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The influence of obstructive sleep apnea and continuous positive airway pressure on the nasal microbiome

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Abstract. – OBJECTIVE: The aim of this study was to investigate the influence of obstructive sleep apnea and continuous positive airway pressure on the nasal microbiome.

PATIENTS AND METHODS: Endonasal swabs from the olfactory groove of 22 patients with moderate and severe obstructive sleep apnea (OSA) and a control group of 17 healthy controls were obtained at the Department of Otorhinolaryngology of the Friedrich-Alexander-Universität Erlangen-Nürnberg. 16S rRNA gene sequencing was performed to further evaluate the endonasal microbiome. In a second step, the longitudinal influence of continuous positive airway pressure (CPAP) therapy on the nasal microbiome was investigated (3-6 and 6-9 months).

RESULTS: Analysis of the bacterial load and β-diversity showed no significant differences between the groups, although patients with severe OSA showed increased α-diversity compared to the control group, while those with moderate OSA showed decreased α-diversity. The evaluation of longitudinal changes in the nasal microbiota during CPAP treatment showed no significant difference in α- or β-diversity. However, the number of bacteria for which a significant difference between moderate and severe OSA was found in the linear discriminant analysis decreased during CPAP treatment.

CONCLUSIONS: Long-term CPAP treatment showed an alignment of the composition of the nasal microbiome in patients with moderate and severe OSA as well as an alignment of biodiversity with that of the healthy control group. This change in the composition of the microbiome could be both part of the therapeutic effect in CPAP therapy and a promoting factor of the adverse side effects of the therapy. Further studies are needed to investigate whether the endonasal microbiome is related to CPAP compliance and whether CPAP compliance can be positively influenced in the future by therapeutic modification of the microbiome.

Key Words:
Nasal microbiome, Microbiome, Obstructive sleep apnea, CPAP, 16S rRNA.

Introduction

Obstructive sleep apnea is a multifactorial disease with multiple genes, environmental influences and development factors that are closely related to this potentially hazardous disease. According to a 1993 study[1], 2-4% of the middle-aged population were affected by obstructive sleep apnea. However, recent epidemiological studies[1-6] show that the prevalence has increased by 14-55% in the last 20 years, and today 13% of the male and 6% of the female population in the USA suffer from obstructive sleep apnea. The prevalence in patients with cardiovascular diseases is about 2 to 3 times higher. Furthermore, the prevalence of obstructive sleep apnea (OSA) in the general population is unfortunately largely underestimated, with about 82% of men and 93% of women remaining undiagnosed[7,8]. The most common therapy for all degrees of obstructive sleep apnea is nocturnal positive airway pressure (PAP) in the form of continuous PAP mode (CPAP, continuous PAP). However, a continuous positive pressure of dry or humidified air in the upper airways is associated with mucosal problems and subsequent airway inflammation. Therefore, subjective and objective reductions in nasal patency, rhinitis,

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sinonasal side effects and associated CPAP mask leakage are common[9]. The nasal cavity as the first part of the respiratory system is responsible for processing and filtering the outside air enriched with organic and inorganic particles[10]. In this regard, the upper respiratory tract is an active part of the immune system and is colonized by various microbes. The sinonasal microbiome is a complex symbiotic organization of bacteria, fungi and the nasal microenvironment. The alteration of microbiomes due to extrinsic and intrinsic factors has been widely discussed in recent literature. Our knowledge of this complex human-microbiological interaction has increased greatly in recent years. Current investigations[11,12] on the nasal and gut microbiome have shown that the microbial milieu is responsible for a healthy state of the mucosa, while dysbiosis can lead to irritation, inflammation and allergies. It thus seems logical that the microbiome plays a significant role in the course of host metabolic and immunological processes[13,14]. A healthy microbiome is important for protecting epithelial integrity, leads to the production of immunoglobulin, induces regulatory T-cells[15] and stimulates the formation of the mucus blanket and nutrient metabolism[16]. It has a significant effect on general inflammatory and allergic disorders[17-21]. A few patterns such as *Staphylococcus epidermidis, Staphylococcus aureus* and *Corynebacterium* have been identified[22-24] and seem to be present in a healthy nasal microbiome. Microbial compositions of the middle meatus and the sphenoethmoidal recess seem to be identical, while the composition of the anterior nose is different, with a greater proportion of *Actinobacteria* and *Firmicutes*[25]. The association of recurrent obstruction in OSA and consecutive airway inflammation with local subepithelial oedema and hypoxia, as well as systemic inflammation, is already well known[10,13,14]. The altered microenvironment can lead to dysbiotic colonization of the airways and even increase the inflammatory process[10,14]. Various clinical studies[26,27] have used scores to show that patients with OSA report significantly more rhinological symptoms. This correlation between clinical symptoms and the severity of OSA may be caused by an altered nasal microbiome. The latest study on the nasal microbiome by Wu et al[10], presented a nasal microbiome enriched with several oral commensals, inflammatory signatures and an increased Shannon diversity index (SDI) in subjects with severe OSA. In a control after only three months of CPAP treatment, no significant alterations in the composition or diversity of the nasal microbiome were observed[10]. Due to the significant influence of the environment and other factors on the microbiome, the aim of our study was to investigate the possible association between obstructive sleep apnea and nasal microbiome and long-term changes in the nasal microbiome caused by long-term CPAP usage in a middle-aged European patient population.

Patients and Methods

Study design and Participants

This single-center prospective controlled study was conducted in accordance with the amended Declaration of Helsinki and was approved by the Ethics Committee of the Friedrich-Alexander-Universität Erlangen-Nürnberg (Ref-No. 284_16 B). Inclusion criteria comprised men and women with untreated obstructive sleep apnea (n=22) and healthy controls with an unremarkable sleep history and without symptoms of acute or chronic rhinosinusitis (n=17). Exclusion criteria comprised patients with central or mixed apnea, patients under the age of 21 or over 90, patients with any acute or chronic condition that would limit the ability to participate in the study, patients with any treatment or surgery of the upper airway system due to OSA, patients with psychological impairment and pregnant woman. Informed consent was obtained from all individual participants included in the study. This diagnostic study was reported according to the STARD requirements.

All patients with OSA were first diagnosed and treated at the Department of Otorhinolaryngology Head and Neck Surgery of the Friedrich-Alexander-Universität Erlangen-Nürnberg without prior CPAP therapy. OSA was defined using diagnostic criteria of the International Classification of Sleep Disorders (ICSD-3): mild OSA apnea-hypopnea index (AHI) $\geq$ 5/h (5 apnea and hypopnea events per hour with sleep-related symptoms and comorbidities), moderate OSA (AHI from 15/h to 29/h), severe OSA (AHI $\geq$ 30/h)[28,29].

Polysomnography (PSG) was carried out using the 33-channel cardiorespiratory SOMNO screen diagnostic system (SOMNOmedics, Randersacker, Germany). The technical implementation of the PSG followed the recommendations of the American Academy of Sleep Medicine (AASM)[30]. The sleep stages and associated events were analyzed and scored visually according to the AASM criteria (version 2.4, 2017)[31]. In

the night following PSG, CPAP titration was performed according to the clinical guidelines of the Positive Airway Pressure Titration Task Force of the AASM[32].

Sample Collection

All samples were collected at the Department of Otorhinolaryngology, Head and Neck Surgery, Friedrich-Alexander-Universität Erlangen-Nürnberg. SalivaGene Buccal Swabs (Stratec molecular, Birkenfeld, Germany) with DNA stabilizer for DNA extraction (Stratec molecular, Birkenfeld, Germany) were taken noninvasively from the deep mucosa of the olfactory groove on the right or left side and frozen at -80°C until DNA extraction.

Concerning the OSA group, follow-up samples were collected after 3-6 and 6-9 months of CPAP therapy. Patients with low CPAP compliance (less than 6 h CPAP therapy per night) were excluded.

DNA Extraction

QIAamp UCP DNA Micro Kit plus Pathogen Lysis Tube S (Qiagen GmbH, Hilden, Germany) were used to extract the total genomic bacterial DNA from the DNA stabilizer and swab heads following the manufacturer's instructions. After DNA extraction all samples underwent a DNA concentration measurement by NanoDrop 200 (Thermo Fisher Scientific, Wilmington, USA) and were stored at -80°C until PCR processing.

Quantitative PCR

To amplify the V3+4 region of bacterial 16S rRNA gene region-specific primers containing dual barcodes and Illumina flow cell adaptor sequences[8] and NEBNext Q5 Hot Start Hifi PCR Kit (New England Biolabs, Ipswich, MA, USA) were employed. For each sample, 10 ng of metagenomic template DNA were used in a reaction consisting of 35 PCR cycles (98°C 15 seconds, 58°C 20 seconds, 72°C 40 seconds). The amplicons were purified with Agencout AMPure XP Beads (Beckmann Coulter, Krefeld, Germany), quantified using a Qbit device (Thermo Fisher, Waltham, MA, USA), normalized, and pooled before sequencing on an Illumina MiSeq device using a 600-cycle paired-end modus. Subsequently, the read FASTQ files were bioinformatically processed (merging, demultiplexing, quality filtering, dereplication, chimera removal) using Usearch 11 according to the Uparse pipeline. Operational taxonomic units (OTUs) were picked at a threshold of 97% similarity and taxonomically classified by comparing the representative OTU sequence to the reference file of the ribosomal database project (RDP version 16, available at: http://rdp.cme.msu.edu).

Statistical Analysis

All data were processed *via* Microbiomeanalyst[33] (available at: https://www.microbiomeanalyst.ca). Paired nonparametric statistics (Mann-Whitney U Test) were used for comparing the alpha diversity between baseline samples and longitudinal samples (pretherapeutic and post 3-6 and 6-9 months). To evaluate differences in β-diversity between the groups, we used permutational multivariate ANOVA (PERMANOVA) and PAST 4.0. To evaluate the taxonomical differences between the groups, we used linear discriminant analysis (LAD) including effect size (LEfSe) and the Mann-Whitney U test for two categories[34]. In the case of longitudinal taxonomical differences, the Wilcoxon rank sum test and LEfSe were used. For demographic data the Kruskal-Wallis' test was used for differences in 3 groups and the Mann-Whitney U test for follow-up analysis. For small numbers Fisher's Z was used instead of Chi-square tests. A p-value <0.05 was considered as statistically significant across all tests.

Results

Study Cohorts

A total of 39 subjects were enrolled in the study: 17 subjects in the control group and 22 subjects in the OSA group. The OSA group could be further divided into the two subgroups moderate (n=9) and severe OSA (n=13) (Table I).

Microbial Signatures of the Study Cohorts

Concerning the overall bacterial load (absolute abundance) from the analyzed cohorts, no significant differences could be detected between the control group and the OSA group or OSA subgroups, respectively (p=0.217) (Figure 1, **Supplementary Table I**).

Analysis of the microbial diversity (α-diversity) of the control group and the OSA group showed no significant differences in terms of the Shannon diversity index (SDI), markers of richness (biodiversity, total number) or the abundance (distribution) of the taxa of a given sample (Mann-Whitney U test; p>0.999). Concerning the analysis of the subgroups, patients suffering from severe OSA had an increased SDI compa-

Table I. Baseline characteristics. Obstructive sleep apnea: OSA, Control Group: CG; Body mass

	CG (N = 17)	Moderate OSA (N = 9)	Severe OSA (N = 13)	Statistical analysis
Sex (♂)	8 (47.1 %)	8 (88.9%)	10 (76.9 %)	$p = 0.062$, V = 0.377
Age	58.00 [49.50; 62.50]	50.00 [43.00; 55.50]	53.00 [47.00; 63.50]	$H_{(2)} = 3.12$, $p = 0.209$
BMI	**25.71 [24.03; 30.74]**	29.76 [25.56; 31.39]	**32.05 [29.23; 39.97]**	$H_{(2)} = 11.05$, $\mathbf{p = 0.004}$
Post-hoc		$p = 0.269$, r = 0.217	$\mathbf{p = 0.001}$, **r = 0.584**	
Baseline AHI		**23.9 [20.45; 27.90]**	**61.70 [43.60; 85.20]**	$\mathbf{p < 0.001}$, **r = 0.834**
AHI during CPAP		4.80 [3.25; 9.35]	6.10 [4.40; 14.20]	$p = 0.229$, r = 0.256
Smoking	15 (88.2 %)	4 (44.4 %)	8 (69.2 %)	
No	0 (0.0 %)	3 (33.3 %)	2 (15.4 %)	$p = 0.125$, V = 0.304
No but previously Yes	2 (11.8 %)	2 (22.2 %)	2 (15.4 %)	

index: BMI; apnea-hypopnea index: AHI, continuous positive airway pressure: CPAP.

red to the control group (Mann-Whitney U test; p=0.202), while patients suffering from moderate OSA showed a decreased SDI (Mann-Whitney U test; p=0.100). The difference between patients with moderate or severe OSA was significant (Mann-Whitney U test; p=0.035) (Figure 2, **Supplementary Table II**).

Analysis of β-diversity based on Bray-Curtis using PAST 4.0 as a measure of differences between the taxonomical microbial abundances from different samples showed no differences between the control group and OSA group (PERMANOVA; F = 0.329, p= 0.9637). Comparison of β-diversity between the control group and moderate (PERMANOVA; p=0.871) or severe OSA group (PERMANOVA; p=0.585) as well as comparison of the moderate and severe OSA groups showed no differences (PERMANOVA; p= 0.574).

Taxonomical Differences

The most abundant taxa across all groups were *Corynebacteriaceae, Carnobacteriaceae* and *Staphylococceae* (Figure 3, **Supplementary Table III**).

To evaluate detailed taxonomical differences between the control group and the OSA group

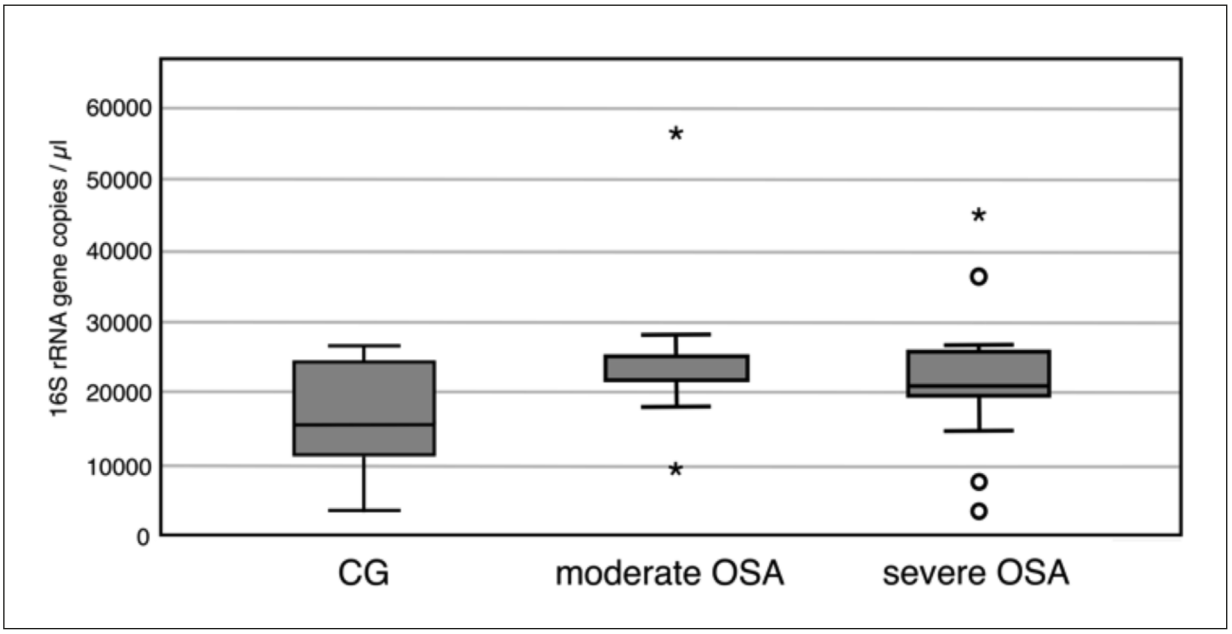

Figure 1. Bacterial load or absolute abundance (16S rRNA gene copies/µl) of the control group and OSA subgroups. Obstructive sleep apnea: OSA, Control Group: CG. Moderate and severe OSA group has a few outliers plotted as small circles for outliers which are more than 1.5 times but less than 3 times the interquartile range above the third quartile or below the first quartile and * for outliers which are more than 3 times the interquartile range above the third quartile or below the first quartile.

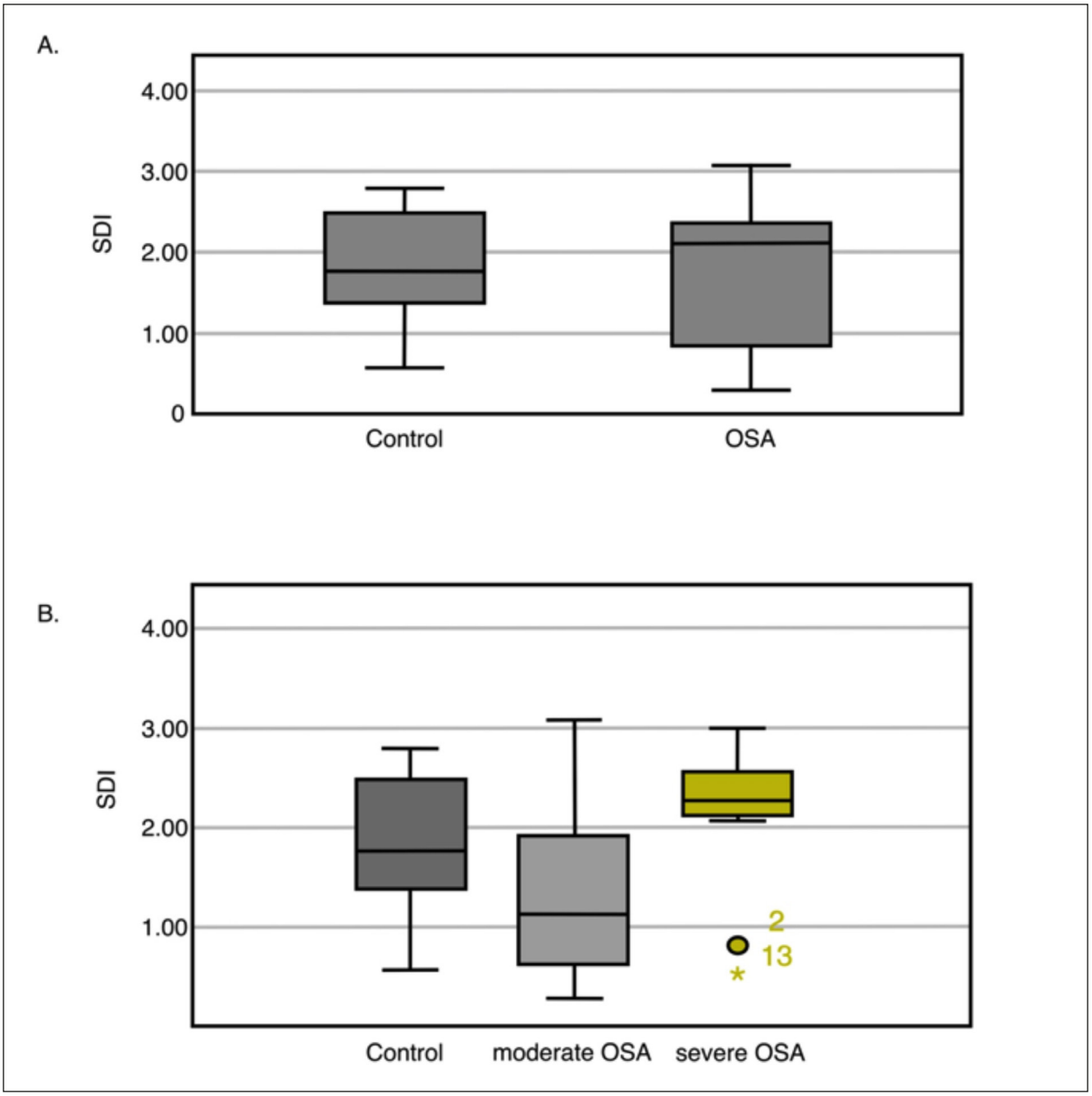

Figure 2. α-diversity of the control group, OSA group and OSA subgroups. **A**, Shannon diversity index (SDI) of the control group and the OSA group. **B**, SDI of the control group and OSA subgroups. Obstructive sleep apnea: OSA; Shannon diversity index: SDI.Severe OSA group has two outliers plotted as small circle for the outlier which is more than 1.5 times but less than 3 times the interquartile range below the first quartile and * for the outlier which is more than 3 times the interquartile range below the first quartile.

linear discriminant analysis (LDA) was used taking the effect size (LEfSE) into consideration[34]. The nasal microbiota of the OSA group compared to the control group was significantly enriched with *Xanthobacteraceae* (Wilcoxon test; p=0.011), while the abundance of *Brucellaceae* (Wilcoxon test; p=0.006), *Burkholderiales incertae sedis* (Wilcoxon test; p=0.002), *Eryspelothrichaceae* (Wilcoxon test; p=0.021) were significantly decreased. The nasal microbiome of patients with moderate OSA was enriched with *Xanthobacteraceae* (Wilcoxon test; p=0.006), while the abundance of *Comamonadaceae* (Wilcoxon test; p=0.014), *Burkholderiales incertae sedis* (Wilcoxon test; p=0.047), *Brucellaceae* (Wilcoxon test; p=0.037) and *Lachnospiraceae* (Wilcoxon test; p=0.035) was decreased compared to the nasal microbiota of the control

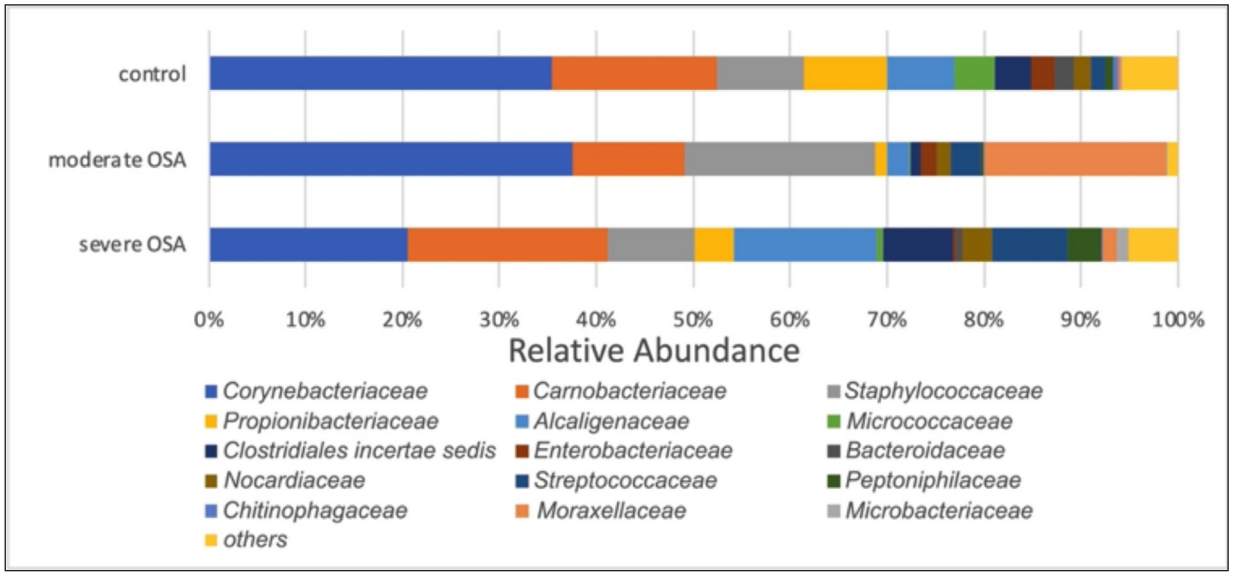

Figure 3. Taxonomical differences between the different groups. Bar chart of the relative abundance of the control group, moderate and severe OSA groups. Obstructive sleep apnea: OSA.

group in subjects with severe OSA. The amount of *Actinomycetaceae* (Wilcoxon test; p=0.048), *Peptoniphilaceae* (Wilcoxon test; p=0.016) and *Xanthobacteraceae* (Wilcoxon test; p=0.033) was increased, while *Brucellaceae* (Wilcoxon test; p=0.017), *Burkholderiales incertae sedis* (Wilcoxon test; p=0.007), *Desulobacteraceae* (Wilcoxon test; p=0.044), *Rhizobiales incertae sedis* (Wilcoxon test; p=0.044), *Sinobacteracteraceae* (Wilcoxon test; p=0.037) and *Erysipelotrichaceae* (Wilcoxon test; p=0.026) were decreased compared to the control group. Moderate OSA subjects compared to severe OSA subjects showed a higher amount of *Actinomycetaceae* (Wilcoxon test; p=0.042), while the amount of *Propionibacteriaceae* (Wilcoxon test; p=0.017) and *Clostridiales incertae sedis* (Wilcoxon test; p=0.035) was reduced compared to severe OSA subjects. (Figure 4, **Supplementary Table IV**).

Effect of CPAP-Treatment on Nasal Microbiome

To investigate the effect of CPAP treatment on the nasal microbiome of OSA patients, nasal swabs were repeated 3-6 months and 6-9 months after baseline measurement in untreated OSA. Evaluation of the longitudinal change in nasal microbiota showed no difference in SDI (Friedman-ANOVA: $\chi^2_{(2)}$=4,308, p=0.116) (Figure 5A, **Supplementary Table II**).

Differentiated by OSA severity, samples from patients with moderate OSA showed a statistically significant increase in α-diversity after 3-6 months of treatment (Mann-Whitney U test; p=0.028, r=0.635), while no further change was detected after 6-9 months (Mann-Whitney U test; p=0.753, r=0.091, Friedman ANOVA: $\chi^2_{(1)}$=9.00, p=0.011). For the group of patients suffering from severe OSA, no statistically significant difference in α-diversity could be shown after 3-6 and 6-9 months of CPAP treatment (Mann-Whitney U test; p=non-significance; Friedman-ANOVA: $\chi^2_{(1)}$=0.286, p=0.867) (Figure 5B, **Supplementary Table II**).

Samples from patients with moderate OSA showed no significant differences in β-diversity after 3-6 months of treatment (PERMANOVA; F=0.518, p=0.902) and no further change after 6-9 months (PERMANOVA; F=0.453, p=0.908). In summary, longitudinal analysis of β-diversity showed no differences in the moderate OSA group during CPAP treatment (PERMANOVA; F=0.526, p=0.971).

In the severe OSA group, no differences in β-diversity were detected either (PERMANOVA; F=0.937, p=0.540). Both follow-up samples showed no statistically significant difference in β-diversity after 3-6 (PERMANOVA F=0.658, p=0.817) and no changes after 6-9 months of CPAP-treatment (PERMANOVA; F=0.801, p=0.662).

Furthermore, longitudinal taxonomical differences before and under CPAP treat-

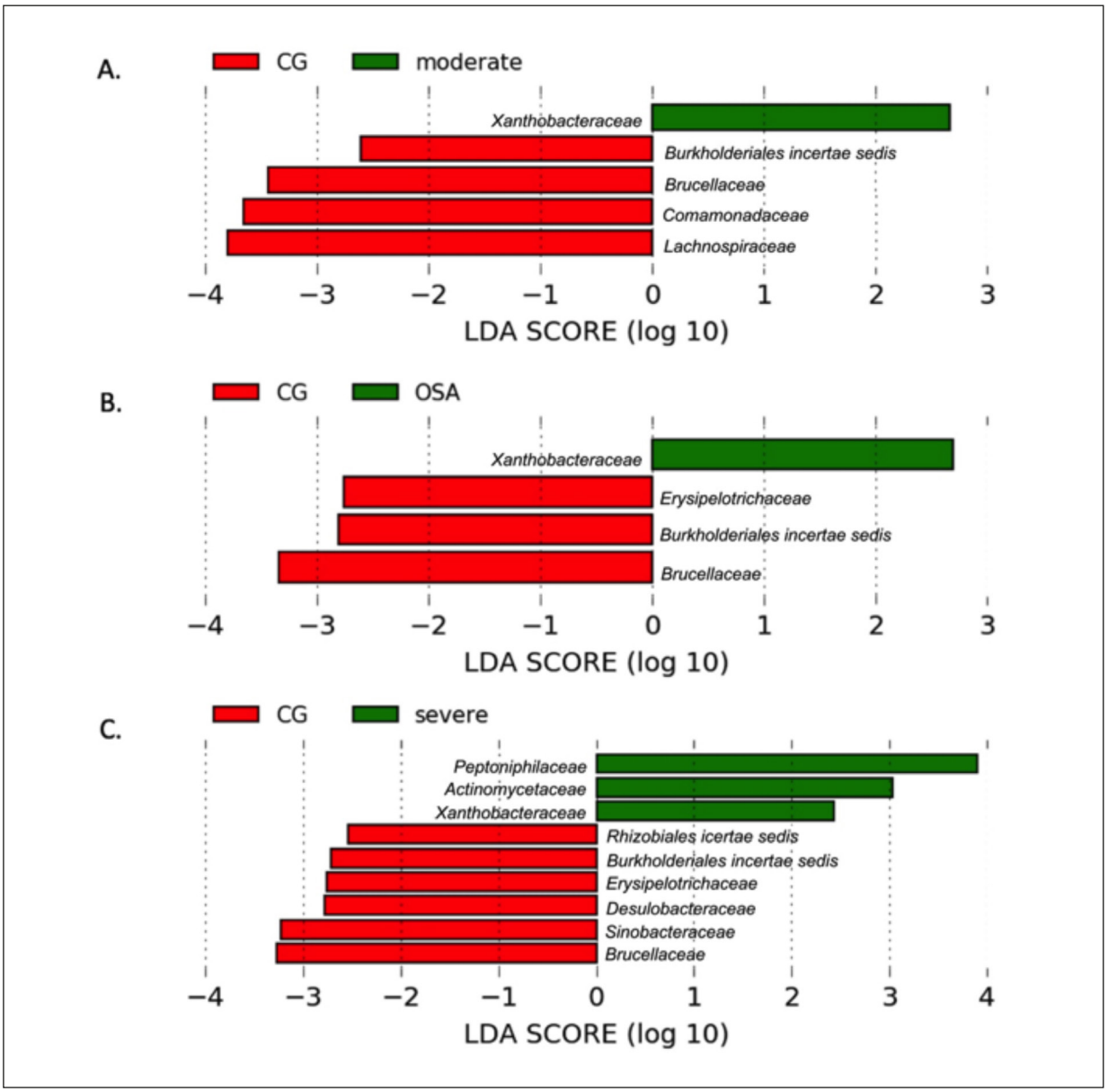

Figure 4. LDA Scores of the OSA group and partitioning into subgroups compared to the control group. **A**, Control group *vs.* OSA, (**B**) Control group *vs.* moderate OSA, (**C**) Control group *vs.* severe OSA. Linear discriminant analysis: LDA; Obstructive sleep apnea: OSA; Control Group: CG.

ment were analyzed. The nasal microbiome of subjects with moderate OSA after 3-6 months of CPAP treatment was enriched with *Propionibacteriaceae, Alcaligenaceae, Clostridiales* and *Streptococcaceae*, while the amount of *Staphylococcaceae, Enterobacteriaceae* and *Carnobacteriaceae* was decreased. After 6-9 months the amount of *Staphylococcaceae* decreased further, while *Alcaligenaceae and Micrococcaceae* increased (Figure 6A, **Supplementary Table III**).

In the severe OSA group the microbiome was enriched with *Streptococcaceae* and *Staphylococcaceae* after 3-6 months of CPAP treatment, while after 6-9 months the nasal microbiome showed a decreased amount of *Streptococcaceae, Clostridiales* and *Peptoniphilaceae* and an increased amount of *Staphylococcaceae* (Figure 6B, **Supplementary Table III**).

In addition, a linear discriminant analysis (LDA) was performed to investigate taxo-

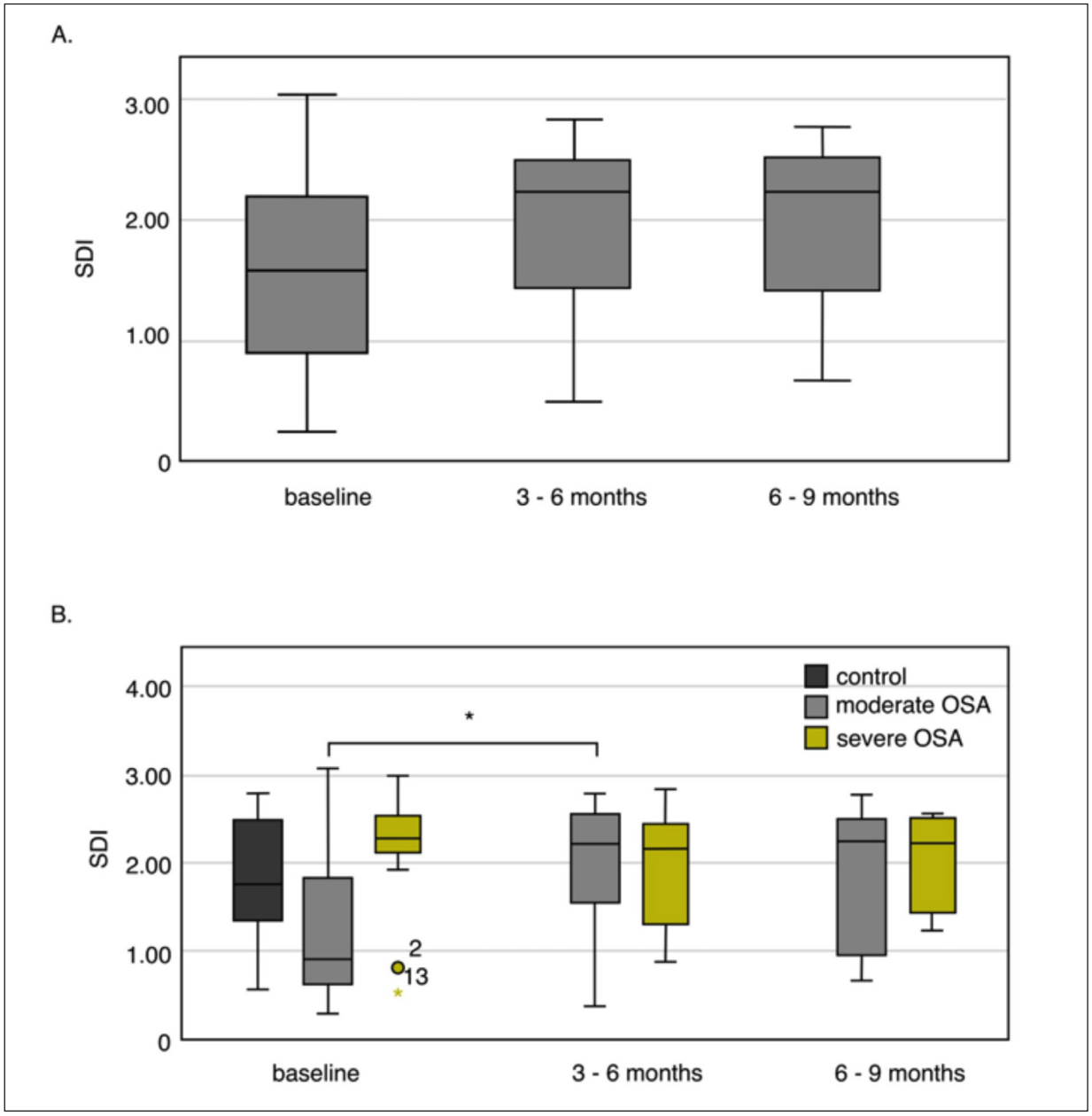

Figure 5. Medium- and long-term impact of CPAP-treatment on α-diversity in patients with OSA. Samples of the baseline microbiome of the OSA group (**A**) and subgroups (**B**) compared to samples after 3-6 and 6-9 months of CPAP-treatment. Obstructive sleep apnea: OSA; Shannon diversity index: SDI. *: p=0.028. Severe OSA group in baseline samples has two outliers plotted as small yellow circle for the outlier which is more than 1.5 times but less than 3 times the interquartile range below the first quartile and yellow * for the outlier which is more than 3 times the interquartile range below the first quartile.

nomical differences in the context of CPAP treatment. A higher absolute LDA score indicates a greater difference in the relative abundance of bacteria. With regard to moderate OSA, 3-6 months of CPAP-treatment resulted in an increase in *Moraxellaceae* (Wilcoxon test; p=0.019), *Ruminococcaceae* (Wilcoxon test; p=0.015), *Brevibacteriaceae* (Wilcoxon test; p=0.028) and *Oxalobacteriaceae* (Wilcoxon test; p=0.034), while *Lavobacteriaceae* (Wilcoxon test; p=0.020) decreased (Figure 7A, **Supplementary Table V**). After 6-9 months of CPAP therapy *Ruminococcaceae* (Wilcoxon test; p=0.004), *Bacteroidaceae* (Wilcoxon test; p=0.012), *Acidaminococcaceae* (Wilcoxon test; p=0.036), *Peptostreptococcaceae* (Wil-

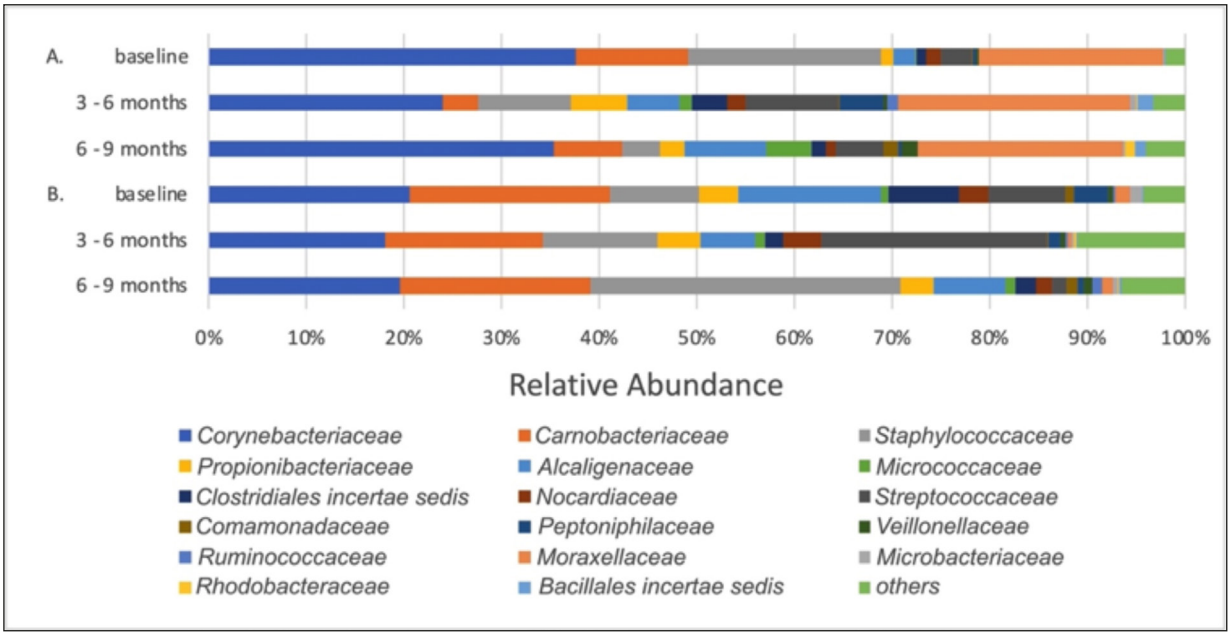

Figure 6. Taxonomical differences at baseline and after 3-6 and 6-9 months of CPAP-treatment. Longitudinal change in relative abundances in patients with moderate (**A**) and severe OSA (**B**).

coxon test; p=0.036), *Bacillaceae* (Wilcoxon test; p=0.043), *Eubacteriaceae* (Wilcoxon test; p=0.036), *Sutterellaceae* (Wilcoxon test; p=0.005), *Helicobacteriaceae* (Wilcoxon test; p=0.043) and *Desulobacteraceae* (Wilcoxon test; p=0.030) were decreased (Figure 7B, **Supplementary Table V**). With regard to severe OSA , 3-6 months of CPAP-treatment resulted in a higher amount of *Methylobacteriaceae* (Wilcoxon test; p=0.046), *Rhodobacteriaceae* (Wilcoxon test; p=0.039), *Pseudomonadaceae* (Wilcoxon test; p=0.013), *Nocardioidaceae* (Wilcoxon test; p=0.027), *Planctomycetaceae* (Wilcoxon test; p=0.036), *Gaiellaceae* (Wilcoxon test; p=0.021) and *Mycobacteriaceae* (Wilcoxon test; p=0.036) (Figure 7C, **Supplementary Table V**). After 6-9 months of CPAP treatment *Burkholderiales incertae sedis* (Wilcoxon test; p=0.018) was increased, while *Geodermatophilaceae* (Wilcoxon test; p=0.024), *Rikenellaceae* (Wilcoxon test; p=0.010), *Oxalobacteriaceae* (Wilcoxon test; p=0.024), *Actinomycetaceae* (Wilcoxon test; p=0.031), *Solirubrobacteraceae* (Wilcoxon test; p=0.024), *Acetobacteraceae* (Wilcoxon test; p=0.024), *Enterobacteriaceae* (Wilcoxon test; p=0.023) and *Lachnospiraceae* (Wilcoxon test; p=0.049) were decreased (Figure 7D, **Supplementary Table V**). Compared to each other, severe OSA samples showed a higher amount of *Gaiellaceae* (Wilcoxon test; p=0.024), while moderate OSA samples showed a higher amount of *Burkholderiales incertae sedis* (Wilcoxon test; p=0.034), *Porphyromonadaceae* (Wilcoxon test; p=0.038) and *Ruminococcaceae* (Wilcoxon test; p=0.017) after 3-6 months of CPAP treatment. After 6-9 months of CPAP treatment, severe OSA samples showed a slightly higher amount of *Burkholderiales incertae sedis* (Wilcoxon test; p=0.03) compared to the moderate OSA group.

Discussion

The results of this study are in line with previous studies[10] demonstrating the association of nasal microbiome and OSA. Subjects with severe OSA showed a higher Shannon diversity-index although the results were not significant. According to prior studies[10], a correlation between biodiversity and the severity of OSA could not be demonstrated in our study. In contrast, however, a significantly lower diversity in the oropharyngeal microbiome has already been described[35] in patients with moderate OSA.

In our study the abundance of *Xanthobacteraceae* was increased in moderate OSA compared to subjects with no OSA, while the abundances of *Burkholderiales incertae sedis, Lachnospira-*

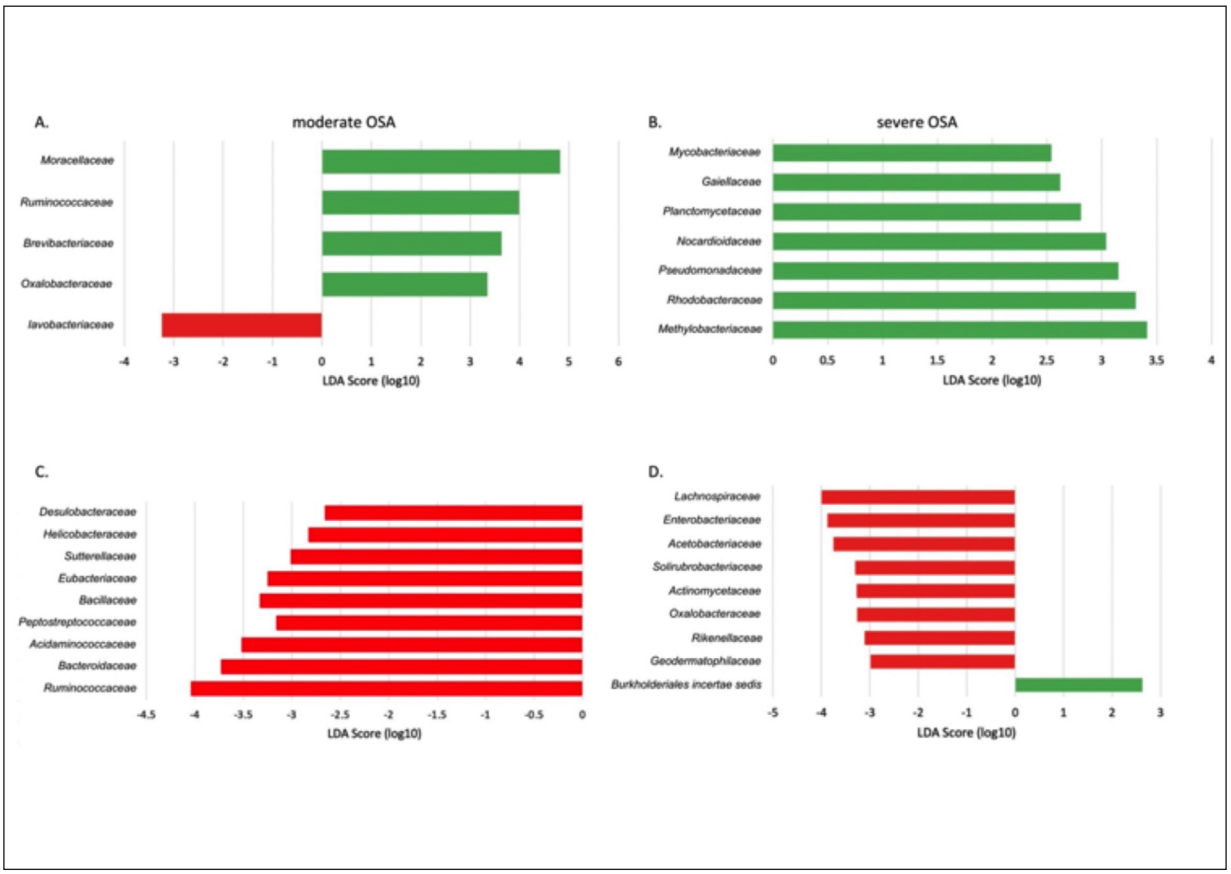

Figure 7. Taxonomical differences in the context of CPAP treatment. A positive LDA score is associated with an increase in the relative abundance of bacteria and a negative LDA score is associated with a decrease in the relative abundance of bacteria. **A**, moderate OSA after 3-6 months of CPAP treatment compared to pretherapeutic samples. **B**, severe OSA after 3-6 months of CPAP treatment compared to pretherapeutic samples. **C**, moderate OSA after 6-9 months of CPAP treatment compared to 3-6 months of CPAP treatment. **D**, severe OSA after 6-9 months of CPAP treatment compared to 3-6 months of CPAP treatment. Linear discriminant analysis: LDA; Obstructive sleep apnea: OSA.

ceae, Comamonadaceae and *Brucellaceae* were decreased. The enrichment of *Xanthobacteraceae* could also be seen in patients with severe OSA besides an additional enrichment of *Actinomycetaceae* and *Peptoniphilaceae*. While *Xanthobacteraceae* are well known as transient commensals in the gastrointestinal microbiome[36], *Burkholderiales incertae sedis* and *Comamonadaceae* have been frequently identified[37] as commensals of the nasal cavity. According to recent studies[38-41], *Eryspelothrichaceae* in gut microbiome is highly coated with IgA, correlates with tumor necrosis factor alpha and seems to affect host cholesterol metabolites. However, the most abundant taxa in all groups were *Corynebacteriaceae, Staphylococcaceae* and *Carnobacteriaceae*. In addition, *Propionibacteriaceae* and *Micrococcaceae* were strongly represented in the control group. Similar to the healthy control group in this study, *Propionibacteriaceae, Corynebacteriaceae* and *Staphylococcaceae* were frequently identified as predominant patterns of a healthy nasal microbiome in various studies[22]. In summary, the recognition of fundamental links between OSA and the nasal microbiome is complicated by the fact that the microbiome depends highly on the exact anatomical sampling site, such as the anterior nares with a keratinized epithelium or paranasal sinuses with a respiratory epithelium and more moisturized air[25]. The enrichment of anaerobic taxa such as *Peptoniphilaceae* may be due to the reduced oxygen content of the airway in OSA or to biofilms leading to a hypoxic environment, as previously described[42] in chronic rhinosinusitis (CRS), whereas more aerobic commensals such as *Brucellaceae, Propionibacteriaceae* and *Burkholderiales* are found[42] in a healthy nasal microbiome. The longitudinal study with subjects suffering from moderate OSA who underwent CPAP treatment showed a significant change

in Shannon diversity towards a higher diversity after 3-6 months of therapy (p=0.018), while the number of bacteria for which a significant difference was found between moderate and severe OSA, in the linear discriminant analysis, decreased during CPAP treatment. After 6-9 months of CPAP treatment, the biodiversity seems to become more similar to that of the control group. Compared to the results of Wu et al[10], the effect of CPAP treatment on the nasal microbiome seems to need more than 3 months of therapy. In general, higher diversities seem to be a marker for a healthier microbiome; higher diversity in the sinonasal microbiome also seems also to be associated with a better postoperative outcome[43] and improved health, whereas lower diversity has been observed[44] in patients with chronic rhinitis. A similar effect could also be observed in studies[45,46] on the gut microbiome. In the severe OSA group, higher numbers of *Rhodobacteriaceae* and *Burkholderiales* were detected, whereas *Enterobacteriaceae* decreased under therapy. As mentioned, *Burkholderiales* are already a known component of the nasal microbiome, and both are considered aerobic bacteria. A possible explanation for the higher concentration of aerobic bacteria on CPAP therapy could be that, on CPAP treatment, obstruction is prevented and better oxygenation of the mucus in the airways is ensured. Thus, several studies[47] have already shown that different oxygen concentrations are an important parameter affecting the microbiota. In subjects suffering from severe OSA, the relative abundance of *Staphylococcaceae* increased under CPAP therapy. Although not significant in the linear discriminant analysis, the increased amount of *Staphylococcaceae* could be caused by several factors already discussed in the literature: higher CPAP pressure and an irritation of the epithelium due to a contamination of the CPAP device with *Staphylococcaceae* during longer treatment or caused by changing environmental conditions such as winter and summer, colder or warmer temperatures[48,49].

In terms of future therapeutic implications, altering and optimizing the microbiome is an important therapeutic target. For example, in mouse models, fecal microbiota transplants, which are already established in gastroenterology, have been shown to help stabilize a healthy gut microbiome and reduce dysbiosis, and are considered a potential factor in the field of psychiatric or neurological disorders. Similarly, it is conceivable that in OSA patients, therapeutic modification of the sinonasal microbiome *via* the probiotics in a nasal spray might reduce the symptoms of rhinitis and sinusitis during CPAP treatment and thus improve compliance[50-52]. The intestinal microbiome is also becoming increasingly important in oncological issues, and the microbial composition seems to be related to both the development of cancer and the specific treatment in terms of immunotherapy efficacy[53].

Limitations

However, some limitations of the study must also be considered. Due to numerous general factors influencing the diversity of the microbiome (gender, age, geographical location, climate, culture, lifestyle habits, nicotine and alcohol consumption, diet, obesity, etc.), the interpretation of individual study results in the context of existing scientific knowledge is considerably complicated or even limited[54]. In addition, viral and fungal populations could also have an impact on the bacterial microbiome and are still not well known. Thus, general conclusions and potential therapeutic implications regarding the microbiome will continue to be limited to an indeterminate degree by the myriad of dispositional and exposure-related influencing factors.

Conclusions

In this study we observed that: 1) There were no significant differences in the absolute bacterial load and β-diversity of the microbiome between the control group and OSA group, although patients with severe OSA showed increased α-diversity compared to the control group, while those with moderate OSA showed decreased α-diversity; 2) The evaluation of longitudinal changes in the nasal microbiota during CPAP treatment showed no significant difference in α- or β-diversity; 3) The most abundant taxa in all groups were *Corynebacteriaceae, Carnobacteriaceae* and *Staphylococcaceae*. In the linear discriminant analysis, *Xanthobacteraceae* were more abundant in the nasal microbiome of OSA patients, while *Brucellaceae, Burkholderiales incertae sedis* and *Eryspelothrichaceae* were more abundant in the nasal microbiome of the healthy control group; 4) In relation to CPAP treatment in patients with moderate and severe OSA, the number of different bacteria, in the linear discriminant analysis, decreased and the biodiversity seems to approach that of the control group. This alignment of the composition of the nasal microbiome in pa-

tients with moderate and severe OSA, as well as an alignment of its biodiversity with that of the healthy control group during CPAP treatment, could be both part of the therapeutic effect in CPAP therapy and a promoting factor of the adverse side effects of the therapy. Further studies are now needed to investigate whether the imbalance of the endonasal microbiome is related to CPAP compliance and whether therapeutic modification of the microbiome in the future with probiotics, microbiota transplantation, different mask surfaces or water reservoirs, would have possible clinical implications in terms of improving CPAP treatment adherence and tolerance.

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Conflict of Interest
The authors declare no competing interests.

Ethics Approval
This study involving human participants was conducted in accordance with the amended Declaration of Helsinki and was approved by the Ethics Committee of the Friedrich-Alexander-Universität Erlangen-Nürnberg (Ref-NO. 284_16 B).

Informed Consent
Informed consent was obtained from all individual participants included in the study.

Authors' Contributions
Christian Lenk, Maximilian Traxdorf and Elena Messbacher contributed substantially to the concept and design of the study. Christian Lenk and Elena Messbacher performed data collection. Christian Lenk and Maximilian Traxdorf were responsible for interpretation, drafting, monitoring and validation of the article. All authors discussed the results, commented on the paper and gave their final approval of the version of the article to be published.

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Conflict of Interest
The authors declare that they have no personal and financial relationships with other people or organizations that could influence this paper. The authors declare that they have no conflict of interest to declare.

Availability of Data and Materials
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

References

1) Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993; 328: 1230-1235.
2) Bixler EO, Vgontzas AN, Lin HM, Ten Have T, Rein J, Vela-Bueno A, Kales A. Prevalence of sleep-disordered breathing in women: effects of gender. Am J Respir Crit Care Med 2001; 163: 608-613.
3) Ip MS, Lam B, Tang LC, Lauder IJ, Ip TY, Lam WK. A community study of sleep-disordered breathing in middle-aged Chinese women in Hong Kong: prevalence and gender differences. Chest 2004; 125: 127-134.
4) Reddy EV, Kadhiravan T, Mishra HK, Sreenivas V, Handa KK, Sinha S, Sharma SK. Prevalence and risk factors of obstructive sleep apnea among middle-aged urban Indians: a community-based study. Sleep Med 2009; 10: 913-918.
5) Sullivan F. Hidden Health Crisis Costing America Billions. Underdiagnosing and Undertreating Obstructive Sleep Apnea Draining Healthcare System 2016; 1-25.
6) Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol 2013; 177: 1006-1014.
7) Rosen RC, Zozula R, Jahn EG, Carson JL. Low rates of recognition of sleep disorders in primary care: comparison of a community-based versus clinical academic setting. Sleep Med 2001; 2: 47-55.
8) Young T, Evans L, Finn L, Palta M. Estimation of the clinically diagnosed proportion of sleep apnea syndrome in middle-aged men and women. Sleep 1997; 20: 705-706.
9) Balsalobre L, Pezato R, Gasparini H, Haddad F, Gregorio LC, Fujita RR. Acute impact of continuous positive airway pressure on nasal patency. Int Forum Allergy Rhinol 2017; 7: 712-717.

10) Wu BG, Sulaiman I, Wang J, Shen N, Clemente JC, Li Y, Laumbach RJ, Lu S, Udasin I, Oanh L, Perez A, Alimokhtari S, Black K, Plietz M, Twumasi A, Sanders H, Malecha P, Kapoor B, Scaglione BD, Wang A, Blazoski C, Weiden MD, Rapoport DM, Harrison D, Chitkara N, Vicente E, Marin JM, Sunderram J, Ayappa I, Segal LN. Severe Obstructive Sleep Apnea Is Associated with Alterations in the Nasal Microbiome and an Increase in Inflammation. Am J Respir Crit Care Med 2019; 199: 99-109.

11) Hekiert AM, Kofonow JM, Doghramji L, Kennedy DW, Chiu AG, Palmer JN, Leid JG, Cohen NA. Biofilms correlate with TH1 inflammation in the sinonasal tissue of patients with chronic rhinosinusitis. Otolaryngol Head Neck Surg 2009; 141: 448-453.

12) Foreman A, Holtappels G, Psaltis AJ, Jervis-Bardy J, Field J, Wormald PJ, Bachert C. Adaptive immune responses in Staphylococcus aureus biofilm-associated chronic rhinosinusitis. Allergy 2011; 66: 1449-1456.

13) Lawley TD, Walker AW. Intestinal colonization resistance. Immunology 2013; 138: 1-11.

14) Frank DN, Zhu W, Sartor RB, Li E. Investigating the biological and clinical significance of human dysbioses. Trends Microbiol 2011; 19: 427-434.

15) Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 2011; 331: 337-341.

16) Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011; 474: 327-336.

17) Johansen FE, Kaetzel CS. Regulation of the polymeric immunoglobulin receptor and IgA transport: new advances in environmental factors that stimulate pIgR expression and its role in mucosal immunity. Mucosal Immunol 2011; 4: 598-602.

18) Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol 2013; 13: 321-335.

19) McLoughlin RM, Mills KH. Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. J Allergy Clin Immunol 2011; 127: 1097-1107; quiz 1108-1099.

20) Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 1997; 159: 1739-1745.

21) Tabas I, Glass CK. Anti-inflammatory therapy in chronic disease: challenges and opportunities. Science 2013; 339: 166-172.

22) Boase S, Foreman A, Cleland E, Tan L, Melton-Kreft R, Pant H, Hu FZ, Ehrlich GD, Wormald PJ. The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection. BMC Infect Dis 2013; 13: 210.

23) Ramakrishnan VR, Feazel LM, Gitomer SA, Ir D, Robertson CE, Frank DN. The microbiome of the middle meatus in healthy adults. PLoS One 2013; 8: e85507.

24) Ramakrishnan VR, Hauser LJ, Frank DN. The sinonasal bacterial microbiome in health and disease. Curr Opin Otolaryngol Head Neck Surg 2016; 24: 20-25.

25) Yan M, Pamp SJ, Fukuyama J, Hwang PH, Cho DY, Holmes S, Relman DA. Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and S. aureus carriage. Cell Host Microbe 2013; 14: 631-640.

26) Kuan EC, Tajudeen BA, Peng KA, Wang MB. Sinonasal outcomes in obstructive sleep apnea syndrome. Laryngoscope 2015; 125: 2617-2620.

27) Moxness MHS, Bugten V, Thorstensen WM, Nordgard S. Sinonasal Characteristics in Patients with Obstructive Sleep Apnea Compared to Healthy Controls. Int J Otolaryngol 2017; 2017: 1935284.

28) Consensus Conference Panel, Watson NF, Badr MS, Belenky G, Bliwise DL, Buxton OM, Buysse D, Dinges DF, Gangwisch J, Grandner MA, Kushida C, Malhotra RK, Martin JL, Patel SR, Quan SF, Tasali E. Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society on the Recommended Amount of Sleep for a Healthy Adult: Methodology and Discussion. Sleep 2015; 38: 1161-1183.

29) Sateia MJ. International classification of sleep disorders-third edition: highlights and modifications. Chest 2014; 146: 1387-1394.

30) Iber CAIS, Chesson AL Jr, Quan SF. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. AASM, Westchester, 2007.

31) Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Davidson Ward SL, Tangredi MM; American Academy of Sleep Medicine. 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: 597-619.

32) Kushida CA, Chediak A, Berry RB, Brown LK, Gozal D, Iber C, Parthasarathy S, Quan SF, Rowley JA. Clinical guidelines for the manual titration of positive airway pressure in patients with obstructive sleep apnea. J Clin Sleep Med 2008; 4: 157-171.

33) Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 2017; 45: W180-W188.

34) Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic bio-

marker discovery and explanation. Genome Biol 2011; 12: R60.

35) Yang W, Shao L, Heizhati M, Wu T, Yao X, Wang Y, Wang L, Li N. Oropharyngeal Microbiome in Obstructive Sleep Apnea: Decreased Diversity and Abundance. J Clin Sleep Med 2019; 15: 1777-1788.

36) Rajilic-Stojanovic M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev 2014; 38: 996-1047.

37) Bassis CM, Tang AL, Young VB, Pynnonen MA. The nasal cavity microbiota of healthy adults. Microbiome 2014; 2: 27.

38) Feazel LM, Robertson CE, Ramakrishnan VR, Frank DN. Microbiome complexity and Staphylococcus aureus in chronic rhinosinusitis. Laryngoscope 2012; 122: 467-472.

39) Zhao Y, Wu J, Li JV, Zhou NY, Tang H, Wang Y. Gut microbiota composition modifies fecal metabolic profiles in mice. J Proteome Res 2013; 12: 2987-2999.

40) Dinh DM, Volpe GE, Duffalo C, Bhalchandra S, Tai AK, Kane AV, Wanke CA, Ward HD. Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. J Infect Dis 2015; 211: 19-27.

41) Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, Ruggiero E, Cho JH, Goodman AL, Flavell RA. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell 2014; 158: 1000-1010.

42) Ivanchenko OA, Karpishchenko SA, Kozlov RS, Krechikova OI, Otvagin IV, Sopko ON, Piskunov GZ, Lopatin AS. The microbiome of the maxillary sinus and middle nasal meatus in chronic rhinosinusitis. Rhinology 2016; 54: 68-74.

43) Ramakrishnan VR, Hauser LJ, Feazel LM, Ir D, Robertson CE, Frank DN. Sinus microbiota varies among chronic rhinosinusitis phenotypes and predicts surgical outcome. J Allergy Clin Immunol 2015; 136: 334-342 e331.

44) Choi EB, Hong SW, Kim DK, Jeon SG, Kim KR, Cho SH, Gho YS, Jee YK, Kim YK. Decreased diversity of nasal microbiota and their secreted extracellular vesicles in patients with chronic rhinosinusitis based on a metagenomic analysis. Allergy 2014; 69: 517-526.

45) Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007; 449: 804-810.

46) Mosca A, Leclerc M, Hugot JP. Gut Microbiota Diversity and Human Diseases: Should We Reintroduce Key Predators in Our Ecosystem? Front Microbiol 2016; 7: 455.

47) Hospodsky D, Pickering AJ, Julian TR, Miller D, Gorthala S, Boehm AB, Peccia J. Hand bacterial communities vary across two different human populations. Microbiology 2014; 160: 1144-1152.

48) David LA, Materna AC, Friedman J, Baptista MI, Blackburn MC, Perrotta A, Erdman SE, Alm EJ. Erratum to: Host lifestyle affects human microbiota on daily timescales. Genome Biol 2016; 17: 117.

49) Poroyko VA, Carreras A, Khalyfa A, Khalyfa AA, Leone V, Peris E, Almendros I, Gileles-Hillel A, Qiao Z, Hubert N, Farré R, Chang EB, Gozal D. Chronic Sleep Disruption Alters Gut Microbiota, Induces Systemic and Adipose Tissue Inflammation and Insulin Resistance in Mice. Sci Rep 2016; 6: 35405.

50) Xu HM, Huang HL, Zhou YL, Zhao HL, Xu J, Shou DW, Liu YD, Zhou YJ, Nie YQ. Fecal Microbiota Transplantation: A New Therapeutic Attempt from the Gut to the Brain. Gastroenterol Res Pract 2021; 2021: 6699268.

51) Boehme M, Guzzetta KE, Bastiaanssen TFS, van de Wouw M, Moloney GM, Gual-Grau A, Spichak S, Olvarría-Ramírez L, Fitzgerald P, Morillas E, Ritz NL, Jaggar M, Cowan CSM, Crispie F, Donoso F, Halitzki E, Neto MC, Sichetti M, Golubeva AV, Fitzgerald RS, Claesson MJ, Cotter Pd, O'Leary OF, Dinan TG, Cryan JF. Microbiota from young mice counteracts selective age-associated behavioral deficits. Nature Aging 2021; 1: 666-676.

52) De Grandi R, Bottagisio M, Di Girolamo S, Bidossi A, De Vecchi E, Drago L. Modulation of opportunistic species Corynebacterium diphtheriae, Haemophilus parainfluenzae, Moraxella catarrhalis, Prevotella denticola, Prevotella melaninogenica, Rothia dentocariosa, Staphylococcus aureus and Streptococcus pseudopneumoniae by intranasal administration of Streptococcus salivarius 24SMBc and Streptococcus oralis 89a combination in healthy subjects. Eur Rev Med Pharmacol Sci 2019; 23: 60-66.

53) Song P, Wang QB, Liang B, Jiang SJ. Advances in research on the relationship between the gut microbiome and cancer. Eur Rev Med Pharmacol Sci 2021; 25: 5104-5112

54) Ramakrishnan VR, Frank DN. Impact of cigarette smoking on the middle meatus microbiome in health and chronic rhinosinusitis. Int Forum Allergy Rhinol 2015; 5: 981-989.