki WR. Feature selection with the Boruta package. J Stat Softw. 2010;36:1–13.
37. Genuer R, Poggi JM, Tuleau-Malot C. VSURF: an R package for variable selection using random forests. R J. 2015;7:19–33.
38. Lê Cao KA, Rossouw D, Robert-Granié C, Besse P. A sparse PLS for variable selection when integrating omics data. Stat Appl Genet Mol Biol. 2008;7. Article 35.
39. García-Hidalgo MC, Benítez ID, Perez-Pons M, Molinero M, Belmonte T, Rodríguez-Muñoz C, et al. MicroRNA-guided drug discovery for mitigating persistent pulmonary complications in critical COVID-19 survivors: a longitudinal pilot study. Br J Pharmacol. 2024.
40. 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:e47.
41. Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. Bioinformatics. 2010;26:2363.
42. de Gonzalo-Calvo D, Martínez-Camblor P, Bär C, Duarte K, Girerd N, Fellström B, et al. Improved cardiovascular risk prediction in patients with end-stage renal disease on hemodialysis using machine learning modeling and circulating microribonucleic acids. Theranostics. 2020;10:8665–76.
43. Pinilla L, Barbé F, de Gonzalo-Calvo D. MicroRNAs to guide medical decision-making in obstructive sleep apnea: a review. Sleep Med Rev. 2021;59:101458.
44. Li K, Wei P, Qin Y, Wei Y. MicroRNA expression profiling and bioinformatics analysis of dysregulated microRNAs in obstructive sleep apnea patients. Medicine (Baltimore). 2017;96:e7917.
45. Yang X, Niu X, Xiao Y, Lin K, Chen X. MiRNA expression profiles in healthy OSAHS and OSAHS with arterial hypertension: potential diagnostic and early warning markers. Respir Res. 2018;19:194.
46. Santamaria-Martos F, Benítez I, Ortega F, Zapater A, Giron C, Pinilla L, et al. Circulating microRNA profile as a potential biomarker for obstructive sleep apnea diagnosis. Sci Rep. 2019;9:13456.
47. Chen C, Lodish HF. Global analysis of induced transcription factors and cofactors identifies Tfdp2 as an essential coregulator during terminal erythropoiesis. Exp Hematol. 2014;42:464–76, e5.
48. Wu L, Rong C, Zhou Q, Zhao X, Zhuansun XM, Wan S, et al. Bone marrow mesenchymal stem cells ameliorate cisplatin-induced renal fibrosis via miR-146a-5p/Tfdp2 axis in renal tubular epithelial cells. Front Immunol. 2021:11.
49. Chen D, Chen Y, Forrest D, Bremner R. E2f2 induces cone photoreceptor apoptosis independent of E2f1 and E2f3. Cell Death Differ. 2013;20:931–40.
50. Mustafa N, Mitxelena J, Infante A, Zenarruzabeitia O, Eriz A, Iglesias-ara A, et al. E2f2 attenuates apoptosis of activated T lymphocytes and protects from immune-mediated injury through repression of Fas and FasL. Int J Mol Sci. 2021;23.
51. Lv R, Liu X, Zhang Y, Dong N, Wang X, He Y, et al. Pathophysiological mechanisms and therapeutic approaches in obstructive sleep apnea syndrome. Signal Transduct Target Ther. 2023;8:218.
52. El Solh AA, Akinnusi ME, Baddoura FH, Mankowski CR. Endothelial cell apoptosis in obstructive sleep apnea: a link to endothelial dysfunction. Am J Respir Crit Care Med. 2007;175:1186–91.
53. Dyugovskaya L, Polyakov A, Lavie P, Lavie L. Delayed neutrophil apoptosis in patients with sleep apnea. Am J Respir Crit Care Med. 2008;177:544–54.
54. Nemeth K, Bayraktar R, Ferracin M, Calin GA. Non-coding RNAs in disease: from mechanisms to therapeutics. Nat Rev Genet. 2024;25:211–32.