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Location-Specific Oral Microbiome Possesses Features Associated With CKD

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Introduction: Chronic kidney disease (CKD), a progressive loss of renal function, can lead to serious complications if underdiagnosed. Many studies suggest that the oral microbiota plays important role in the health of the host; however, little is known about the association between the oral microbiota and CKD pathogenesis.

Methods: In this study, we surveyed the oral microbiota in saliva, the left and right molars, and the anterior mandibular lingual area from 77 participants (18 with and 59 without CKD), and tested their association with CKD to identify microbial features that may be predictive of CKD status.

Results: The overall oral microbiota composition significantly differed by oral locations and was associated with CKD status in saliva and anterior mandibular lingual samples. In CKD patients, we observed a significant enrichment of *Neisseria* and depletion of *Veillonella* in both sample types and a lower prevalence of *Streptococcus* in saliva after adjustment for other comorbidities. Furthermore, we detected a negative association of *Neisseria* and *Streptococcus* genera with the kidney function as measured by estimated glomerular filtration rate. *Neisseria* abundance also correlated with plasma interleukin-18 levels.

Conclusion: We demonstrate the association of the oral microbiome with CKD and inflammatory kidney biomarkers, highlighting a potential role of the commensal bacteria in CKD pathogenesis. A better understanding of the interplay between the oral microbiota and CKD may help in the development of new strategies to identify at-risk individuals or to serve as a novel target for therapeutic intervention.

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Chronic kidney disease (CKD) is defined as decreased kidney function that persists for more than 3 months.[1,2] CKD is a global public health problem, affecting 10% of the population worldwide. In the United States alone, the overall prevalence of CKD is approximately 14%, affecting 31 million adults[3]; among these, 9 of 10 cases remain undiagnosed. Without diagnosis and treatment, CKD can lead to serious complications.[2] Numerous CKD risk factors have been reported, with hypertension and diabetes topping the list.[4] Other risk factors[5] include family history of CKD, male gender, older age,[4] smoking,[6,7] African ancestry,[4] low socioeconomic status,[8–10] history of cardiovascular disease,[11] obesity, nephrotoxicity, and acute kidney injury.[12] Moreover, recent studies have suggested a link between CKD and periodontal disease.[13] Although periodontal disease is an infection localized to the oral cavity, increasing evidence suggests that periodontal disease results from a chronic systematic inflammatory dysregulation mediated by the oral microbiome.[13,14] The oral microbes and the resultant inflammatory mediators may infiltrate the systemic bloodstream, interact with host cells of target organs, and induce a systematic inflammatory reaction that may exacerbate kidney dysfunction.[14] A recent

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human study showed that the elevated serum antibody to a periodontal pathogen, *Porphyromonas gingivalis*, was significantly associated with decreased kidney function.[15] Yet, the underlying mechanism linking periodontal disease to CKD remains largely unknown.

Recent advances in next-generation sequencing technology have helped to link the host microbiota composition and kidney function. It has been shown that microbial dysregulation in the gut exacerbated uremia, leading to further progression of CKD and inflammation (reviewed by Khoury *et al.*[16]). However, even though many studies have demonstrated that the human oral microbiota represents 1 of the most diverse microbial communities in the body and plays an important role in the health and disease of the host,[17,18] and despite the established link between CKD and periodontal health, little is known about the role of the oral microbiota in CKD pathogenesis. Therefore, in the present study, we explored the association of the bacterial composition in several oral locations, including saliva and dental plaques, with biomarkers of kidney function and CKD status using an electronic medical record (EMR)–linked biobank. Understanding the connection between the oral microbiome and kidney function could help develop noninvasive strategies to identify individuals at risk for developing CKD or, for those with established disease, experiencing CKD progression, and may serve as a novel therapeutic target.

MATERIALS AND METHODS

Study Subjects

This study was approved by the Institutional Review Board (IRB) of the Icahn School of Medicine at Mount Sinai. Mount Sinai serves the diverse local communities of upper Manhattan, including Central Harlem (86% African American), East Harlem (88% Hispanic/Latino), and Upper East Side (88% European white) with broad health disparities.[19] Study participants were initially recruited to the Bio*Me* Biobank Program of the Charles Bronfman Institute for Personalized Medicine at Mount Sinai, New York, and provided a blood sample at entry. We cross-referenced the list of Bio*Me* participants with that of patients registered in the Mount Sinai Dentistry database (Dentrix) to identify active patients with upcoming dental appointments to recruit for our study.

The inclusion criteria for our study were to be a Bio*Me* participant, >18 years, no antibiotic use for ≥3 months before sampling, no missing molars as determined during a dental appointment, as well as agreement and ability to consent to participate in this study. A short questionnaire was administered to collect demographic and health status information on each subject. The CKD status of study participants was determined from a previously described electronic phenotyping algorithm that has been shown to outperform CKD definition through the International Classification of Diseases, 10th Revision (ICD-10) codes alone and demonstrated > 90% validity.[20] All CKD cases were also validated by a content expert through chart reviews. In the Bio*Me*, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to determine estimated glomerular filtration rate (eGFR) from serum creatinine and age, sex, and race.[3] The median time lag between the the closest EMR eGFR measurement to the oral sampling and the oral sample collection date was 0.33 year (interquartile range = 0.6 year) and did not differ by CKD status.

Cytokine Biomarkers

Plasma samples taken at the time of enrollment to the Bio*Me* Biobank Program were stored at −80°C and were used to derive the biomarker measures. Concentrations of tumor necrosis factor receptor−1 (TNFR1) and tumor necrosis factor receptor−2 (TNFR2) were measured via the 2-plex 96-well prototype cytokine array from Mesoscale Diagnostics (Meso Scale Discovery, Gaithersburg, MD). The average intra-assay coefficient of variation was <5% for the calibrators as well as for the quality control sample. The interassay coefficient of variation for TNFR1 was 13% and for TNRF2 was 11%. The average lower limit of detection obtained from multiple runs was 2.84 pg/ml for TNFR1 and 0.40 pg/ml for TNFR2. Concentrations of kidney injury marker−1 (KIM1), interleukin-18 (IL18), monocyte chemoattractant protein−1 (MCP1), and chitinase 3-like-1 gene product (YKL40) were also measured via a custom 4-plex assay from Mesoscale Diagnostics. The intra-assay coefficient of variation for the calibrators was <10% and the inter-assay coefficients of variation ranged from 6% to 12% for the 4 biomarkers. The average lower limit of detection obtained from multiple runs was 0.43, 0.14, 0.08, and 0.49 pg/ml for the 4 biomarkers, respectively.

Oral Samples

A total of 277 samples from saliva, the left and right molars, and the anterior mandibular lingual area along with clinical information were collected using a standard protocol by a trained dentist from 77 consecutive participants (18 with CKD and 59 without) at the time of recruitment to our study. For saliva, samples were collected and stabilized using OMNIgene Discover kit (DNA Genotek, Ottawa, ON, Canada). For dental plaque, the scrapings of dental plaque were collected at the gum–tooth interface of

selected teeth and pooled in a 1.5-ml sterile Eppendorf tube containing 200 μl of sterile water. All collected samples were then stored at −80°C immediately after sampling. Because the oral samples were collected from the participants who were already enrolled in the Bio*Me* Biobank Program with the blood samples collected at the entry, there was on average a 48-month gap between the blood sample and oral sample collection.

Bacterial 16S Ribosomal RNA Polymerase Chain Reaction and Sequencing

Oral plaque and saliva DNA was extracted using the PowerSoil DNA isolation kit following the protocol from the manufacturer (Mobio, Carlsbad, CA). The phylogenetically informative V3 to V4 region of the 16S rRNA gene was amplified using universal primer 347F/803R.[17,21] We designed a dual-barcoding approach to label the 16S ribosomal RNA (rRNA) gene amplicons from each sample.[22] The primers were synthesized by IDT (Integrated DNA Technology, Coralville, IA) with sequences published previously.[22] The integrity of the amplicons was verified by agarose gel electrophoresis. The resulting ∼460 bp amplicons were pooled and then sequenced on the Illumina MiSeq 2x300 paired-end sequencing platform.

Bacterial 16S rRNA Data Analysis

The pair−end sequences were merged using PANDAseq.[23] High-quality reads (length <400 or the quality score <Q30 at >1% of bases) were further split by barcode and trimmed of primer regions using QIIME1.9.0.[24] Duplicate measurements of 10 samples were sequenced using different barcodes and batches to test the sequencing reproducibility. We used the command *pick_open_reference_otus.py* in QIIME with the default cutoff of 97% to cluster sequencing reads to operational taxonomic units (OTUs) using Uclust.[25] The program further built a biom-formatted OTU table with assigned taxonomic information for each OTU. Using Chimera Slayer,[26] chimera sequences arising from the polymerase chain reaction amplification were detected and excluded from the aligned representative sequences and the OTU table.

The microbial diversity within each sample, or α diversity, was calculated using the Shannon index and Inverse Simpson index as metrics and represented the measure of diversity at the genus level.[27,28] The overall microbiome dissimilarities among all samples, or β diversity, were assessed using the Bray−Curtis distance matrices and visualized by a nonmetric multidimensional scaling plot.[29] The pairwise permutational multivariate analysis of variance (PERMANOVA) procedure,[24,30] using the *Adonis* function of the *R* package *vegan* 2.0-5[31] with the maximum number of permutations = 999, was performed to test the significance of the overall microbiome differences between the oral microbiota grouped by sampling locations and subject clinical status.

Using the linear discriminant analysis effect size method,[32] we further selected the microbiome features significantly associated with CKD at various taxonomic ranks with the absolute value of the linear discriminant analysis score >3.0. Several abundant differential genera were further analyzed to compare the mean and variance of the relative abundance. We applied receiver operating characteristic analysis using the *pROC* package[33] in R to assess the performance of CKD classification based on selected microbiome features. The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)[34] was used to predict the metagenome functional content based on the close reference-based OTU table generated using the QIIME pipeline with our 16S rRNA sequencing data.

Statistical Analysis

All categorical variables were expressed as count and percentage. All numeric variables were expressed as mean and SD. Statistical significance was assessed by *t* test or nonparametric Wilcoxon test for numeric variables and χ^2 test for categorical variables. A P value < 0.05 was considered significant after the false discovery rate (FDR) adjustment for multiple comparisons, unless specified otherwise. The Spearman correlation analysis was used to identify correlations of eGFR and plasma biomarkers with bacterial diversity and OTU relative abundance. A P value was calculated for each pairwise comparison using an R command *corr.test* with both a q value (R package *qvalue*)[35] <0.05 and the Spearman |rho|>0.3 required to declare statistical significance. Spearman partial correlation analysis was performed using R package *ppcor*[36] to correct for eGFR.

Datasets

16S rRNA sequencing information has been deposited into National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with submission ID *SUB2430778* and BioProject ID *PRJNA376427*.

RESULTS

Study Population

We recruited 77 consecutive subjects who participated in the Mount Sinai, New York Bio*Me* Biobank Program and had upcoming appointment at a dental clinic. The subjects' mean age was 49 ± 13 years; 64% were women. Of the subjects, 43% were African American, 47% Hispanic/Latino, 4% white, and 7%

other. In all, 23% had CKD. As expected, CKD was more prevalent in patients with hypertension, history of coronary heart disease, or periodontal disease (Table 1). CKD patients were more likely to be treated with antihypertensive medications and insulin.

Survey of Oral Microbiome

From study participants, we collected 277 oral samples, of which 75 were from the anterior mandibular lingual area, 68 from the left molar, 69 from the right molar, and 65 from saliva. Fifteen plaque samples from 5 subjects were excluded from further analysis because of low DNA yield. In total, we obtained 26 million high-quality 16S rRNA sequencing reads from 262 samples, or ~ 100,000 reads per sample. After assigning taxonomic identification to each sample, we found that the dominant phylum in anterior mandibular lingual area, left molar, right molar, and saliva included *Actinobacteria* (9.0% ± 4.6%, 10.0% ± 6.9%, 11.6% ± 11.3%, and 8.6% ± 5.4%, respectively), *Bacteroidetes* (15.5% ± 9.3%, 23.5% ± 9.4%, 23.3% ± 8.7%, and 30.7% ± 10.4%, respectively), *Firmicutes* (15.6% ± 17.2%, 31.6% ± 10.6%, 32.0% ± 10.2%, and 32.5% ± 9.7%, respectively), *Fusobacteria* (22.1% ± 12.5%, 16.7% ± 8.1%, 15.4% ± 8.5%, and 4.7% ± 3.3%, respectively), and *Proteobacteria* (11.1% ± 8.9%, 10.4% ± 7.2%, 10.3% ± 8.2%, and 17.6% ± 9.9% respectively). The nonmetric multiple dimensional scaling plot (Figure 1a) visualizes the dissimilarity of the overall oral microbiome abandance among the oral samples (β diversity) by sampling location. The pairwise PERMANOVA test suggested significant differences in the microbiota composition among all sampling locations (all P values $\leq$ 0.001 with permutation = 999) except for the left and right molars (P = 0.74 with permutation = 999). The Student t test to compare the mean overall diversity (α diversity) measured by Shannon or inverse Simpson index showed that the anterior mandibular lingual samples possessed the highest, while saliva the lowest, α diversity (Figure 1b).

Association Between CKD Status and Location-Specific Oral Microbiome

The overall microbiome of saliva and anterior mandibular lingual samples showed significant association with CKD status in both univariate (P = 0.009 and P = 0.014, respectively; Supplementary Figure S1) and multivariate PERMANOVA test after the adjustment for type 2 diabetes, coronary heart disease, periodontal disease, hypertension, and BMI (P = 0.01 and P = 0.012, respectively; data not shown). In samples from right or left molar plaques, a trend for association of the oral microbiota composition with CKD status was observed in univariate but not in multivariate analyses (P = 0.087 and P = 0.21, respectively).

Table 1. Demographic and clinical characteristics of the study cohort

Variable	Total	Non-CKD	CKD	*P* value
Sample size	77	59	18	
Age, yr	49 (12.8)	47.5 (12.7)	53.8 (12.2)	0.07
Gender, female	49 (63.6%)	40 (67.8%)	9 (50%)	0.17
Ethnicity, n (%)				0.52
African American	33 (42.9%)	24 (40.7%)	9 (50%)	
White	3 (3.9%)	3 (5.1%)	0 (0%)	
Hispanic	36 (46.8%)	29 (49.2%)	7 (38.9%)	
Other	5 (6.5%)	3 (5.1%)	2 (11.1%)	
Smoking status, yes	26 (33.4%)	22 (37.3%)	4 (22.2%)	0.24
Body mass index, kg/m^2	31.7 (1.0)	31.0 (8.6)	34.1 (9.1)	0.20
Medical conditions, n (%)				
Type 2 diabetes	22 (28.6%)	15 (28.8%)	7 (46.7%)	0.19
Hypertension	49 (63.6%)	32 (54.2%)	17 (94.4%)	**0.0019**
Coronary heart disease	23 (29.9%)	11 (18.6%)	12 (66.7%)	**<0.0001**
Medications, n (%)				
Antihypertensive medication	50 (64.9%)	33 (56.9%)	17 (94.4%)	**0.0027**
Lipid-lowering medications	30 (39.0%)	20 (33.9%)	10 (55.6%)	0.099
Diabetes medication	14 (18.2%)	10 (17.0%)	4 (22.2%)	0.61
Insulin	22 (28.6%)	11 (18.6%)	11 (61.1%)	**0.0004**
Blood pressure, mm Hg				
Systolic blood pressure	126.8 (17.1)	126.4 (16.1)	128 (20.4)	0.74
Diastolic blood pressure	73.4 (10.6)	72.5 (10.7)	76.1 (10.3)	0.22
Blood levels				
Low-density lipoprotein, mg/dl	99.4 (36.1)	105 (36.8)	81 (27.5)	**0.014**
High-density lipoprotein, mg/dl	52.0 (16.0)	52.4 (16.5)	50.6 (14.5)	0.68
Total cholesterol, mg/dl	177.5 (40.0)	183.3 (41.12)	158.4 (29.82)	**0.02**
Triglycerides, mg/dl	133.4 (64.2)	133.6 (66.8)	132.7 (56.7)	0.96
Hemoglobin A1C, %	6.1 (1.4)	6.1 (1.5)	6.0 (1.0)	0.92
Dental health, n (%)				
Periodontal disease	43 (66.2%)	30 (58.8%)	13 (92.9%)	**0.017**
Plasma biomarkers, pg/ml				
TNFR1	4852 (1078)	2993 (1073)	10,532 (4150)	**8.2e-6**
TNFR2	6505 (790)	4951 (398)	11,254 (2724)	**0.0004**
KIM1	205 (27)	147 (16)	382 (89)	**0.0003**
MCP1	207 (24)	178 (10)	296 (92)	0.07
YKL40	82,622 (22,198)	80,713 (28,998)	88,455 (17,300)	**0.003**
IL18	554 (56)	515 (67)	674 (92)	**0.029**
eGFR (ml/min per 1.73	71.0 (30.0)	83.2 (23.1)	44.1 (25.4)	**4.2e-10**

eGFR, estimated glomerular filtration rate; IL18, interleukin-18; KIM1, kidney injury marker−1; MCP1, monocyte chemoattractant protein−1; TNFR1, tumor necrosis factor receptor−1; TNFR2, tumor necrosis factor receptor−2; YKL40, chitinase 3-like−1 gene product.
Data are presented as n (percentage) or mean (SD).
Bold indicates $P < 0.05$.

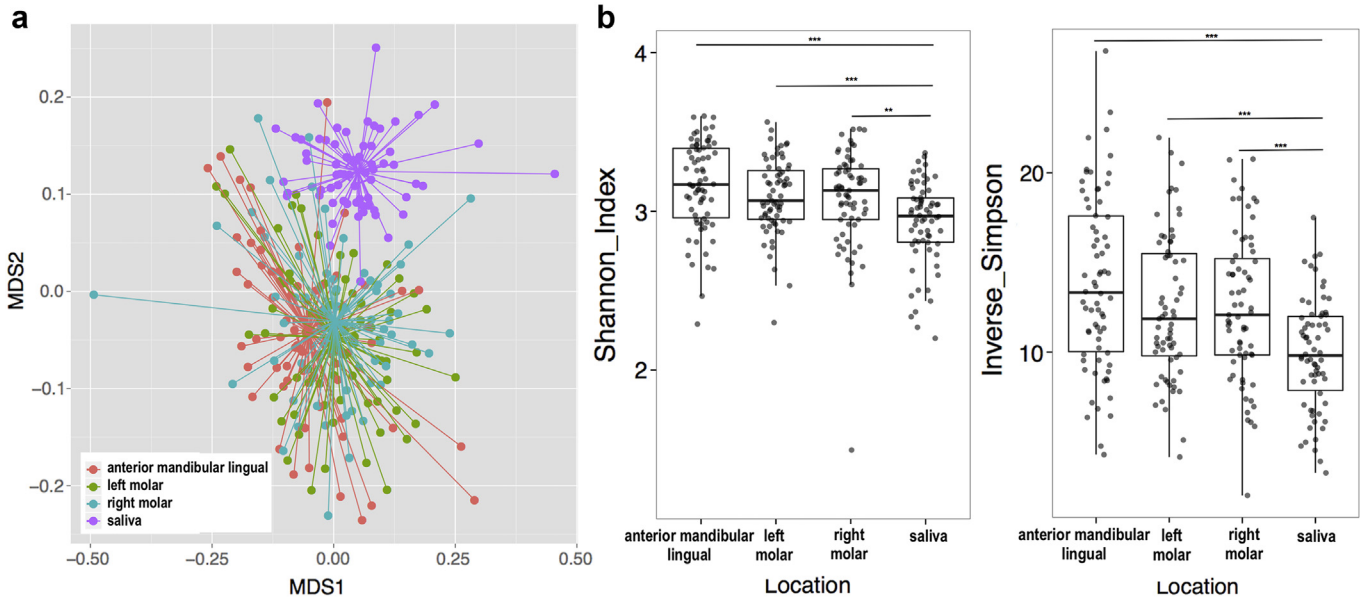

Figure 1. Overall oral microbiome diversity by sampling location. (a) Comparison of the relative microbiota abundance (β diversity) between oral locations by the permutational multivariate analysis of variance (PERMANOVA) test. *P* values ≤ 0.001 with 999 permutations for all comparisons except for left and right molar ($P = 0.74$ with 999 permutations). The Bray–Curtis distance matrices were visualized using a nonmetric multiple dimensional scaling plot. (b) Comparison of the overall bacterial diversity (α diversity) between sampling locations (**$P < 0.01$, ***$P < 0.001$ by Student *t* test).

Bacterial Taxa Features Associated With CKD Status

The linear discriminant analysis effect size test was performed using 16S sequencing data from saliva and anterior mandibular lingual samples, the 2 locations to show significant differences in the overall microbiome composition by CKD status (Supplementary Figure S1), in order to select particular discriminative features of CKD. The results showed that the microbiota of CKD patients in both locations had significantly higher prevalence of *Proteobacteria*, in particular, *Neisseria*, and lower prevalence of *Veillonella* and *Atopobium* (Figure 2a and b). However, many selected features were location specific. For instance, among the most significant features, we found increased *Gammaproteobacteria* and *Burkholderoales* only in anterior mandibular lingual samples of CKD patients, but not in saliva samples. In contrast, decreased *Lactobacillales* and *Streptococcus* were found in CKD patients' saliva, but not in anterior mandibular lingual samples. We further quantified the relative abundances of those CKD-associated genera in saliva and anterior mandibular lingual locations (Figure 2b) using the sequencing counts of the selected genus divided by the total counts. Although the person-to-person variation in the relative abundance of these taxa was high, particularly for the *Neisseria* genus, we found that those genera were not rare (>0.1% mean abundance), suggesting that their abundance can potentially be quatified to predict the CKD status. In the receiver operating characteristic analysis aimed to assess the performance of CKD classification based on selected microbiome features, we found that the *Neisseria*/*Veillonella* ratio in anterior mandibular lingual samples and *Neisseria*/*Streptococcus* in saliva samples were able to correctly predict the CKD status in 81% and 77% cases, respectively, outperforming the prediction using *Neisseria*, *Veillonella* or *Streptococcus* alone (Figure 2c).

Differential Predicted Functional Metagenome Features and Their Association With Oral Location and CKD Status

We performed the PICRUSt analysis to predict the full metagenomic content of microbial communities using 16S gene surveys[34] and compared the predicted metagenomic pathways by oral location and CKD status (Figure S2). The nonmetric multidimensional scaling plot (Figure S2a) suggested that the predicted metagenomic pathways of saliva microbiota were significantly different from those of the other 3 locations ($P = 0.001$ by PERMANOVA). In the saliva and anterior mandibular lingual samples, we found several pathways to be differentially abundant by CKD status ($P < 0.05$ after false discovery rate [FDR] adjustment; Figure S2b). Among those pathways, the ether lipid metabolism in saliva samples showed a nearly 2-fold reduction in CKD patients. We also observed increased abundance of fatty acid metabolism and

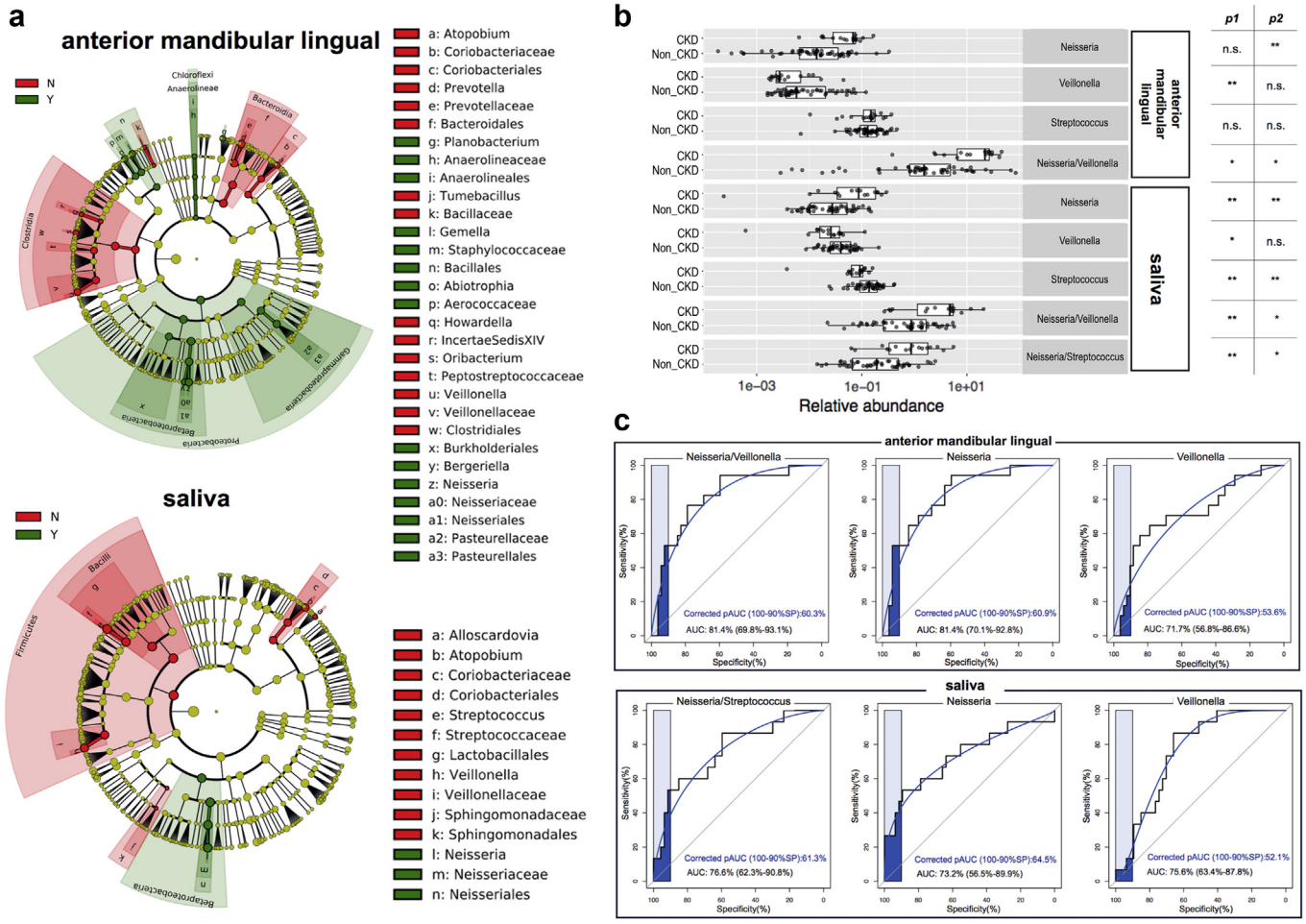

Figure 2. Oral microbial features associated with chronic kidney disease (CKD). (a) The cladoplots depict differential oral microbial features selected by linear discriminant analysis effect size analysis by CKD status in anterior mandibular lingual and saliva samples. Differential taxa between CKD and no CKD are demonstrated in color for the most abundant class: green indicating increase and red indicating reduction in CKD patients. (b) Comparison of the relative abundance of selected microbial features by CKD status. *P* value 1 (*P1*) was obtained from a Wilcoxon–Mann–Whitney test. *P* value 2 (*P2*) was obtained from a multivariate regression assuming a γ distribution for taxa and normal distribution for ratios while adjusting for type 2 diabetes, hypertension, coronary heart disease, periodontal disease, and body mass index. After multivariable adjustment, the association of CKD status with bacterial genera and ratios remained significant. (c) Receiver operating characteristic (ROC) curves and area under the curve (AUC) values to indicate the diagnostic accuracy of the selected features to predict CKD status.

genetic information processing in anterior mandibular lingual samples and decreased abundance of the antibiotic biosynthesis, cellular processes, and phosphotransferase pathways in saliva of patients with CKD compared to those with no CKD (Figure S2b). No significant differences by CKD status were found in samples from the left and right molar sites (data not shown).

Correlation Between Plasma Biomarkers and CKD-Associated Oral Microbiota Features

We measured plasma levels of 6 kidney biomarkers that represent different but complementary pathways for CKD development and progression: TNFR1 and TNFR2, representing global inflammation; KIM1, representing tubular injury; IL18; MCP1, representing both inflammation and injury; and YKL40, representing fibrosis/repair. The biomarker levels were significantly higher in individuals with CKD (Table 1) and negatively correlated with eGFR (Spearman correlation rho < -0.3) (Figure 3a and b). eGFR also showed a strong positive correlation with the relative abundance of the taxa features selected from linear discriminant analysis effect size analysis, including *Alloscardovia* and *Streptococcus,* and a strong negative correlation with *Neisseria* (Figure 3a). Moreover, inflammatory biomarkers showed a positive correlation between IL18 and *Neisseria* genus and a negative correlation between TNFR1 and *Streptococcus* (Figure 3a). At the OTU level, 3 OTUs from the *Streptococcus* genus and 1 OTU from the *Atopobium* genus were strongly positively associated with eGFR (Figure 3b). Also, negative correlations of IL18 and TNFR1 with

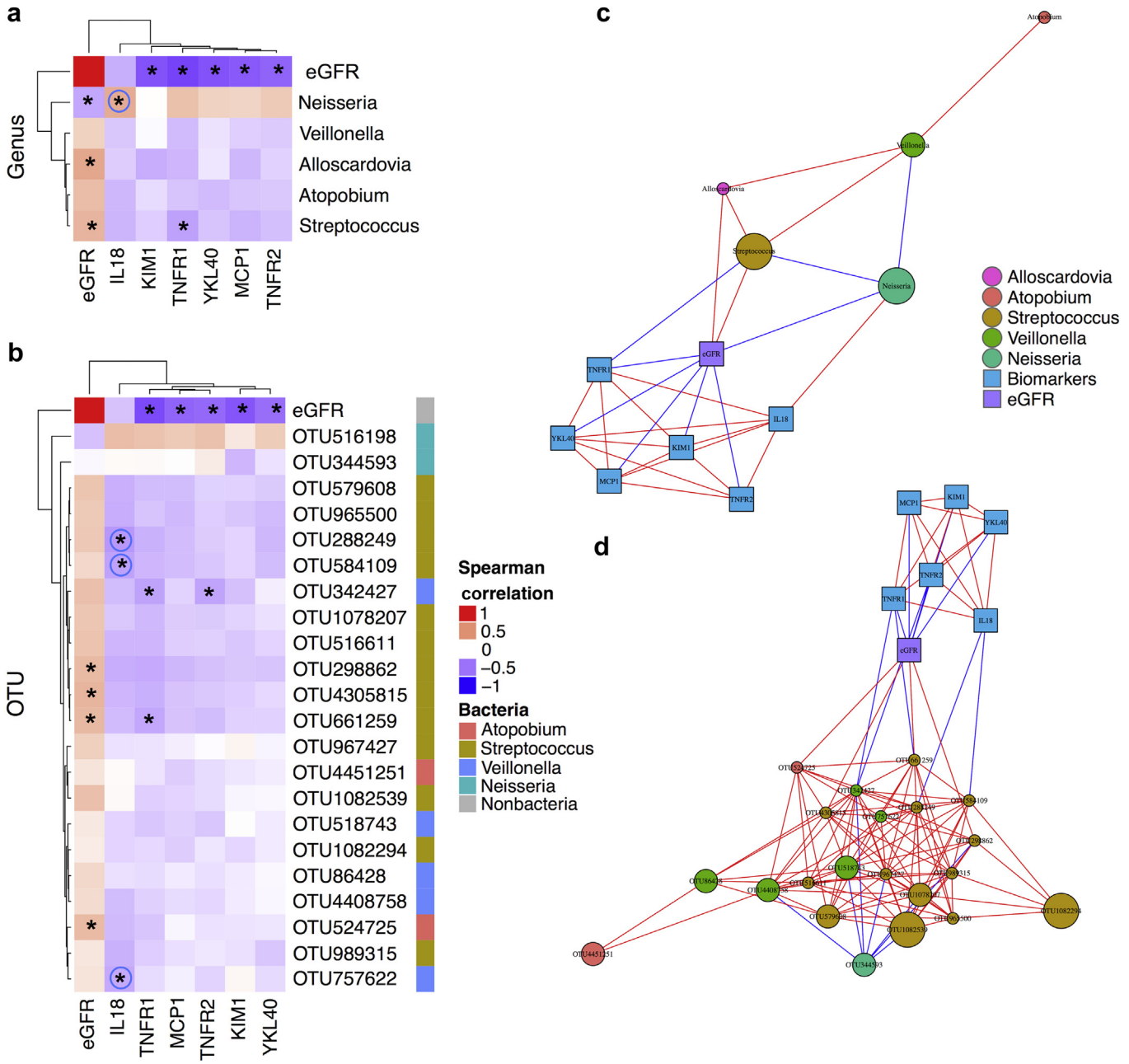

Figure 3. Correlation analysis of the saliva microbiome with serum biomarkers. (a,b) Spearman correlation analyses conducted among the following: (a) the 5 most differential genera selected from the linear discriminant analysis effect size analysis, 6 plasma biomarkers, and estimated glomerular filtration rate (eGFR), and (b) 22 operational taxonomic units (OTUs) from the 5 genera, 6 plasma biomarkers, and eGFR. The results are presented as a heatmap and are grouped using unsupervised clustering. The scale ranges from +1.0 (red) to −1.0 (blue). An asterisk (*) indicates a Spearman rho > 0.3 or rho < -0.3. Circled in blue are correlations that survived the correction for eGFR using Spearman partial correlation analysis. (c,d) Correlation network constructed using the Fruchterman−Reingold layout in the R [*Igraph*] package. The nodes of the network represent the genera (c) or OTUs (d), plasma biomarkers and eGFR, where the edges (i.e., connections) correspond to a significant ($P < 0.05$, $q < 0.05$) and negative (blue, Spearman rho < -0.3) or positive (red, Spearman rho > 0.3) correlation between the nodes. The size of the nodes represents relative abundance of bacterial taxa.

Veillonella and *Streptococcus*, and of TNFR2 with *Veillonella*, were observed (Figure 3b). Pairwise Spearman correlations between CKD-associated microbiota features (5 genera), 6 host kidney biomarkers, and eGFR were further visualized in a correlation network consisting of 30 edges (correlations) and 12 nodes (bacterial genera and host parameters) (Figure 3c and d). We found eGFR to be the hub of the entire network, with 2 subnetworks created for plasma biomarkers and oral microbiota features (Figure 3c). To further test whether the observed correlations were independent of eGFR, we performed the Spearman partial correlation between circulating biomarkers and the

oral microbial genera while correcting for eGFR. We found that the significant positive correlation of IL18 with *Neisseria* genus was independent of eGFR ($P = 0.0074$ and $P = 0.026$, with and without the adjustment for eGFR, respectively), whereas the negative correlation of TNFR1 with *Streptococcus* was diminished ($P = 0.019$ and 0.31, respectively), indicating no independent relationship between *Streptococcus* and TNFR1. At the OTU level, we detected similar results with 2 subnetworks clustered by biomarkers and bacterial OTUs connected through eGFR (Figure 3d). Importantly, after correcting for eGFR, several OTUs remained significantly associated with IL18, indicating the association that is independent of eGFR, whereas the association between other OTUs and biomarkers did not survive the adjustment (Figure 3a and b). Interestingly, strong correlations between the 2 subnetworks were observed only for OTUs with low relative abundance.

DISCUSSION

In this study, we used a biobanking setting, EMR, and dental record systems to analyze existing blood samples and collect additional oral samples and dental information during a regular dental visit for a cohort of patients participating in the Mount Sinai Bio*Me* Biobank Program. For the first time, we compared the microbiota from 4 oral locations (saliva, mandibular anterior lingual area, and plaques from both left and right molar) between individuals with and without CKD and detected significant differences in the overall oral microbiome diversity, mostly pronounced in saliva and anterior mandibular lingual samples. As expected, the abundance of specific taxa was site specific. In particular, at the genus level, we detected the enrichment of *Neisseria* (*Proteobacteria* phylum) and depletion of *Veillonella* and *Streptococcus* (both *Firmicutes* phylum) in CKD patients. The high abundance of *Neisseria* and the lower abundance of *Streptococcus* and *Alloscardovia* (*Actinobacteria* phylum) also correlated with lower eGFR.

Although most of the direct functional evidence points to the pathogenic *Neisseria* and *Streptococcus* species, such as *N. gonorrhoeae*, *N. meningitides*, *S. sanguinis*, *S. mutans* and others, playing a role in the dysregulation of the host inflammation response,[37] the commensal *Neisseria*, *Veillonella* and *Streptococcus* are known to represent the major proportion of the core saliva microbiota.[38] Yet, several recent studies have shown that the commensal *Neisseria* and *Streptococcus* could modulate the host inflammation[39–41] and further contribute to the dysbiosis observed in the salivary microbiota of patients with inflammatory bowel disease[42] and cancers.[43,44] Moreover, *Veillonella* and *Streptococcus* have been reported in the majority of atherosclerotic plaques, where their combined abundances correlated with those in the oral cavity.[45] At the phylum level, our findings are consistent with previous studies in the gut demonstrating that pediatric patients with end-stage renal disease (ESRD) had a decreased relative abundance of *Firmicutes* and an increase in *Proteobacteria*,[46] further suggesting the role for these taxa in kidney dysfunction. Also, a lower abundance of *Streptococcus* taxa have been reported in 1 previous study of the subgingival microbiome of patients with ESRD on dialysis.[47] Like in our study, in Araújo *et al.*,[47] CKD and control groups did not differ in the individual proportions of periodontitis-associated taxa. However, the major differences between the ESRD and control groups were driven by *Prevotella dentalis* (*Bacteroidetes* phylum) and *Abiotrophia defective* (*Firmicutes* phylum)—the taxa not enriched in our CKD patients. There are several potential explanations why we did not fully replicate these findings, including the fact that none of our CKD patients were on dialysis and that different adapter-specific primers and sequencing platforms were used by our study, leading to dissimilarities in bacterial identification. Future studies are warranted to validate our results using comparable technologies.

Interestingly, we found that the *Neisseria*/*Veillonella* ratio in anterior mandibular lingual and *Neisseria*/*Streptococcus* in saliva samples were the best oral microbiome biomarkers to predict CKD diagnosis, correctly predicting CKD status in 81% and 77% cases, respectively. If independently replicated, this information could help detect disease in individuals without prevalent CKD, or identify those whose disease will eventually progress.

Given that CKD is prevalent in individuals with diabetes, hypertension, cardiovascular disease, and periodontal disease, the disorders that have been previously associated with altered oral microbiome, we adjusted for CKD-related comorbidities in our analyses. We found that the association between the oral microbiota and CKD status remained significant after the adjustment, suggesting that the observed associations may reflect biological mechanisms largely independent of common CKD risk factors.

Analysis of inferred metagenomes indicated that the altered oral microbiome is associated with CKD through influencing a number of metabolic pathways, while simultaneously having consequences for cellular processing and genetic replication and repair. Specifically, we revealed decreased abundance of the

ether lipid metabolism, antibiotic biosynthesis, and phosphotransferase pathways, as well as increased abundance of fatty acid metabolism and phosphatidylinositol biosynthesis pathways in saliva or mandibular anterior lingual samples of patients with CKD compared to those with no CKD. The link to the lipid and fatty acid metabolism is particularly interesting, given that patients with CKD have dyslipidemia (reviewed by Tsimihodimos *et al.*[48]), while recent translational research has shown that deranged fatty acid metabolism in renal tubular cells is linked to profibrotic pathways responsible for kidney disease progression.[49,50] Thus, the link between oral microbiota, dysregulated metabolic pathways, and CKD development/progression should be evaluated in detail further.

There are several potential mechanisms that may explain the altered oral microbial ecology in CKD patients, including dysregulated inflammatory and metabolic pathways, higher concentration of urea in the saliva, mucosal inflammation due to ammonia accumulation, decrease in the salivary mucin coating increasing vulnerability to infections, and medications specific to CKD patients.[47] Moreover, in addition to altered oral microbiota, we also tested the relationship between the oral microbiota and 6 plasma cytokines linked to CKD and representing inflammatory, injury-related, and fibrosis/repair pathways. All of these biomarkers have been associated with development or progression of kidney disease in several distinct settings including diabetes, acute injury, and transplantation.[51–55] We found that the high abundance of *Neisseria* genus was associated with high IL18 plasma levels. The opposite trend was observed for the *Streptococcus* genus, whereby the lower abundance correlated with higher IL18 and TNFR1 levels. At the OTU level, higher abundance of *Veillonella* was correlated with higher TNFR1 and TNFR2 levels. However, only the associations with IL18 survived the correction for eGFR. Therefore, although the common pathway between deranged microbiota and CKD could possibly be inflammatory in nature, most of these associations are mediated through eGFR. Whether the bacterial link with IL18 is causal and whether altering microbiota could change CKD risk need to be further explored.

This study has several strengths. First, it proved the utility of the biobank setting for comprehensive oral microbiome studies, whereby individuals (who already consented for biobanking and provided blood samples, and whose extensive clinical data are available through EMR) were approached at a dental clinic for oral sample collection and dental health record verification. Moreover, to the best of our knowledge, this is the first study that reportedly investigated the association between the oral microbiome and CKD prevalence. Sampling of multiple oral locations also allowed parsing out the contribution of site-specific taxa to disease risk. Furthermore, associations were also observed with inflammatory plasma biomarkers linked to kidney dysfunction, providing potential mechanisms for the obtained results and reducing the chances of our results being false positive.

Our study also has some limitations. First, although we included race/ethnicity, age, and some clinical variables as covariates in regression models, our moderate sample size restricted us from further exploring the role of other potential confounders (e.g., medications, diet) or stratifying by CKD severity. In addition, we did not collect information on whether participants had dry mouth. Since half of CKD patients are known to have a dry oral environment, this could represent an unmeasured confounder.[56] Therefore, a validation of our findings in a larger cohort is warranted. Second, in this study, because the oral samples were collected from the participants who were already enrolled in the Bio*Me* Biobank Program with the blood samples collected at the entry, there was on average a 48-month gap between the blood sample collection used to measure plasma cytokines and oral sample collection for microbiome analysis, which could inaccurately reflect the biomarker levels at the time of oral sampling. Although there are no data available on changes over a 4-year period for these cytokines, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial has shown changes over a period of 24 months that ranged between 0.6% and 8.2%, with IL18 decreasing by only 2.3%.[57] Also, even though we pulled out from the EMR the eGFR estimates closest to the oral sample collection dates and observed significant correlations between eGFR and the plasma biomarkers, there is still a possibility that we underestimated the relationship between CKD-associated microbial taxa and biomarker levels. In addition, we excluded individuals who received antibiotics $\leq$ 3 months prior to oral sampling; however, one may argue that this time is not sufficient for the flora to recover. Yet, prior studies have shown that, as oppose to the gut, where short-term antibiotic treatment may shift the microbiota to long-term alternative dysbiotic states,[58] the salivary microbiome recovers quickly and is surprisingly robust toward antibiotic-induced disturbance.[59] Moreover, our study subjects were pooled from the Biobank that represents the diverse local communities of upper Manhattan served by the Mount Sinai Medical Center, with $\sim$90% African American and

Hispanic/Latino and <4% whites; therefore, our results may not be generalizable to the general population. Also, our study design did not allow determining whether the altered oral microbiome is a risk factor for CKD or its consequence. Future prospective studies will be needed to address these issues and to assess the predictive value of the oral microbiome composition reported here on CKD development and progression. Finally, although the 16S sequencing is very informative and cost-efficient for microbiome survey, its amplicon size is merely 460 base pairs, limiting our ability to identify bacterial taxa at the strain level, otherwise provided by metagenomic sequencing. Nevertheless, we used an imputation tool to predict functional differences in the oral microbiota by CKD status.

In summary, we detected distinctive microbiota composition in saliva and anterior mandibular lingual samples associated with CKD diagnosis. A better understanding of the role of the oral microbiome in kidney function may shed new light on the pathogenesis of renal diseases and help in the designing of novel interventions aimed at restoring oral symbiosis to treat or prevent CKD.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Comparison of the oral microbiota between study subjects with and without chronic kidney disease (CKD) in mandibular anterior lingual and saliva samples using nonmetric multidimensional scaling ordination.

Figure S2. PICRUSt prediction of the metagenomic functional pathways of the oral microbiota.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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