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Obstructive Sleep Apnea Associated with Periodontitis: Evidence from Mendelian Randomization and Salivary Proteomics

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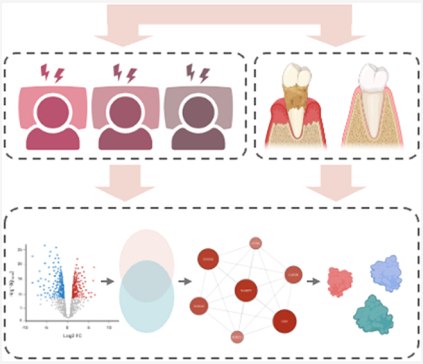
ABSTRACT: Clinical evidence supports a link between obstructive sleep apnea (OSA) and periodontitis. However, whether OSA exerts a causal effect on periodontitis and the underlying protein biomarkers remains unclear. This study aimed to investigate their causal relationship and to identify salivary protein markers involved in the shared pathogenesis. First, a bidirectional two-sample Mendelian randomization (MR) analysis using genome-wide association study (GWAS) statistics was conducted to evaluate the causal relationship. Second, data-independent acquisition (DIA)-based salivary proteomics was applied to identify differentially expressed proteins in periodontally healthy patients with OSA (n = 30) and controls (n = 10). The OSA-associated upregulated proteins were screened for consistency with OSA severity and overlap with an independent periodontitis cohort. Network and hub protein analyses were performed. Genetic analysis in the MR suggested that OSA could statistically contribute to periodontitis development, but not in the reverse direction. Salivary proteomics revealed severity-dependent expression changes in OSA, with upregulated proteins enriched in the antigen processing and presentation pathway. Eight proteins detected in OSA patients showed increased expression with periodontal destruction. NAMPT, GSN, and S100A8/A9 emerged as hub proteins, validated by ELISA in independent OSA-associated periodontitis patients. The integrative genetic and proteomic investigations support a potential causal and proteome association between OSA and periodontitis, highlighting NAMPT, GSN, and S100A8/A9 as candidate mediators that may underpin the inflammatory interplay.



KEYWORDS: *sleep-disordered breathing, periodontal inflammation, protein, biomarker, causal association*

1. INTRODUCTION

Periodontitis is a chronic inflammatory disease that causes progressive destruction of the tissues supporting teeth, leading to alveolar bone resorption and, in severe cases, tooth loss.[1] As a constant potential source of infection, periodontitis has been considered a risk factor for cardiovascular diseases, cerebrovascular diseases, and respiratory diseases.[2] Moreover, periodontitis was also reported to affect the onset and development of systemic inflammatory conditions, including arthritis and type 2 diabetes mellitus.[3]

Obstructive sleep apnea (OSA) and periodontitis are both chronic diseases that cause a tremendous global burden.[4,5] OSA is characterized by repetitive upper airway collapse during sleep, leading to intermittent hypoxia, systemic inflammation, and oxidative stress.[6] Emerging clinical evidence suggests a potential link between OSA and periodontitis, with OSA patients exhibiting increased risk of periodontal tissue destruction.[7−9] Previous studies have mainly reported the coexistence of periodontitis and OSA; however, whether OSA exerts a causal influence on periodontitis and the underlying molecular mechanisms remains to be elucidated.

Previous studies have demonstrated that OSA can trigger systemic inflammatory pathways, while periodontitis is driven by dysregulated immune responses and altered oral microenvironments.[10] The treatment of OSA has systemic improvements in inflammation, which could benefit the oral microenvironment and periodontal treatment efficacy.[11] On the other hand, healthy periodontal structures could provide enough anchorage for mandibular advancement devices (MADs) and the nasal mask of positive airway pressure (PAP) to treat OSA.[12] Collectively, these observations suggest that OSA and periodontitis may be interconnected through shared pathophysiological pathways and mutually influential clinical management strategies. However, the extent of this relationship and the specific molecular mediators involved remain largely unexplored.

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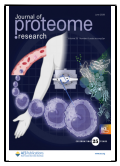

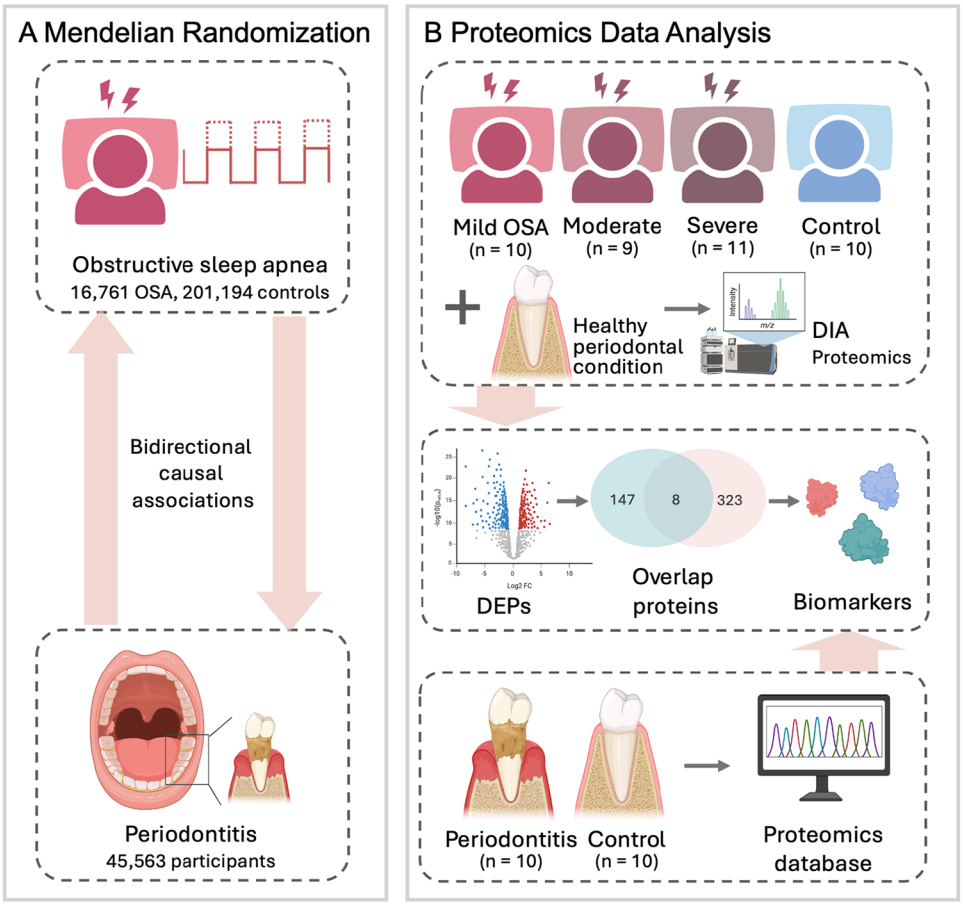

Figure 1. Overview of the study design OSA: obstructive sleep apnea; DIA: data-independent acquisition; DEPs: differentially expressed proteins. Created in BioRender. Yu, V. (2026) https://BioRender.com/xorai03.

To address these gaps, first, we applied bidirectional Mendelian randomization (MR) analyses to assess the causal relationship between OSA and periodontitis using genetic instruments derived from genome-wide association studies (GWAS). Second, we performed salivary proteomic profiling in patients with OSA and healthy periodontal conditions to identify proteins that are also upregulated in independent periodontitis proteomic data sets. By integrating causal inference and molecular profiling, this study aims to provide insights into the relationship between OSA and periodontitis, and to identify overlapping upregulated proteins as potential biomarkers linking the two diseases.

2. EXPERIMENTAL SECTION

2.1. Study Design

The study design is illustrated in Figure 1, which outlines (1) a bidirectional MR study analyzing the causal association between OSA and periodontitis; (2) based on the causal direction, salivary proteome characteristics in patients with OSA using data-independent acquisition (DIA) mass spectrometry (MS), and overlap of consistent upregulated proteins with periodontitis patients from the proteomics identification database (PRIDE).[13]

2.2. Mendelian Randomization

The causal association from OSA to periodontitis was investigated using the genetic variants on sleep apnea obtained from the FinnGen Consortium (G6), which was derived from 16,761 cases with OSA and 201,194 controls (https://www.finngen.fi). The diagnosis of OSA was made by the hospital discharge or cause of death registration with the International Classification of Diseases (ICD-10 G47.3 and ICD-9 3472). The periodontitis summary genetic variants were derived from the Gene-Lifestyle Interaction in the Dental End points (GLIDE) Consortium by Shungin et al.,[14] with 17,353 cases and 28,210 controls. Periodontitis was diagnosed using criteria by the Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP),[15] or two or more sextants had a probing depth of at least 5.5 mm, or data retrieved from diagnosis codes in hospital registers.

The causal association from periodontitis to OSA was assessed using the genetic variants on the FinnGen Consortium, which consisted of 4434 chronic periodontitis cases and 259,234 controls. The diagnosis of periodontal diseases was defined using the ICD-10 K05.3 codes by dentists or dental hygienists. The OSA summary genetic variants were derived from a meta-analysis of GWAS (n = 5727) by Chen et al.,[16] using single nucleotide polymorphisms (SNPs) associated with AHI in European Americans.

The instrument selection is detailed in the Supporting Methods. The random-effect inverse variance weighted (IVW) method was used as the primary analysis to identify significant causal associations with $p < 0.05$.[17,18] MR-Egger[19] and weighted median estimation[20] were referred to provide more robust estimates in a broader set of scenarios to improve the IVW estimates. The presence of heterogeneity was assessed with the Cochran's Q test, with Cochran-Q derived $p < 0.05$ and $I^2 > 25\%$ recognized as existing heterogeneity.[21] The pleiotropy was tested using the MR-Egger intercept and MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO). Leave-one-out analysis, the Steiger directionality test, and radial MR were performed as sensitivity analyses.

2.3. Study Participants

This research was conducted at the Department of Orthodontics, Center for Oral Therapy of OSA, Peking University Hospital of

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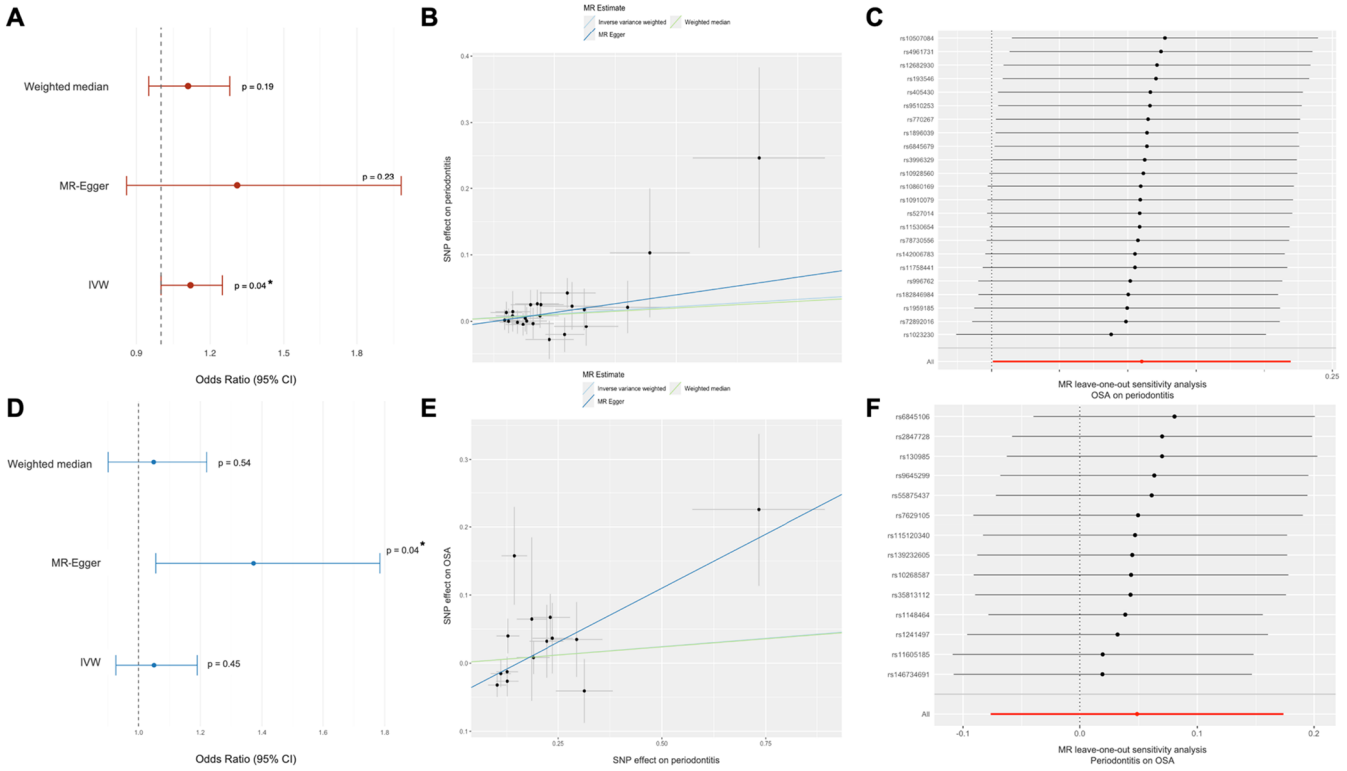

Figure 2. Bidirectional causal effect of OSA and periodontitis by Mendelian randomization analysis (A) Forest plot showing genetically predicted OSA causally associated with increased risk for periodontitis, reaching statistical significance in the IVW method, consistent direction in all three methods; (B) The scatter plot of SNP-specific effects with IVW, weighted median, and MR-Egger regression lines for the causal effect of OSA on periodontitis.; (C) The leave-one-out sensitivity analysis for the causal effect of OSA on periodontitis; (D) Forest plot showing insignificant causality of periodontitis on OSA in the IVW method; (E) The scatter plot of SNP-specific effects with IVW, weighted median, and MR-Egger regression lines for the causal effect of periodontitis on OSA; (F) The leave-one-out sensitivity analysis for the causal effect of periodontitis on OSA. OSA: obstructive sleep apnea; IVW: inverse-variance weighted.

Stomatology, with ethical approval from the Institutional Review Board of Peking University School of Stomatology (PKUSSIRB-2019115009) and registration in the Chinese Clinical Trial Registry (ChiCTR-IND-17013232). The study followed the principles of the Declaration of Helsinki, and all participants provided written informed consent.

Patients with OSA were diagnosed with Type 1 polysomnography (PSG) at the Sleep Division of Peking University People's Hospital, from October 2018 to July 2019. Details of the PSG recording methodology have been described previously.[22] The apnea-hypopnea index (AHI) was calculated as the number of apnea and hypopnea events per hour of sleep. Patients with AHI 5 to 15, 15 to 30, and >30 events/h were classified as mild, moderate, and severe OSA, respectively. The nonsnoring control group included volunteers matching the OSA group in age and sex. The inclusion criteria of the OSA group were as follows: (1) age >18y, male, diagnosed with OSA, without coexisting sleep disorders; (2) without systemic diseases (hypertension, diabetes, anxiety, etc.); (3) consent to participate. For the control group, the criteria included: (1) no self-reported or previously diagnosed sleep disorders, such as insomnia or poor sleep quality; (2) no evidence of sleep disorders on Type 3 home sleep apnea tests (HSAT); and (3) consent to participate. Participants with inflammatory oral mucosa, bleeding gums, and dental defects were excluded.

Patients with periodontitis (n = 10) and controls (n = 10) were retrieved from the study by Belstrom et al.,[23] in which periodontitis was defined as bleeding on probing ≥25% of total sites, a minimum of two teeth with clinical attachment level ≥4 mm, and a minimum of two teeth with probing depth ≥6 mm.

2.4. DIA Proteomics

Saliva samples were collected between 6:00 and 7:00 AM immediately after PSG or HSAT monitoring, following protocols consistent with previous studies.[24−27] All the samples were analyzed in the same run to minimize batch effects.

The detailed DIA-MS proteomics methods were described elsewhere.[28] Briefly, proteins were extracted using the SDT buffer (4%SDS, 100 mM Tris−HCl, pH 7.6), quantified by the BCA assay, and evaluated by SDS-PAGE. Protein digestion was performed with trypsin following the filter-aided sample preparation (FASP) protocol. Pooled peptides were fractionated into 10 components with a high-pH reversed-phase kit (Thermo), desalted using C18 cartridges, lyophilized, and reconstituted in 0.1% formic acid. Peptide concentration was measured by OD280, and indexed retention time (iRT) standards were added before liquid chromatography (LC)-MS analysis.

For library construction, DDA data were acquired using an EvoSep One system coupled to a timsTOF Pro mass spectrometer (Bruker). Ionization was in positive mode (100−1700 Da), with a mobility range of 0.75−1.35 Vs/cm^2 and PASEF settings of eight MS/MS scans and 24 s dynamic exclusion. Quantitative DIA analysis was conducted on the same LC-MS platform under 4D-DIA mode (four TIMS windows, 100 ms accumulation each). Collision energy was linearly ramped from 59 eV ($1/K_0$ = 1.30 Vs/cm^2) to 20 eV ($1/K_0$ = 0.85 Vs/cm^2). DDA spectra were searched in Spectronaut (v14.4, Biognosys) against the UniProt human proteome appended with iRT peptides. Search parameters: enzyme = trypsin, max 1 missed cleavage, fixed = carbamidomethyl (C), variable = oxidation (M) and acetyl (N-term), false discovery rate (FDR) < 1% and a 99% confidence level for protein identification. DIA raw data were analyzed using the same software with dynamic iRT, MS2 interference

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Table 1. Demographic and Polysomnographic Characteristics in Patients with OSA[d]

parameters	control (n = 10)	mild OSA (n = 10)	moderate OSA (n = 9)	severe OSA (n = 11)	OSA (n = 30)
Age (y)	38.6 ± 4.5	34.7 ± 4.3	43.2 ± 11.0	42.5 ± 7.5	40.1 ± 9.4
BMI (kg/m^2)	24.8 ± 2.2	25.9 ± 2.6	28.8 ± 3.1[a]	28.6 ± 3.7[a]	27.8 ± 3.5[a]
ESS	7.8 ± 4.8	8.2 ± 4.1	10.9 ± 7.1	15.1 ± 3.6[a,b]	11.5 ± 5.8
AHI (events/h)	3.7 ± 1.3	10.5 ± 1.6	23.4 ± 4.3[a,b]	47.1 ± 15.6[a,b,c]	27.8 ± 18.4[a]
$LSpO_2$ (%)	91.6 ± 2.2	80.6 ± 24.2	86.3 ± 3.8	70.7 ± 12.3[a,c]	78.7 ± 17.3[a]

[a]p < 0.05, comparisons between control and mild OSA. [b]p < 0.05, comparisons between moderate/severe OSA and mild OSA. [c]p < 0.05, comparisons between severe and moderate OSA. [d]OSA: obstructive sleep apnea; BMI: body mass index; ESS: Epworth sleepiness scale; AHI: apnea-hypopnea index; $LSpO_2$: lowest oxygen saturation during sleep.

correction, and cross-run normalization enabled. Peptides with Q < 0.01 were retained, and protein abundances were calculated using the MaxLFQ algorithm. For missing values in the DIA proteomics data, zero imputation was applied. ELISA kits were applied to detect the levels of potential hub proteins (USCN and RUIXIN biotech) following the manufacturer's protocol.

The LC-MS proteomics data of periodontitis were downloaded from the ProteomeXchange Consortium via the PRIDE[13] partner repository with the data set identifier PXD004319. The LC-MS proteomics data of OSA were deposited via iProX[29] with the Project ID IPX001463200.

2.5. Bioinformatics Analysis

Proteins exhibiting a fold change (FC) greater than 1.5 or less than 0.67 and a q-value using the Storey method below 0.05 were regarded as significantly altered, to control the FDR. A permutation test was conducted to evaluate the significance of group differences in protein expression between the control and severe OSA groups. For each protein, a two-sample t-test was performed on the original data, followed by 1000 permutations of group labels to generate a null distribution of t-statistics. Differentially expressed proteins (DEPs) were visualized through volcano plot representations. Functional enrichment of DEPs was carried out with Blast2GO (https://www.blast2go.com/), focusing on level-2 Gene Ontology (GO) terms across three categories: biological process, molecular function, and cellular component. Pathway annotation was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://geneontology.org/). The hub value of a protein was defined as the sum of its correlation coefficients with all other proteins in the data set. Intergroup functional differences were evaluated using Fisher's exact test, with statistical significance set at p < 0.05. The statistical power for the identified hub proteins was calculated using Cohen's d and two-sample t-tests.

2.6. Statistical Analysis

All the statistical analyses were performed using R 4.3.0 (the R Foundation for Statistical Computing, Vienna, Austria). Intergroup comparisons were conducted using one-way ANOVA with Tukey's multiple comparison or an independent t-test. Spearman correlation was analyzed between protein abundance and clinical variables, with the Benjamini-Hochberg false discovery rate for multiple testing. A p-value less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Mendelian Randomization

The results of the MR analysis are shown in Figure 2. In total, 23 SNPs were selected to predict OSA (F-statistics ranging from 20.85 to 44.31), and 14 SNPs for periodontitis (F-statistics ranging from 20.87 to 28.14). The harmonized data were summarized in Tables S1 and S2. Genetically predicted SNPs associated with OSA were statistically associated with periodontitis in the IVW method (p = 0.04, OR = 1.12), but not in other methods (Figure 2A,B). However, the directions and magnitude among the three MR methods were consistent, indicating that patients with OSA might have an increased risk of periodontitis. No significant heterogeneity (Cochran's Q p = 0.94) or pleiotropy was observed (MR-Egger intercept p = 0.46, MR-PRESSO global test p = 0.95). The leave-one-out analysis showed that the causal estimates remained consistent in direction and magnitude after sequentially removing each SNP (Figure 2C). Most SNPs demonstrated a consistent directionality in the Steiger directionality test (Table S1). In the radial MR analysis, exclusion of the top contributing variants did not materially alter the IVW estimate (p = 0.039, OR = 0.125).

However, the causality from periodontitis to OSA did not reach statistical significance in the IVW method (p = 0.45), although the directionality in all three methods was consistent (Figure 2D,E). No significant heterogeneity (Cochran's Q p = 0.05) or pleiotropy was observed (MR-Egger intercept p = 0.47, MR-PRESSO global test p = 0.07). The leave-one-out analysis showed that estimates remained consistent in direction and magnitude (Figure 2F).

3.2. Study Participants

The demographic and polysomnographic characteristics of the patients with OSA are demonstrated in Table 1. Patients with OSA were aged 40.1 ± 9.4y, with an average AHI of 27.8 ± 18.4 events/h and the lowest oxygen saturation ($LSpO_2$) of 78.7 ± 17.3%. There were 10, 9, and 11 patients in the mild, moderate, and severe OSA groups, respectively. The mean age of the periodontitis group was 53.5y (range: 42 to 70y), and the average age of the control group was 27.1y (range: 24 to 39y). The characteristics of study participants in the periodontitis group were detailed elsewhere.[30]

3.3. Characterization of Salivary Proteins in OSA

Using DIA technology, a total of 405 proteins were identified, corresponding to 90.2% of the proteins in the DDA library. The principal component analysis (PCA) plot showed partial separation between OSA and the control group, particularly as the severity of OSA increased (Figure 3A). There were 97 proteins that demonstrated a permutation test p-value below 0.05. DEPs between severe OSA and the control group were identified with an FC > 1.5 or <0.67, and Storey q-value <0.05, and are illustrated in Figure 3B. There were 30 and 6 proteins significantly upregulated and downregulated, respectively. The top 20 upregulated proteins are summarized in Table S3. As Figure 3C shows, proteins with consistent relative abundance across various severities of OSA groups were further selected. Fifteen proteins, including S100A8, S100A9, NAMPT, HSPA5, SH3BGRL, SPINK5, KLK13, MYL6, HSPE1, CTSS, SH3BGRL3, CAPZB, H2BC12, PRDX1, and GSN, were selected, although intergroup comparisons did not reach statistical significance in all proteins (Figure 3D).

GO and KEGG analyses were employed to characterize the potential function of the selected 15 DEPs (Figure 3E). The

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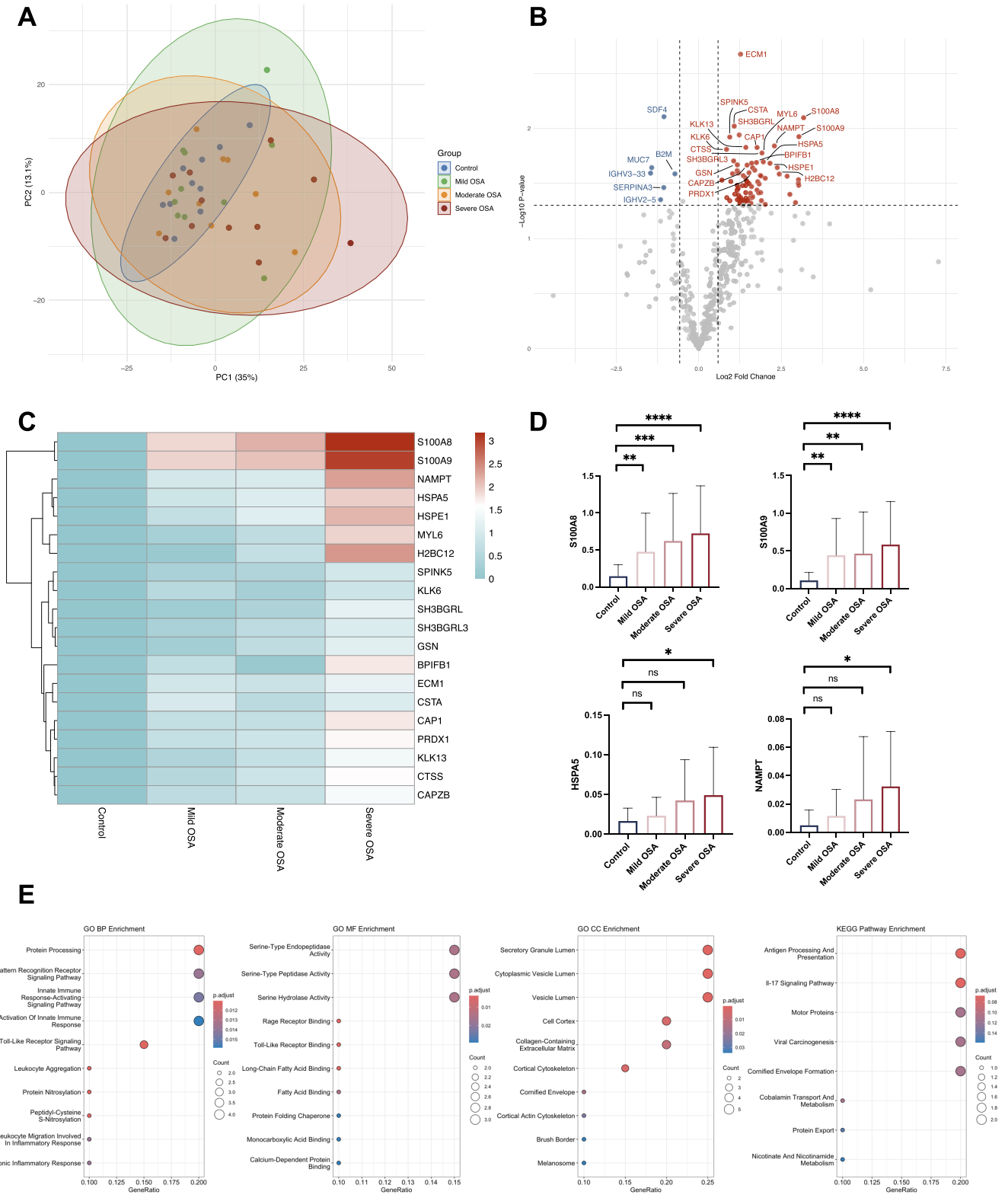

Figure 3. Salivary proteomic characterization in patients with OSA (A) The principal component analysis (PCA) of the OSA and control groups showing partial intergroup separations; (B) Differentially expressed proteins between severe OSA and control groups illustrated by a volcano plot (Storey q-value <0.05, fold change >1.5 or <0.67); (C) Heatmap of relative abundance of upregulated proteins across various severities of OSA; (D) Relative abundance of S100A8, S100A9, HSPA5, and NAMPT in mild to severe OSA; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; ns: nonsignificant; (E) Selected upregulated proteins enrichment analysis revealing the potential biological process (BP), molecular function (MF), cellular component (CC), and KEGG pathways enriched in the severe OSA group compared to the control group. OSA: obstructive sleep apnea.

biological process of GO analysis revealed dysregulated proteins related to protein processing and the toll-like receptor signaling pathway. Molecular function analysis indicated dysregulated proteins were enriched in serine-type endopepti-

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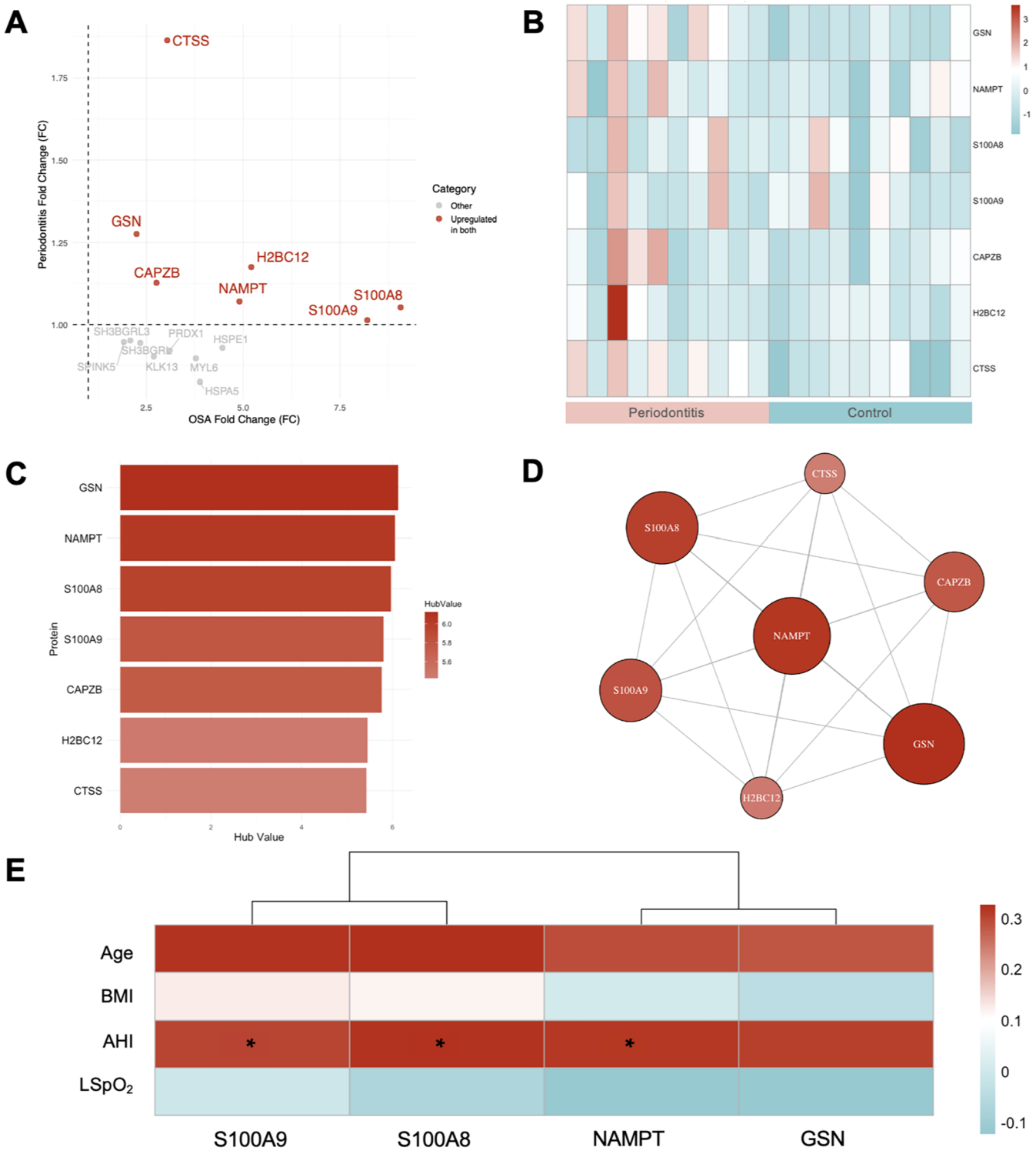

Figure 4. Identification of marker proteins in OSA-associated periodontitis (A) Scatter plot illustrating the overlap between salivary proteomic alterations in OSA and periodontitis, the subset of dual-upregulated proteins (n = 8) marked in red; (B) Heatmap of dual-upregulated proteins in periodontitis and control groups; (C) Bar plot showing the hub values of the dual-upregulated proteins derived from the salivary proteomic coexpression network; (D) Protein−protein interaction network highlighting hub proteins based on intramodular connectivity; (E) Correlation heatmap between protein abundance and clinical variables showing positive association between AHI and protein abundance. OSA: obstructive sleep apnea; BMI: body mass index; AHI: apnea-hypopnea index; $LSpO_2$: lowest oxygen saturation. *$p < 0.05$.

dase activity. Additionally, cellular components analysis showed that DEPs were localized mainly in the secretory granule lumen and the cell cortex. KEGG pathway enrichment analysis revealed significant antigen processing and presentation, and the IL-17 signaling pathway.

3.4. Marker Proteins in OSA-Associated Periodontitis

The fold change of the selected 15 OSA-upregulated proteins in periodontitis is demonstrated in Figure 4A,B, which show S100A8, S100A9, NAMPT, H2BC12, CAPZB, GSN, and

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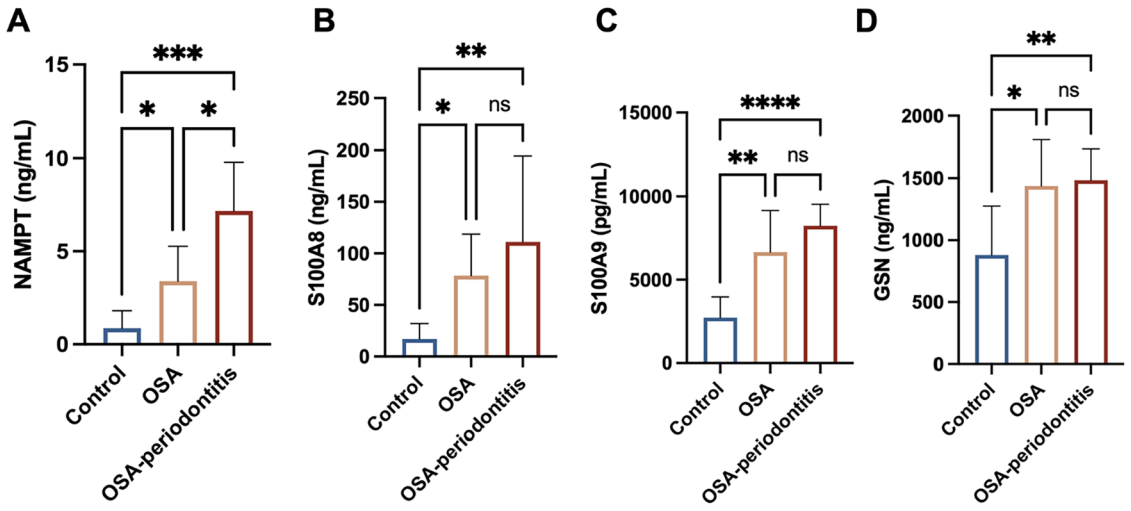

Figure 5. Salivary protein levels in independent patient groups by ELISA Salivary levels detected by ELISA of NAMPT (A), S100A8 (B), S100A9 (C), and GSN (D). (Control n = 10, aged 35.3 ± 10.6y, M/F = 4:6; OSA n = 8, aged 38.8 ± 11.6y, M/F = 5:3; OSA-associated periodontitis n = 6, aged 37.2 ± 13.6y, M/F = 3:3). OSA: obstructive sleep apnea; ns: nonsignificant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

CTSS were upregulated in both conditions. The bar plot (Figure 4C) and network plot (Figure 4D) show that these dual-upregulated proteins are closely associated with each other. GSN, NAMPT, and S100A8/A9 showed the highest hub values in the OSA-periodontitis interaction. Correlation analysis revealed positive associations between these proteins' abundance and AHI (Figure 4E), reaching statistical significance in NAMPT (r^2 = 0.317, p = 0.038), S100A8 (r^2 = 0.321, p = 0.044), and S100A9 (r^2 = 0.303, p = 0.037). However, there were no significant correlations between AHI, age, BMI, $LSpO_2$, and protein abundance after multiple testing. The statistical power for the identified hub proteins (NAMPT, S100A8, S100A9, GSN) showed results from 0.66 to 0.84 (Table S4), indicating moderate to high sensitivity to detect the observed effect sizes for these proteins.

The salivary levels of NAMPT, S100A8, S100A9, and GSN from independent patient groups with OSA-associated periodontitis, OSA, and control are demonstrated in Figure 5, which shows that protein levels were significantly elevated in both the OSA and OSA-associated periodontitis groups compared to controls, with a trend toward higher expression in the comorbid group. However, no statistically significant difference was observed between the OSA and OSA-associated periodontitis groups, except for NAMPT.

4. DISCUSSION

In this study, we integrated MR and salivary proteomics to elucidate the relationship between OSA and periodontitis. First, we observed significant causal associations of OSA with periodontitis, but not in the reverse direction, in the MR analyses using genetic instruments derived from large-scale GWAS. Second, based on the potential causal direction, the salivary proteomic profiling revealed distinct proteome signatures in OSA, characterized by 8 proteins that were concurrently upregulated in both OSA and periodontitis. Among them, GSN, NAMPT, and S100A8/A9 emerged as central hub proteins within the shared proteomic network, with expression levels validated by ELISA.

Clinical evidence has reported inconsistent associations between OSA and periodontitis. A meta-analysis including 30,994 participants has observed that periodontitis has a direct association with OSA (OR = 2.17) based on clinical research findings.[31] Similar findings were duplicated in another meta-analysis covering 43,414 individuals.[32] However, some investigations with a smaller sample size resulted in a negative association.[9,33] Therefore, the current study conducted a bidirectional MR analysis, trying to minimize potential confounding factors using robust genetic instruments, and explored a statistically significant causality. Genetic evidence suggested a potential causal association of OSA with periodontitis, but not vice versa. The causal estimates from IVW, weighted median, and MR-Egger were all directionally consistent, suggesting a relatively stable effect direction across different analytical assumptions without statistical heterogeneity or pleiotropy. Most SNPs showed consistent directionality in the Steiger test, supporting the exposure-to-outcome effect. Radial MR indicated that excluding influential SNPs did not materially change the results. The F-statistics of all SNPs were greater than 20, indicating that the SNPs were strong instruments. The current genetic evidence suggests that OSA may act as a causal factor contributing to the development of periodontitis. Nonetheless, further validation is warranted due to potential residual pleiotropy or confounding.

Based on the MR analysis, this study utilized a DIA-MS-based quantitative proteomic approach to identify the potential salivary protein markers in periodontally healthy patients with OSA. The protein expression profiles between various severities of OSA and the control groups were clearly separated. Thirty DEPs were found to be significantly upregulated in severe OSA, and the KEGG functional analysis revealed significant antigen processing and presentation, and the IL-17 signaling pathway. These pathways were well-recognized immune signatures in periodontitis,[34] suggesting that OSA might induce similar antigen-presenting mechanisms in the oral environment as an inflammatory interplay between the two conditions. However, further validation is warranted. The upregulated proteins were further screened for consistency with OSA severity and overlap with an independent periodontitis cohort, identifying 8 concurrently upregulated proteins as potential protein markers linking the two diseases.

Among the dual-upregulated proteins, several candidates might be biologically plausible mediators of the inflammatory

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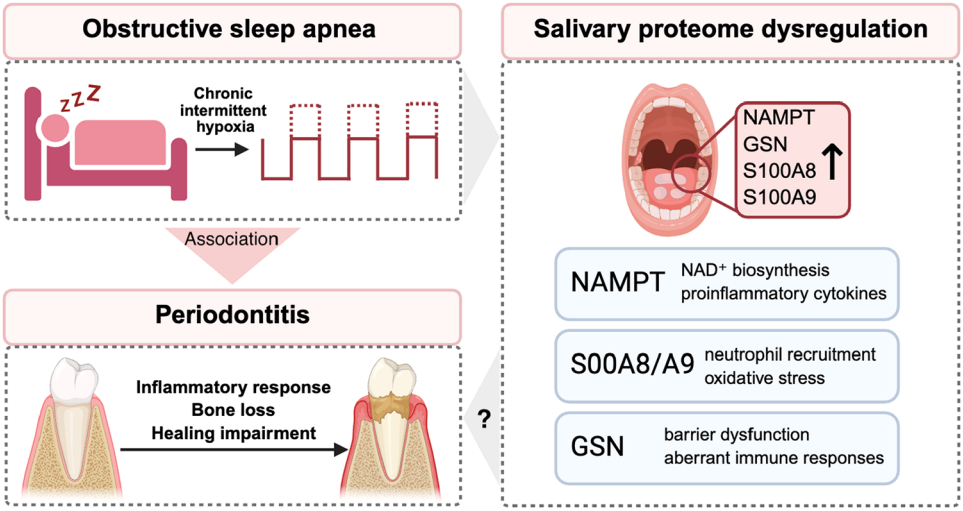

Figure 6. Potential associations of marker proteins-driven obstructive sleep apnea-associated periodontitis created in BioRender. Yu, V. (2026) https://BioRender.com/210sijo.

crosstalk between OSA and periodontitis. NAMPT, a key regulator of immunometabolism, may serve as a molecular bridge linking hypoxia to innate immune activation by promoting nicotinamide adenine dinucleotide (NAD^+) biosynthesis and the release of proinflammatory cytokines.[35] In periodontitis, NAMPT expression is markedly elevated in endothelial cells, with its levels increasing in parallel with disease severity.[36] Excessive NAMPT not only impairs the regenerative potential of periodontal ligament cells[37] but also contributes to periodontal inflammation and alveolar bone destruction through its enzymatic activity.[38] Moreover, NAMPT has been implicated as an essential mediator in rheumatoid arthritis-associated periodontitis,[39] suggesting its broader role in inflammatory bone loss and systemic immune dysregulation.

S100A8, frequently coexpressed with S100A9, acts as a potent alarmin that amplifies neutrophil recruitment and oxidative stress, thereby perpetuating tissue inflammation. S100A8/A9 is intensely upregulated during infection, metabolic, immune system dysfunction, and degenerative diseases-induced inflammation.[40] Regarding periodontal inflammation, S100A8 was significantly higher in saliva but lower in gingival cervical fluid (GCF) in patients with periodontitis.[41] However, Kojima et al.[42] found S100A8/A9 in GCF to be potential markers for periodontitis. Gelsolin (GSN), a cytoskeletal remodeling protein, is involved in actin filament dynamics and epithelial integrity, and its dysregulation has been associated with both barrier dysfunction and aberrant immune responses,[43] which have been reported to participate in several diseases' progression, including cancer, Alzheimer's disease, and arthritis.[44] Patients with hereditary gelsolin amyloidosis have an increased risk for periodontitis even when the oral self-care is adequate,[45] possibly by regulating actin cytoskeleton dynamics in osteoclasts, thereby modulating alveolar bone resorption.[46]

Together, these proteins may constitute a shared pathogenic axis whereby intermittent hypoxia in OSA augments systemic oxidative stress and marker proteins-driven inflammation, which in turn exacerbates local periodontal tissue destruction (Figure 6). This integrated mechanism may provide a potential biological explanation for the comorbidity between OSA and periodontitis. Furthermore, ELISA validation showed that the lack of a significant difference between the OSA and OSA-associated periodontitis groups may suggest that these proteins are primarily driven by shared inflammatory pathways. OSA alone may already induce substantial upregulation, resulting in a ceiling effect whereby additional periodontal inflammation leads only to a modest, nonsignificant increase. Alternatively, the limited sample size may have reduced the statistical power to detect intergroup differences. The current findings require further validation.

This study has several strengths. First, this study provides an integrative framework combining genetic causal inference and proteomic profiling, which strengthens the biological plausibility of the observed association between OSA and periodontitis. Second, the DIA-based salivary proteomics enabled high-throughput and quantitative characterization of molecular alterations in a noninvasive biofluid, offering translational potential for clinical screening and monitoring of OSA-associated periodontal inflammation. Third, the subsequent cross-disease overlap and network analyses identified hub proteins such as NAMPT, GSN, and S100A8/A9, which occupy central positions in the OSA-periodontitis network. These findings not only provide mechanistic insight into the inflammatory and metabolic crosstalk between the two diseases but also highlight promising protein targets for future therapeutic intervention.

However, there are also some limitations to the study. First, although the MR analysis provided evidence supporting a causal association of OSA with periodontitis, the interpretation remains limited by the reliance on a single primary MR method, statistical assumptions, populations and ethnic groups. Further validation in independent and multiethnic cohorts is required to confirm the generalizability of the findings. Second, while the bioinformatic and network analyses identified potential hub proteins mediating OSA-periodontitis interaction, the overlap analysis involves data sets generated from different platforms and laboratories, which may introduce technical variability. These findings are observational, and experimental verification using *in vitro* and *in vivo* models will be essential to elucidate the precise biological mechanisms. Third, significant demographic differences, including age, sex, and BMI, were observed among the study groups, which may represent a potential confounding factor in the interpretation

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of salivary protein alterations. Although correlation analyses did not reveal significant associations between age, BMI and the identified hub proteins, the influence cannot be completely excluded. Therefore, future studies with larger sample sizes and more balanced baseline characteristics are warranted to further validate these findings.

5. CONCLUSION

This study provides integrative genetic and proteomic evidence suggesting a potential causal and protein link between OSA and periodontitis. MR analyses indicated a possible causal association of OSA with periodontitis, while salivary proteomic profiling revealed a shared upregulation of inflammatory and metabolic proteins. Hub proteins such as NAMPT, GSN, and S100A8/A9 may serve as key mediators in OSA-associated with periodontitis, although further experimental validation is warranted to elucidate their precise roles and mechanisms.

■ ASSOCIATED CONTENT

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supporting file. The salivary proteomic data of patients with OSA will be publicly accessible upon publication on iProX (project ID IPX001463200).

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.5c01158.

Methods; the harmonized data of obstructive sleep apnea on periodontitis in the Mendelian Randomization study (Table S1); the harmonized data of periodontitis on obstructive sleep apnea in the Mendelian Randomization study (Table S2); the top 20 upregulated proteins in severe obstructive sleep apnea (Table S3); the power calculation of the identified protein markers (Table S4) (PDF)

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Notes

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