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Gut and oral microbiome modulate molecular and clinical markers of schizophrenia-related symptoms: A transdiagnostic, multilevel pilot study

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Abstract

Although increasing evidence links microbial dysbiosis with the risk for psychiatric symptoms through the microbiome-gut-brain axis (MGBA), the specific mechanisms remain poorly characterized. In a diagnostically heterogeneous group of treated psychiatric cases and nonpsychiatric controls, we characterized the gut and oral microbiome, plasma cytokines, and hippocampal inflammatory processes via proton magnetic resonance spectroscopic imaging (^{1}H-MRSI). Using a transdiagnostic approach, these data were examined in association with schizophrenia-related symptoms measured by the Positive and Negative Syndrome Scale (PANSS). Psychiatric cases had significantly greater heterogeneity of gut alpha diversity and an enrichment of pathogenic taxa, like *Veillonella* and *Prevotella*, in the oral microbiome, which was an accurate classifier of phenotype. Cases exhibited significantly greater positive, negative, and

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general PANSS scores that uniquely correlated with bacterial taxa. Strong, positive correlations of bacterial taxa were also found with cytokines and hippocampal gliosis, dysmyelination, and excitatory neurotransmission. This pilot study supports the hypothesis that the MGBA influences psychiatric symptomatology in a transdiagnostic manner. The relative importance of the oral microbiome in peripheral and hippocampal inflammatory pathways was highlighted, suggesting opportunities for probiotics and oral health to diagnose and treat psychiatric conditions.

Keywords

psychosis; neuroimaging; hippocampal inflammation; peripheral inflammation; cytokines; gut microbiome; oral microbiome; symptom domains

1. Introduction

The contribution of human commensal microbes to chronic health conditions has been well characterized (Clemente et al., 2018; Gevers et al., 2014; Jie et al., 2017; Zeevi et al., 2015), with a more recent focus on mental health and behavioral disorders. Both human and animal studies demonstrate associations between microbiota and psychosis (Kraeuter et al., 2020), depressive symptoms (Valles-Colomer et al., 2019), social behavior (Gacias et al., 2016), Alzheimer's disease (Chen et al., 2020), and even the effectiveness of drug treatments for multiple sclerosis (Katz Sand et al., 2019) and Parkinson's disease (Maini Rekdal et al., 2019). However, the mechanisms by which microbial imbalances, i.e. dysbiosis, modulate brain phenotypes remain unclear. Dysbiosis has been associated with pro-inflammatory metabolites that recruit immune cells, which in turn produce pro-inflammatory cytokines and chronic, low-grade inflammation (Clemente et al., 2018). This process also impacts the neurotransmitter systems implicated in behavioral disorders, including serotonin, dopamine, and glutamate pathways (A. H. Miller et al., 2013; Treadway et al., 2019).

The gut and brain are highly interconnected with pathophysiologic actions presumed to be conveyed through the microbiome-gut-brain-axis (MGBA). The gut and brain have bidirectional relationships from earliest life via peripheral inflammation and the vagus nerve, with the postnatal microbiota playing an essential role in establishing immune responses and CNS development (Hoffman et al., 2020). This mechanism is particularly relevant to schizophrenia-related psychopathology, as many exposures associated with the risk for psychosis also influence the microbiota (Hoffman et al., 2020). It is appealing to consider the MGBA over the life course, as the microbiota modulates systemic and local inflammation, and robust evidence supports inflammatory underpinnings for psychiatric conditions (Hoffman et al., 2020). Inflammation has been observed in the hippocampus, which is robustly linked to psychosis (Harrison, 2004; Lieberman et al., 2018). In addition to consistent reports of altered anatomy, perfusion, and activation (Kirov et al., 2013; Samudra et al., 2015; Tamminga et al., 2010), there is increased pro-inflammatory gene expression and basal perfusion following the emergence of psychosis (Malaspina et al., 1999, 2004; Schobel et al., 2009; Talati et al., 2015). In schizophrenia cohorts, magnetic resonance spectroscopic imaging (MRSI) of inflammatory metabolites has identified reduced neuronal integrity, as well as associations of dysmyelination and excitatory neurotransmission with

psychotic and manic symptom severity (Joe et al., 2021; Kraguljac et al., 2021; Schwerk et al., 2014; Steen et al., 2005; Tsai & Coyle, 2002).

The microbiota from other body sites, including the oral cavity, can also contribute to psychiatric disease pathogenesis and progression, but oral samples remain undercharacterized (Maitre et al., 2020; Martin et al., 2022; Scassellati et al., 2021). Oral microbes are particularly relevant given their proximity to the brain and the oft-reported relationships between brain disorders and oral health (Cormac & Jenkins, 1999), dating back to the pre-medication era (Noll, 2004). In addition, the oral cavity can serve as a reservoir for bacteria that exert pathogenic effects in other tissues (Atarashi et al., 2017; Segal et al., 2016).

A recent review and meta-analysis demonstrated a transdiagnostic depletion of anti-inflammatory butyrate-producing bacteria and enrichment of pro-inflammatory bacteria across patients with depression, bipolar disorder, schizophrenia, and anxiety diagnoses (Nikolova et al., 2021), although it did not characterize the MGBA mechanisms underpinning specific symptom domains. We investigate the MGBA in a cohort of cases with psychotic and nonpsychotic psychiatric diagnoses and controls with no mental illness, all characterized for schizophrenia-related symptoms. We estimate gut and oral microbiome diversity and identify associations of the microbiome with symptom domains and multi-level measures of the MGBA, including peripheral and hippocampal MRSI inflammatory markers.

2. Methods

2.1. Subjects and clinical assessments

This study was approved by the IRB at the Icahn School of Medicine at Mount Sinai in New York. Eligible participants were persons having a psychotic disorder, including any schizophrenia-related psychosis and psychotic bipolar disorder (n = 9) or having a nonpsychotic affective disorder (n = 6), or healthy controls having no psychiatric disorder and no personal or family history of psychosis (n = 8). Exclusion criteria included active major medical and neurological disorders, traumatic brain injury, significant risk of harm to self and others, and body metal that precluded imaging (Fendrich et al., 2022). All participants signed written informed consent and underwent research diagnostic assessments with the Diagnostic Interview for Genetics Studies (DIGS) (Nurnberger et al., 1994) and the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) administered by trained and reliable clinical interviewers. The PANSS rates 30 different schizophrenia symptoms on a 7-point scale (1 = absent, 7 = extreme) to generate separate scores for positive, negative, and general psychopathology subscales.

2.2. Microbiome sample acquisition and analysis

2.2.1. Sample preparation and 16S rRNA gene library construction: All subjects self-collected fecal samples using a stool collection container (Catalog number 02–544-208, Fisherbrand™) and oral mucosa samples using sterile swabs as previously described (Dominguez-Bello et al., 2016). Samples were kept at 4°C and shipped overnight

to the Clemente Lab at Mount Sinai, where they were frozen at −80°C upon arrival. Genomic DNA was extracted from all samples using the PowerSoil DNA Isolation Kit (QIAGEN) as previously described (Clemente et al., 2015). The V4 region of the 16S rRNA DNA gene was amplified in triplicate, pooled, and sequenced on the Illumina MiSeq platform (paired-end 250bp) following standard protocols (Caporaso et al., 2011). All specimens were sequenced except for two stool samples (one lost to follow up, one with insufficient quality). We obtained an average of 43,926 ± 157,173 sequences per sample (median ± standard deviation) and a total of 1,967 amplicon sequence variants (ASVs), indicating a sequencing depth sufficient to represent most taxa present in our samples.

2.2.2. Microbiome data analysis: Sequenced 16S rRNA gene data were analyzed with QIIME2 using default parameters unless stated otherwise. The raw sequencing reads were demultiplexed and an ASV table was constructed using DADA2 (Callahan et al., 2016). Bacterial contaminants were identified based on microbial taxa found in negative controls following Salter et al. (Salter et al., 2014) and subsequently removed from analysis. Alpha diversity was measured using “observed features” and beta diversity using unweighted UniFrac distances (Lozupone & Knight, 2005) on tables rarefied at 1,000 sequences per sample. After rarefaction, one oral control sample was discarded due to low sequencing coverage.

2.3. Cytokine Profiling

Whole blood was collected into EDTA tubes and centrifuged at 1,520 x g for 10 minutes at 4°C. Plasma was then isolated and stored at −80°C. We obtained blood samples from 14 cases and 7 controls and quantitatively determined the plasma levels of a panel of circulating inflammatory cytokines that included CCL2, CCL3, CCL4, CCL5 (RANTES), CCL7, CCL11 (eotaxin), CCL22 (macrophage-derived chemokine [MDC]), CXCL1 (growth-regulated alpha protein [GRO]), CXCL10 (IP-10), CX3CL1 (fractalkine), epidermal growth factor (EGF), fibroblast growth factor 2 (FGF-2), Fms-related tyrosine kinase 3 ligand (Flt3L), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-α2, IFN-γ, IL-1β, IL-6, IL-1α, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12β, IL-12(p70), IL-13, IL-15, IL-17A, platelet-derived growth factor (PDGF), soluble CD40 ligand (sCD40L), TGF-α, TNF-α, TNF-β, and vascular endothelial growth factor (VEGF). The assessment was performed using the MILLIPLEX MultiAnalyte Profiling (MAP) Human Cytokine/Chemokine Kit for 96-well Assay (MilliporeSigma) run on the Luminex platform.

2.4. Magnetic resonance acquisition and post-processing

Hippocampal proton magnetic resonance spectroscopic imaging (^{1}H-MRSI) and whole brain MRI were conducted at 3 T in a whole-body 3.0 T MRI scanner (Trio, Siemens AG, Erlangen, Germany) as previously described (Joe et al., 2021; Meyer et al., 2016). We conducted imaging for 9 cases and 5 controls, with remaining subjects lost to follow up, claustrophobia, or excluded for body metal. The advantage of three-dimensional ^{1}H-MRSI is bilateral coverage, i.e. capture of both left and right hippocampi, of the entire irregularly-shaped structure in a single measurement at 1 mL nominal spatial resolution. Hippocampal inflammatory processes were assessed by measuring the

following metabolites: *N*-acetylaspartate (NAA) for neuronal integrity, creatine (Cr) for energy metabolism, choline (Cho) for dysmyelination, glutamine/glutamate ratio (Glx) for excitatory neurotransmission, and *myo*-inositol (mI) for astrogliosis. MATLAB 18 (MathWorks, Framingham, MA) was used to calculate the fraction of gray matter, white matter, and CSF in each voxel, retaining those with: (i) at least 30% hippocampus tissue volume; (ii) < 30% CSF, (iii) Cramer-Rao lower bounds < 20% for a given metabolite (enabling to reject just a single metabolite from an otherwise "acceptable" group that meet this criterion in that voxel); and (iv) 4 Hz < linewidths < 13 Hz (Kreis, 2004). This multi-voxel approach affords spatial resolution for voxel signal-averaging and regression analyses not possible with single voxel studies, as previously described (Tal et al., 2012). Metabolites passed quality control criteria as follows: NAA, Cho, and Cr for 9 cases and 4 controls; Glx for 6 cases and 3 controls; and mI for 7 cases and 4 controls.

2.5. Statistical analyses

Group differences were assessed using the Mann-Whitney U Test and F Test for quantitative variables, including cytokines and magnetic resonance measures, and Fisher's Exact Test for binary variables, including sex and medication status. Analysis of microbial alpha and beta diversity were performed using the Mann-Whitney U Test and PERMANOVA, respectively, and microbial differential analysis was performed using LEfSe (Segata et al., 2011). Microbial relative abundances were correlated with PANSS scores, cytokines, and neuroimaging measures using Correlations Under the Influence (CUTIE), a method that increases sensitivity while removing potentially false correlations (Bu et al., 2022). Spearman's rank coefficients were reported for correlation analysis, and Pearson coefficients were utilized for the Fisher z-transformation for differential correlation analysis. Random forest was conducted using the scikit-learn package in Python. A P value threshold of 0.05 was used to determine statistical significance for all analyses, which were performed using R v3.6.0 and Python v3.7.0.

3. Results

3.1. Clinical characterization of individuals with psychiatric disorders

An overview of the analyses to characterize MGBA-associated changes in a diagnostically heterogeneous cohort is shown in Figure 1. The 15 psychiatric cases consisted of 9 individuals with psychotic disorders (5 schizophrenia, 2 schizoaffective, 2 psychotic bipolar disorder) and 6 others with nonpsychotic disorders (5 depression and 1 nonpsychotic bipolar disorder). The 8 healthy controls have no personal or family history of psychosis and no psychiatric conditions. Clinical characteristics are described in Table 1. The case and control groups did not significantly differ by age, sex, or BMI. Group status was not associated with antibiotic, probiotic, or proton pump inhibitor usage, and oral hygiene habits and cigarette smoking did not significantly differ between groups. Symptom severity was assessed using the PANSS, the gold standard for measuring schizophrenia symptom severity (Freitas et al., 2019). As expected, cases exhibited significantly more severe positive, negative, and general psychopathology as measured by the PANSS subscales (P = 0.009, 0.001, < 0.0001), as well as significant enrichment of psychotropic medication usage (P < 0.0001), specifically antipsychotics (P = 0.019).

3.2. Microbiome composition is altered in psychiatric cases independent of medication effects

We first characterized the gut and oral microbiome by estimating alpha diversity, which measures the number of unique microbes within each sample (Figure 2A), and beta diversity, which estimates the distance between two samples based on microbiome composition (Figure 2B). In the oral microbiome, alpha diversity did not significantly differ between groups; however, oral beta diversity did (PERMANOVA, $P = 0.010$). In the gut microbiome, alpha and beta diversity did not significantly differ between groups, but the variance of the alpha diversity was significantly greater among cases (F test, $P = 0.036$). A power analysis estimated that a sample size of 30 subjects per group would allow us to detect significant differences in the mean gut alpha diversity with 80% power and alpha of 0.05. Importantly, potential confounders such as BMI and medications did not significantly impact alpha diversity (Mann-Whitney U test, $P > 0.05$) and beta diversity (PERMANOVA, $P > 0.05$) in the gut and oral microbiome, nor peripheral and hippocampal inflammation (Mann-Whitney U test, $P > 0.05$).

Next we analyzed the taxonomic composition of the gut and oral microbiome (Figure 2C). *Firmicutes* and *Bacteroidetes* were the predominant phyla in the gut microbiome, whereas *Firmicutes* and *Proteobacteria* were the most predominant in the oral microbiome. Differential enrichment analysis at the genus level identified several microbial taxa that significantly differed between cases and controls (Figure 2D). In the gut microbiome, *Lactobacillus* and *Coprobacillus* were significantly enriched among cases (LDA = 3.722, 3.304), whereas *Alistipes* and unidentified *Ruminococcaceae* and *Peptostreptococcaceae* were the most significantly enriched among controls (LDA = 4.162, 3.918, 3.714). In the oral microbiome, *Veillonella*, *Prevotella*, and *Actinomyces* were the most significantly enriched among cases (LDA = 4.461, 4.306, 4.132) and an unidentified *Pasteurellaceae*, *Haemophilus*, and *Actinobacillus* were the most significantly enriched among controls (LDA = 4.938, 4.746, 4.617).

Additional multilevel data of the MGBA were used to train random forest models to determine their predictive value, specifically plasma cytokines to measure peripheral inflammation, and hippocampal MRSI metabolites and whole brain MRI volumes to measure hippocampal inflammation (Figure 2E). When the model was trained on microbial features alone, the oral microbiome accurately predicted cases (AUC = 0.735) but the gut microbiome did not (AUC = 0.526). While cytokines and neuroimaging data alone were poor classifiers (AUC = 0.214, 0.633), adding them to the oral microbiome classifier increased the overall performance (AUC = 0.790).

3.3. Microbial taxa associated with schizophrenia symptomatology

We performed correlation analysis between microbial taxa and PANSS scores in cases using CUTIE, a tool for robust detection of significant correlations (Bu et al., 2022). As different symptom domains are theorized to have distinct underpinnings in the NIMH Research Domain Criteria (RDoC) (Cuthbert & Morris, 2021), microbial features were correlated with the positive, negative, and general PANSS subscales (Figure 3). Oral *Weeksellaceae* was significantly correlated with PANSS General scores (r = 0.671; $P = 0.006$), whereas gut

Acidaminococcus and *Blautia* were significantly correlated with PANSS Positive scores (r = 0.637, −0.667; $P = 0.010$, 0.006). Gut *Lachnospiraceae* was significantly correlated with PANSS Positive and General scores (r = −0.721, −0.626; $P = 0.002$, 0.012).

3.4. Differences in inflammatory profiles are associated with microbiome changes

Controls had significantly higher plasma levels of the hematopoietic growth factor Flt3L (Mann-Whitney U Test, $P = 0.003$) and greater variance of plasma levels for most of the cytokines in our panel compared to cases (F Test, $P < 0.05$; Table 2). In psychiatric cases, we correlated the microbiome with immune profiles using CUTIE, which identified significant correlations between plasma cytokines and individual taxa (Supplementary Figure 1). We highlight several clinically relevant correlations in Figure 4A. IP-10 (CXCL10), a Th1-chemokine, significantly correlated with the oral abundance of *Capnocytophaga* (r = 0.727, $P = 0.003$). FGF-2, a cytokine that promotes neurogenesis, significantly correlated with gut *Blautia* (r = 0.711, $P = 0.004$). Plasma levels of TNF-α and IL-1β, key mediators of the inflammatory response, significantly correlated with the gut abundances of *Clostridium* and *Lachnospiraceae*, respectively (r = 0.723, 0.770; $P = 0.003$, 0.001).

We next conducted differential correlation analysis of the microbiome with inflammatory profiles to compare whether they differed between cases and controls, i.e. whether the correlation coefficients were significantly different between groups. Given its higher predictive power to discriminate cases from controls (Figure 2E), the oral microbiome data is focused on in this analysis (Figure 4B). Two clusters of significantly differential correlations were identified. Oral *Treponema* and an unidentified *Mogibacteriaceae*, *Pasteurellaceae*, and *Bacillaceae* formed the first cluster, and *Haemophilus*, *Prevotella*, *Atopobium*, and an unidentified *Weeksellaceae* and *Veillonellaceae* formed a second cluster. Controls exhibited greater correlations than controls with MDC, eotaxin (CCL11), GRO, and sCD40L in the first cluster, with MCP-1 in the second cluster, and with IP-10 in both clusters. Of note, *Haemophilus* correlated with GRO more greatly in cases than controls in the second cluster. Controls also had significantly greater correlations of an unidentified *Pasteurellaceae* with most of the interleukins, MIP-1α (CCL3), MIP-1β (CCL4), TNFβ, TGF-α, G-CSF, GM-CSF, fractalkine, EGF, VEGF, IFN-α2, and IFN-γ. Differential correlation analysis of the gut microbiome also identified two clusters, albeit with non-significant associations (Supplementary Figure 2).

3.5. Differences in hippocampal ^{1}H-MRSI and MRI inflammatory markers are associated with microbiome changes

Hippocampal ^{1}H-MRSI was used to measure choline (Cho), *N*-acetylaspartate (NAA), glutamine and glutamate (Glx), and *myo*-inositol (mI), and whole brain MRI was used to measure the fractional gray matter (fGM), white matter (fWM), and cerebrospinal fluid (fCSF) volumes for post-processing volume correction. MRSI and MRI measures did not differ between groups, as we have observed in several different cohorts (Kirov et al., 2013; Meyer et al., 2016). Hippocampal inflammatory markers were correlated with the abundance of gut and oral microbial taxa in psychiatric cases using CUTIE (Supplementary Figure 3), with several clinically relevant relationships highlighted in Figure 5A. The oral abundance of *Bifidobacterium* significantly correlated with NAA, a measure of neuronal

integrity (r = 0.814, $P = 0.008$). Oral *Bulleidia* significantly correlated with Cho, a sum of phosphocholine and glycerophosphocholine that measures membrane turnover, particularly of myelin, to predict dysmyelination (r = 0.828, $P = 0.006$). In the gut microbiome, *Clostridium* significantly correlated with Glx, a measure of excitatory neurotransmission (r = 0.943, $P = 0.005$), and *Lachnospira* significantly correlated with mI, a marker of astrogliosis (r = 0.955, $P = 0.001$).

Differential correlation analysis found that the oral microbiome correlated with specific MRSI measures of hippocampal inflammation (Figure 5B), whereas the gut microbiome significantly correlated with MRI measures of whole brain volumes (Supplementary Figure 4). Specifically, oral microbial features formed two distinct clusters with regards to hippocampal Cho, NAA, and Cr. Oral *Haemophilus*, *Filifactor*, *Treponema*, *Actinomyces*, *Neisseria*, and an unidentified *Mogibacteriaceae* and *Prevotella* composed a cluster of correlations with Cho, NAA, and Cr concentrations that was significantly greater in cases. Oral *Prevotella*, *Atopobium*, *Tannerella*, *Campylobacter*, *Veillonella*, *Actinobacillus*, *Pseudomonas*, and an unidentified *Weeksellaceae*, *Pasteurellaceae*, and *Bacillaceae* formed a second cluster of correlations with Cho, NAA, and Cr that was significantly greater in controls. Hippocampal mI, a marker of gliosis, clustered independently of the aforementioned genera with *Prevotella*, *Atopobium*, *Tannerella*, *Campylobacter*, *Veillonella*, *Actinobacillus*, *Pseudomonas*, *Haemophilus*, *Filifactor*, *Treponema*, and an unidentified *Pasteurellaceae* and *Bacillaceae*.

4. Discussion

Compelling evidence points towards a key role of microbial dysbiosis, peripheral inflammation, and hippocampal inflammation in psychosis pathophysiology. This pilot study tested the hypothesis that the microbiome influences schizophrenia-related symptoms through the inflammatory pathways of the MGBA. Our multilevel approach is the first to characterize the MGBA integrating microbiome, hippocampal inflammation, and axial inflammatory pathways, and illustrates a framework to better understand schizophrenia-related psychopathology across psychiatric diagnoses. Our results identify microbial alterations across diagnoses, similarly to a recent meta-analysis of over 30 studies that analyzed gut microbiome composition across multiple psychiatric disorders (Nikolova et al., 2021). Our transdiagnostic approach is in accordance with the NIMH RDoC to address the heterogeneity of DSM-5 diagnoses (Insel et al., 2010; Kelly et al., 2018; Nikolova et al., 2021).

Prior gut microbiome studies have reported inconsistent changes in alpha diversity in persons with schizophrenia, bipolar disorder, and depression (Nikolova et al., 2021). The increased variability in gut alpha diversity in our cases is consistent with the etiological heterogeneity of DSM-5 diagnoses like schizophrenia, and may reflect pathophysiological differences of our diagnostically mixed but rigorously assessed cohort (Malaspina et al., 2012). As for the oral cavity, a recent schizophrenia study of the salivary microbiome reported significantly altered and lower beta diversity distances in first episode schizophrenia patients compared to healthy controls (Qing et al., 2021).

The oral microbiome has been studied in several neuropsychiatric disorders, like depression, anxiety and autism, but only recently in three schizophrenia studies, including a case study by our group on this cohort (Al Bataineh et al., 2022; Aleti et al., 2022; Joe et al., 2021; Qing et al., 2021; Ragusa et al., 2020; Simpson et al., 2020; Yolken et al., 2021). Individuals with severe mental illness have long been reported to have worse oral health (Kisely et al., 2011; Yang et al., 2018). Acute psychosis has also been observed in patients with oral infections for over 100 years (Noll, 2004). The lack of significant differences in oral hygiene habits between cases and controls in our cohort suggest that other pathological processes are associated with psychiatric disease. The oral cavity can harbor pro-inflammatory pathogens that trigger gut inflammation, cause infection, and even spread to the brain (Kitamoto et al., 2020; Park et al., 2022), as shown in glaucoma and Alzheimer's disease (Astafurov et al., 2014; Dominy et al., 2019). Many of the oral taxa enriched in our psychiatric cases are sulfate-reducing, such as *Veillonella*, *Actinomyces*, *Atopobium*, *Campylobacter*, and some members of the *Veillonellaceae* family, whose abundance has been negatively correlated with cognitive performance (Liu et al., 2020). Qing and colleagues reported an enrichment of sulfate-reducing oral bacteria, which can damage the mucosal epithelium as reported in periodontitis and ulcerative colitis (Dordević et al., 2020; Kushkevych et al., 2020; Qing et al., 2021). Further studies will be required to elucidate the role of sulfate reduction and other mechanisms by which known oral taxa may contribute to local and systemic inflammatory processes.

We identified differential associations of oral *Weeksellaceae* and gut *Acidaminococcus*, *Blautia*, and *Lachnospiraceae* with the PANSS subscales in our transdiagnostic analysis. Oral *Weeksellaceae* was depleted in schizophrenia (Figure 2D) in concordance with a recent study of schizophrenia and mania (Yolken et al., 2021). Given its positive association with the PANSS General score, this taxon could be enriched in schizophrenia patients with more severe general psychopathology. In contrast, *Acidaminococcus* is enriched in the gut microbiome of persons with schizophrenia, depression, and, in some studies, bipolar disorder (Nikolova et al., 2021). The positive association of gut *Acidaminococcus* with PANSS Positive scores suggests it may have potential as a transdiagnostic marker of psychotic and manic symptoms. While gut *Lachnospiraceae* and *Blautia* negatively correlated with PANSS Positive scores, *Lachnospiraceae* is consistently depleted and *Blautia* is inconsistently altered in psychotic and affective disorders like schizophrenia and depression (Nikolova et al., 2021; Vindegaard et al., 2021; Zhang et al., 2020). Overall, these findings suggest that perturbations in the abundance of certain oral and gut taxa may play a role in diverse psychiatric presentations of psychotic individuals.

Cytokine profiling identified significantly reduced levels of Flt3L, a B cell-specific cytokine that has been associated with Sjögren's syndrome and may contribute to oral dysbiosis-mediated psychiatric symptoms. Rare case reports of Sjogren's syndrome with schizophrenia-like symptoms are theorized to result from salivary gland inflammation spreading to the brain (Cox & Hales, 1999; Lin, 2016; Tobón et al., 2013). Our cases were generally in an anti-inflammatory state, which may be a consequence of treatment with antipsychotics, antidepressants, and mood stabilizers. These medications can exert anti-inflammatory effects on peripheral cytokines (Fonseka et al., 2016; Goldstein et al., 2009; Kenis & Maes, 2002). In psychiatric disease, dysbiosis has been linked to

immune aberrations like food allergies and chronic, low-grade inflammation (Caputi et al., 2021; Severance et al., 2015). Dysbiosis may induce psychiatric symptoms via circulating antibodies mediating cellular cytotoxicity, even if cytokine profiles become anti-inflammatory with treatment.

We also identified significant microbial correlations with peripheral cytokines. Oral *Capnocytophaga* is a commensal microbe enriched in gingivitis, oral cancer, and carcinoma (Jolivet-Gougeon & Bonnaure-Mallet, 2021). Its relationship with plasma IP-10, reportedly decreased in treated schizophrenia patients (Asevedo et al., 2013; Noto et al., 2015), may illustrate a pathologic inflammatory process. *Blautia*, which is associated with higher cognitive performance and depleted in depression (Liu et al., 2020; J. Yang et al., 2020), positively correlated with FGF-2, which promotes neurogenesis. FGF-2 expression is in fact induced by antipsychotics and associated with schizophrenia and negative symptom severity (Dremencov et al., 2021; Hashimoto et al., 2003; Li et al., 2018). *Blautia* and FGF-2 may therefore have a neuroprotective relationship that is attenuated in negative schizophrenia-related symptomatology. Gut *Clostridium* and *Lachnospiraceae* correlated with TNF-α and IL-1β, respectively. These microglia-derived, pro-inflammatory cytokines have been linked to psychosis (B. J. Miller et al., 2011) and can activate the tryptophan-kynurenine pathway, which regulates the glutamatergic system and is dysregulated in schizophrenia and depression (Müller & Schwarz, 2007; Noyan et al., 2021). This pathway may be one such mechanism by which gut dysbiosis-mediated inflammation contributes to schizophrenia pathogenesis, although additional studies will be required to confirm this hypothesis.

The association of oral *Bifidobacterium*, which can improve periodontitis and gingivitis (Invernici et al., 2018), with hippocampal NAA suggests a potential role for oral probiotics for neuronal health and integrity. We have previously reported that hippocampal Cho, a marker of dysmyelination, is associated with manic and positive schizophrenia symptoms (Malaspina et al., 2021). Hippocampal Glx, a measure of neuronal excitation, has also been associated with psychosis severity and theorized to indicate a hyperglutamatergic state (Kraguljac et al., 2021; Tsai & Coyle, 2002). These inflammatory processes may be driven respectively by *Bulleidia* and *Clostridium*, which were both enriched in our cases. Further, in our differential correlation analysis, the oral microbiota clustered distinctly with Cho, NAA, and Cr, whereby Cr may indicate neurotoxic hypermetabolism leading to myelin disruptions and neuronal damage, as respectively measured by Cho and NAA. Astrogliosis indicated by mI helps repair CNS insults and could potentially ameliorate symptoms, given our findings that gut abundance of *Lachnospira* correlates with both mI and improved general psychopathology.

4.1. Limitations

Unlike previous studies, we did not observe major alterations in overall gut microbiome composition, though we found higher variability in alpha diversity and enrichment of specific taxa (Figure 2A, 2D). While medications like antipsychotics and antidepressants have been reported to exert antimicrobial effects *in vitro* (Ait Chait et al., 2020; Maier et al., 2018), medications did not significantly alter the microbiome of our cohort, suggesting that they may not exert the same effects *in vivo*. While we acknowledge the small sample

size of this pilot, we note that we were able to capture significant changes in microbial diversity, composition, and its relation to immune and hippocampal inflammation, especially in the oral cavity. Our power analysis suggests that a modest increase in sample size (n = 30 per group) would allow us to identify additional differences in gut microbiome between the groups. Further, the diagnostic heterogeneity of our cohort allowed us to identify symptoms associated with these MGBA biomarkers across diagnostics.

4.2. Conclusions

Our findings highlight the need for more comprehensive characterization of the MGBA in psychiatric disease. The gut microbiome has been an actionable target in human health, such as fecal microbiome transplants (Hirten et al., 2019; Kang et al., 2019; Moayyedi et al., 2015) and pre- and probiotics (Ansari et al., 2020; Wieërs et al., 2019). We expand this scope by characterizing the oral microbiome, which is relatively understudied in psychiatric disease, using a multilevel, transdiagnostic approach to study the MGBA and investigate potential mechanisms underlying schizophrenia-related symptoms across psychiatric diagnoses. The gut and especially oral microbiome are highlighted in this study with the goal of identifying microbial biomarkers to ultimately aid in improving diagnosis and developing future therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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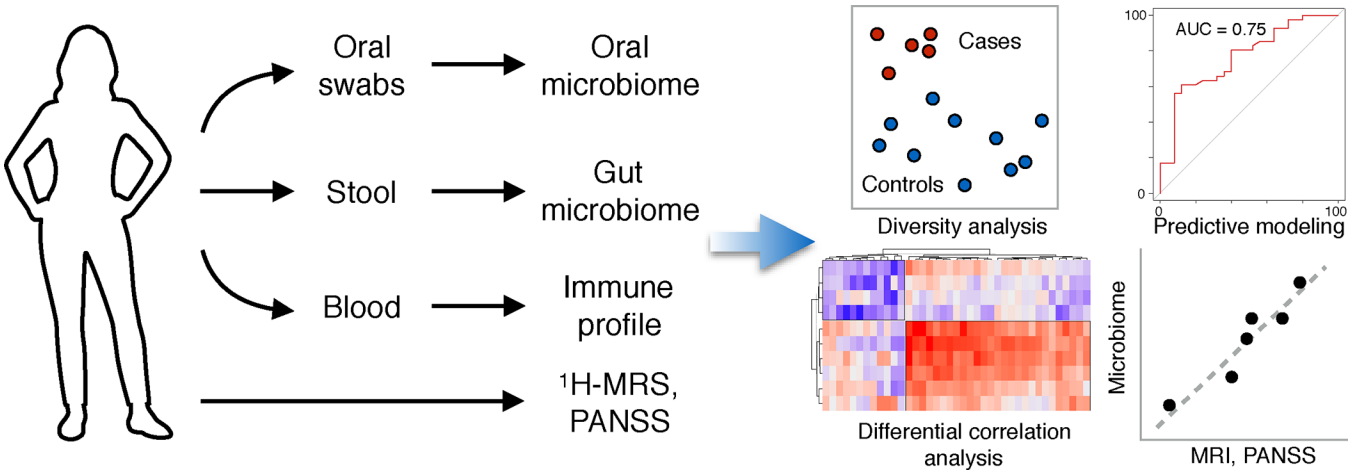

Figure 1. Study design.

Stool, oral swabs, and blood were collected from patients to characterize the gut and oral microbiome and immune profiles. Clinical symptom scores (i.e. PANSS) and ^{1}H-MRSI were also obtained from each patient. Microbiome diversity analysis, differential correlation analysis, predictive modeling, and correlation analysis were conducted to assess differences between cases and controls and identify potential biomarkers associated with psychiatric disease.

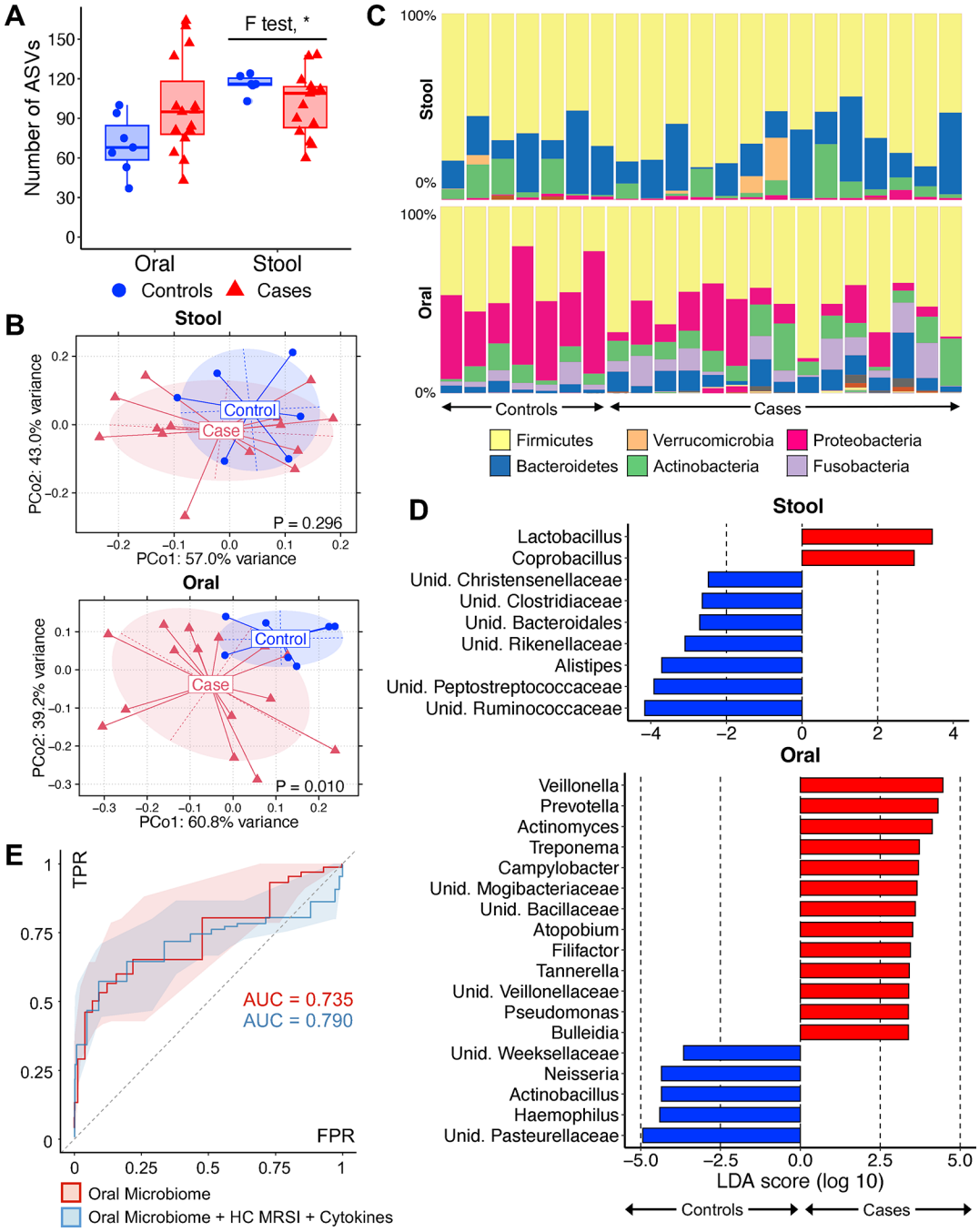

Figure 2. Characterization of the oral and gut microbiome.

(A) Alpha diversity was estimated using the number of observed ASVs in the gut and oral microbiome of cases (n_{stool} = 15, n_{oral} = 15) and controls (n_{stool} = 6, n_{oral} = 7), hereafter indicated in red and blue, respectively. (B) Principal coordinate analysis plots were based on unweighted UniFrac distances for the gut and oral microbiome of cases (n_{stool} = 15, n_{oral} = 15) and controls (n_{stool} = 6, n_{oral} = 7). (C) Taxonomic composition at the phylum level of the gut and oral microbiome of cases (n_{stool} = 15, n_{oral} = 15) and controls (n_{stool} = 6, n_{oral} = 8). (D) Fold enrichment of bacterial taxa at the genus level in the gut and oral microbiome of

cases (n_{stool} = 15, n_{oral} = 15) and controls (n_{stool} = 6, n_{oral} = 8), whereby bar length indicates effect size. (E) ROC curves and corresponding AUC constructed from a Random Forest classifier built using oral microbiome data alone in blue (n_{cases} = 15, $n_{controls}$ = 8), and in conjunction with cytokine data (n_{cases} = 14, $n_{controls}$ = 7) and neuroimaging data (n_{cases} = 9, $n_{controls}$ = 5) in red. Abbreviations: AUC, area under the curve; ASV, amplicon sequence variant; LEfSe, Linear discriminant analysis Effect Size; PCo, principle coordinate; ROC, receiver operating characteristic.

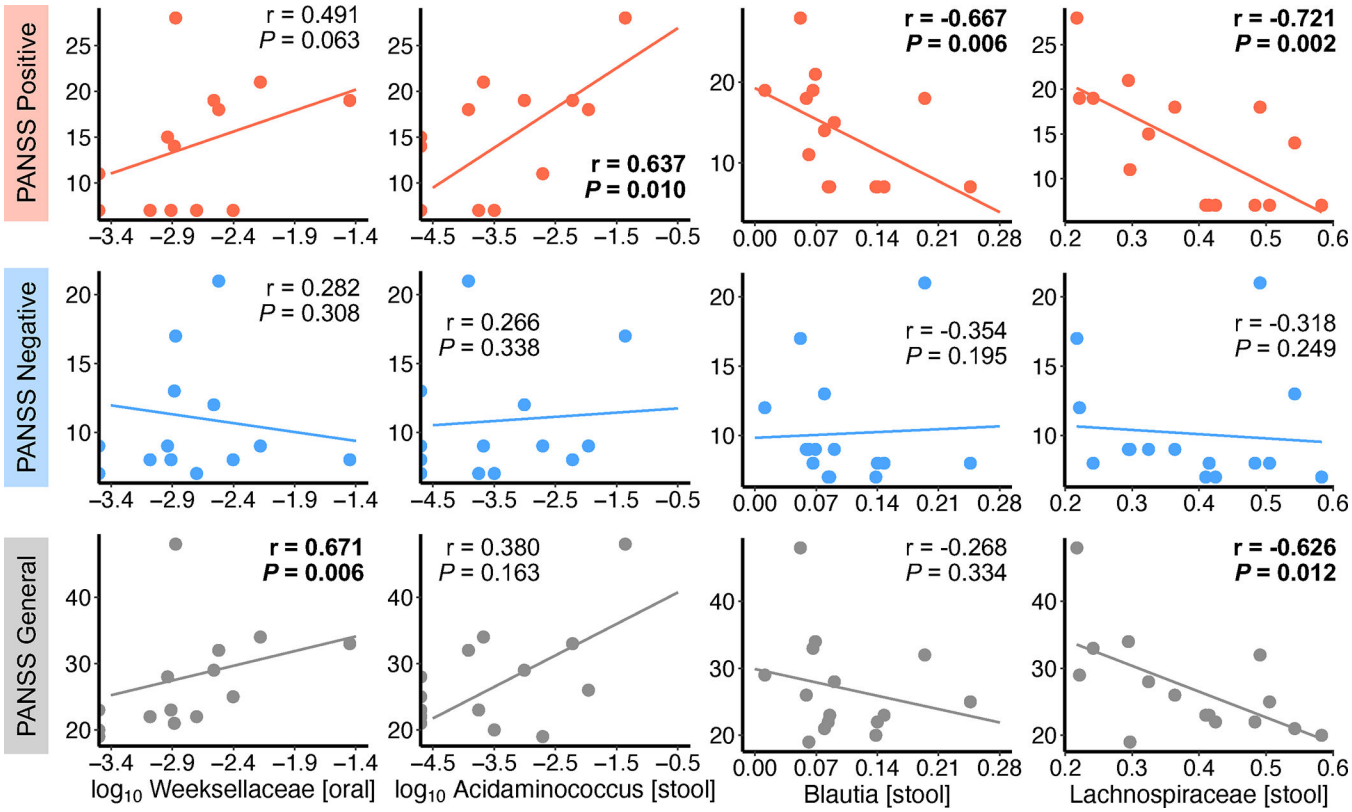

Figure 3. Differential microbial associations with the symptom domains of schizophrenia.

Correlations between the PANSS Positive, Negative, and General symptom scores with the relative abundances of oral *Weeksellaceae*, gut *Acidaminococcus*, gut *Blautia*, and gut *Lachnospiraceae* in cases only (n_{stool} = 15, n_{oral} = 15). Significant correlations are in bold.

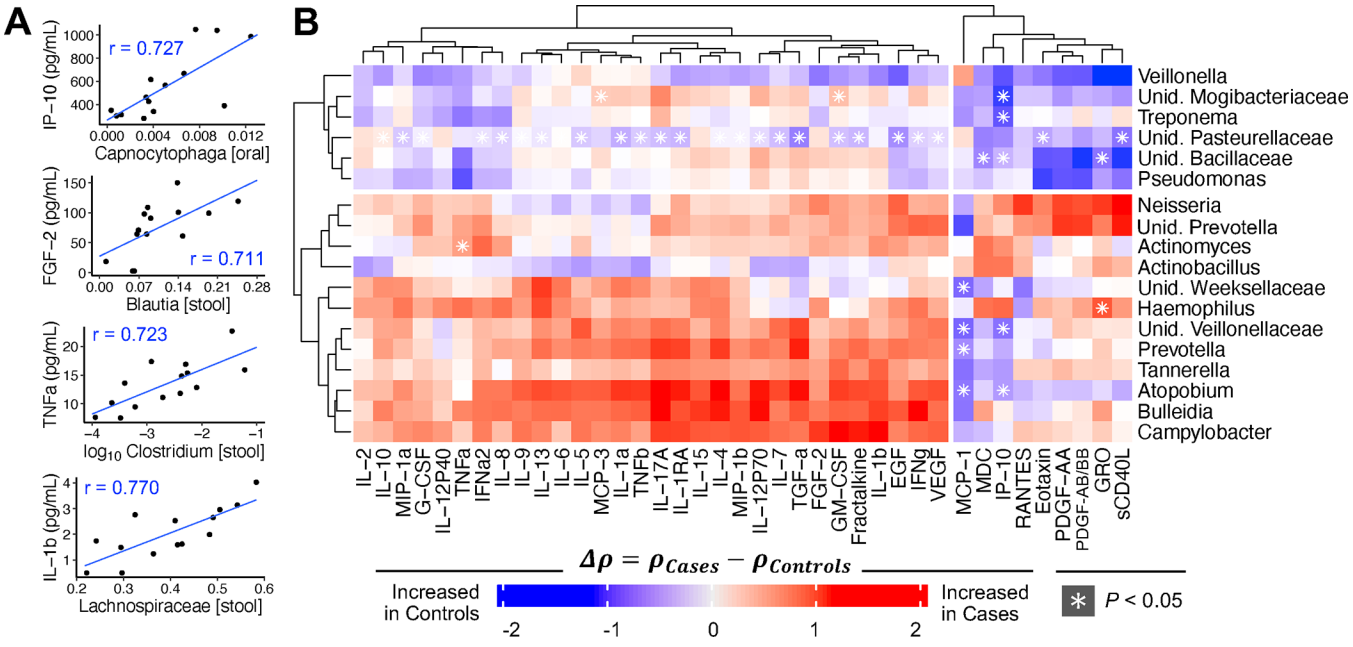

Figure 4. Differential microbial associations with the immune profiles of cases and controls.

(A) Correlations between the microbial relative abundances and plasma cytokine levels of gut *Capnocytophaga* and IP-10, gut *Blautia* and FGF-2, *Clostridium* and TNF-α, and *Lachnospiraceae* and IL-1β in cases only (n = 14). (B) Heatmap of differential correlations between oral microbial taxa and cytokines and in cases (n = 14) and controls (n = 7), where red and blue indicate more positive rho values in cases and controls, respectively.

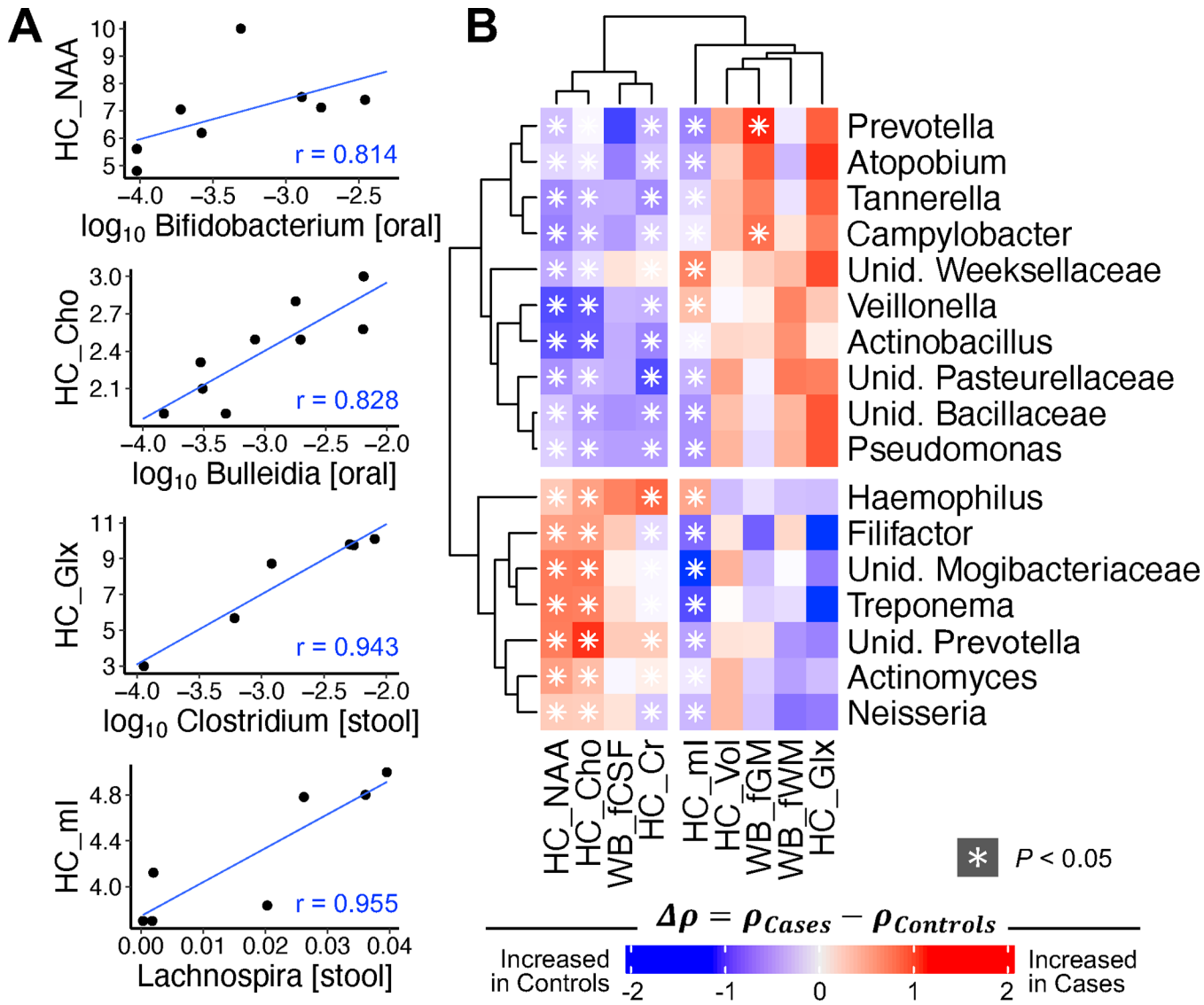

Figure 5. Differential microbial associations with brain anatomy and hippocampal metabolites in cases and controls.

(A) Correlations between the microbial relative abundances and hippocampal metabolites, specifically oral *Bifidobacterium* and NAA (n = 9), oral *Bulleidia* and Cho (n = 9), gut *Clostridium* and Glx (n = 6), and gut *Lachnospira* and mI (n = 7) in cases only. (B) Heatmap of differential correlations of oral microbial taxa with hippocampal ^{1}H-MRSI and whole brain MRI measures, with red and blue respectively indicating more positive rho values in cases (n = 9) and controls (n = 5). Abbreviations: Cho, choline; Cr, creatine; fCSF, fractional cerebrospinal fluid; fGM, fractional gray matter; fWM, fractional white matter; Glx, glutamate and glutamine; HC, hippocampal; mI, *myo*-inositol; NAA, *N*-acetylaspartate; Unid., unidentified; WB, whole brain.

Table 1.

Cohort Characteristics

	Cases (n = 15)	Controls (n = 8)	*P* value[a]
Demographic characteristics			
Age, years; mean ± SD	39.1 ± 9.9	36.9 ± 10.3	0.497
Female sex; count (%)	10 (66.7)	5 (62.5)	1
BMI, kg/m^2; mean ± SD	29.2 ± 6.0	26.6 ± 5.1	0.420
Clinical characteristics; mean ± SD			
PANSS Positive subscale	13.7 ± 6.7	7.0 ± 0	**0.009**
PANSS Negative subscale	10.1 ± 4.0	7.0 ± 0	**0.001**
PANSS General subscale	27.0 ± 7.5	16.0 ± 0	**<0.0001**
Tooth brushing frequency[b]	1.6 ± 0.7	1.4 ± 0.7	0.414
Tooth flossing frequency[c]	2.5 ± 1.9	3.2 ± 1.2	0.281
Cigarette smoking frequency[d]	3.2 ± 1.9	4.5 ± 1.4	0.086
Concomitant medications; count (%)			
Antibiotics	1 (0.1)	0	1
Proton pump inhibitors	1 (0.1)	0	1
Probiotics	0	1 (0.1)	0.348
Psychotropic medications[e]	13 (86.7)	0	**<0.0001**
Antipsychotics	8 (53.3)	0	**0.019**
Antidepressants	6 (0.4)	0	0.058
Mood stabilizers	5 (0.3)	0	0.122

[a] *P* values are given for group comparisons using the Mann-Whitney *U* Test (demographic and clinical characteristics excluding sex) and Fisher's exact test (sex, concomitant medications).

[b] 1. >2 times/day | 2. 1–2 times/day | 3. 1 time/day | 4. Never

[c] 1. Daily | 2. 3–5 times/week | 3. 1–2 times/week | 4. Few times/month | 5. Never

[d] 1. Daily | 2. 3–5 times/week | 3. 1–2 times/week | 4. Few times/month | 5. Never

[e] Subjects taking antipsychotics, antidepressants, or mood stabilizers

Table 2.

Cytokine Levels Among Cases and Controls

	Level, pg/mL, Mean ± SD		*P* value	
Cytokines	**Cases (n = 14)**	**Controls (n = 7)**	**Mann-Whitney *U* Test**	***F* Test**
EGF	22.61 ± 19.08	39.03 ± 60.24	0.970	**6.23E-04**
FGF-2	75.13 ± 43.69	212.66 ± 374.17	0.793	**5.72E-09**
Eotaxin	79.52 ± 72.33	95.02 ± 70.60	0.433	9.84E-01
TGF-α	7.44 ± 23.40	26.51 ± 64.39	0.706	**2.38E-03**
G-CSF	48.40 ± 52.79	147.39 ± 262.26	0.500	**4.33E-06**
Flt-3L	1.62 ± 0.00	35.60 ± 78.75	**0.003**	**0.00E+00**
GM-CSF	10.45 ± 8.99	47.60 ± 88.18	0.279	**1.04E-09**
Fractalkine	51.59 ± 59.84	267.86 ± 552.68	0.764	**2.22E-09**
IFN-α2	12.36 ± 12.29	75.43 ± 156.56	0.310	**3.73E-11**
IFN-γ	9.98 ± 9.04	49.88 ± 87.15	0.454	**1.29E-09**
GRO	427.02 ± 503.07	632.46 ± 740.92	0.911	2.28E-01
IL-10	7.86 ± 7.53	160.92 ± 395.81	0.525	**4.10E-19**
MCP-3	50.74 ± 90.03	96.56 ± 117.08	0.560	4.01E-01
IL-12P40	11.74 ± 15.57	143.86 ± 351.88	0.939	**2.34E-14**
MDC	674.27 ± 231.50	731.50 ± 395.54	0.852	9.96E-02
IL-12P70	4.92 ± 5.12	93.20 ± 232.71	0.546	**2.74E-18**
PDGF-AA	93.06 ± 129.14	107.03 ± 130.07	0.576	9.15E-01
IL-13	44.10 ± 115.45	138.96 ± 295.98	0.759	**4.55E-03**
PDGF-AB/BB	1344.98 ± 1381.70	2541.73 ± 2570.93	0.433	5.74E-02
IL-15	5.39 ± 3.32	26.73 ± 56.79	0.852	**8.62E-13**
sCD40L	305.94 ± 321.38	615.56 ± 850.30	0.682	**3.42E-03**
IL-17A	3.41 ± 2.78	21.88 ± 43.75	0.354	**2.51E-12**
IL-1RA	256.05 ± 690.71	828.29 ± 1653.67	0.653	**8.28E-03**
IL-1α	104.01 ± 324.70	207.65 ± 350.36	0.354	7.64E-01
IL-9	16.15 ± 34.17	35.32 ± 62.77	0.778	6.25E-02
IL-1β	2.05 ± 1.01	16.98 ± 35.97	0.389	**6.68E-17**
IL-2	2.07 ± 1.12	15.58 ± 35.18	0.398	**3.14E-16**
IL-3	1.50 ± 0.00	5.51 ± 10.62	0.189	**0.00E+00**
IL-4	112.48 ± 324.88	135.62 ± 212.85	0.737	3.07E-01
IL-5	9.63 ± 23.73	31.69 ± 67.74	0.450	**1.68E-03**
IL-6	21.29 ± 53.95	35.56 ± 57.41	0.613	7.94E-01
IL-7	5.37 ± 8.73	24.60 ± 51.68	0.286	**5.39E-07**
IL-8	8.07 ± 17.20	14.05 ± 21.30	0.411	4.86E-01
IP-10	557.03 ± 279.07	972.90 ± 1041.45	0.737	**1.09E-04**
MCP-1	291.08 ± 107.09	341.71 ± 143.17	0.478	3.58E-01
MIP-1α	2.48 ± 1.78	7.18 ± 12.66	0.505	**5.67E-08**
MIP-1β	19.57 ± 16.76	77.77 ± 158.10	0.709	**1.70E-09**

	Level, pg/mL, Mean ± SD		*P* value	
Cytokines	**Cases (n = 14)**	**Controls (n = 7)**	**Mann-Whitney *U* Test**	***F* Test**
RANTES	4604.07 ± 3528.02	4756.65 ± 3236.01	0.852	8.80E-01
TNF-α	13.37 ± 4.18	63.34 ± 129.43	0.628	**4.01E-16**
TNF-β	103.20 ± 259.57	213.60 ± 397.94	0.321	1.85E-01
VEGF	65.14 ± 58.30	185.14 ± 356.85	1.000	**3.61E-07**