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ORIGINAL RESEARCH

Inflammatory and Redox Blood Gene Expression Fingerprint of Severe Obstructive Sleep Apnoea in Patients With Mild Alzheimer's Disease

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Introduction: Obstructive sleep apnoea (OSA) is the sleep disorder most frequently found in patients with Alzheimer's disease (AD). The intermittent hypoxia (IH) caused by OSA may participate in AD pathogenesis through increase in oxidative damage and inflammation. We aimed to identify inflammatory and redox genes differentially expressed in the blood from AD patients with severe OSA compared with those with nonsevere OSA.

Methods: We included 40 AD patients diagnosed based on clinical manifestations and AD biomarker levels in cerebrospinal fluid (CSF). Severe or nonsevere OSA (apnoea–hypopnea index $\geq$ 30/h and $<$ 30/h, respectively) was diagnosed through overnight polysomnography (PSG). The expression levels of 136 inflammation-related and 84 redox-related genes were evaluated by whole blood targeted transcriptomics.

Results: Three inflammatory and six redox genes were upregulated in the blood of AD patients with severe OSA. Three of them correlated with PSG parameters. A pathway enrichment analysis showed a strong enrichment of the serotonergic synapse pathway in severe OSA AD patients.

Discussion: Our results show an upregulation of nine genes involved in NF-κB-mediated inflammation and redox metabolism in the blood of patients with mild AD with severe OSA. Therefore, severe OSA may worsen the inflammation and oxidative damage that are already altered in patients with AD.

Keywords: Alzheimer's disease, obstructive sleep apnea, oxidative stress, inflammation, gene expression, NF-κB signaling

Introduction

Alzheimer's disease (AD) is the most common type of dementia and is characterized by a progressive memory impairment that can ultimately affect other mental abilities, such as cognition and behaviour.[1] AD is a multifactorial disease, and evidence suggests that genetic, metabolic, lifestyle and environmental factors may have a role in the onset and progression of the disease.[2] Although some amyloid-targeting therapies have produced appreciable slowing of clinical AD progression,[3–5] no treatment is available to cure the disease. Therefore, identifying treatable risk factors is of special importance.

In the last decade, different sleep disorders have been considered risk factors for the development of AD. Of all of them, obstructive sleep apnoea (OSA) is the one that most consistently has been found in different studies.[6,7] OSA is a treatable

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condition characterized by repeated events of complete or incomplete pharyngeal collapses during sleep, which in turn produces intermittent hypoxia (IH) and sleep fragmentation.[8] The presence of OSA in AD patients ranges between 45 and 90%, reaching 40% for severe OSA.[9] OSA shares several risk factors with AD, such as hypertension and metabolic syndrome, including obesity, diabetes mellitus, and dyslipidaemia.[10] In addition, OSA has been related to increased levels of amyloid β-42 (Aβ-42) and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF), two specific pathological features of AD.[11]

Different hypotheses have been proposed to explain the increased risk of AD in OSA patients, such as dysfunction of the glymphatic system or damage to the arterial wall.[12,13] On the other hand, IH caused by OSA has been shown to increase oxidative damage and the inflammatory response,[14–16] which are among the first pathophysiological alterations present in the AD continuum.

Oxidative stress is caused by the imbalance between the production of reactive oxygen species (ROS) and the ability of the body to neutralize or eliminate them.[17] ROS are generated by aerobic metabolism and can damage cell components by oxidizing major biomolecules such as nucleic acids and proteins.[18] OSA is also characterized as a disrupter of the pro-/antioxidant balance due to the repetition of hypoxia-reoxygenation cycles.[8] Evidence indicates that oxidative stress may play an important role in the pathogenesis of AD. For example, researchers have recently suggested that increased mitochondrial oxidative stress, lipid oxidation and bioenergetic failure in the entorhinal cortex evolves to neurodegeneration, which in turn leads to the onset and progression of AD.[19] However, analysing the levels of oxidative stress at the systemic level or in CSF of AD patients has resulted in contradictory results.[17,20,21]

On the other hand, inflammation is also present in the AD brain from the preclinical stages of the disease and has been shown to play a pivotal role in AD pathogenesis.[22] OSA is associated with low-grade systemic inflammation, which in fact correlates with the severity of nocturnal hypoxemia.[23] Given the fundamental role of dysregulation of inflammation and oxidative stress pathways in OSA and AD, previous studies have related several alterations at the gene expression level that are common between both pathological conditions.[24] Therefore, considering that OSA may affect AD via induction of oxidative stress and inflammatory response, it is expected that this possible affectation is also linked to OSA severity in AD patients. Following this hypothesis, our objectives were (i) to identify dysregulations of oxidative stress and inflammatory genes in the blood from AD patients with severe OSA; (ii) to find possible correlations between the expression of the investigated genes with the MMSE (Mini-Mental State Examination) score and the CSF levels of AD biomarkers as well as with PSG parameters; (iii) to analyse the inter- and intrapathway relationships of significantly associated genes. For this study, the expression of 136 inflammatory and 84 redox key genes was analysed in AD patients with different OSA severities using quantitative real-time polymerase chain reaction (qRT–PCR). These panels include the major inflammatory and redox key genes involved in OSA and AD conditions.[25,26]

Materials and Methods

Study Population

This project is an ancillary study of a prospective trial designed to evaluate the influence of OSA on the cognitive decline of AD patients after one year of follow-up (NCT02814045).[27] Forty patients with mild AD were recruited at the Cognitive Disorders Unit of the Hospital Universitari Santa Maria (Lleida, Spain) between 2015 and 2019 and diagnosed according to the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria.[28]

Eligibility criteria for this case–control study were as follows: males and females older than 60 years, acetylcholinesterase inhibitor-naïve and with a new diagnosis of mild AD (MMSE score ≥ 20).

The exclusion criteria were as follows: (1) diagnosis of dementia other than AD or any somatic, psychiatric, or neurological disorder that might cause cognitive impairment (2) presence of any previous diagnosis of OSA treated with CPAP; (3) presence of any previously diagnosed sleep disorder such as narcolepsy, severe insomnia, or chronic lack of sleep; (4) existence of visual or hearing problems that, in the investigator's judgement, could render compliance with the study procedures difficult; (5) comorbidities such as cancer, severe depression, severe renal or hepatic insufficiency, and severe cardiac or respiratory failure; (6) acute episodes of inflammation within 1 month prior to the investigation; (7) excessive alcohol intake (>280 g/week); (8) magnetic resonance imaging (MRI) evidence of stroke, hydrocephalus, a space-occupying lesion, or any clinically relevant central nervous system disease other than AD; (9) existence of

untreated (or treated for less than 3 months prior to the screening visit) vitamin B12 or folate deficiency; and (10) presence of untreated thyroid disease.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Care Ethics Committee (CE-1218). An informed consent form was signed by all patients and responsible caregivers (and/or if applicable, the legal representative if different from the responsible caregiver).

Study Design and Clinical Variables

Patients who met the inclusion criteria and none of the exclusion criteria and consented to participate in the study were subjected to a detailed interview concerning their personal history, a general clinical examination for associated conditions and comorbidities and anthropometric data collection. The variables collected were as follows: age, sex, years of education, unhealthy habits (alcohol consumption and smoking), vascular risk factors (diabetes mellitus, hypertension, dyslipidaemia, heart diseases and stroke) and personal psychiatric history.

The cognitive state of the included subjects was assessed using the MMSE. This questionnaire evaluates multiple cognitive domains, including place and time orientation, attention, word recall, calculation, language, and visual construction. The obtained score ranges from 0 to 30, and a higher MMSE score indicates better cognitive function.[29]

Participants underwent an overnight polysomnographic (PSG) study in the sleep laboratory of the Sleep Unit at the Hospital Universitari Santa Maria (Lleida, Spain). The sleep staging and classification of the breathing cessation events that compose the AHI were performed according to the American Academy of Sleep Medicine guidelines.[30,31] The analysis was carried out by an experienced sleep technician blinded to this study. Participants were classified as having nonsevere OSA (apnoea–hypopnea index (AHI) < 30/h) or severe OSA (AHI ≥ 30/h) based on the study findings. The following morning, CSF and blood samples were collected between 8:00 and 10:00 a.m. to avoid variations related to circadian rhythm.

APOE Genotyping

Venous blood (2.5 mL) was collected from each patient in EDTA-containing tubes. Blood samples were centrifuged at 1500 rpm for 20 min, and the buffy coat was used for DNA extraction and APOE genotyping. DNA was extracted using a Max-well® RCS blood DNA kit (Promega, USA), and APOE genotyping was performed using RT–PCR with TaqMan® SNP genotyping assays (C_3038793_20 and C_904973_10) according to the manufacturer's user guide (publication no. MAN0009593, revision B.0).

CSF AD Biomarkers

CSF samples were collected in polypropylene tubes, centrifuged at 2000 × g for 10 min at 4°C, and stored at −80°C. CSF levels of Aβ42 (Innotest® β-Amyloid (1–42)), total tau (t-tau) (Innotest® hTAU Ag) and p-tau (Innotest® Phospho-Tau (181P)) were assessed using the enzyme immunoassay method according to the manufacturer's instructions (Fujirebio Europe, Ghent, Belgium). All samples were measured in duplicate, and the results are reported in pg/mL. The cut-off values for pathological levels of CSF biomarkers were established using an independent cohort of AD patients and controls in our laboratory and were as follows: ≤ 600 pg/mL for Aβ42, > 425 pg/mL for t-tau, and > 65 pg/mL for p-tau.[32] Samples were obtained with support from IRBLleida Biobank (B.000682) and Biobank and Biomodels Platform ISCIII PT23/00032.

Polysomnography (PSG)

PSG studies were performed in the sleep laboratory unit of the Sleep Unit at the Hospital Universitari Santa Maria de Lleida (Lleida, Spain). The parameters assessed were: electroencephalogram (EEG) (C3/A2-C4/A1 O2/A1 and O1A2, according to the international electrode placement system), bilateral electrooculogram (EOG), chin electromyogram (EMG), chest and abdominal respiratory belt, air-flow measurement (using oral and nasal thermocouple and nasal pressure records), pulse oximetry, electrocardiogram (ECG), body-position recording, snore microphone and bilateral piezoelectric sensors to detect leg movements. The median [IQR] of effective sleep in our patients was 303.0 min [223.3, 339.3].

PSG was performed according to international guidelines for all study participants with the following devices: Embletta® sleep monitor (Embla, Canada), a Sibelmed Exea Series 5 (Sibel SAU, Spain), a Philips Respironics Alice 6 LDx (Philips, USA) and an ApneaLink Resmed (Resmed, Canada).

Apnoea was defined as the absence or reduction of airflow of at least 90% lasting for more than 10 seconds, while hypopnea was defined as a reduction in airflow (30–90%) that lasted more than 10s and led to arousal or oxygen desaturation (a decrease in oxygen saturation greater than 3%). The AHI was defined as the number of apnoea and hypopnea events per hour during the time spent sleeping (events/h). The arousal index was determined as the number of awakening events per hour after sleep onset, and CT90 was determined as the percentage of cumulative sleep time with oxyhaemoglobin saturation (SpO2) < 90%. The oxygen desaturation index ≥3% (ODI-3%) was determined by calculating the average number of desaturation episodes per hour of recording time, where desaturation episodes were defined as a decrease in mean oxygen saturation of ≥3% lasting at least ten seconds.[33]

Gene Expression

Blood was collected in PAXgene Blood RNA Tubes (Qiagen, Venlo, the Netherlands), and total RNA was isolated with a PAXgene Blood miRNA Kit (Qiagen) according to the manufacturer's protocol. The purity and quantity of the total RNA isolated were spectrophotometrically evaluated using NanoDrop 2000 equipment (Thermo Scientific, Waltham, Massachusetts, U.S). Then, 400 ng of RNA was used to perform reverse transcription with the RT^2 First Strand Kit (Qiagen). Using the RT^2 Profiler^{TM} PCR Array Human NF-κB Signaling Pathway (PAHS-025Z, Qiagen) and the RT^2 Profiler^{TM} PCR Array Human NFκB Signaling Targets (PAHS-225Z, Qiagen), the expression of 136 inflammatory genes mainly involved in NF-κB signalling and their target genes, respectively, were analysed (Supplementary Tables 1 and 2). The expression of 84 key genes involved in oxidative stress and the antioxidant response was also evaluated with the RT^2 Profiler^{TM} PCR Array Human Oxidative Stress Plus (PAHS-065Y, Qiagen) (Supplementary Table 3). These panels were chosen because they include the major inflammatory and redox key genes involved in OSA and AD conditions.[25,26] SYBR Green chemistry was performed using an ABI-7500 fast instrument (Applied Biosystems, Waltham, Massachusetts, U.S). The expression level of each transcript was normalized to the geometric mean of two housekeeping genes (*HPRT1* and *RPLP0*) whose stability in the whole blood was previously validated.[34] Gene expression levels were expressed as $2^{-\Delta CT}$ values, and the fold change (FC) was calculated as the $2^{-\Delta CT}$ mean values for patients with severe OSA divided by $2^{-\Delta CT}$ mean values for patients with nonsevere OSA.

Enrichment Analysis

A pathway enrichment analysis considering the genes differentially expressed between the two groups was performed using KEGG 2021 human tool of the web-based open source Enrichr software.[35–37]

Statistical Analysis

Descriptive statistics were calculated to summarize the characteristics of the study population. The mean ± standard deviation (SD) was reported for the continuous variables when data were normally distributed, while for nonnormally distributed data, the median and interquartile range (25th percentile, 75th percentile) were reported. The normality of the distributions was determined with the Kolmogorov–Smirnov and Shapiro–Wilk tests. Categorical data were summarized as frequencies (percentages). Patients were stratified in two groups according to the OSA status (AHI ≥ 30/h vs AHI < 30/h). Clinical and sociodemographic characteristics of patients were compared between groups using the *t* test (or an equivalent nonparametric test) or the chi-square test, depending on the type of variables (quantitative or categorical, respectively). Since the gene expression data were not normally distributed, differences in expression levels between the two groups were assessed with the nonparametric Mann–Whitney *U*-test. Only changes in gene expression with an FC > 1.5 and $p < 0.05$ were considered significant in this study. The Spearman rank correlation coefficient (ρ) was calculated to determine correlations between gene expression levels and polysomnography parameters or AD biomarkers. All statistical analyses were performed using IBM SPSS Statistics 28 (Chicago, IL, USA).

Results

Characteristics of the Cohort

Forty patients with mild AD who had available clinical data and whole blood samples were included in the study. The median [IQR] age of the population was 78.5 [73.0, 80.8] years; 30 (75.0%) participants were women, and the mean (SD) MMSE score was 23.5 (2.51). The most frequent vascular risk factor was arterial hypertension, which was present in 22 (55.0%) patients, followed by hypercholesterolemia in 16 (40.0%) participants. Depression and diabetes were present in 10 (25.0%) and 9 (22.5%) patients, respectively. With respect to the sleep parameters, the mean (SD) Epworth Sleepiness Scale score was 5.95 (4.64), and the median [IQR] AHI was 16.3 [8.63, 41.5]. Regarding ODI, the median [IQR] was found to be 6.46 [1.7, 23.9]. Of the total 40 participants, 27 were considered to have nonsevere OSA (AHI < 30/h), and 13 patients were classified as having severe OSA (AHI ≥ 30/h). The demographic characteristics, medical disorders and AD biomarkers of the patients were similar between both OSA severity groups, with the exception of sex. The characteristics of the study population separated by OSA status are summarized in Table 1.

Table 1 Demographic and Clinical Characteristics of AD Patients Stratified According to Their OSA Status

	All (n=40)	**Nonsevere OSA (AHI < 30/h) (n=27)**	**Severe OSA (AHI ≥ 30/h) (n=13)**	***p***
Demographic characteristics				
Age at baseline visit (years), median [IQR]	78.5 [73.0; 80.8]	78.0 [73.0; 81.0]	79.0 [74.0; 80.5]	0.706
Sex (female), n (%)	30 (75.0)	23 (85.2)	7 (53.8)	**0.032**
Education				0.526
No education, n (%)	5 (12.5)	3 (11.1)	2 (15.4)	
Primary studies, n (%)	29 (72.5)	21 (77.8)	8 (61.5)	
Secondary studies, n (%)	6 (15.0)	3 (11.1)	3 (23.1)	
BMI (kg/m2), mean (SD)	28.5 (3.8)	27.3 (3.2)	29.9 (3.6)	0.135
Family history of AD (yes), n (%)	15 (37.5)	10 (37.0)	5 (38.5)	0.931
Medical disorders				
Hypertension (yes), n (%)	22 (55.0)	15 (55.6)	7 (53.8)	0.919
Diabetes (yes), n (%)	9 (22.5)	5 (18.5)	4 (30.8)	0.385
Hypercholesterolaemia (yes), n (%)	16 (40.0)	9 (33.3)	7 (53.8)	0.215
Depression (yes), n (%)	10 (25.0)	7 (25.9)	3 (23.1)	0.845
Smoker				0.736
Nonsmoker, n (%)	36 (90)	24 (88.9)	12 (92.3)	
Current smoker, n (%)	0 (0)	0 (0)	0 (0)	
Former smoker, n (%)	4 (10)	3 (11.1)	1 (7.7)	
MMSE score, mean (SD)	23.5 (2.5)	22.9 (2.7)	24.4 (2.0)	0.133

(*Continued*)

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Table 1 (Continued).

	All (n=40)	Nonsevere OSA (AHI < 30/h) (n=27)	Severe OSA (AHI ≥ 30/h) (n=13)	*p*
Polysomnography parameters				
AHI (events/h), median [IQR]	16.3 [8.6; 41.5]	12.0 [6.0; 16.7]	52.1 [41.0; 56.5]	**<0.001**
CT90, median [IQR] (%)	3 [0; 10]	1 [0; 4]	7.0 [1.0; 33.5]	0.082
Mean SaO2, median [IQR] (%)	93 [91; 93]	93 [92; 94]	93 [90; 93]	0.478
Minimum SaO2, median [IQR] (%)	84 [81; 87]	85 [84; 88]	84 [79.5; 85.0]	0.107
Arousal index, events/h	12 [4.0; 22.8]	15 [3; 29]	8 [3.5; 16.5]	0.133
Oxygen Desaturation index, events/h	6.46 [1.7; 23.9]	4.60 [1.5; 13.6]	18.2 [4.0; 38.1]	**0.016**
Epworth Sleepiness Scale, mean (SD)	5.95 (4.64)	5.26 (3.81)	6.31 (5.3)	0.873
AD biomarkers				
Aβ42 CSF (pg/mL), mean (SD)	486.5 (145.1)	487.4 (146.8)	484.9 (148.0)	0.974
Total tau CSF (pg/mL), median [IQR]	487 [401; 609]	494 [422; 627]	464 [394; 576]	0.469
Phospho-tau CSF (pg/mL), median [IQR]	76.50 [67.3; 92.8]	75 [67; 93]	81 [65.5; 92.5]	0.987
ApoE ε4 (carrier), n (%)	22 (55.0)	13 (50.0)	9 (69.2)	0.254
Medications				
ACE inhibitors, %	15 (37.5%)	9 (33.3%)	6 (46.2%)	0.433
Beta-blockers, %	4 (10.0%)	2 (7.40%)	2 (15.4%)	0.431
Diuretic agents, %	13 (32.5%)	8 (29.6%)	5 (38.5%)	0.576
Calcium-channel blockers, %	4 (10.0%)	3 (11.1%)	1 (7.70%)	0.736
Lipid-lowering agents, %	16 (40.0%)	8 (29.6%)	8 (61.5%)	0.054
Insulin, %	0 (0.00%)	0 (0.00%)	0 (0.00%)	–

Note: Bold Text Indicates p Statistically Significant ($p < 0.05$).

Abbreviations: OSA, obstructive sleep apnoea; AHI, apnoea–hypopnea index; IQR, interquartile range; BMI, body mass index; SD, standard deviation; AD, Alzheimer's disease; CT90, cumulative percentage of the time spent at saturations below 90%; SaO2, oxygen saturation; MMSE, Mini-Mental State Examination; Aβ42, amyloid beta-42; CSF, cerebrospinal fluid; ApoE, apolipoprotein E; ACE-Angiotensin-Converting Enzyme.

Identification of Differentially Expressed Genes Depending on OSA Severity

The first objective of the study was to evaluate the expression of inflammatory and redox genes in AD patients with severe OSA. Therefore, a pathway-focused array of 136 inflammatory and 84 redox genes was analysed using RT–PCR. Our results indicated that three inflammatory (*RAF1, RELB*, and *TNFSF14*) and six redox genes (*ALOX12, DUSP1, GSR, PDLIM1, PTGS2*, and *SOD2*) were upregulated in the blood of AD patients with severe OSA (FC ≥ 1.5, $p < 0.05$) (Table 2 and Figure 1).

Correlation Between Differentially Expressed Genes and AD Biomarkers, MMSE Scores and PSG Parameters

Spearman's ranked correlation analysis between the differentially expressed genes and the AD biomarkers, MMSE score, and PSG parameters was performed in all patients while controlling for age and sex. Our results showed that the *GSR* gene had a significantly positive correlation with AHI ($r = 0.390$, $p = 0.023$), and *TNFSF14* and *ALOX12* both had

Table 2 Differentially Expressed Genes Between AD Patients With and Without Severe OSA (FC > 1.5 and p values < 0.05)

	Nonsevere OSA (AHI < 30) (n=27)		Severe OSA (AHI ≥ 30) (n=13)		FC	p value
	Mean*	SD	Mean*	SD		
RAF1	0.755	0.461	1.139	0.416	1.5	0.010
RELB	0.088	0.054	0.131	0.051	1.5	0.015
TNFSF14	0.047	0.034	0.072	0.033	1.5	0.004
ALOX12	0.045	0.022	0.068	0.026	1.5	0.048
DUSP1	2.338	1.156	3.928	1.778	1.7	0.010
GSR	0.029	0.009	0.044	0.020	1.5	0.023
PDLIM1	0.229	0.108	0.348	0.142	1.5	0.014
PTGS2	0.104	0.043	0.156	0.059	1.5	0.040
SOD2	16.511	7.758	24.190	8.064	1.5	0.030

Note: *Mean of 2-ΔCT values.

Abbreviations: OSA, obstructive sleep apnoea; AHI, apnoea-hypopnea index; SD, standard deviation; FC, fold change; RAF1, Raf-1 proto-oncogene; serine/threonine kinase; RELB, RelB proto-oncogene; NF-κB subunit; TNFSF14, TNF superfamily member 14; ALOX12, Arachidonate 12-lipoxygenase; 12S type; DUSP1, Dual Specificity Phosphatase 1; GSR, Glutathione-disulfide Reductase; PDLIM1, PDZ and LIM domain 1; PTGS2, Prostaglandin-endoperoxidase synthase 2; SOD2, Superoxide Dismutase 2.

positive correlations with the arousal index (r = 0.366, $p = 0.033$ and r = 0.424, $p = 0.013$, respectively) (Figure 2). The levels of the investigated genes were not correlated with the CSF levels of AD biomarkers, MMSE score, or the other PSG parameters (Supplementary Table 4).

In addition, the Spearman correlation analysis between the differentially expressed inflammatory and redox genes indicated significant interpathway connections between most of these genes (Table 3).

Pathway Enrichment Analysis of Differentially Expressed Genes

A pathway enrichment analysis of the differentially expressed genes was performed using Enrichr software. The top 8 pathways from the KEGG 2021 human database with significantly overlapping genes are shown in Figure 3 (adjusted p value < 0.05). *DUSP1, ALOX12, RAF1* and *PTGS2* were the genes that overlapped in the serotonergic synapse, which was the main enriched pathway (adjusted p value = 0.00001250). The C-type lectin receptor (adjusted p value = 0.0003925) and NF-kappa B signalling pathways (adjusted p value = 0.0003925) were the most significantly enriched.

Discussion

In this study, we determined the differential expression of inflammatory and redox genes in whole blood from AD patients with severe and nonsevere OSA. We identified three inflammatory genes and six redox genes that were upregulated in AD patients with severe OSA. Of all upregulated genes, *TNFSF14* and *ALOX12* (inflammatory and redox genes, respectively) were positively correlated with the arousal index. *GSR*, a redox gene, was positively correlated with the AHI. Finally, we found good correlations between the three inflammatory genes and six redox genes, indicating potential functional associations between them.

OSA is a potentially treatable and highly prevalent entity that increases the risk of AD.[38,39] Inflammation and oxidative stress caused by episodes of hypoxia and reoxygenation are among the mechanisms through which OSA can increase the risk of AD. However, to date, no studies have been reported that evaluate whether severe OSA modifies gene expression profile in the blood of patients with AD. Through an integrated bioinformatic analysis, Wu et al identified key common genes that are mainly involved in cellular responses to oxidative stress and neuroinflammation, in both AD and

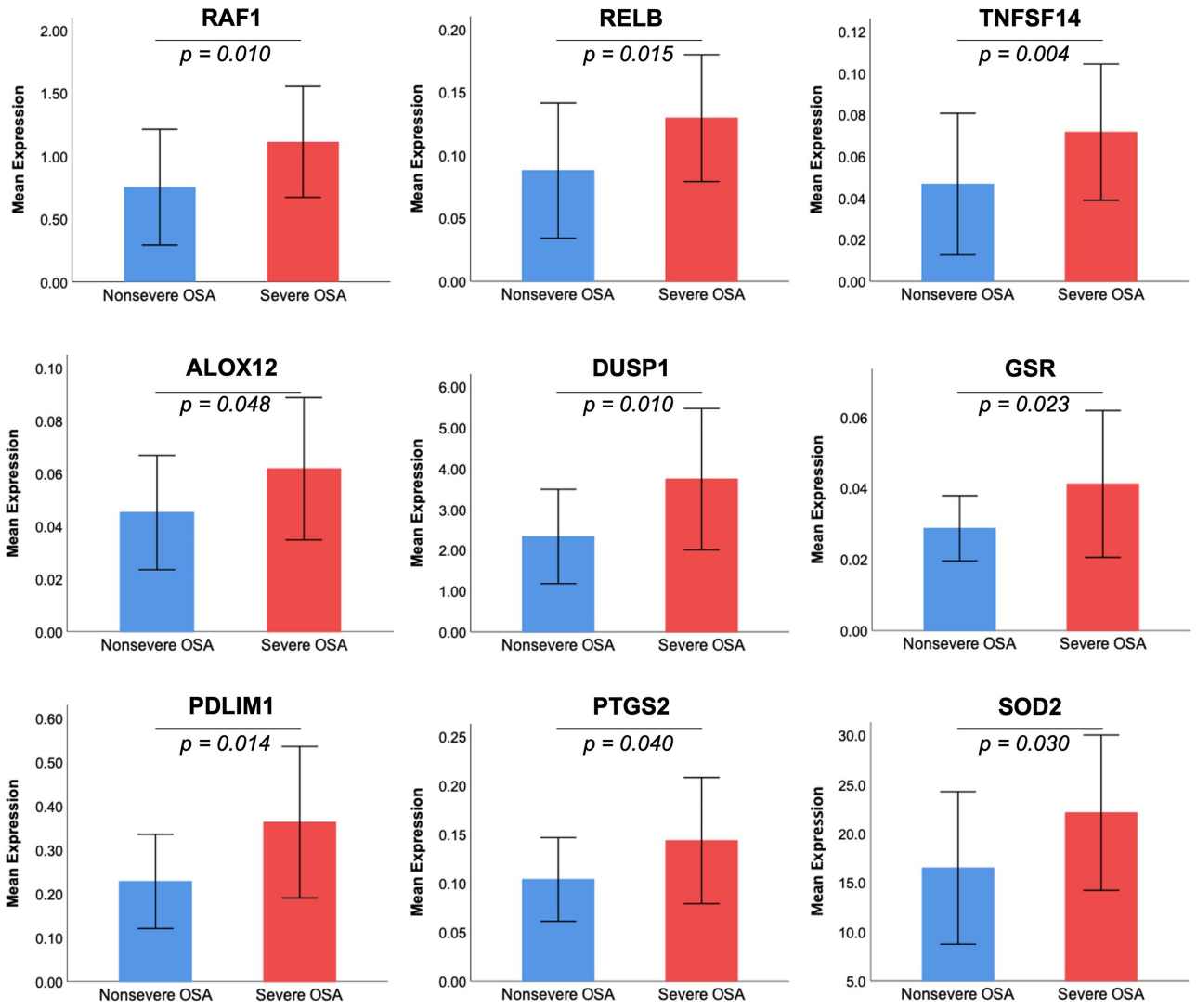

Figure 1 Means and standard deviations between OSA severity groups in AD patients in statistically significant differentially expressed genes.

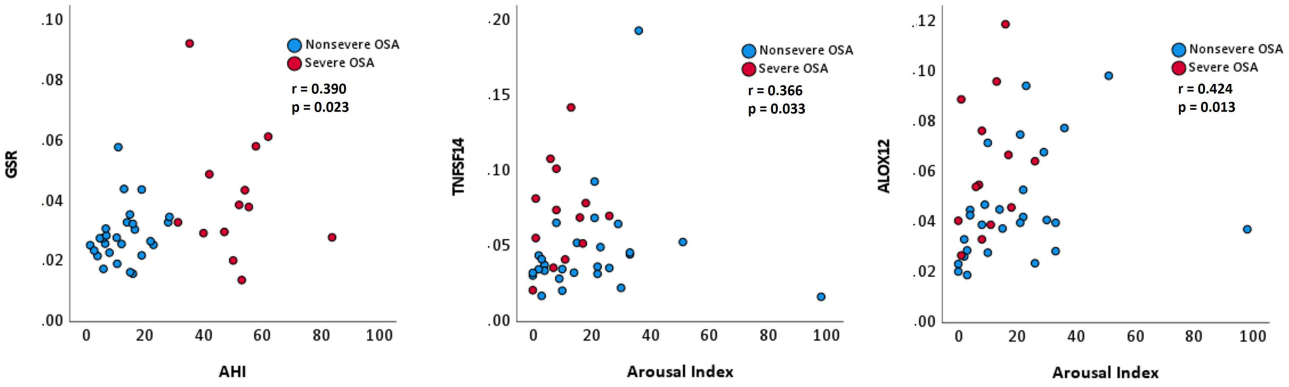

Figure 2 Statistically significant correlations between differentially expressed genes, AHI and the arousal index after adjusting for age and sex. Blue dots: nonsevere OSA patients; red dots: patients with severe OSA.

Table 3 Statistically Significant Correlations Between Differentially Expressed Inflammatory and Redox Genes. The Color Intensity Indicates the Level of Correlation Between Genes (More Intensity, More Correlation)

		Redox genes					
		ALOX12	**DUSP1**	**GSR**	**PDLIM1**	**PTGS2**	**SOD2**
Inflammatory genes	**RAF1**	0.508	0.566	0.417	0.358	0.554	0.623
	RELB	0.512	0.541	0.558	0.621	0.481	0.470
	TNFSF14	0.469	0.433			0.450	0.479

Abbreviations: RAF1, Raf-1 proto-oncogene, serine/threonine kinase, RELB, RelB proto-oncogene, NF-κB subunit, TNFSF14, TNF superfamily member 14, ALOX12, Arachidonate 12-lipoxygenase, 12S type, DUSP1, Dual Specificity Phosphatase 1, GSR, Glutathione-disulfide Reductase, PDLIM1, PDZ and LIM domain 1, PTGS2, Prostaglandin-endoperoxidase synthase 2, SOD2, Superoxide Dismutase 2.

OSA.[24] To our knowledge, our study is the first attempt to evaluate the OSA-associated differential expression by qRT-PCR of a large panel of inflammatory and redox genes in the context of AD.

Regarding inflammation, we found three upregulated genes in patients with severe OSA that are involved in NF-κB pathways: the *RELB* gene, which encodes a transcription factor in the noncanonical NF-κB pathway; the *RAF1* proto-oncogene, encoding a serine/threonine protein kinase in the MAPK/ERK pathway; and *TNFSF14*, which encodes a TNF ligand family member. The upregulation of these genes suggests a major activation of the NF-κB pathway in the blood of patients with severe OSA compared with those with nonsevere form of OSA. OSA is related to low-grade systemic inflammation,[16] and NF-κB is a central factor in the inflammatory cascade. Supporting this notion, several studies link OSA to NF-κB activation and subsequent neuroinflammation and OSA-associated cognitive dysfunction in nonAD patients.[40–43] In addition, our study showed that in AD patients with severe OSA, *ALOX12* and *PTGS2*, both of which are inflammatory and redox genes involved in prostaglandin production, were upregulated. It has been reported that intermittent hypoxia leads to an increase in the synthesis of COX-2 (the protein encoded by *PTGS2*) which results in the increase of prostaglandin E2 synthesis.[44]

Concerning oxidative stress, we observed overexpression of *DUSP1*, the gene encoding dual specificity phosphatase 1, which plays an important role in the cellular response to environmental stress; *GSR*, the gene encoding glutathione reductase, which is critical for resisting oxidative stress and maintaining the reducing environment of the cell; *PDLIM1*, the gene encoding regulatory adapter proteins for the cytoskeleton; and *SOD2*, the gene encoding superoxide dismutase 2, which plays an antiapoptotic role to protect against oxidative stress by clearing mitochondrial ROS.[45] Most of the upregulated redox genes

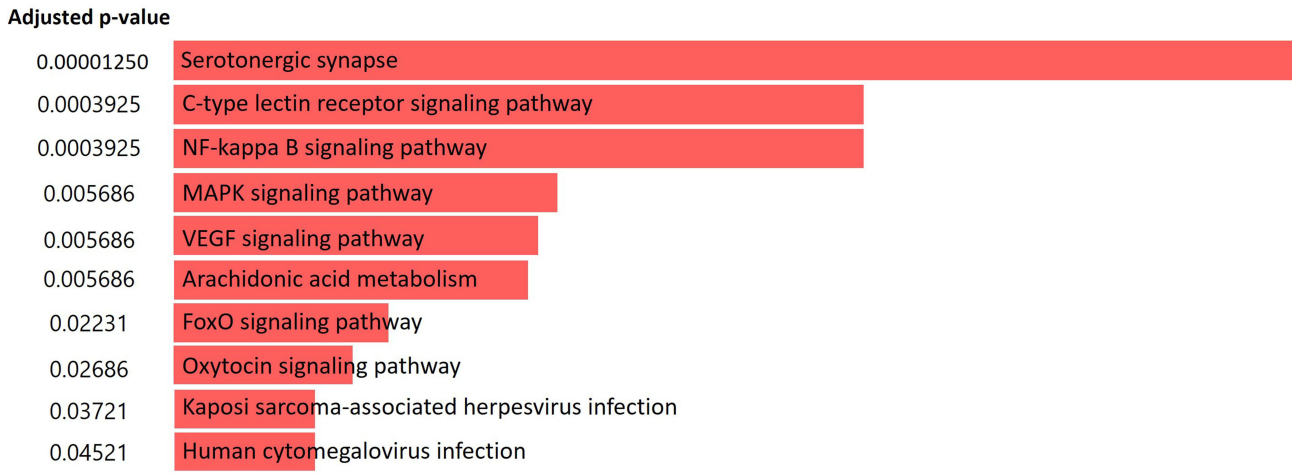

Figure 3 Pathway analysis of differentially expressed genes (FC ≥ 1.5 and *p* values < 0.05).

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in patients with severe OSA are related to the cellular response to environmental stress, which suggests a direct relationship between OSA severity and the level of oxidative stress. Cells potentially increase the expression of genes related to resistance to oxidative stress as a defense strategy because of a major need for antioxidant protection. Interestingly, *DUSP1* was previously found as a hub gene between OSA and AD pathologies.[24] Analogously, *SOD2* was also found to be upregulated in blood samples from nonAD patients with OSA compared with healthy controls.[46]

Among the differentially expressed genes, *GSR, TNFSF14* and *ALOX12* significantly correlated with PSG parameters. However, no significant associations were observed for AD biomarkers or the MMSE score, suggesting that AD pathological hallmarks were not related to the differential expression of these genes.

After conducting a pathway enrichment analysis, we found a strong enrichment of the serotonergic synapse pathway. This result is consistent with those of Jagannathan et al, who used the same analytical tool and found that the serotonin pathway was the most altered OSA-related pathway in the nonAD population.[47] Besides its role as a neurotransmitter, serotonin also plays other physiological roles such as the contribution to vasodilation or the affectation of the central respiratory drive.[48,49] Serotonin plays important roles in ventilatory stimulation and the regulation of sleep/wake cycles.[50] Therefore, our results suggest a possible link between this neurotransmitter and the severity of OSA. Due to the serotonin multiple physiological and psychological implications, this link would deserve future research. Differentially expressed genes were also found to be significantly enriched in the C-type lectin receptor signalling pathway, which is related to immune responses and has been shown to activate the NF-κB pathway.[51]

We think that our findings in differential expression of genes between OSA severities in AD patients can potentially be valuable for diagnostic purposes. The PSG is the gold standard for diagnosis of OSA but has some limitations because of the difficulties in its performance, especially in patients with cognitive deterioration. Therefore, a screening analysis through whole blood samples will be a more convenient and less costly procedure for diagnosis of severe OSA in AD. Moreover, OSA is not the only sleep disorder related to AD, and other studies have previously described abnormal expression of genes or shared hub genes between sleep disorders and AD pathology.[52,53]

Our study has some strengths, such as the use of PSG as a diagnostic method for OSA. This method is the gold standard for the detection of OSA and permitted us to evaluate the correlation between the differentially expressed genes and the PSG parameters of the patients. In addition, our study population was assessed for CSF AD biomarkers, ensuring that the patients had both clinical and biological evidence of AD. However, the sample size is relatively small, and thus the results of the present study should be validated in a larger cohort of AD patients with different AD severities. Our results can be affected by confounding factors such as lifestyle and obesity that were not considered in this study. We evaluated neither the downstream protein expression nor longitudinal alterations. These assessments could support our findings and clarify how gene expression changes influence the progression of AD patients with severe OSA. However, our results could represent a starting point for developing future studies to evaluate whether treatment of severe OSA could reverse the upregulation of these genes in patients with AD. Friščić et al as well as Al-Halawani et al found a positive effect of CPAP therapy on chronic inflammatory states.[54,55] In other studies, CPAP treatment also decreased the blood levels of inflammatory and oxidative stress markers in severe OSA patients.[56,57] However, Christensson et al demonstrated that although changes in whole blood transcriptome in patients can be normalized after three months of CPAP treatment, there is a return to untreated OSA gene expression after 12 months of effective CPAP treatment.[58] Therefore, our findings regarding the differential expression of genes between OSA severities in AD patients could also have significant clinical relevance.

In conclusion, our findings highlight the upregulation of a panel of genes involved in NF-κB-mediated inflammation and redox metabolism in the blood of patients with mild AD diagnosed with severe OSA, suggesting that severe OSA can worsen the inflammation and oxidative damage already altered in patients with AD. Therefore, OSA treatment could perhaps reduce the impact of these two pathological events on the AD population, which suffers from a high prevalence of OSA.

Data Sharing Statement

The data reported in this manuscript are available within the article and/or its supplementary data. Additional data from NCT02814045 will be shared by the corresponding author with request from any qualified investigator.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Care Ethics Committee (CE-1218).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests in this work.

References

1. Lane CA, Hardy J, Schott JM. Alzheimer's disease. *Eur J Neurol*. 2018;25:59–70. doi:10.1111/ene.13439
2. Ferrer I. Hypothesis review: Alzheimer's overture guidelines. *Brain Pathol*. 2023;33(1). doi:10.1111/BPA.13122
3. Budd Haeberlein S, Aisen PS, Barkhof F, et al. Two randomized phase 3 studies of aducanumab in early Alzheimer's disease. *J Prev Alzheimers Dis*. 2022;9(2):197–210. doi:10.14283/JPAD.2022.30
4. van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. 2023;388(1):142–143. doi:10.1056/NEJMOA2212948
5. Sims JR, Zimmer JA, Evans CD, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA*. 2023;330(6):512–527. doi:10.1001/JAMA.2023.13239
6. Leng Y, McEvoy CT, Allen IE, Yaffe K. Association of sleep-disordered breathing with cognitive function and risk of cognitive impairment: a systematic review and meta-analysis. *JAMA Neurol*. 2017;74(10):1237. doi:10.1001/JAMANEUROL.2017.2180
7. Emamian F, Khazaie H, Tahmasian M, et al. The association between obstructive sleep apnea and Alzheimer's disease: a meta-analysis perspective. *Fron Aging Neurosci*. 2016;8:78. doi:10.3389/fnagi.2016.00078
8. Arnaud C, Bochaton T, Pépin JL, Belaidi E, Belaidi E. Obstructive sleep apnoea and cardiovascular consequences: pathophysiological mechanisms Syndrome d'apnées obstructives du sommeil et conséquences cardiovasculaire: mécanismes physiopathologiques. *Arch Cardiovasc Dis*. 2020;113:350–358. doi:10.1016/j.acvd.2020.01.003
9. Gaeta AM, Benítez ID, Jorge C, et al. Prevalence of obstructive sleep apnea in Alzheimer's disease patients. *J Neurol*. 2020;267:1012–1022. doi:10.1007/s00415-019-09668-4
10. Bonsignore MR, Baiamonte P, Mazzuca E, Castrogiovanni A, Marrone O. Obstructive sleep apnea and comorbidities: a dangerous liaison. *Multidiscip Respir Med*. 2019;14:8. doi:10.1186/s40248-019-0172-9
11. Osorio RS, Ayappa I, Mantua J, et al. The interaction between sleep-disordered breathing and apolipoprotein E genotype on cerebrospinal fluid biomarkers for Alzheimer's disease in cognitively normal elderly individuals. *Neurobiol Aging*. 2014;35(6):1318–1324. doi:10.1016/j.neurobiolaging.2013.12.030
12. Buratti L, Viticchi G, Falsetti L, et al. Vascular impairment in Alzheimer's disease: the role of obstructive sleep apnea. *J Alzheimer's Dis*. 2014;38:445–453. doi:10.3233/JAD-131046

13. Roy B, Nunez A, Aysola RS, Kang DW, Vacas S, Kumar R. Impaired glymphatic system actions in obstructive sleep apnea adults. *Front Neurosci*. 2022;16:884234. doi:10.3389/FNINS.2022.884234
14. Arnaud C, Beguin PC, Lantuejoul S, et al. The inflammatory preatherosclerotic remodeling induced by intermittent hypoxia is attenuated by RANTES/CCL5 inhibition. *Am J Respir Crit Care Med*. 2011;184(6):724–731. doi:10.1164/RCCM.201012-2033OC
15. Gras E, Belaidi E, Briançon-Marjollet A, Pépin JL, Arnaud C, Ribuot DG. Endothelin-1 mediates intermittent hypoxia-induced inflammatory vascular remodeling through HIF-1 activation. *J Appl Physiol*. 2016;120(4):437–443. doi:10.1152/JAPPLPHYSIOL.00641.2015/ASSET/IMAGES/LARGE/ZDG0041617150005.JPEG
16. Jullian-Desayes I, Joyeux-Faure M, Tamisier R, et al. Impact of obstructive sleep apnea treatment by continuous positive airway pressure on cardiometabolic biomarkers: a systematic review from sham CPAP randomized controlled trials. *Sleep Med Rev*. 2015;21:23–38. doi:10.1016/J.SMRV.2014.07.004
17. Dakterzada F, Jové M, Cantero JL, Pamplona R, Piñoll-Ripoll G. Plasma and cerebrospinal fluid nonenzymatic protein damage is sustained in Alzheimer's disease. *Redox Biol*. 2023;64:102772. doi:10.1016/J.REDOX.2023.102772
18. Checa J, Aran JM. Reactive oxygen species: drivers of physiological and pathological processes. *J Inflamm Res*. 2020;13:1057–1073. doi:10.2147/JIR.S275595
19. Jové M, Mota-Martorell N, Torres P, et al. Life the causal role of lipoxidative damage in mitochondrial bioenergetic dysfunction linked to Alzheimer's disease pathology. *Life*. 2021;11(5):388. doi:10.3390/life11050388
20. Nantachai G, Vasupanrajit A, Tunvirachaisakul C, Solmi M, Maes M. Oxidative stress and antioxidant defenses in mild cognitive impairment: a systematic review and meta-analysis. *Ageing Res Rev*. 2022;79:101639. doi:10.1016/j.arr.2022.101639
21. Schrag M, Mueller C, Zabel M, et al. Oxidative stress in blood in Alzheimer's disease and mild cognitive impairment: a meta-analysis. *Neurobiol Dis*. 2013;59:100–110. doi:10.1016/J.NBD.2013.07.005
22. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement*. 2018;4:575–590. doi:10.1016/J.TRCI.2018.06.014
23. Yokoe T, Minoguchi K, Matsuo H, et al. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. *Circulation*. 2003;107(8):1129–1134. doi:10.1161/01.CIR.0000052627.99976.18
24. Wu L, Wang W, Tian S, Zheng H, Liu P, Wu W. Identification of hub genes in patients with Alzheimer disease and obstructive sleep apnea syndrome using integrated bioinformatics analysis. *Int J Gen Med*. 2021;14:9491–9502. doi:10.2147/IJGM.S341078
25. Milanesi E, Dobre M, Cucos CA, et al. Whole blood expression pattern of inflammation and redox genes in mild Alzheimer's disease. *J Inflamm Res*. 2021;14:6085–6102. doi:10.2147/JIR.S334337
26. Gharib SA, Hayes AL, Rosen MJ, Patel SR. A pathway-based analysis on the effects of obstructive sleep apnea in modulating visceral fat transcriptome. *Sleep*. 2013;36(1):23–30. doi:10.5665/sleep.2294
27. Jorge C, Targa A, Benítez ID, et al. Obstructive sleep apnoea and cognitive decline in mild-to-moderate Alzheimer's disease. *Eur Respir J*. 2020;56(5):2000523. doi:10.1183/13993003.00523-2020
28. Mckhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement*. 2011;7(3):263–269. doi:10.1016/j.jalz.2011.03.005
29. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12(3):189–198. doi:10.1016/0022-3956(75)90026-6
30. Berry RB, Budhiraja R, Gottlieb DJ, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM manual for the scoring of sleep and associated events. deliberations of the sleep apnea definitions task force of the American Academy of Sleep Medicine. *J Clin Sleep Med*. 2012;8(5):597–619. doi:10.5664/jcsm.2172
31. Berry RB, Brooks R, Gamaldo C, et al. AASM scoring manual updates for 2017 (Version 2.4). J. *Clin Sleep Med*. 2017;13(5):665–666. doi:10.5664/jcsm.6576
32. Ortega RL, Dakterzada F, Arias A, et al. Usefulness of CSF biomarkers in predicting the progression of amnesic and nonamnesic mild cognitive impairment to Alzheimer's disease. *Curr Aging Sci*. 2019;12(1):35–42. doi:10.2174/1874609812666190112095430
33. Iber C, Ancoli-Israel S, Chesson A, Quan SF. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specification. *J Clin Sleep Med*. 2007;2007:1–49.
34. Milanesi E, Manda G, Dobre M, et al. Distinctive under-expression profile of inflammatory and redox genes in the blood of elderly patients with cardiovascular disease. *J Inflamm Res*. 2021;14:429–442. doi:10.2147/JIR.S280328
35. Chen EY, Tan CM, Kou Y, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinf*. 2013;14:128. doi:10.1186/1471-2105-14-128
36. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. 2016;44(1):W90–W97. doi:10.1093/NAR/GKW377
37. Xie Z, Bailey A, Kuleshov MV, et al. Gene set knowledge discovery with enrichr. *Curr Protoc*. 2021;1(3). doi:10.1002/CPZ1.90
38. Osorio RS, Gumb T, Pirraglia E, et al. Sleep-disordered breathing advances cognitive decline in the elderly. *Neurology*. 2015;84(19):1964–1971. doi:10.1212/WNL.0000000000001566
39. Yaffe K, Laffan AM, Harrison SL, et al. Sleep-disordered breathing, hypoxia, and risk of mild cognitive impairment and dementia in older women. *JAMA*. 2011;306(6):613–619. doi:10.1001/JAMA.2011.1115
40. Shen C, Liu X, Liu T, Ou Y, Ouyang R. IL-33/ST2 mediating systemic inflammation and neuroinflammation through NF-kB participated in the neurocognitive impairment in obstructive sleep apnea. *Int Immunopharmacol*. 2023;115:109604. doi:10.1016/j.intimp.2022.109604
41. Greenberg H, Ye X, Wilson D, Htoo AK, Hendersen T, Liu SF. Chronic intermittent hypoxia activates nuclear factor-κB in cardiovascular tissues in vivo. *Biochem Biophys Res Commun*. 2006;343:591–596. doi:10.1016/j.bbrc.2006.03.015
42. Htoo AK, Greenberg H, Tongia S, et al. Activation of nuclear factor κB in obstructive sleep apnea: a pathway leading to systemic inflammation. *Sleep Breath*. 2006;10:43–50. doi:10.1007/s11325-005-0046-6
43. Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation*. 2005;112(17):2660–2667. doi:10.1161/CIRCULATIONAHA.105.556746

44. Picado C, Roca-Ferrer J. Role of the cyclooxygenase pathway in the association of obstructive sleep apnea and cancer. *J Clin Med*. 2020;9(10):1–19. doi:10.3390/JCM9103237
45. Suthammarak W, Somerlot BH, Opheim E, Sedensky M, Morgan PG. Novel interactions between mitochondrial superoxide dismutases and the electron transport chain. *Aging Cell*. 2013;12(6):1132–1140. doi:10.1111/ACEL.12144
46. Hoffmann MS, Singh P, Wolk R, Romero-Corral A, Raghavakaimal S, Somers VK. Microarray studies of genomic oxidative stress and cell cycle responses in obstructive sleep apnea. *Antioxid Redox Signal*. 2007;9(6):661–669. doi:10.1089/ARS.2007.1589
47. Jagannathan R, Seixas A, St-Jules D, et al. Systems biology genetic approach identifies serotonin pathway as a possible target for obstructive sleep apnea: results from a literature search. *Sleep Disord*. 2017;2017:6768323. doi:10.1155/2017/6768323
48. Halperin D, Reber G. Influence of antidepressants on hemostasis. *Dialogues Clin Neurosci*. 2007;9(1):47–59. doi:10.31887/DCNS.2007.9.1/dhalperin
49. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. *Annu Rev Med*. 2009;60:355–366. doi:10.1146/annurev.med.60.042307.110802
50. Maierean AD, Bordea IR, Salagean T, et al. Polymorphism of the serotonin transporter gene and the peripheral 5-hydroxytryptamine in obstructive sleep apnea: what do we know and what are we looking for? A systematic review of the literature. *Nat Sci Sleep*. 2021:13–125. doi:10.2147/NSS.S278170.
51. Kingeter LM, Lin X. C-type lectin receptor-induced NF-κB activation in innate immune and inflammatory responses. *Cell mol Immunol*. 2012;9(2):105–112. doi:10.1038/CMI.2011.58
52. Niu L, Zhang F, Xu X, et al. Chronic sleep deprivation altered the expression of circadian clock genes and aggravated Alzheimer's disease neuropathology. *Brain Pathol*. 2022;32(3):e13028. doi:10.1111/bpa.13028
53. Liang L, Yan J, Huang X, et al. Identification of molecular signatures associated with sleep disorder and Alzheimer's disease. *Front Psychiatry*. 2022;13:925012. doi:10.3389/fpsyt.2022.925012
54. Friščić T, Perčić M, Vidović D, Štajduhar A, Galić E. Impact of CPAP therapy on new inflammation biomarkers. *J Clin Med*. 2022;11(20):6113. doi:10.3390/jcm11206113
55. Al-Halawani M, Kyung C, Liang F, et al. Treatment of obstructive sleep apnea with CPAP improves chronic inflammation measured by neutrophil-to-lymphocyte ratio. *J Clin Sleep Med*. 2020;16(2):251–257. doi:10.5664/jcsm.8176
56. Karamanlı H, Özol D, Ugur KS, et al. Influence of CPAP treatment on airway and systemic inflammation in OSAS patients. *Sleep Breath*. 2014;18(2):251–256. doi:10.1007/s11325-012-0761-8
57. Jurado-Gámez B, Fernandez-Marin MC, Gómez-Chaparro JL, et al. Relationship of oxidative stress and endothelial dysfunction in sleep apnoea. *Eur Respir J*. 2011;37(4):873–879. doi:10.1183/09031936.00027910
58. Christensson E, Mkrtchian S, Ebberyd A, et al. Whole blood gene expression signature in patients with obstructive sleep apnea and effect of continuous positive airway pressure treatment. *Respir Physiol Neurobiol*. 2021;294:103746. doi:10.1016/j.resp.2021.103746