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Oral and Gut Dysbiosis in Migraine

Oral Microbial Signatures as Biomarkers of Migraine

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Abstract

Background and Objectives

Emerging evidence suggests that oral health conditions may exacerbate migraine, and saliva is a potential source of biomarkers for migraine. The 3-way interaction of the oral-gut-brain axis has been implicated in several neurologic disorders, but has rarely been studied in migraine. This study examined the oral and gut microbiomes simultaneously and identified several key oral microbes that may influence migraine.

Methods

In this cross-sectional case-control study, participants were divided into 3 groups: episodic migraine (n = 55), chronic migraine (n = 55), and healthy control (HC) (n = 55). Demographic and clinical characteristics; lifestyle factors; and biological samples including saliva, stool, and blood were collected. Composition, function, and community type of the oral and gut microbiomes were compared among the 3 groups.

Results

Oral dysbiosis was more pronounced than gut dysbiosis in the migraine groups, with 13 oral genera significantly enriched or depleted compared with HCs. The migraine groups showed increased abundance of *Gemella*, *Streptococcus*, *Granulicatella*, and *Rothia* and decreased abundance of *Alloprevotella*, *Veillonella*, *Haemophilus*, *Selenomonas*, *Campylobacter*, *Cardiobacterium*, *Megasphaera*, and *Kingella* after adjustment for demographic and lifestyle factors including diet. The enriched oral genera within the migraine groups were associated with carbohydrate metabolic pathways, whereas the depleted oral genera were associated with pathways related to nitrogen. A significant proportion of the oral microbial signatures of migraine included genera capable of reducing nitrate and/or nitrite. Some of these oral microbial signatures of migraine had a relative abundance that was positively or negatively associated with the number of headache days per 30 days and formed distinct microbial clusters in both the oral cavity and gut. Machine learning classifiers using the oral microbiome effectively classified migraine status, with an area under the receiver-operating characteristic curve of 0.83–0.88.

Discussion

Our findings suggest that oral dysbiosis may be involved in the development of migraine and highlight specific oral microbes as potential diagnostic biomarkers and therapeutic targets for migraine.

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Supplementary Material

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Glossary

ASV = amplicon sequence variant; **AUC** = area under the receiver operating characteristic curve; **BMI** = body mass index; **CGRP** = calcitonin gene-related peptide; **CM** = chronic migraine; **DMM** = Dirichlet Multinomial Mixtures; **EM** = episodic migraine; **HC** = healthy control; **HIT-6** = Headache Impact Test-6; **KEGG** = Kyoto Encyclopedia of Genes and Genomes; **LEfSe** = linear discriminant analysis effect size; **MIDAS** = migraine disability assessment; **PC** = principal component; **ROC** = receiver-operating characteristic; **SCFA** = short-chain fatty acid.

Introduction

Migraine is a prevalent and debilitating neurologic disorder affecting over 1 billion people, significantly affecting individuals' quality of life and imposing a considerable burden on health care systems.[1,2] Some pivotal mediators, such as calcitonin gene-related peptide (CGRP), have been identified for migraine; however, a complete mechanism of its pathophysiology remains elusive.[3,4] The lack of biomarkers for migraine often results in suboptimal treatment, causing delays in diagnosis and increasing the burden of migraine on patients.

A growing body of research indicates that saliva contains a number of potential biomarkers for migraine, which can provide valuable insight into the physiologic changes observed in individuals with migraine.[5-11] Notably, recent studies on salivary CGRP have highlighted the potential of saliva as a medium for investigating biomarkers relevant to the diagnosis and treatment of migraine.[10,11]

On the other hand, emerging evidence suggests that the gut-brain axis, and by extension, the oral-gut-brain axis, may influence the pathogenesis of several neurologic diseases.[12-15] The gut-brain axis is a bidirectional communication between the gastrointestinal system and CNS,[12] which is significantly influenced by the gut microbiota and their metabolites, such as neurotransmitters and short-chain fatty acids (SCFAs).[16] This concept is extended in the oral-gut-brain axis to include the oral microbiome, which is the second largest microbiome after the gut and is mechanically and chemically linked to the gut.[17] Furthermore, the oral microbiota and their by-products directly influence the brain through the trigeminal, olfactory, and facial nerves and bloodstream.[13,18] Alternatively, they indirectly affect the brain by inducing systemic inflammation or disrupting the gut microbial composition by translocation of microorganisms from saliva to the gut.[17,19]

Disruptions in the balance of the oral and gut microbiome may be associated with the pathogenesis of migraine. Some previous studies have shown alterations in the oral or gut microbiome in individuals with migraine.[20-23] Nevertheless, the findings were heterogeneous across the studies, which made it challenging to ascertain the specific factors that are responsible for migraine. Therefore, there is a need to investigate the oral and gut microbiomes simultaneously and analyze their intricate inter-relationships. We concurrently examined the oral and gut microbial dysbiosis in participants with episodic migraine (EM) and chronic migraine (CM) compared with healthy controls (HCs). Furthermore, we identified potential taxonomic and functional signatures of migraines using oral microbiome.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all participants, and this study was approved by the institutional review board of Severance Hospital (IRB No. 2021-3925-001).

Participants

We recruited participants with EM and CM from the neurology outpatient clinic of a tertiary care university hospital (Seoul, Republic of Korea) between September 2022 and May 2023. The inclusion criteria for participants with EM and CM were as follows: (1) age 19–65 years and (2) meeting the clinical criteria of the third edition of the International Classification of Headache Disorders (ICHD-3) for EM (code 1.1 or 1.2) or CM (code 1.3).[24] The exclusion criteria were as follows: (1) medication overuse headache; (2) receipt of preventive treatment of migraine; (3) a current or history of treatment for oral, gastrointestinal, medical, or psychiatric disorders, except for anxiety, depression, or fibromyalgia; (4) significant dietary changes in the 3 months before the study; (5) probiotic or antibiotic use in the 6 months before the study; and (6) pregnancy or lactation. The clinical characteristics and common comorbidities of migraine including anxiety, depression, and fibromyalgia were assessed in participants with EM and CM (eMethods).

Furthermore, we recruited controls through advertisement after matching for age, sex, and body mass index (BMI) distribution in the EM and CM groups. The controls were eligible for the study if they had not experienced any headaches in the previous year and had not had a migraine or probable migraine attack in their lifetime. In addition, exclusion criteria (3)–(6) were applied to the control group. All study participants completed a lifestyle survey, including questions regarding cohabitation, smoking habit, sleep pattern, and dietary habit.

Sample Size Calculation

We calculated the sample size for this study using the original data set from our previous study (eMethods, eFigure 1, and

eFigure 2).[22] The total sample size required was calculated as 165; we included 55 samples per group in this study.

Sample Collection and 16S rRNA Gene Sequencing

We collected unstimulated saliva using a 50-mL sterile Eppendorf conical tube between 9 AM and noon on the day of consent. Participants were asked to refrain from eating, drinking, chewing, smoking, and brushing their teeth for at least 1 hour before the saliva was collected. On the same day, blood was drawn from the antecubital vein into a Becton, Dickinson Vacutainer Serum Separator Tube (Becton, Dickinson and Company, USA). The blood sample was maintained at room temperature for 15 minutes to allow clotting and centrifuged at 1500 g for 15 minutes at 4°C to separate the clot from serum. The saliva and serum were immediately stored at −70°C. A stool collection kit (SPL Life Sciences, Korea) was used to collect stool within 3 days of consent (preferably the earliest day after consent). Stool samples collected at home were sealed and immediately frozen at −20°C, followed by delivery to the study site with the ice pack provided within 14 days of collection. Then, the samples were stored at −70°C until the analysis.

We performed targeted sequencing of the microbial 16S rRNA gene extracted from saliva and stool samples. Detailed procedures for DNA extraction, PCR amplification, and sequencing are provided in eMethods.

Determination of SCFAs

We determined serum acetate, butyrate, and propionate levels using liquid chromatography-tandem mass spectrometry (eMethods, eTable 1 and eTable 2).

Diversity and Taxonomic and Functional Abundance Analysis

We performed amplicon sequence variant (ASV)-based taxonomic profiling using a pipeline based on internal plugins of QIIME2 (version 2022.11). Taxonomic assignment was performed using the feature classifier plugin with the EzBioCloud database (CJ Bioscience Inc., Korea) as a reference.[25]

The number of taxa and Shannon Diversity Index were calculated for alpha diversity, and Bray-Curtis dissimilarity was calculated for beta diversity. The SIAMCAT R package was used for differential abundance tests to identify oral and gut microbial signatures of the EM and CM groups compared with those of the HC group at the genus level.[26] The analysis included preprocessing options of prevalence filtering with a cutoff of 0.1 and multiple testing correction with the Benjamini-Hochberg procedure at a significance cutoff of 0.05. Moreover, we constructed a logistic regression model to determine whether the identified microbial signatures remained significant after adjusting for potential confounding covariates, including age, sex, BMI, and lifestyle factors.

We used the PICRUSt algorithm to estimate the Kyoto Encyclopedia of Genes and Genomes (KEGG) functional pathway profiles based on taxonomic profiles.[27] Spearman's correlation coefficient was used to examine the correlations between the relative abundances of oral genera and metabolic pathway signatures, which were visualized using a heatmap generated with Seaborn Python library.

Association of Microbial Signatures With Clinical Characteristics

Poisson regression analysis was used to assess the associations between the relative abundance of oral microbial signatures and the number of headache days per 30 days in participants with migraine to further explore the clinical implications of the oral microbial genera identified in the comparative analysis. Age, sex, BMI, lifestyle factors, anxiety, depression, and fibromyalgia were adjusted for as covariates.

Correlation Network Analysis and Envirotyping

We conducted correlation network analysis on the microbial communities within each body site to identify bacterial modules that represent important microbial associations (eMethods). Orotypes and enterotypes—distinct clusters of communities with a similar oral and gut microbial composition, respectively—were identified using Dirichlet Multinomial Mixtures (DMM) clustering.[28] The fitting of the model based on the Laplace approximation was used to determine the optimal number of clusters. We used the linear discriminant analysis effect size (LEfSe) to discern signature genera that stratify the microbiomes into distinct clusters, using LDA 3.0 as the cutoff.[29]

Oral-Gut Transmission Analysis

The ASVs shared between the oral cavity and gut were used to determine the potential oral-to-enteric transmission. An ASV was classified as shared if it was identified in both the oral and gut microbiomes of each individual. Otherwise, it was designated as exclusive to either the oral or gut microbiome. Subsequently, the average percentage abundance of the shared microbiome in the oral cavity and gut was compared among the CM, EM, and HC groups. Furthermore, the occurrence of oral microbial signatures in the gut was examined across the groups.

Statistical Analysis

The moonbook R package with the default options was used for statistical analysis. Continuous variables were compared using independent *t*-test or analysis of variance for normally distributed data and Wilcoxon rank-sum test or Kruskal-Wallis test for nonparametric data. Categorical data were compared using Pearson's χ^2 or Fisher's exact test. We performed principal component analysis for the lifestyle survey data using the sklearn Python library. Eight principal component (PC) axes were ultimately selected and used as covariates in the modeling to adjust for potential confounding factors in the relationship between the microbiome and migraine (eMethods and eFigure 3).

Table 1 Demographic and Clinical Characteristics of Participants

	CM (n = 55)	EM (*n* = 55)	HC (*n* = 55)	*p* Value
Sex, female, n (%)	51 (92.7)	49 (89.1)	51 (92.7)	0.732
Age, y	45.9 ± 10.8	42.2 ± 9.9	44.5 ± 11.3	0.181
BMI, kg/m^2	22.8 ± 3.5	22.8 ± 3.3	23.1 ± 3.6	0.892
Number of headache days per 30 d	21.1 ± 6.2	5.0 ± 2.8		<0.001[a]
Severe pain intensity, n (%)	46 (83.6)	45 (81.8)		>0.999
Unilateral location, n (%)	30 (54.5)	38 (69.1)		0.170
Pulsating quality, n (%)	54 (98.2)	49 (89.1)		0.118
Aggravation by routine physical activity, n (%)	46 (83.6)	49 (89.1)		0.578
Nausea, n (%)	49 (89.1)	50 (90.9)		>0.999
Vomiting, n (%)	26 (47.3)	33 (60.0)		0.251
Photophobia, n (%)	36 (65.5)	30 (54.5)		0.330
Phonophobia, n (%)	41 (74.5)	35 (63.6)		0.302
Total HIT-6 score	63.6 ± 7.7	58.0 ± 9.3		0.001[a]
Total MIDAS score	40.4 ± 46.7	15.6 ± 17.0		<0.001[a]
Anxiety, n (%)	33 (60.0)	15 (27.3)		0.001[a]
Depression, n (%)	33 (60.0)	15 (27.3)		0.001[a]
Fibromyalgia, n (%)	25 (45.5)	5 (9.1)		<0.001[a]

Abbreviations: BMI = body mass index; CM = chronic migraine; EM = episodic migraine; HC = healthy control; HIT-6 = Headache Impact Test; MIDAS = Migraine Disability Assessment.
For age, sex, and BMI, CM, EM, and HC groups are compared. For other clinical variables, CM and EM groups are compared.
[a] $p < 0.05$.

Machine Learning Modeling for the Classification of Migraine

First, we constructed machine learning–based random forest models for migraine classification (i.e., migraine [CM or EM] vs HC, CM vs HC, and EM vs HC) using all oral microbiome features after prevalence filtering with a cutoff of less than 10%. A feature importance analysis identified genus-level oral microbial markers as high-ranking features for classifying migraines that showed significant enrichment or depletion in the CM or EM groups compared with the HC group (eFigure 4).

Then, we developed machine learning–based random forest models for migraine classification using 3 different sets of features (host, microbial, and host and microbial features). Age, sex, BMI, and lifestyle factors (i.e., PC 1–8) were used as host features. Oral microbial signatures were incorporated as microbial features. We performed 10 random repeats of five-fold cross-validation for each dataset and assessed the performance of the classification models using the area under the receiver-operating characteristic (ROC) curve (AUC). We further conducted feature importance analyses for models from microbial features.

Data Availability

Data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

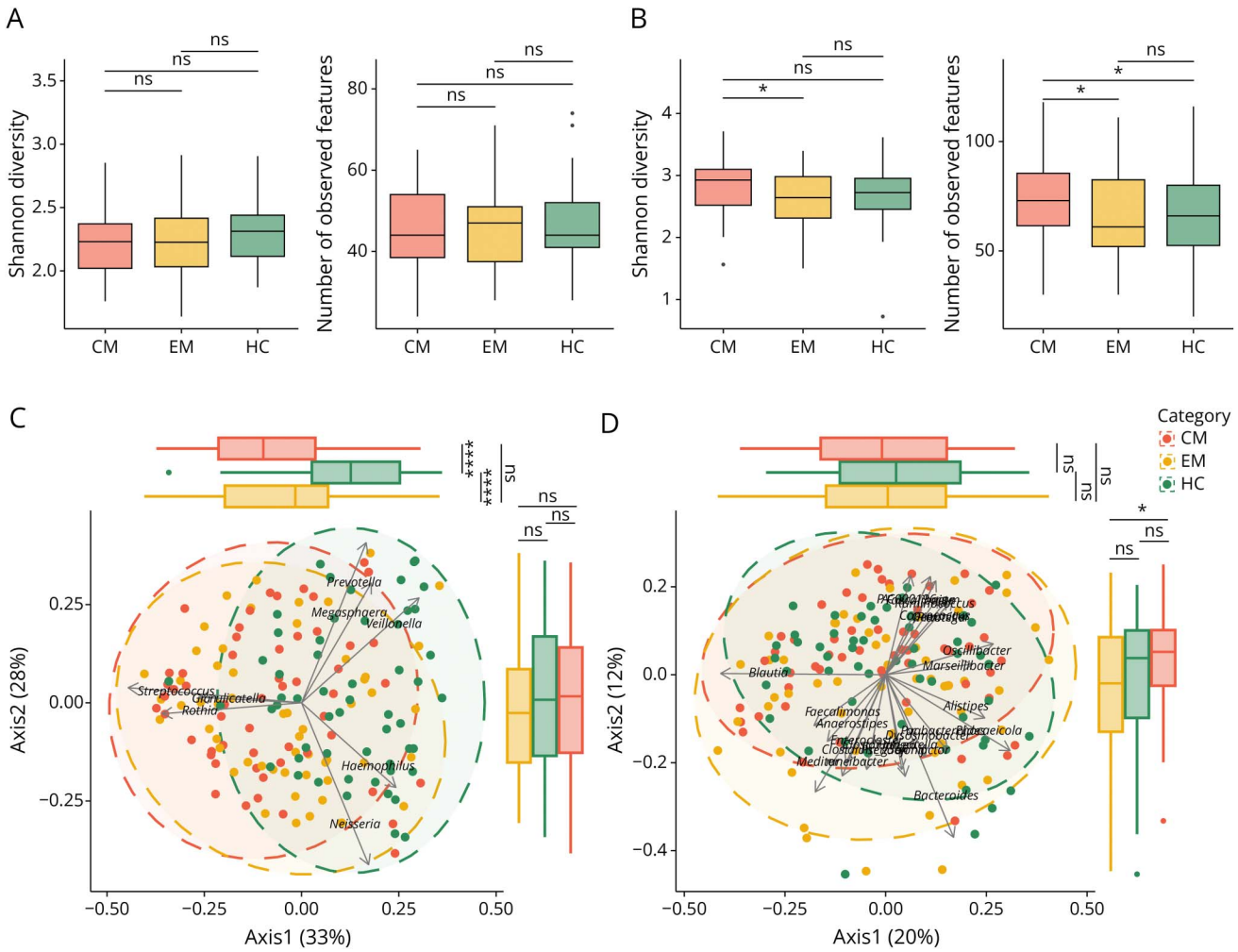
Characteristics of Participants With EM and CM and Healthy Controls

In total, 165 participants were enrolled, with 55 participants each in the EM, CM, and HC groups. The demographic and clinical characteristics are presented in Table 1. Sex, age, and BMI did not significantly differ among the 3 groups. However, the number of headache days per 30 days; total Headache Impact Test-6 (HIT-6) score; total Migraine Disability Assessment (MIDAS) score; and prevalence of anxiety, depression, and fibromyalgia significantly differed between the EM and CM groups.

Overall Composition and Microbial Diversity

The overall composition of the oral and gut microbiomes of the 3 groups was examined at the phylum and genus levels, respectively (eFigure 5). Alpha diversity did not significantly differ at the genus level of the oral microbiome (Figure 1A). However, for the gut microbiome, the CM group had a higher Shannon diversity than the EM group ($p = 0.027$) and a greater number of observed features than the EM ($p = 0.034$) and HC ($p = 0.040$) groups (Figure 1B). Genus-level beta diversity analysis of the oral and gut microbiomes revealed significant differences among the 3 groups ($p = 0.001$

Figure 1 Oral and Gut Microbiome Diversity in the CM, EM, and HC Groups



Alpha diversity (left) and number of taxa observed at the genus level (right) of (A) oral and (B) gut microbiomes. Beta diversity of PCoA based on Bray-Curtis dissimilarity at the genus level of (C) oral and (D) gut microbiomes. ****$p < 0.0001$; *$p < 0.05$. CM = chronic migraine; EM = episodic migraine; HC = healthy control; ns = not significant; PCoA = principal coordinate analysis.

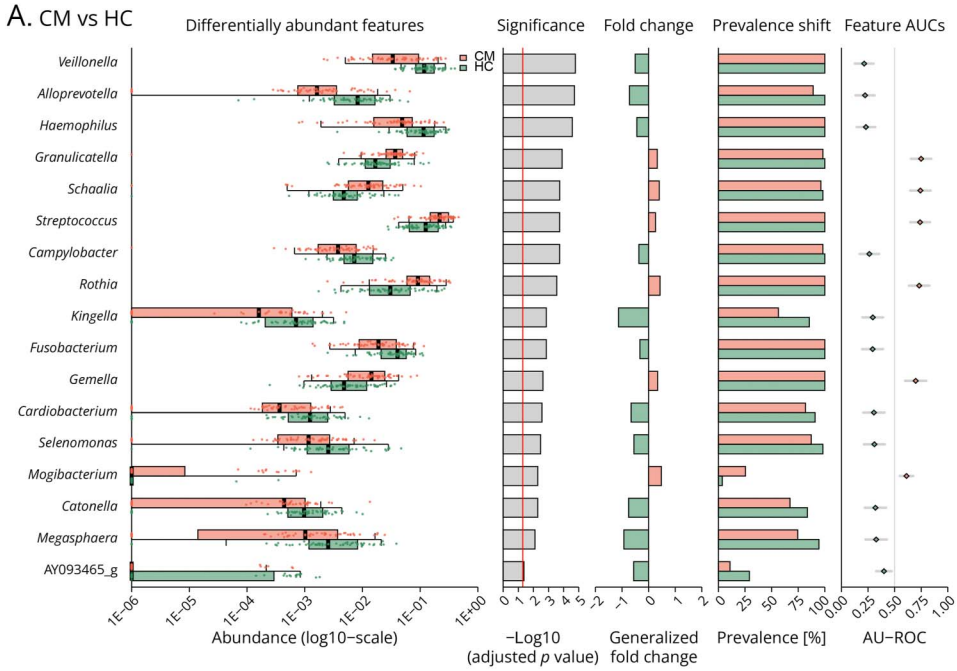
and 0.039 for the oral and gut microbiomes, respectively), with a more pronounced difference observed in the oral microbiome (Figure 1, C and D).

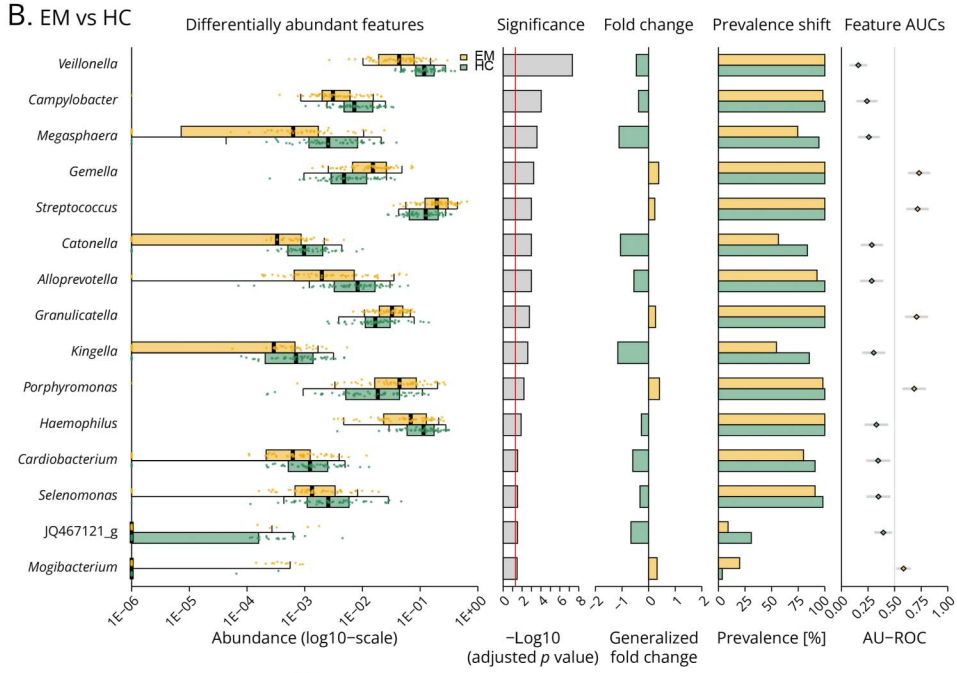
Taxonomic and Functional Signatures of Oral Microbiome

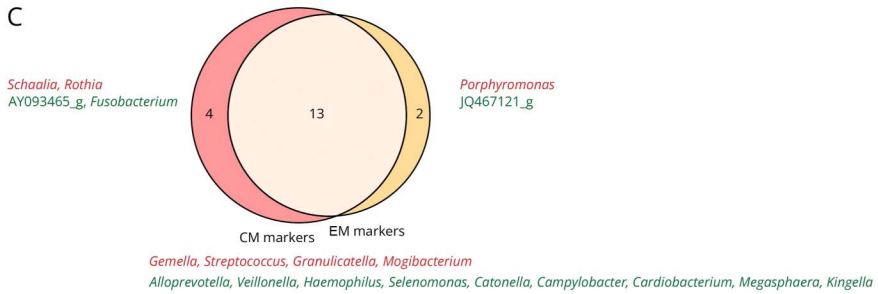
We identified 17 and 15 oral microbial signatures at the genus level for the CM and EM groups, respectively, which exhibited either significant enrichment or depletion compared with that in the HC group (Figure 2, A and B). We observed a significant increase in the abundance of *Gemella*, *Streptococcus*, *Granulicatella*, *and Mogibacterium* and a significant reduction in the abundance of *Alloprevotella*, *Veillonella*, *Haemophilus*, *Selenomonas*, *Catonella*, *Campylobacter*, *Cardiobacterium*, *Megasphaera*, and *Kingella* in both the CM and EM groups (Figure 2C). Furthermore, the CM group exhibited enrichment of *Schaalia* and *Rothia*. However, the relative abundance of the gut microbiota did not significantly differ between the CM and EM groups compared with that in the HC group. Statistical significance was generally retained for the oral microbiome after logistic regression modeling was applied with age, sex, BMI, and lifestyle factors as adjustment variables (eTable 3). Furthermore, compared with the HC group, the EM group was significantly enriched with *Rothia* after the adjustment.

A total of 62 and 52 KEGG metabolic pathways were identified as significant functional markers of the oral microbiome for the CM and EM groups, respectively. Of these, 48 were identified as overlapping markers (Figure 3, A and B). Carbohydrate metabolism, including fructose/mannose, galactose, starch/sucrose, and glycolysis/gluconeogenesis, in addition to glycan biosynthesis and metabolism, was enhanced in both the CM and EM groups. By contrast, the metabolic pathways of butanoate, propanoate, and nitrogen metabolism were depleted in the migraine groups. Notably, the abundance of oral microbial and metabolic signatures exhibited a significant correlation and clustering (Figure 3C).

Figure 2 Differential Abundance of the Oral Microbiome at the Genus Level Between (A) CM and HC, (B) EM and HC, and (C) Venn Diagram of Overlapping Altered Taxa

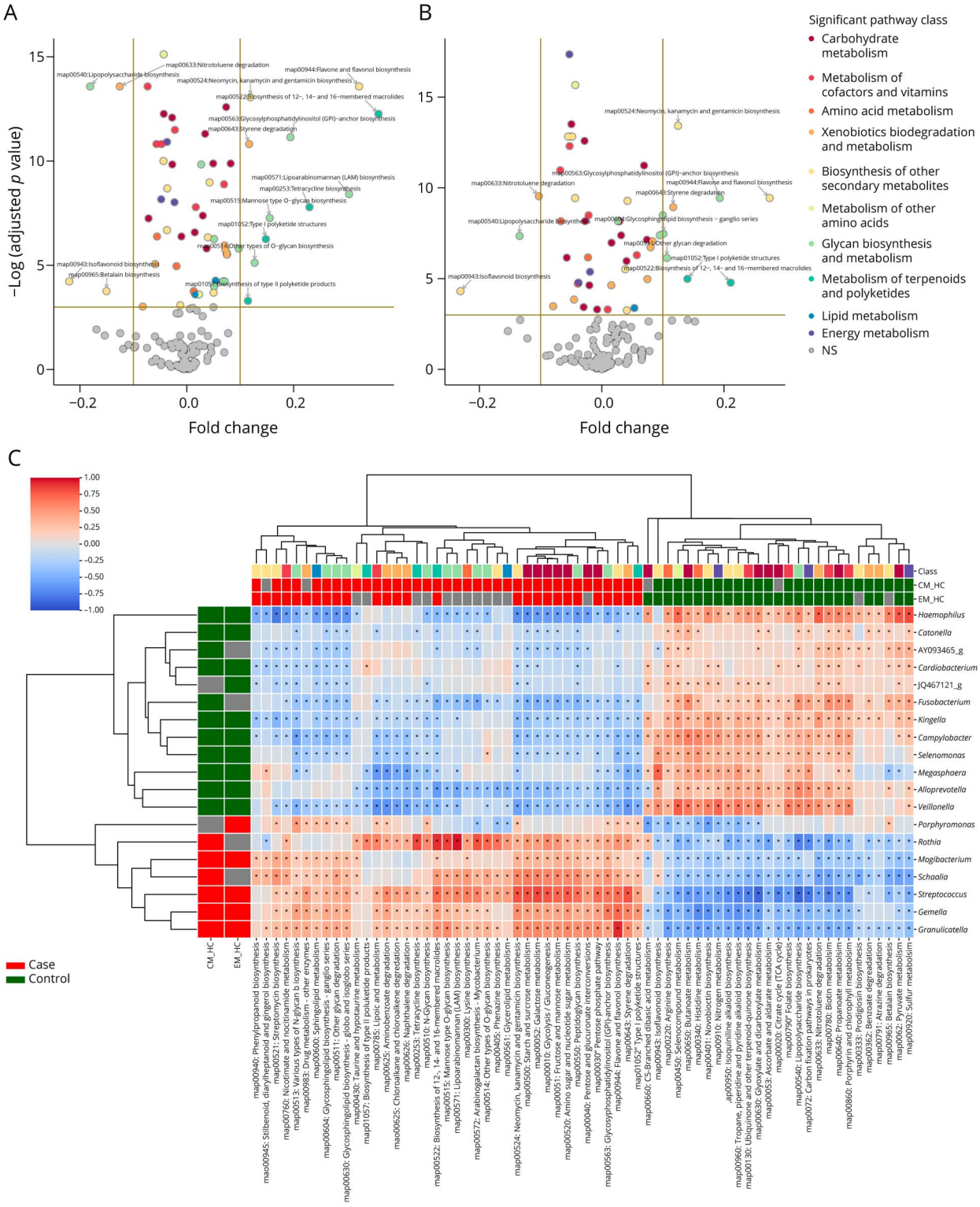






(C) Venn diagram of oral microbial signatures increased or decreased in the CM and/or EM groups compared with those in the HC group. Magenta represents taxa enriched in participants with migraine, and green represents taxa enriched in controls. AUC = area under the receiver-operating characteristic curve; CM = chronic migraine; EM = episodic migraine; HC = healthy control.

Figure 3 Differential Abundance of Functional Metabolic Pathway Profiles of the Oral Microbiome Between (A) CM and HC or (B) EM and HC Groups



In the volcano plot, the x-axis represents the fold change (FC) between 2 comparison groups, while the y-axis represents the *p* value. The features with an adjusted $p < 0.05$ are colored according to their pathway class, and the significant features with FC ≥ 0.1 are additionally labeled with text. (C) A heatmap of Spearman correlation results of percentage abundances of pathways and taxa to represent the association between taxonomic oral signatures and estimated functional KEGG metabolic pathway signatures. Spearman's rho values, are represented by heatmap colors, and $p < 0.05$ are highlighted with an asterisk. CM = chronic migraine; EM = episodic migraine; HC = healthy control; KEGG = Kyoto Encyclopedia of Genes and Genomes.

Table 2 Poisson Regression Analysis of the Association Between Headache Days per 30 Days and Oral Microbial Signatures Significantly Enriched or Depleted in the Migraine Groups Compared With the Control Group

Oral microbial signatures at genus level	Estimate	Standard error	z value	*p* Value	Significance
Rothia	0.017	0.004	4.445	<0.001	***
Catonella	0.707	0.154	4.587	<0.001	***
Haemophilus	−0.022	0.005	−4.487	<0.001	***
Megasphaera	0.196	0.06	3.252	0.001	**
Mogibacterium	3.921	1.207	3.249	0.001	**
Schaalia	0.037	0.014	2.599	0.009	**
Granulicatella	0.033	0.014	2.465	0.014	*
Alloprevotella	−0.057	0.026	−2.155	0.031	*
Selenomonas	−0.202	0.094	−2.15	0.032	*
Cardiobacterium	−0.439	0.21	−2.086	0.037	*
AY093465_g	−2.879	1.487	−1.936	0.053	ns
Fusobacterium	−0.02	0.011	−1.862	0.063	ns
Kingella	0.401	0.323	1.241	0.215	ns
Porphyromonas	−0.004	0.005	−0.689	0.491	ns
JQ467121_g	0.705	1.059	0.665	0.506	ns
Gemella	−0.008	0.017	−0.501	0.616	ns
Campylobacter	−0.028	0.065	−0.423	0.673	ns
Veillonella	0.001	0.005	0.25	0.803	ns
Streptococcus	<0 · 001	0.003	0.015	0.988	ns

Abbreviations: BMI = body mass index; ns = not significant.
Age, sex, BMI, lifestyle factors (i.e., principal components 1–8), anxiety, depression, and fibromyalgia were adjusted as covariates.
***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

The genera *Streptococcus*, *Granulicatella*, *Gemella*, and *Schaalia* were positively associated with pathways associated with sugar metabolism. By contrast, the genera *Veillonella*, *Selenomonas*, and *Campylobacter* were positively associated with metabolic pathways related to SCFAs and nitrogen.

Association Between Oral Microbial Signatures and Headache Days per 30 Days

The relative abundance of *Rothia*, *Granulicatella*, *Schaalia*, *Megasphaera*, *Catonella*, and *Mogibacterium* had significant positive associations with the number of headache days per 30 days, whereas *Cardiobacterium*, *Selenomonas*, *Alloprevotella*, and *Haemophilus* had negative associations (Table 2 and eFigure 6).

Serum SCFA Levels

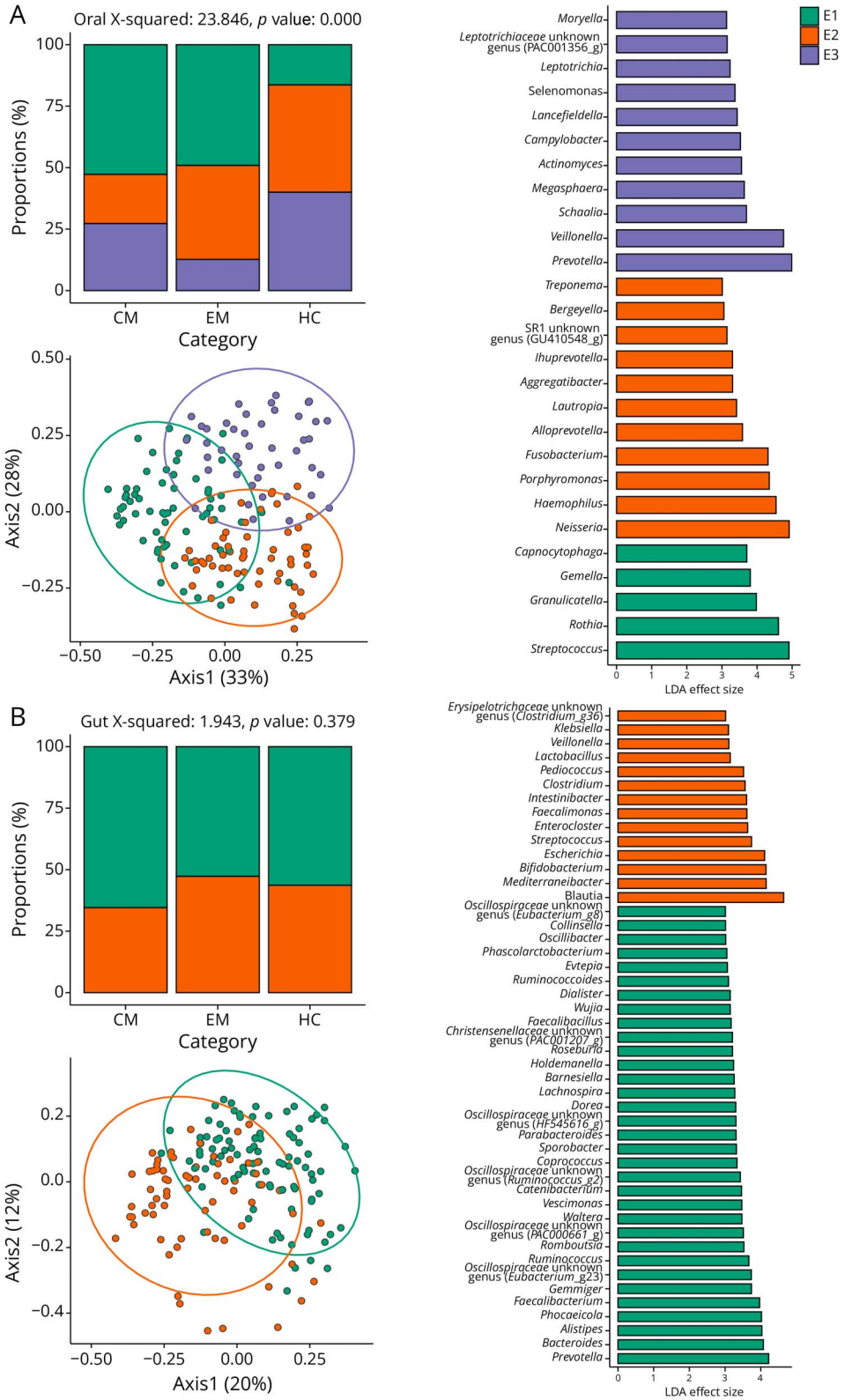
The level of serum propionic acid was significantly reduced in the CM group in comparison with both the EM ($p < 0.001$) and HC ($p < 0.001$) groups. However, no significant differences were observed in serum acetic acid and butyric acid levels across the 3 groups (eFigure 7).

Microbial Network and Community-Level Orotype and Enterotype

The correlation network analysis identified a number of distinct microbial clusters within each of the sampled body sites (eFigure 8). Among the genera enriched in migraine, *Rothia*, *Gemella*, *Streptococcus*, and *Granulicatella* clustered together. Moreover, distinct subnetwork clusters were identified in the gut taxa, with 1 notable gut bacterial cluster comprising oral microbial signatures of migraine, including *Rothia*, *Gemella*, *Streptococcus*, *Granulicatella*, and *Veillonella*.

On the other hand, the community-wide ecological structures in the oral cavity and gut were evaluated using the DMM clustering method. The 3 oral clusters showed different microbial profiles: Cluster E1, with a higher abundance of *Rothia*, *Gemella*, *Streptococcus*, and *Granulicatella*; Cluster E2, with a higher abundance of *Neisseria*, *Haemophilus*, *Porphyromonas*, and *Fusobacterium*; and Cluster E3, with a higher abundance of *Prevotella*, *Veillonella*, *Schaalia*, and *Megasphaera*. A significant difference in the prevalence of oral clusters was observed between the CM, EM, and HC groups,

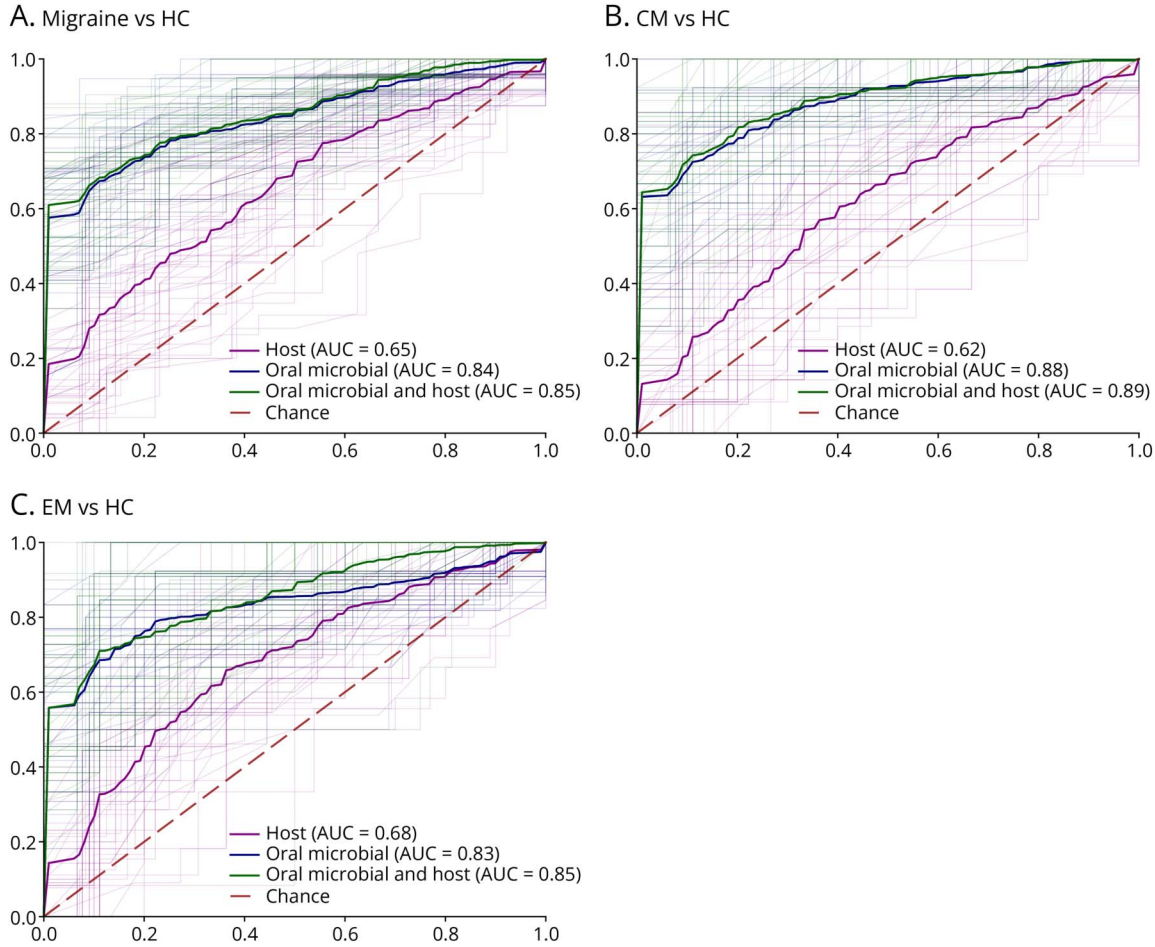
Figure 4 Ecological Structures of the (A) Oral and (B) Gut Microbiome at the Genus Level



Relative proportions of ecological structure types across groups for the (A, upper left) oral and (B, upper left) gut microbiomes. PCoA plot based on Bray-Curtis dissimilarity by ecological structures of the (A, lower left) oral and (B, lower left) gut microbiomes. LEfSe analysis comparing the genus profiles of the (A, right) oral and (B, right) gut microbiomes by ecological structure. LEfSe = linear discriminant analysis effect size; PCoA = principal coordinate analysis.

with the HC group having a significantly lower proportion of E1 than both the CM and EM groups ($p < 0.001$) (Figure 4A). For the gut microbiome, 2 distinct clusters were identified. Cluster E1 was characterized by a higher abundance of *Prevotella*, *Bacteroides*, *Alistipes*, and *Phocaeicola*, whereas Cluster E2 was distinguished by a higher abundance

Figure 5 Machine Learning–Based Classification of Migraine—(A) Migraine, (B) CM, and (C) EM—Using Host-Derived Features, Oral Microbial Signatures, and Combinations of Host-Derived Features and Oral Microbial Signatures



CM = chronic migraine; EM = episodic migraine.

of *Blautia*, *Bifidobacterium*, and *Escherichia*. Nevertheless, there were no significant differences in the proportion of the 2 clusters across the 3 groups ($p = 0.379$) (Figure 4B).

Oral-Gut Transmission Analysis

The mean percentage abundance of shared ASVs was not significantly different across the 3 groups ($p = 0.147$ and $p = 0.133$ for oral and gut, respectively). The 8 most commonly co-occurring taxa were *Streptococcus*, *Rothia*, *Veillonella*, *Haemophilus*, *Granulicatella*, *Schaalia*, *Gemella*, and *Megasphaera* (eFigure 9 and eTable 4).

Machine Learning–Based Classification of Migraine Using Oral Microbiome

Machine learning–based random forest models for the classification of migraine (CM or EM) yielded an AUC of 0.84 ± 0.06 using microbial features (i.e., oral microbial signatures of migraine) and 0.85 ± 0.05 using combinations of host and microbial features (Figure 5A). For the classification of CM, the best performance was achieved with an AUC of 0.89 ± 0.07 using both host and microbial features, followed by 0.88 ± 0.07 using microbial features (Figure 5B). For the classification of EM, the models showed an AUC of 0.85 ± 0.08 using both host and microbial features and 0.83 ± 0.09 using microbial features (Figure 5C). The feature importance values for *Veillonella* and *Alloprevotella* were high for all 3 migraine classifications (eFigure 10).

Discussion

In this study, we conducted a simultaneous investigation of the oral and gut microbiomes in participants with EM, CM, and controls. The main findings of the study were as follows: (1) Significant alterations were identified in the oral microbiome of the migraine groups compared with the HC group; however, the gut microbiome did not significantly differ. (2) The increased relative abundance of *Gemella*, *Streptococcus*, *Granulicatella*, and *Rothia* and the decreased relative abundance of *Alloprevotella*, *Veillonella*, *Haemophilus*, *Selenomonas*, *Campylobacter*, *Cardiobacterium*, *Megasphaera*, and *Kingella* in both the EM and CM groups remained statistically significant

after adjustment for demographic and lifestyle factors. (3) The relative abundance of some of these oral genera was significantly associated with the number of headache days per 30 days after adjustment for demographic and lifestyle factors and psychiatric comorbidities. (4) The enriched oral genera within the migraine groups were associated with carbohydrate metabolic pathways, whereas the depleted oral genera were associated with pathways related to SCFAs and nitrogen. (5) The oral microbial signatures of migraine were observed simultaneously in the oral cavity and gut, forming several distinct clusters. (6) Machine learning classifiers using the oral microbiome effectively classified the migraine status, with a high AUC of 0.83–0.88.

Participants with migraine in this study were divided into EM and CM groups based on clinical and biological distinctions recognized in current headache research. According to the ICHD-3, CM is defined as headache occurring on at least 15 days per month for more than 3 months, with migraine features present on at least 8 days.[24] In addition to frequency, CM is increasingly recognized as a distinct clinical entity, characterized by greater disease burden, higher rates of psychiatric comorbidities, and an increased risk of medication overuse.[30,31] Furthermore, CM involves pathophysiologic alterations, including increased cortical excitability, sustained neuroinflammation, and heightened central sensitization, which contribute to impaired pain modulation compared with EM.[32] These distinctions provided the rationale for examining EM and CM separately in this study. Recent research has also shown that patients with CM, particularly those with medication overuse, exhibit increased gut permeability and systemic inflammation, which may disrupt the gut-brain axis and promote migraine chronification.[33]

Despite a number of studies demonstrating oral dysbiosis in several neurologic diseases,[13-15] there is a paucity of research concerning the oral microbiome in migraine.[21,34] On the other hand, previous studies have reported that oral health conditions, such as periodontitis, can directly and indirectly exacerbate migraine and increase the risk of chronification.[35,36] We hypothesized an association between oral dysbiosis and migraine and identified distinct taxonomic and functional signatures of the oral microbiome for migraine. Given the anatomical proximity of the trigeminovascular system to the oral cavity, significant changes in oral conditions, including local inflammation and oral dysbiosis, may directly or indirectly influence the development of migraine. Moreover, previous studies have suggested that saliva may provide valuable insights into migraine, highlighting several biomarkers such as enzymes, hormones, and neuropeptides in saliva.[5-11] Our findings revealed that the salivary microbiome may serve as an additional promising biomarker for migraine.

Furthermore, the oral microbiome is known to play a critical role in the first step of a nitrate-nitrite-nitric oxide pathway.[37] Nitrate from diet or endogenous metabolism accumulates in saliva and is reduced to nitrite by oral bacteria. Nitric oxide has been implicated in the pathophysiology of migraine by influencing cerebral vasodilation, neurogenic inflammation, and nociceptive processing in the CNS.[38] We observed a significant increase in the abundance of the genera *Gemella*, *Streptococcus*, and *Granulicatella* in both the CM and EM groups and an increase in the abundance of the genera *Rothia* and *Schaalia* in the CM group compared with controls. In line with the previous study,[34] all of these genera have species with the capacity to reduce nitrate and/or nitrite. However, some of the genera which were significantly depleted in both the CM and EM groups—the genera *Veillonella*, *Haemophilus*, *Selenomonas*, and *Kingella*—also include nitrate-reducing species. The findings were comparable with those observed in the previous study, which demonstrated that dietary nitrate increased the relative abundance of certain nitrate-reducing bacteria (e.g., *Rothia*) while simultaneously reducing the abundance of other nitrate reducers (e.g., *Veillonella*).[39] Further studies with a longitudinal design are needed to clarify the implications of the findings.

This study identified several distinct clusters of genera within the oral microbiome, with a notable prevalence of the cluster comprising *Rothia*, *Gemella*, *Streptococcus,* and *Granulicatella* in participants with migraine. Moreover, these oral bacteria formed a cluster in the gut, suggesting that they may possess distinct characteristics or niche habits within the bacterial community. It has been demonstrated that oral bacteria generally exhibit limited colonization in a healthy intestine. However, in pathologic conditions such as inflammatory bowel disease, there is an increased likelihood of oral pathogens becoming more abundant in the gut, thereby reinforcing the connection between oral and gut microbiomes.[19] We observed that the number and abundance of shared taxa between the oral and gut microbiomes were low; however, rare but selective taxa including these 4 oral genera were identified in the gut microbiome. This observation underscores the importance of these oral microbes in that they are abundant in the disease group and therefore have greater potential to be used as diagnostic and therapeutic targets.

This study has several limitations. First, although the 16S rRNA amplicon approach is cost-effective, it has a limited resolution for the identification of bacterial species. To determine the species-level composition, further studies using shotgun metagenomics are required. Second, the cross-sectional design of this study precludes the possibility of establishing a causal relationship between the observed differences in relative abundance in taxonomic and functional profiles and migraine. Third, although participants with ongoing or past treatment of oral or gastrointestinal disease were excluded, those with asymptomatic forms such as apical periodontitis or subclinical inflammatory bowel disease may have been included. Finally, the machine learning–based classification models developed for migraine were validated internally. Future studies may need to use independent cohorts to provide more robust evidence for the potential use of oral microbial signatures as a diagnostic marker for migraine.

This study has the following strengths: (1) The study was designed to achieve the sample size required for statistical power based on our previous research.[22] (2) The study concurrently examined the oral and gut microbiomes in individuals with migraine. (3) The study investigated and adjusted for the confounding host variables, including demographic, lifestyle, and psychiatric factors, which are known to contribute to variability across microbiota studies.

Anxiety, depression, and fibromyalgia are particularly prevalent among individuals with migraine,[30,40] with independent associations reported between these conditions and alterations in the oral and gut microbiome.[41-43] In our cohort, anxiety, depression, and fibromyalgia were reported in 27%, 27%, and 9% of patients with EM, respectively, and in 60%, 60%, and 46% of patients with CM, respectively. Acknowledging the potential for confounding effects, we carefully evaluated and adjusted for these conditions in the analyses. Although subgroup analyses excluding participants with comorbidities could have provided additional insights, the limited number of eligible participants precluded such analyses without compromising statistical validity. Furthermore, given the high prevalence of these conditions among individuals with migraine, results derived from a strictly comorbidity-free cohort would likely have limited generalizability to the broader real-world migraine population. Nevertheless, the observed alterations in the oral microbiome remained consistent after adjustment, supporting the significance of oral microbial signatures in relation to the migraine disease state.

In conclusion, our findings suggest that oral dysbiosis may play a role in the development of migraine, highlighting specific oral microbiota taxa as potential diagnostic biomarkers and therapeutic targets for migraine.

Author Contributions

S. Cho: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. Y. Jung: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. H.-S. Oh: major role in the acquisition of data; analysis or interpretation of data J. Yum: analysis or interpretation of data. S. Song: analysis or interpretation of data. J.W. Jeong: analysis or interpretation of data. W.-S. Ha: analysis or interpretation of data. K.M. Kim: analysis or interpretation of data. W.-J. Kim: analysis or interpretation of data. M.K. Chu: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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