European Review for Medical and Pharmacological Sciences 2015; 19: 4293-4299

Expression data analysis to identify key target genes in visceral fat tissue associated with obstructive sleep apnea

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Abstract. – OBJECTIVE: The purpose of this study was to screen key genes related to mechanisms and consequences of obstructive sleep apnea (OSA)-induced perturbations in visceral fat tissue depots.

MATERIALS AND METHODS: Microarray data of GSE38792, comprising 10 visceral fat samples from OSA patients and 8 visceral fat samples from control subjects, was obtained from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were identified in visceral fat samples from OSA patients compared with controls using Bioconductor package limma. Gene Ontology (GO) and pathway enrichment analyses were carried out to identify significantly altered biological functions. Furthermore, a protein-protein interaction (PPI) network was constructed with STRING database and visualized with Cytoscape software. Additionally, the transcriptional regulatory relationships were screened using UCSC ENCODE Genome Browser.

RESULTS: A total of 380 DEGs were identified, of which 188 were up-regulated and 192 were down-regulated. The DEGs were involved in different GO terms and pathways, mainly associated with metabolism such as proteolysis. PPI network analysis revealed that Actin, Alpha 1, Skeletal Muscle (*ACTA1*), Histone Deacetylase 2 (*HDAC2*), and Small Ubiquitin-Like Modifier 1 (*SUMO1*) were hub proteins. In addition, *HDAC2* was shown to encode a transcription factor (TF) and it could regulate 3 DEGs.

CONCLUSIONS: Genes such as *ACTA1*, *HDAC2*, and *SUMO1* were presumed to play critical roles in the mechanisms and consequences of OSA-induced perturbations in visceral fat tissue depots, which may be useful for deeply studying the mechanisms underlying OSA.

Key Words:

Obstructive sleep apnea, Metabolism, Differentially expressed genes, Protein-protein interaction network, Transcription factor.

Introduction

Obstructive sleep apnea (OSA) is a common disorder characterized by repeated episodes of the upper airway obstruction during sleep accompanied by intermittent hypoxia, arousal, and sleep fragmentation[1]. OSA is known to be a risk factor for road and workplace accidents, and is also associated with an increased likelihood of several systemic illnesses including cardiovascular disease, hypertension and other metabolic disorders[2-4]. OSA has now been widely recognized as a major public health concern, affecting at least 2% to 4% of the adult population[3]. However, the underlying mechanisms are not entirely understood.

In recent years, studies have provided support that sleep fragmentation may promote the adverse metabolic consequences of OSA by perturbing normal function of visceral adipose tissue and altering insulin sensitivity[5-7]. Because of the known importance of visceral fat tissue depots in regulating metabolism, inflammation and insulin resistance[8], understanding the pathophysiologic consequences of OSA on changes in adipocyte biology is a significant step in elucidating mechanisms linking OSA with systemic effects. Recently, the work of Gharib et al[9] reported that a short exposure to sleep fragmentation in mice perturbed the transcriptional profiling of visceral adipocytes and induced metabolic dysregulation. Besides, another study had demonstrated that OSA was associated with gene expression alterations in visceral fat and a critical metabolic pathway, the peroxisome proliferator activated receptor (PPAR), was found to be down-regulated in subjects with OSA[10]. However, key genes associated with the mechanisms and consequences of OSA-induced perturbations in regulatory work of adipocytes remain unclear.

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Microarray data analysis enables the identification of gene regulatory networks and crucial genes associated with disease[11]. In the present study, we carried out a bioinformatics analysis of dataset GSE38792, the same microarray data used by Gharib et al[10]. We identified the differentially expressed genes (DEGs) in visceral fat samples obtained from OSA patients, followed by Gene Ontology (GO) and pathway enrichment analyses and protein-protein interaction (PPI) network construction. Besides, transcriptional regulatory relationships were screened. We sought to find involvement of critical genes in visceral adipocytes which may be important modulators of disturbances associated with OSA.

Materials and Methods

Affymetrix Microarray Data

The microarray data of GSE38792, deposited by Gharib et al[10], was downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The platform information is GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]. Ten visceral fat samples obtained intraoperatively from the omentum of 10 OSA patients and another 8 visceral fat samples obtained from the omentum of 8 normal controls were included in this dataset.

Data Preprocessing and Screening of DEGs

The normalized microarray data were downloaded. According to the annotation information, gene expression value was calculated as the mean value of its corresponding probe values. Linear Models for Microarray data (limma) is an R/Bioconductor software package which can provide an integrated solution for data analysis from gene expression experiments[12]. In this study, DEGs in OSA samples compared with normal controls were identified using the limma package[12]. A p value < 0.01 was selected as the threshold for screening DEGs.

GO and Pathway Enrichment Analyses of DEGs

GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were carried out for the identified DEGs using GO-function R package[13] and *org.Hs.eg.db* and *GO.db* annotation packages[14]. The significant GO terms or KEGG pathways were enriched by more than 2 genes with the threshold of p value < 0.05.

PPI Network Construction

To perform network analyses upon the identified DEGs, a PPI network was constructed in this study by retrieving relatively high confidence protein interactions from STRING which is a comprehensive PPI database[15]. The interaction pairs with the PPI combined score > 0.4 were chosen, which corresponded to a medium-confidence network[16]. The resulting network was visualized with Cytoscape[17].

Screening of Transcriptional Regulatory Relationships

The University of California Santa Cruz (UCSC) genome browser (http://genome.ucsc.edu) can serve as the central repository for genome annotations and genomic data generated by the Encyclopedia of DNA Elements (ENCODE) project[18] which aims to identify all functional elements in the human genome sequence. In the present study, to gain further insights into the molecular functions of the identified DEGs, differentially expressed transcription factors (TFs) among the DEGs and the gene regulatory relationships between differentially expressed TFs and DEGs were screened based on human TF binding sites data and genetic coordinate position information which were available at the UCSC ENCODE Genome Browser[18]. In particular, we searched TF binding sites between the range of 1000 base pair (bp) upstream and 500 bp downstream in the transcription start site of each gene, and the identified TF was considered to be associated with this DEG.

Results

DEGs Screening

Compared with the normal controls, we identified 380 DEGs with significantly altered expression in OSA samples, of which 188 were up-regulated and 192 were down-regulated. The heat map of DEGs was shown in Figure 1. The results showed that the DEGs expression pattern could significantly distinguish the OSA samples from

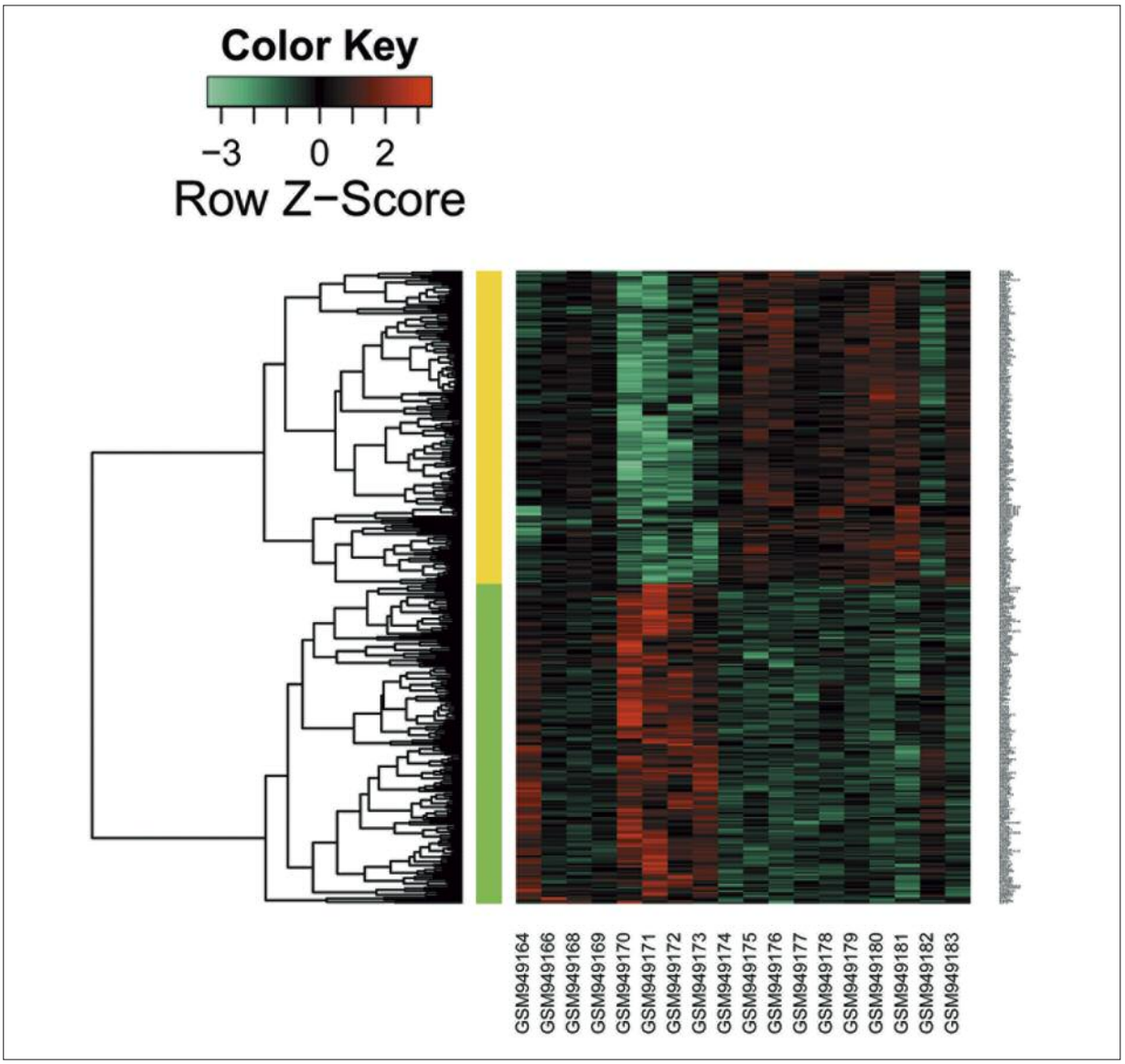

Figure 1. Heat map representing up-regulated and down-regulated genes in OSA samples compared with controls. The yellow row-side color represents the up-regulated genes in OSA samples. The bright green row-side color indicates the down-regulated genes in OSA samples. Red or green colors indicate either higher or lower expression levels of DEGs.

normal controls.

Functional and Pathway Enrichment Analyses of DEGs

GO and pathway enrichment analyses indicated that DEGs in OSA samples were significantly enriched in different GO terms and KEGG pathways. In particular, a large amount of GO terms were enriched. The top 5 enriched GO terms in each category as biological process (BP), molecular function (MF), and cellular component (CC) of the identified DEGs were shown in Table I. The results revealed that DEGs were mainly involved in the GO terms associated with metabolism such as proteolysis. On the other hand, the results of pathway enrichment analysis showed that a total of 9 KEGG pathways were enriched, for example, starch and sucrose metabolism, pentose and glucuronate interconversions, and protein processing in endoplasmic reticulum (Table II).

Construction and Analysis of PPI Network

The PPI network was constructed with the information from STRING database, comprising 162 nodes and 294 interactions (Figure 2). As

Table I. The top 5 enriched GO terms of each category.

GO-ID	Category	Term	Count	*p*-value
GO:0006508	BP	Proteolysis	42	8.97E-05
GO:0072378	BP	Blood coagulation, fibrin clot formation	4	0.000461
GO:0032722	BP	Ribosomal large subunit export from nucleus	5	0.000491
GO:0000055	BP	JUN phosphorylation	2	0.001523
GO:0007258	BP	Keratinocyte apoptotic process	2	0.001523
GO:0044421	CC	Extracellular region part	82	0.000995
GO:0042272	CC	Nuclear RNA export factor complex	2	0.001526
GO:0005576	CC	Extracellular region	92	0.004218
GO:0031982	CC	Vesicle	76	0.005259
GO:0031988	CC	Membrane-bounded vesicle	74	0.005489
GO:0004705	MF	JUN kinase activity	2	0.000794
GO:0016909	MF	SAP kinase activity	2	0.000794
GO:0015036	MF	Disulfide oxidoreductase activity	4	0.001212
GO:0015037	MF	Peptide disulfide oxidoreductase activity	2	0.003841
GO:0019966	MF	Interleukin-1 binding	2	0.003841

shown in the PPI network, the hub proteins with degree more than 10 were Actin, Alpha 1, Skeletal Muscle (*ACTA1*) (degree=15), Histone Deacetylase 2 (*HDAC2*) (degree=14), Small Ubiquitin-Like Modifier 1 (*SUMO1*) (degree=13), Actin-Like 6A (*ACTL6A*) (degree=11).

Transcriptional Regulatory Relationships Screening

Regulatory relationships were predicted between identified differentially expressed TFs and DEGs. The results showed that HDAC2 was identified as a differentially expressed TF and HDAC2 could interact with other 3 DEGs, namely, EFR3 Homolog A (*EFR3A*), Male Germ Cell-Associated Kinase (*MAK*), Prolyl 4-Hydroxylase, Alpha Polypeptide I (*P4HA1*), forming 3 regulatory pairs.

Discussion

OSA is caused by a conglomeration of complex pathophysiological processes and this common disease has an association with significant morbidities which affect the neurocognitive cardiovascular, and metabolic systems[19]. In this study, we identified 188 up-regulated and 192 down-regulated genes in visceral fat tissue of OSA patients compared with normal controls. GO and pathway enrichment analyses indicated that DEGs were mainly involved in the GO terms or pathways associated with metabolism. Besides, *ACTA1*, *HDAC2*, and *SUMO1* were identified to be hub proteins in the PPI network. In addition, HDAC2 could act as a TF and HDAC2 was identified to regulate other 3 DEGs.

ACTA1 belongs to the highly conserved actin family of proteins, which play a role in cell motility, structure and integrity[20]. *ACTA1* is a gene involved in integrin pathway[21]. Studies[22] have shown that there are many integrin family members with different functions. For example, β1 integrin could induce interleukin-8 production in human natural killer cells which is involved in inflammatory response[23]. Besides, Taylor et al[24] had demonstrated that adipose tissue might be an critical source of inflammatory mediators in OSA. Additionally, our study identified that the up-regulated gene *ACTA1* found in adipocytes from OSA patients was a hub protein in the PPI network with the highest degree. Collectively, we suggested that *ACTA1* might play an essential role in the regulation of adipocyte inflammation affected by OSA.

The product of *HDAC2* belongs to the histone deacetylase family which is responsible for the deacetylation of lysine residues on the N-terminal part of the core histones[25]. Park et al[26] had reported that intermittent hypoxia which was involved in the mechanisms of OSA-related cardiovascular diseases might influence the activity of *HDAC*. Besides, the findings of Peng et al[27] showed that HDAC2 could selectively regulate some gene transcription during oxidative stress-induced neuronal cell death. Moreover, in the current study, we found that *HDAC2* was a hub protein in the PPI network and HDAC2 could act as a TF, which was in line with the previous investigations, suggesting that *HDAC2* may play a central role in the mechanisms and consequences of OSA-induced perturbations in visceral fat tis-

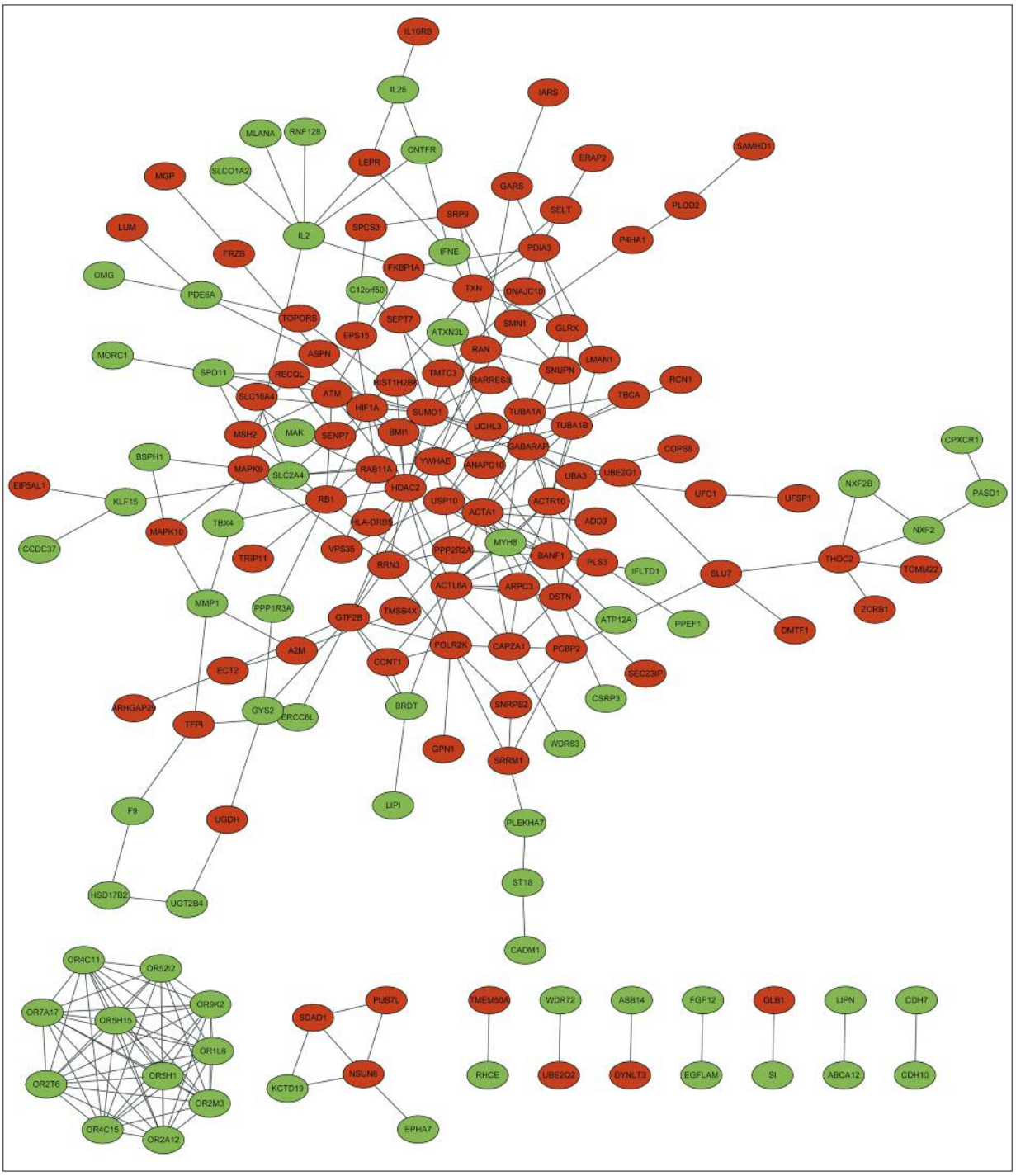

Figure 1. Protein-protein interaction (PPI) network of differentially expressed genes (DEGs). The up-regulated genes and down-regulated genes are displayed in red and green nodes, respectively.

sue depots.

In addition, SUMO1 is a member of the SUMO protein family which is bound to target proteins as part of a post-translational modification system in a manner similar to ubiquitin[28]. Recent studies have demonstrated that SUMO1 can promote nuclear accumulation[29] and SUMO-1 can regulate body weight and adipogenesis via the nuclear receptor peroxisome proliferator-acti-

Table II. The enriched KEGG pathways of DEGs.

KEGG-ID	Pathway	Count	*p*-value	Genes
3013	RNA transport	9	0.002237	RAN, SMN1, NXF2, SNUPN, NXF2B, EIF4G2, THOC2, SUMO1, SRRM1
500	Starch and sucrose metabolism	5	0.003477	GYS2, UGDH, UGT2A3, UGT2B4, SI
53	Ascorbate and aldarate metabolism	3	0.01278	UGDH, UGT2A3, UGT2B4
4141	Protein processing in endoplasmic reticulum	8	0.01315	PDIA3, DNAJC10, LMAN1, UBE2G1, MAPK9, ATXN3L, MAPK10, EIF2AK2
40	Pentose and glucuronate interconversions	3	0.022453	UGDH, UGT2A3, UGT2B4
4740	Olfactory transduction	13	0.032578	OR11A1, OR52I2, OR2M3, OR7A17, OR1L6, OR8K1, OR4C15, OR4C11, OR9K2, OR2T6, OR10X1, CLCA4, OR2A12
4920	Adipocytokine signaling pathway	4	0.040258	LEPR, MAPK9, MAPK10, SLC2A4
5219	Bladder cancer	3	0.045349	DAPK2, MMP1, RB1
531	Glycosaminoglycan degradation	2	0.049972	HYAL4, GLB1

vated receptor γ (PPARγ) in male and female mice[30]. Besides, Agbor et al[31] had showed that *SUMO1* promoted glycolysis in hypoxia. Additionally, Drager et al[32] had reviewed that OSA exacerbated the cardiometabolic risk in obesity and the metabolic syndrome. In accordance with the previous studies, our results found that *SUMO1* was associated with RNA transport pathway and *SUMO1* had a higher node degree in the PPI network. Therefore, we suggested that *SUMO1* might be associated with the mechanisms and consequences of OSA-induced perturbations in metabolism of adipocytes.

Conclusions

We identified several key genes (*ACTA1*, *HDAC2* and *SUMO1*) involved in the regulation of adipocyte metabolism and inflammation associated with OSA. *ACTA1* may play a role in the regulation of adipocyte inflammation affected by OSA. Besides, *HDAC2* may act as a TF and be crucial in consequences of OSA on adipocytes. Moreover, *SUMO1* may be associated with metabolism dysregulation of adipocytes induced by OSA. However, some limitations still remain in this study. First, the sample size for microarray analysis was small and a larger sample cohort was needed to verify the identifications. Furthermore, this study was lack of experimental verification. Thus, further genetic and experimental investigations with a larger sample size are still needed to confirm the results in the future, which may provide an useful information for deeply studying the mechanisms and consequences of OSA from a molecular-level perspective.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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