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Antidepressant usage is associated with alterations in gut microbiota diversity and abundance in Parkinson's Disease patients

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SUMMARY Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor dysfunction with co-presentation of neuropsychiatric and gastrointestinal symptoms prior to therapeutic treatments. Studies have established a relationship between PD and gut microbiome dysbiosis. Furthermore, recent studies show a relationship between mental health disorders and gut microbiome dysbiosis. Little is known on how the presence of neuropsychiatric disorders in PD patients can impact the gut microbiome in the absence of PD medication or gastrointestinal disorders. In this study, we first aimed to determine if antidepressant usage in PD patients was associated with alterations in the gut microbiota of PD patients. We then further investigated differences in gut microbiome profiles between PD patients who use antidepressants and those who do not. Our results indicate a significant difference in the beta diversity of microbiomes between PD patients who take antidepressants compared to those who do not. We further show that lower alpha diversity is present in groups that used antidepressants, and that antidepressant use was linked to enrichment of certain bacterial and archaeal families associated with gut dysbiosis in both PD and control groups. Finally, we show that PD subjects who used antidepressants were associated with a lower abundance of the Prevotellaceae bacterial family compared to PD patients who did not use antidepressants. This research can allow us to further understand associations between the presence of PD neuropsychiatric disorders and the gut microbiome of PD patients and, in turn, have clinical implications in the treatment of neuropsychiatric illnesses in PD.

INTRODUCTION

Parkinson's disease (PD) is a common proteinopathic neurodegenerative disorder clinically characterized by bradykinesia, rest tremors, rigidity, and postural disturbances (1). These motor dysfunctions result from the progressive formation of Lewy Bodies, aggregates of protein alpha-synuclein, in the substantia nigra pars compacta, which in turn leads to loss of dopaminergic neurons in those regions and abnormal dopaminergic neurotransmission in the basal ganglia motor circuit (1, 2). In addition to the classical motor symptoms, PD can also lead to various neuropsychiatric symptoms such as sleep difficulties, depression, anxiety, motivational deficits (apathy), and fatigue, as well as gastrointestinal disorders including constipation, gastroparesis, and reduced colonic transit time (3).

Previous studies have well established associations of PD with disruptions in the homeostasis, diversity, or functional distribution of microbes in the gut, known as gut microbiota dysbiosis (4). For instance, *Helicobacter pylori* are much more prevalent in PD patients and have shown to impede treatment of PD motor symptoms by hindering PD drug

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absorption (5). In another study, fecal samples observed in PD patients showed a significant reduction in the bacterial family Prevotellaceae, which correlate to diminished levels of beneficial short chain fatty acids (SCFAs), and an increase in Enterobacteriaccae, which have associations with the severity of postural instability and gait trouble (6). Further, Cirstea *et al*. discovered notable bacterial clusters that correlated with gut function in PD. Their results identify novel inverse associations of reduced butyrate producing bacteria in PD patients with increased constipation and colonic transit time (7). Furthermore, they found positive correlations between elevated levels of p-cresol and phenylacetylglutamine, two protein degradation byproducts by the gut microbiota, in PD patients, with firmer stools and constipation severity (7). However, when considering the associations between PD and gut dysbiosis, these studies are limited as they involve confounding variables that include the use of PD medication, unknown dietary intake, and the presence of gastrointestinal disorders at the time of microbial assessment. Previous research has elucidated a bidirectional mode of communication between the gut microbiota and the brain, known as the gut-brain axis (GBA) (8). Bilateral Information flow across the GBA can have implications on gut flora make-up and overall mental health, where disturbances or fluctuations on one end can have adverse effects on the other. This is evident in studies that link gut dysbiosis with several mental illnesses including anxiety and depression (8). Parks *et al.* showed that induction of anxiety and depression-like behaviors in mice were associated with altered gut microbiome profiles (9). Kelly *et al.*, show that depression is associated with decreased microbiota richness and diversity, and that transplantation of gut microbe profiles from depressed patients into depleted microbiome rat models induced features and behaviors that were characteristic of anxiety and depression. However, the findings of this study are limited as the majority of subjects in this study were on antidepressants (10). McGovern *et al.* show *in vivo* evidence, in animal models, of antidepressants having the capacity to exert antimicrobial pressures, which may have an effect on the gut microbiome diversity (11). Zhang *et al.* show that antidepressants may have impacts on gut microbe diversity in animal models (12). However, very limited studies have evaluated the direct effects of antidepressants on intestinal microbiota in humans. Whether the impacts of antidepressants on the gut microbiome is direct or indirect via host pathways is yet to be understood. The use of medication represents a confounding variable in many studies regarding the association between depression and gut dysbiosis. There is a knowledge gap regarding the study of depressed patients who are unmedicated at the time of microbial assessment (13). Research on the GBA is still ongoing and has mostly focused on gastrointestinal functions impacting the GBA (14). Less research has been conducted on the role that neuropsychiatric disorders play on the gut microbiota, especially in the context of PD. Understanding more about this bidirectional interplay between the gut microbiota and mental health in PD patients can provide opportunities to better manage neuropsychiatric disorders and improve mental health in PD therapy. Therefore, we aim to determine if the neuropsychiatric disorders of PD, specifically fatigue, sleep disorders, antidepressant use and apathy, play a role on the gut microbiota of PD patients.

Here, we use PD metadata retrieved from the Cirstea *et al*. paper to conduct our research (7). Due to the close association between gut microbiota dysbiosis and the onset of neurological disorders via the GBA (8), we hypothesize that PD patients who have the neuropsychiatric disorders of fatigue, sleep problems, apathy or antidepressant use will be associated with altered gut microbial communities. If the hypothesis is correct, then the microbiota of PD patients who have neuropsychiatric disorders are expected to correlate with reduced microbiome abundance and diversity. To determine a causal link between the neuropsychiatric disorders of PD and significant differences in the gut microbiome, future studies should conduct a longitudinal study. Researching the effects of neuropsychiatric disorders on the gut microbiome is important as it can reveal gut bacterial biomarkers associated with the onset of neuropsychiatric conditions. This can further inform therapeutic strategies aimed in preventing and treating neuropsychiatric and mental health conditions, especially in the realm of PD management.

METHODS AND MATERIALS

Parkison's Disease dataset. We obtained our Parkinson's metadata from a cross sectional cohort study by Cirstea *et al.* on three hundred participants (197 Parkinson's patients and 103 controls), aged 58-71 years, to determine associations between the microbiota and gastrointestinal disorders commonly observed in PD patients (7). Fecal samples were collected from the participants for microbiome sequencing. DNA was extracted using QIAamp PowerFecal DNA Kits (QIAGEN 12830); further details of the DNA extraction protocol were not mentioned by the authors (7). The bacterial 16S rRNA V4 region was amplified using barcoded 515F/806R primers (forward sequence: 5'GTGCCAGCMGCCGCGGTAA-3', reverse sequence: 5'GGACTACHVHHHTWTCTAAT-3') and was sequenced on an Illumina MiSeq platform. Using R v4.1.2 (15), we filtered the metadata to only include the neuropsychiatric categories of fatigue, antidepressant use (type and dosage of antidepressants were not reported), sleep problems and apathy. We then converted the numerical data of the fatigue and apathy categories into binary "yes-no" data. We determined fatigue cutoff scores using the Fatigue Severity Scale (FSS), a scale recommended by the Movement Disorder Society, where a cut-off score of 4 or more is considered indicative of problematic fatigue (16). We determined Apathy cut off scores using the reliable and valid Apathy Scale (AS) where scores of 14 or more are indicative of clinical apathy (17). We performed all analysis in this study using this revised metadata.

Data processing and analysis in QIIME 2. In order to perform diversity analysis by analyzing the amplicon sequencing data for PD and control subjects, we followed the QIIME2 workflow and analyzed both groups separately (18). We imported and demultiplexed the data and then determined the amplicon sequence variants (ASVs) using a truncation length of 251 nucleotides in order to preserve the entire sequences (19). We filtered out mitochondrial and chloroplast sequences from the sequencing data, and subsequently filtered the metadata for PD subjects only and control subjects only. We then ran them independently to look at the diversity metrics within each group (20, 21). We followed the same steps for each set of patients, with all the PD subjects being run first and then the control subjects. The data for the subjects within the group was further filtered based on the variable being examined, with separate feature tables being generated for fatigue, apathy, sleep problems, or antidepressant usage. This caused the total number of samples based on each neuropsychiatric variable to be different. This was done because not all the subjects had data on all of the four neuropsychiatric variables, hence filtering for them all together would have greatly reduced the sample size. Of all the 197 PD subjects, 84 had sleep problems and 112 did not and the rest did not report; 66 had fatigue and 85 did not and the rest did not report; 57 consumed antidepressants and 139 did not and the rest did not report; 25 had apathy and 68 did not and the rest did not report. Of all the 103 control subjects, 27 had sleep problems and 62 did not and the rest did not report; 17 had fatigue and 73 did not and the rest did not report; 7 consumed antidepressants and 83 did not and the rest did not report; 5 had apathy and 29 did not and the rest did not report. For each variable, we generated a tree for phylogenetic diversity analysis. We then ran alpha and beta diversity metrics at a sampling depth of 6000 reads (22) and significance was determined for alpha-groups and beta-groups within each variable. We ran these steps for all the variables within the PD subjects and control subjects separately, with 8 sets of diversity metrics being generated in total. In this study, we evaluated Pielou's Evenness, Faith's Phylogenetic Diversity, Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance, and Weighted UniFrac distance (23-27). Pielou's evenness index is an alpha diversity metric that measures evenness by considering the abundance of each species in a given environment (28). Faith's Phylogenetic Diversity is another alpha diversity metric that assesses the phylogenetic relatedness among the species in a given environment (29). Jaccard index is a beta diversity metric that considers the presence or absence of species to compare diversity of environments (30). Bray-Curtis index is another beta diversity metric that accounts for the abundance of the species in each environment (30). Unweighted UniFrac is a beta diversity metric that accounts for both presence and absence of species and their phylogenetic relatedness when comparing diversity between environments (27). Weighted UniFrac is another beta diversity metric that accounts for species abundance

as well as their presence and absence and phylogenetic relatedness (31). We calculated significance for the alpha-diversity metrics using pairwise Kruskal-Wallis test, conducted through the QIIME2 program. We calculated significance for the beta-diversity metrics using PERMANOVA through the QIIME2 program, running 999 permutations for each test. Prior to calculating p-values to determine significance, we determined Pseudo-F values for each metric. These results are available in Supplementary Tables 2 and 3.

Statistical analyses in R

We performed all statistical analysis using R (version 4.1.2; 15). We used the following packages in R to perform the taxonomic analysis and the relative and differential abundance analysis: here (32), tidyverse (33), microeco (34), cowplot (35), file2meco (34), picante (36), GUniFrac (37), ggalluvial (38), ggh4x (39), ggpubr (40), randomForest (41), igraph (42), rgexf (43), htmlwidgets (44), phyloseq (45), indicspecies (46), ape (47), DESeq2 (48), vegan (49), and qiime2R (50).

Taxonomic analysis of PD and control subjects based on their antidepressant use. To gain insight into taxonomic relationships (i.e. shared and unique taxa) between PD and control subjects based on antidepressant use and to test if antidepressant use had an effect on PD and control subjects, we performed a four-way taxonomic Venn analysis using R. We used the trans_venn class of the microeco package to analyze the number of shared features or ASVs between categories.

Indicator taxa analysis of PD and control subjects based on antidepressant use. To determine if there was an enrichment of certain taxa depending on the intake of antidepressants in PD patients and control individuals, we performed an indicator taxa analysis at the family level using the IndVal method (51). This statistical approach allows for identifying taxa that may be strongly associated with presence in a particular type of environment (46), in this case allowing us to identify bacterial families in the gut whose presence may be strongly associated with consumption of antidepressants. In particular, the IndVal method calculates an indicator value for each taxon based on the specificity (calculated as A-value, representing the degree to which a taxon is only found in a particular type of environment) and fidelity (calculated as B-value, representing how likely it is for a of the taxon to be found in other environments of similar type) for a particular environment (52). We imported the filtered features table, the taxonomic classification data, the metadata file, and the rooted phylogenetic tree into R as a phyloseq object and used the indicspecies package (46) to conduct the indicator taxa analysis. To assess statistical significance, the indicspecies package conducts permutational test to calculate a p-value for each indicator value. We considered bacterial families with an p-value of less than 0.05.

Differential and relative abundance analysis of PD and control subjects based on their antidepressant use. Prior to performing the relative abundance and differential abundance analysis, we used the tidyverse package to remove subjects that had unreported antidepressant use, and to facilitate the relative abundance analysis by adding a new column to the metadata which contained the disease state and antidepressant use status of each sample (e.g. a control subject with no antidepressant use would have Control_no as its value for this column). We then imported Qiime artifacts containing the ASV data, the phylogenetic tree, and the taxonomy data, into microeco, and eliminated samples with less than 6000 sequencing depth, retaining 94.3% of samples.Features with less than 0.05% abundance were also eliminated in order to remove any potential noise in the data. The relative abundance data was not used for making statistical comparisons but to reveal the top 10 most abundant families in each group. The Wald test was used in the differential abundance analysis to make statistical comparisons between the groups.

RESULTS

PD patients that use antidepressants have significantly different gut microbiome compositions compared to PD patients that do not use antidepressants. In order to determine which neuropsychiatric factors could impact the gut microbiome, we ran diversity

analyses using the QIIME2 pipeline. After performing alpha and beta diversity analysis, we determined significant results by generating p-values for each of the metrics tested. The results of this analysis are displayed in Table 1. Through a pairwise Kruskal-Wallis test, we found that microbial species richness was significantly different between PD patients who had high apathy and PD patients who had low apathy (p-value = 0.048), as indicated by Pielou's Evenness. Most notably, however, multiple metrics (Bray Curtis p-value = 0.027, Jaccard p-value = 0.017, and Weighted UniFrac p-value = 0.04) indicated that species diversity is significantly different between PD patients who use antidepressants compared to those who do not. Therefore, we concentrated on the antidepressant use variable for further analysis. We also ran diversity metrics for the same variables within control subjects, but no significant results were found (Supplementary Table 1). It is important to note that the sample size for each variable was not equal between the control and PD group (e.g. proportion of control subjects with sleep problems was different from the proportion of PD subjects with sleep problems); therefore, the lack of significant results in the control subjects may have been due to this sampling bias.

TABLE. 1 Alpha- and beta-diversity analysis results within PD patients. Neuropsychiatric symptoms (sleep problems, apathy, antidepressant use, and fatigue) and diversity metrics within PD patients. The alpha diversity metrics (Pielou's evenness and Faith's Phylogenetic Diversity) were assessed for significance using pairwise Kruskal-Wallis test and the beta diversity metrics (Bray-Curtis, Jaccard, Unweighted UniFrac, and Weighted UniFrac) were assessed for significance using PERMANOVA. P-values for each diversity analysis have been displayed comparing presence or absence of the neuropsychiatric symptoms. Double asterisks (**) indicate significant results ($p<0.05$).

	Alpha Diversity		**Beta Diversity**			
	Pielou's Evenness	Faith's PD	Bray Curtis	Jaccard	Unweighted UniFrac	Weighted UniFrac
Sleep Problems	0.542	0.228	0.164	0.735	0.092	0.221
Apathy	0.048**	0.440	0.545	0.840	0.996	0.723
Antidepressant Use	0.142	0.909	0.027**	0.017**	0.164	0.040**
Fatigue	0.698	0.378	0.776	0.848	0.579	0.722

Fewer unique taxa present in PD and control subjects using antidepressants. Following alpha and beta diversity analysis, we aimed to analyze the taxonomic relationships between PD patients and control subjects on antidepressants and those not on antidepressants. To do so, we looked for unique and shared taxa across these four conditions, and visualized our findings in a four-way Venn Diagram (Fig. 1). We found unique and shared taxa across all four conditions. Most notably, we observed less unique taxa present in PD and control subjects using antidepressants (15% and 1.9%, respectively), compared to PD patients and controls not using antidepressants (36.5% and 31.5%, respectively).

Antidepressant use is associated with the enrichment of certain bacterial families in control and PD subjects. Given that unique taxa were found for each of the four groups (Fig. 1), we performed an indicator taxa analysis at the family level in order to identify any bacterial families that were strongly associated with each group (p-value ≤ 0.05). The analysis returned 2 bacterial (Desulfovibrionaceae and Campylobacteraceae) families for PD subjects with antidepressant use, and 2 bacterial (Fusobacteriaceae and Bacteroidaceae) and 1 archaeal (Methanomassiliicoccaceae) families for control subjects with antidepressant use (Table 2). All of the identified indicator families were exclusive to one group and were not shared with other groups. No indicator families were identified for control or PD subjects with no antidepressant use.

Differential abundance analysis reveals significantly lower abundance of the Prevotellaceae family in PD subjects with antidepressant use. We compared the relative abundance of each group based on disease state and antidepressant use. Upon visual inspection, the relative abundance plot of the top 10 most abundant families across all 4

groups revealed noticeable differences in the percent relative abundance of the Prevotellaceae and Bacteroidaceae families (Fig. 2). In particular, both control and PD subjects with

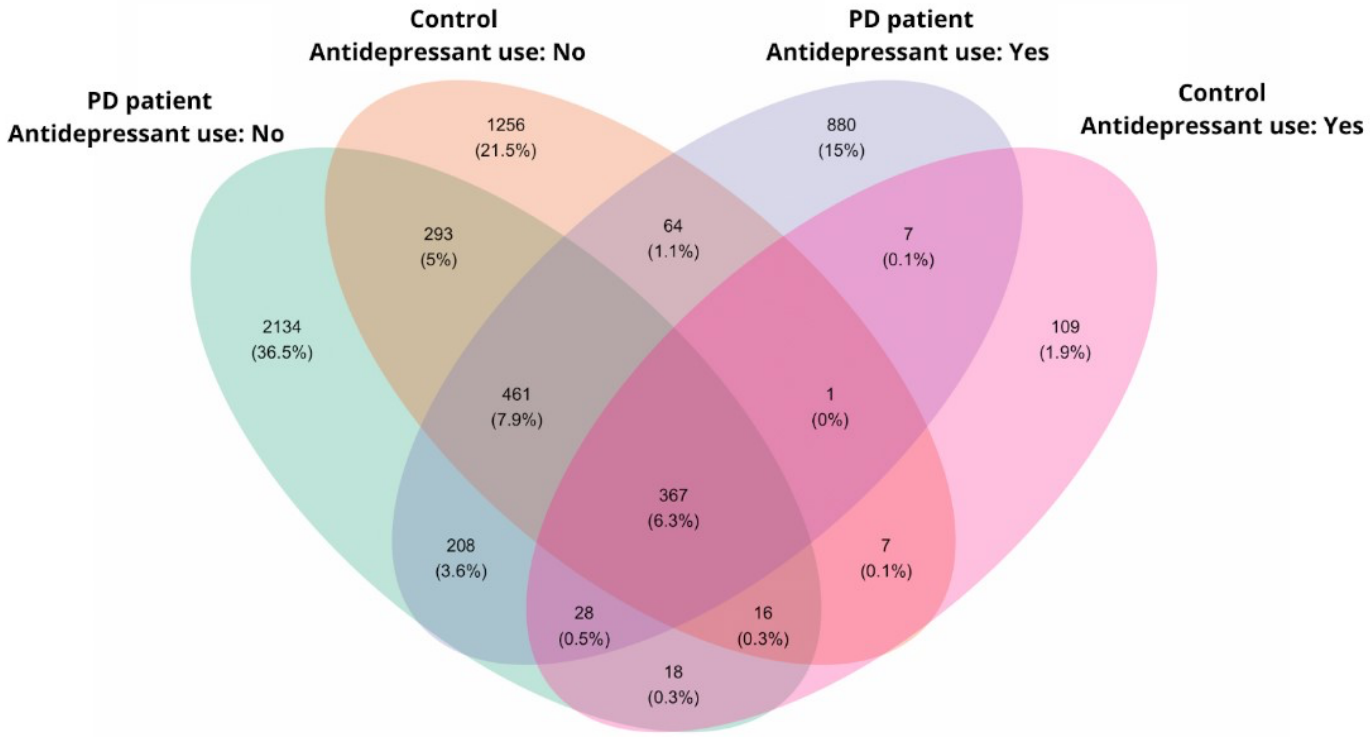

FIG. 1 Taxonomic analysis of PD and control subjects based on their antidepressant use. A four-way Venn diagram analyzing shared and unique ASVs between PD patients not on antidepressants (green), control individuals not on antidepressants (orange), PD patients on antidepressants (blue) and control individuals on antidepressants (red). The trans_venn class of the microeco package in R was used to create the four-way venn diagram based on the number of shared features or ASVs between each group.

TABLE. 2 Indicator taxa of PD and control subjects that were on antidepressants. A-value (representing specificity), B-value (representing fidelity) and indicator value for each indicator taxon from each test group have been displayed. To assess significance, the P-value for each indicator value was calculated using the permutation test through the indicspecies package in R. All displayed p-values indicate significant results (p-value ≤ 0.05).

Group	Indicator taxa (Phylum; Order; Family)	A-value	B-value	Indicator value	p-value
Control patients that were on antidepressants	Bacteria; Bacteroidales; Bacteroidaceae	0.3427	1	0.3427	0.04
	Archaea; Methanomassiliicoccales; Methanomassiliicoccaceae	0.8645	0.1429	0.1236	0.015
	Bacteria; Fusobacteriales; Fusobacteriaceae	0.6656	0.1429	0.0951	0.045
PD patients that were on antidepressants	Bacteria; Desulfovibrionales; Desulfovibrionaceae	0.4867	0.9123	0.4440	0.035
	Bacteria; Campylobacterales; Campylobacteraceae	0.9564	0.0877	0.0839	0.05

antidepressant use seemed to have lower abundance of the Prevotellaceae family when compared to subjects without antidepressant use. In addition, control subjects with antidepressant use seemed to have higher abundance of the Bacteroidaceae family when compared to control subjects without antidepressant use. These results suggest that there may be differences in the abundance of particular families in each group based on their antidepressant use; however, statistical comparisons would need to be made in order to assess

whether these observed differences are significant and further studies are required to determine whether these observations are an effect of antidepressant consumption or not.

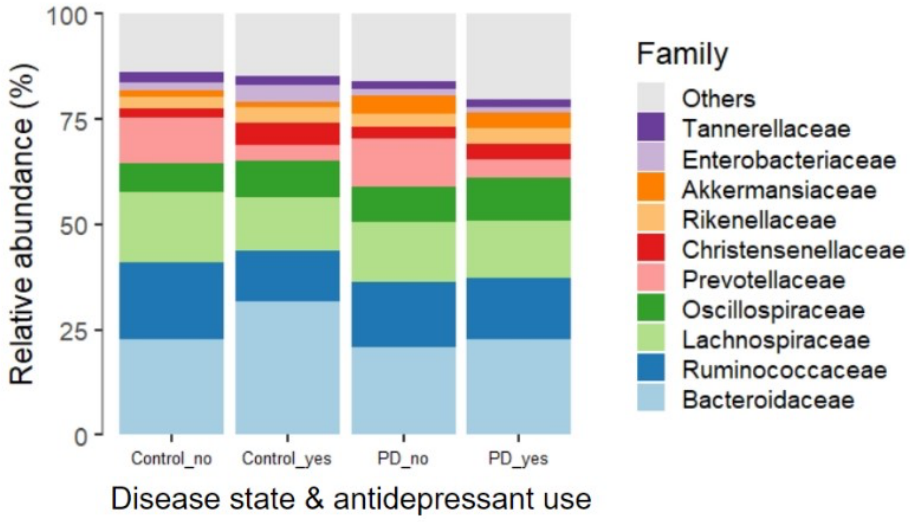

FIG. 2 Relative abundance analysis for top 10 most abundant families across all subjects based on their disease state and antidepressant use. Top 10 most abundant families were identified based on the families with the highest mean abundance across each four groups. Each bar is labeled based on the subject's diseased state followed by their antidepressant use status (e.g. Control_no represents control subjects who do not use antidepressants). The microeco package in R was used to process and display the data.

To determine whether these observed differences were statistically significant, we performed differential abundance analysis at the family level for each group based on their antidepressant use. The results revealed that indeed, the PD subjects with antidepressant use have significantly lower abundance of the Prevotellaceae family compared to PD subjects without antidepressant use (p-value < 0.001). However, this difference was not found to be significant for control subjects with antidepressant use. No other families that were among the top 10 most abundant ones across the 4 groups (including Bacteroidaceae) were found to be differentially abundant when comparing subjects with antidepressant use with subjects without antidepressant use within each disease state.

DISCUSSION

In this study, we aimed to determine whether certain neuropsychiatric disorders of PD have a significant effect on the gut microbiome of PD patients and control subjects. Research has well established a bidirectional communication between the gut and the brain via the GBA, however this research is mostly focused on gastrointestinal functions (9). Less papers have been published on the role that neuropsychiatric disorders play on the gut microbiome, especially in the context of PD. Studying neuropsychiatric associations and impacts on gut bacteria composition can provide more opportunities to prevent and treat neurological diseases. The results from our study show a connection between mental health of PD patients and their gut microbiomes, and open the door to future studies that could further shed light on the relationship between these two aspects of their health.

Antidepressant use is associated with differences in gut microbiome diversity in PD patients. Through QIIME2 diversity analysis, we were able to determine whether any of the neuropsychiatric symptoms we were studying (sleep problems, apathy, antidepressant use, or fatigue) led to differences in the alpha or beta diversity of the gut microbiota composition of the subjects. The results of our diversity analysis indicate that PD subjects who were using antidepressants had significantly different microbial communities compared to PD subjects who were not taking antidepressants. Multiple metrics support this difference, with significant differences found in Bray-Curtis, Jaccard, and Weighted UniFrac measures of beta-diversity. These results suggest that antidepressant use in PD patients may cause changes in both microbial abundance and phylogenetic diversity of the gut microbiota. Changes in microbial diversity due to antidepressant use have also been reported by other studies. For example, one study by Lukić *et al.* examined the effects of five commonly used antidepressants on the gut microbiota in mice and they found that antidepressants reduced the richness and increased the beta diversity of gut bacteria compared to controls (53). A different study by Zhang *et al.*

found that there was a significant difference in microbial diversity according to Weighted and Unweighted UniFrac distances between antidepressant-treated rats and those who had chronic unpredictable mild stress induced depression (12). Overall, our results are consistent with what has been found in the literature, however, we did not find any significant differences in richness or in Unweighted UniFrac. This discrepancy may be explained in part by the different model systems, sample sizes, and confounding variables involved in each study.

Antidepressant use is associated with the enrichment of certain bacterial and archaeal families associated with gut dysbiosis. Having established earlier that PD patients who were on antidepressants had a significantly different microbial diversity when compared to those who were not on antidepressants, we performed a taxonomic analysis to determine the taxonomic relationships between both groups of PD patients, and included the control individuals for comparison purposes. Our findings indicate that there were unique and shared taxa across all four conditions. This result suggests that the unique taxa in each group might be a driver of the microbial diversity difference found in both our beta diversity analysis, and Cirstea *et al.* (7). Moreover, our findings also show that there was less unique taxa present in both PD and control subjects using antidepressants, than in PD patients and control individuals that were not on antidepressants. In order to further identify the taxa that drove this result, we performed indicator taxa analysis. The results of the analysis suggested that antidepressant use may be associated with alterations in gut microbial composition, involving the enrichment of certain bacterial and archaeal families (Table 2). More specifically, the Bacteroidaceae, Methanomassiliicoccaceae, and Fusobacteriaceae families were found to be enriched in control individuals that were on antidepressants. On the other hand, the Desulfovibrionaceae and Campylobacteraceae families were found to be enriched in PD patients that were on antidepressants. While each of the identified indicator families were found to be exclusive to their respective group, there are important differences in their specificity (i.e. A value) and fidelity (i.e. B value) parameters. For control subjects that were on antidepressants, the Bacteroidaceae family has the highest fidelity but lowest specificity, while the archaeal Methanomassiliicoccaceae family has the highest specificity, but equally low fidelity as the Fusobacteriaceae family. The Bacteroidaceae family may be considered as the most reliable indicator family for this group because it has the highest indicator value, which considers both the specificity and fidelity of each family. Regarding PD patients that were on antidepressants, the Desulfovibrionaceae family has the highest fidelity but the lowest specificity in the group, whereas the Campylobacteraceae family has the highest specificity but lowest fidelity in the group. Since the Desulfovibrionaceae family has the higher indicator value, it may be considered as the most reliable indicator family for PD subjects with antidepressant use. The fact that no indicator families were found for both PD and control subjects that were not on antidepressants could mean that there were no bacterial or archaeal families that showed specificity and/or fidelity for either of those two groups. Because more diverse groups of organisms are present at higher taxonomic ranks, running the indicator taxa analysis at higher taxonomic ranks could increase the chance of finding indicator taxa in the groups that were not on antidepressants.

Furthermore, we found in the literature that all of the indicator families identified in control and PD patients that were on antidepressants have been shown to be associated with gut dysbiosis. According to Huang *et al*. the Bacteroidaceae family was strongly expanded in WT mice that had dextran sulphate sodium induced colitis (54). As reported by Pozuelo *et al.* untreated patients with irritable bowel syndrome and constipation had significantly higher levels of the Methanomassiliicoccaceae archaeal family (55). Moreover, the Fusobacteriaceae family was found to be enriched in patients with gastric cancer according to a study performed by Castaño-Rodriguez *et al.* (56). Additionally, the Campylobacteraceae family was shown to increase the risk of acquiring inflammatory bowel disease in a meta-analysis also performed by Castaño-Rodriguez *et al.* (57). Likewise, the genus *Desulfovibrio*, which belongs to the Desulfovibrionaceae family, has been shown to take part in the pathogenesis of intestinal inflammatory disorders, as reported by to Jiang *et al.* (58). With regards to how antidepressant use relates to gut dysbiosis, the systematic review by Letchumanan *et al*. suggested that the chronic use of antidepressants could cause adverse effects in patients due to antidepressant-associated gut dysbiosis (59). Although the precise impact of antidepressant treatments on gut microbiota composition remains largely unexplored, our results suggest that

the enrichment of only indicator bacterial families that are associated with gut dysbiosis is likely to be due to the intake of antidepressants in both PD and control patients. The fact that the use of antidepressants dysregulates the microbiome highlights the importance of improving the clinical efficacy of the current antidepressant therapies.

Reduction in abundance of the Prevotellaceae family in subjects with antidepressant use. Given that there are taxonomic differences based on antidepressants use, we investigated the changes in abundance of certain taxa in both PD and control patients. Our differential abundance analysis results showed a significant decrease (Wald test p-value < 0.05) in the abundance of the Prevotellaceae family in PD patients with antidepressant use when compared to PD patients with no antidepressant use; in addition, we also found a non significant reduction (Wald test p-value > 0.05) in the abundance of this family in control subjects with antidepressant use when compared to control subjects with no antidepressant use. The significantly lower abundance of the Prevotellaceae family in PD subjects with antidepressant use may be hinting at potential associations between this family and the use of antidepressants in PD subjects. In addition, given that this difference was not found in control subjects with antidepressant use, it could also be hinting at potential associations between consumption of drugs for Parkinson's Disease and the abundance of the Prevotellaceae family; however, this paper will be focusing on potential associations with the use of antidepressants.

While we did not find any previous studies that investigated the effect of antidepressant use on microbiota abundance of PD patients specifically, we did find a number of studies that performed abundance analysis on effect of antidepressant on the microbiota through *in vitro* and *in vivo* models. In a meta-analysis done by McGovern *et al.* in 2019 it was found that selective serotonin reuptake inhibitors (SSRIs), as a major class of antidepressants, exhibit antimicrobial effects in animal models (11). In particular, a study done by Cussotto *et al.* in 2019 found a depletion of the genus *Prevotella* (found in the Prevotellaceae family) in male rats treated with Fluoxetine (a SSRI) (60). While the mechanism through which SSRIs exhibit antimicrobial activity is not well understood (61), *in vitro* studies done on *Staphylococcus aureus* and *Escherichia coli* show that SSRIs act as efflux pump inhibitors in these bacteria (62, 63). Hence, while the original publication on our dataset (7) does not report which type(s) of antidepressants were used by the participants, a potential reason behind the reduction in abundance of the Prevotellaceae family in PD patients who use antidepressants could be due to efflux pump inhibition in the members of this family (assuming the administered antidepressants were SSRIs). Overall, our abundance analyses results highlight the need for studies that investigate the effect of antidepressant use on PD patients exclusively, as well as studies that further investigate the mechanisms through which antidepressants may exhibit antimicrobial activity in particular families of bacteria such as Prevotellaceae.

Limitations Other than the disease state and neuropsychiatric measures, the original dataset that was used in this study included measures of multiple other physiological and dietary variables for each participant such as sex, age, BMI, or alcohol consumption. Therefore, one of the main limitations of our analysis was not controlling for these confounding variables, hence limiting our ability to derive more robust causal links between antidepressant use and microbial diversity and abundance. However, controlling for all of the confounding variables would have greatly decreased the sample size of our study and it would have come at the expense of the reliability of our results.

In our study, we did not have data available to us showing the composition of the microbiotas of depressed patients before taking antidepressants. It has been shown in studies that depression itself can alter the gut microbiome community composition, even if the patient is not taking antidepressants (64). Because of this, it is possible that the dysbiosis observed in patients is due in part to their antidepressant medication, and due in another part to their depression. In order to parse apart these interactions, and determine which effects were due to the medication and which due to the depression, we would have to conduct a longitudinal study to compare the microbiome compositions at various points in these patients' treatments. However, we think that with the data we did have available, we were able to show that there is a distinction between the microbial compositions of patients taking antidepressants and

those not taking antidepressants, supporting the need for future studies into the effects of the different variables involved.

Another limitation was the non-uniformity in the sample size of our case and control groups. For example, there were only 7 control subjects who used antidepressants while there were 83 control subjects who did not use antidepressants; and there were 57 PD patients who did not use antidepressants while there were 139 PD patients who did use antidepressants. Therefore, such differences in the sample size of our case and control groups may have deviated aspects of our results.

Lastly, another limitation of our analyses was the binary classification of our neuropsychiatric data. Variables such as sleep problems or antidepressant use were recorded in binary (i.e. yes or no) by origin, however, variables such as the FSS Fatigue Score or Apathy Score were converted into binary categories by us. Given that these neuropsychiatric symptoms exhibit a variety of levels in terms of their dosage or severity, converting them into binary categories for the sake of feasibility would come at the expense of generating less accurate or nuanced results.

Conclusions In this study, we aimed to analyze the effect of neuropsychiatric symptoms (namely, fatigue, sleep problems, antidepressant use, and apathy) on the gut microbial diversity and abundance of PD patients. Overall, our results suggest that the use of antidepressants in PD patients is associated with the microbiota beta-diversity of the gut and may also be associated with changes in the abundance of certain taxa, namely, the Prevotellaceae family. In addition, our results revealed that antidepressants may be associated with changes in the composition of the gut microbiome given that less unique taxa were found in both PD and control subjects that were on antidepressants, when compared to those individuals who were not, and that indicator families associated with gut dysbiosis were identified in both PD and control subjects that were on antidepressants. However, given the limitations of our study and the lack of studies investigating the effects of antidepressants exclusively on PD patients, more research is required to draw further conclusions. Nonetheless, our results have laid the foundation for better understanding the scope of effect that antidepressants may have on the gut microbiota.

Future Directions Future investigations could attempt to better capture potential causal relationships between neuropsychiatric symptoms and microbial diversity. This could be done by better controlling for confounding variables such as dietary or physiological factors as well as maintaining better sample size uniformity among case and control groups. This would require access to a more extensive dataset than ours so that controlling for confounding variables would not come at the expense of a small sample size. Furthermore, future projects could zoom into the effect of antidepressant use on microbial diversity by focusing on specific types of antidepressants or specific doses of antidepressants, hence allowing for deriving more detailed results on this topic. In addition, future projects could investigate other neuropsychiatric symptoms (such as anxiety, agitation, or hallucinations) and analyze the data using other diversity metrics (such as Shannon's index which accounts for both richness and evenness (65)), hence allowing for extending the scope of analysis in this topic.

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CONTRIBUTIONS

All four members of the team contributed equally to running the analyses for the project and writing the manuscript was a collective effort. In general, the following tasks were done by the following members:
Parsa Abrishamkar did the relative and differential abundance analyses, wrote its pertaining methods, results, and discussion section. He also wrote the study limitations, conclusion, future directions, and acknowledgments section of the manuscript.
Claudia Barreto was assigned to do the indicator taxa analysis for both PD and control individuals, wrote the corresponding methods, results, and discussion sections for it. She also contributed to the overall writing of the discussion.
Baria Choudry performed the diversity analysis using QIIME2, wrote the related methods, results, and discussion section, and generated the significance tables. She also wrote the abstract of the manuscript.
Steven Or did the introduction and the methods and results pertaining to taxonomic Venn analysis.

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