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Antidepressant use and age affects the diversity of the gut microbiome in Parkinson's disease patients

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SUMMARY Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects mobility and brain coordination. Most cases of PD are observed in people above 60 years of age with some developing the disease before 50. Due to the impact of clinical symptoms such as depression on patient well-being, antidepressants are commonly prescribed. Studies have analyzed the gut microbiome and its association with PD, however, factors contributing to altered gut microbiota in PD patients remain largely unexplored. We performed an *in silico* analysis using 16S rRNA gut microbiome sequences to assess whether age and antidepressant use affect the gut microbiome diversity in PD patients. As such, we determined that there was no relationship between antidepressant use and age. Subsequently, we aimed to analyze the effects of age and antidepressant use on the gut microbiome in PD patients. We found that both age and antidepressant usage significantly alters the gut microbial diversity. We found the microbial community diversity and abundance were significantly lower in the antidepressant use group, and PD patients above 60 years old had a higher microbial diversity than younger PD patients. Additionally, we found indicator taxa associated with older PD patients only. Furthermore, there were lower abundances of the bacteria family Prevotellaceae, which is commonly present in gut microbiota and results in idiopathic rapid eye movement behavioural sleep disorder, in PD patients who used antidepressants. Our findings suggest that age and antidepressant usage strongly influence the diversity of PD microbiota. Further investigation is needed to analyze factors like the BDI depression score and age, specifically the younger-onset, to detect gradual shifts in the gut microbial community with respect to antidepressant consumption.

INTRODUCTION

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by tremors, stiffness, and effects on motion and cognition (1). There are significant clinical implications for PD development through the progression of mental and behavioural changes like depression, sleep disruptions, memory loss, and fatigue (1). PD is present in more than 10 million people worldwide with most cases observed in people above 60 years old (2, 3). Although PD is primarily a disease prevalent in the elderly, approximately 5% to 10% of people develop the disease before age of 50 (2). PD is caused by a loss of nerve cells in the substantia nigra part of the brain, which could lead to a decrease in the number of dopamine levels, affecting brain coordination (4). Furthermore, it is a multifactorial disorder involving the interplay of aging, genetics, and environmental factors (5). The impact of this disease on quality of life is significant enough to warrant an in-depth analysis of this disease.

The gut microbiota is emerging as an important modulator of neurodegenerative diseases like PD, due to the early involvement of the gastrointestinal tract resulting in motor symptoms (6). In order to investigate how intestinal bacteria are associated with gastrointestinal health in PD patients, Cirstea *et al*. (2020) analyzed fecal samples from PD patients and healthy individuals. The researchers identified significant taxonomic differences in the intestinal bacterial microbiome composition between PD patients and healthy individuals (7). The dataset also provided extensive additional data on antibiotic usage, alcohol consumption, antidepressant usage, and other consumables for each patient (7). Food and beverage consumption is a major factor in determining the composition of our intestinal bacterial microbiome (8). In addition, past studies have investigated the effects of other substances including coffee consumption and antibiotic usage on microbial community structure (9),

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alcohol consumption and body mass on the gut microbiome (10), and dietary fibre intake on the metabolism of specific gut bacterial taxa (11). However, the effects of antidepressant usage and age on gut bacterial microbiome diversity remains to be determined.

Growing evidence suggests that there is a correlation between PD and depression (12). It is estimated that at least 50% of those diagnosed with PD experience some form of depression during their illness (2). Antidepressants are a class of drugs that decrease symptoms of depressive disorders by maintaining the chemical imbalance of neurotransmitters (13). Specifically, selective serotonin reuptake inhibitors are the most commonly prescribed antidepressants for the treatment of depression in PD, due to their more favourable side-effect profile (14). Preliminary studies on the correlation between antidepressant usage and gut microbiota suggested that antidepressant usage altered the gut microbiota in mice models (15). Moreover, antidepressants reduced the richness and increased beta diversity of mouse gut bacteria (15). Chaitg *et al.* (2020) investigated the *in vitro* antimicrobial activity of antidepressants from different therapeutic classes against strains of human gut microbiota. The findings determined that the human gut microbiota can be altered in response to antidepressant usage that can have a wide impact on health (16).

Age is the largest risk factor for the development and progression of PD, which can impact cognition and other functions leading to the pathogenesis of PD (17). Growing evidence suggests that there is a two-way relationship between the gut microbiome and many age-associated changes (18). Wilmanski *et al.* (2021) analyzed the relationship between the gut microbiota and its correlation to healthy aging and determined that gut microbial composition and diversity change over time with age (19). However, the correlation between the age of the PD patients and the gut microbial composition remains to be explored.

Specific bacterial taxa have been found to be associated with PD. Preliminary studies observed enrichment of the genera *Lactobacillus*, *Akkermansia*, and *Bifidobacterium* and depletion of bacteria belonging to the family Lachnospiraceae and the genus *Faecalibacterium* (20). Additionally, the family Enterobacteriaceae has been reported to be more abundant in fecal samples from PD patients compared with matched controls from the previous studies (21). The presence of increased endotoxin from the *Enterobacteriaceae* in the body of PD patients promotes the release of inflammatory cytokines in the body and, with blood circulation through the blood-brain barrier, further induces nerve inflammation and nerve death (21).

Taking into consideration the results, we aimed to further explore the impact of antidepressant usage and age, associated with the altered gut microbiota in PD patients by using a dataset generated by Cirstea *et al.* (2020) from the Finley lab at the University of British Columbia (UBC). The dataset contains fecal samples from 197 PD patients and 103 healthy controls, along with their associated age and antidepressant usage data. We tested the hypothesis that age and antidepressant usage will have a significant effect on the diversity of the gut microbiota within PD patients. We predicted that both of these factors will alter certain taxonomic groups as the variables are both highly relevant during PD development. To test our hypothesis, we 1) determined if there is a relationship between age and antidepressant usage in terms of the PD status, 2) explored the effects of age and antidepressant usage on the gut microbiome in PD patients, and 3) conducted indicator taxa and differential abundance analysis relative to age and antidepressant usage in PD patients, respectively. An understanding of gut microbiota composition in PD patients and the identification of the effects of antidepressant usage and age on gut microbial community structure could allow for the development of potential diagnostic and therapeutic treatment methods which could be critical in combating PD.

METHODS AND MATERIALS

Patient demographic and sample collection. The dataset for our patients utilized for this project was obtained from research conducted by Cirstea *et al.* (2020) who collected dietary, medical, and mental wellbeing information for all patients. A total of 300 patients aged between 40 to 85 (197 PD patients, 103 control patients) were used for their study and those diagnosed with PD were recruited from the Parkinson's Research Centre (PPRC) at UBC, Canada. Cirstea *et al.* (2020) extracted DNA from patient-provided fecal samples and

amplified the V4 region of the 16S rRNA with barcoded 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVHHHTWTCTAAT-3') primers. Amplified 16S rRNA was sequenced using an Illumina MiSeq platform to obtain raw sequences that we used for our analysis. Further information regarding the research conducted by Cirstea *et al.* (2020) can be found in their main published paper (7). Steps regarding QIIME2 parameters are listed in "QIIME2_code" (22).

Metadata filtering. Our research had a focus on how antidepressant use and age alters gut microbiota within PD patients. Metadata was filtered for the 197 patients that had PD and filtered again based on antidepressant usage (Yes/No) or age group (Above/Below) where the two variables were treated independently. For antidepressant use, there were 57 patients who were categorized as "Yes" who used antidepressants and 139 patients who were categorized as "No" who did not use antidepressants. One patient did not have their antidepressant use information recorded and only their age was used for this analysis. For the age group, there were 144 patients who were categorized as "Above" which indicated an age greater than 60 and 53 patients who were categorized as "Below" which indicated an age less than or equal to 60. The average age to develop PD was determined to be around 60 and was chosen as a threshold for analysis based on the information provided by the Parkinson's Canada organization as patients were recruited within Canada (23). To summarize, we used the metadata in two different ways 1) we filtered for patients with PD and 2) analyzed for either age or antidepressant usage. Age will be used for indicator analysis and antidepressant use will be used for differential abundance analysis. The additional column for "Age_group" is listed in the updated metadata file denoted as "modded_parkinsons_metadata.txt" where "parkinsons_metadata.txt" was the original copy.

QIIME2 workflow and pipeline. When the metadata was filtered for PD and antidepressant use or age group, the alpha rarefaction curves were similar, and a sampling depth of 6000 was chosen. At our chosen depth, we retained 185 PD patients and 98 control patients. Further information regarding specific parameter details for QIIME2 are listed in "QIIME2_code" (22).

Sequence processing and taxonomic assignment. Raw sequence files were demultiplexed in QIIME2 (v2021.11) and were truncated to a length of 251 using DADA2 pipeline (22, 24). Following denoising, taxonomic assignment on amplicon sequence variants (ASVs) were conducted using a naïve Bayes trained 16S rRNA SILVA database (SILVA 138 SSU Ref NR 99) (25). As mentioned by Cirstea *et al.* (2020), the amplified V4 region was aligned against the trained SILVA database using the q2-feature plugin tool as part of QIIME2. Phylogenetic trees were generated from the SILVA database by comparing ASVs against its reference sequences for beta diversity analysis. Further analysis of taxonomy was conducted in R version 4.1.2 (26). Steps regarding QIIME2 parameters are listed in "QIIME2_code".

Beta diversity analysis. To test our hypothesis that age and antidepressant usage will have a significant effect on the diversity of the gut microbiota within PD patients, we calculated diversity metrics on both sets of data in QIIME2 for the PD-filtered age group and PD-filtered antidepressant use group. Weighted UniFrac distance and unweighted UniFrac distance were run for both sets of conditions and displayed as Principal Coordinate Analysis (PCoA) plots in QIIME2 and R. Weighted UniFrac distance and unweighted UniFrac distance was chosen to determine if microbial abundance was relevant in gut microbial diversity. The packages used for carrying over and plotting QIIME2 data in R included *phyloseq* (27), *qiime2R* (28), and *ggplot2* (29). Data was further filtered using the *tidyverse* (30) package. Using permutational analysis of variance (PERMANOVA), statistical significance was determined based on the FDR-adjusted p-value ($q < 0.05$) with 999 permutations. Further information regarding specific parameter details in QIIME2 can be found in "QIIME2_code" and "Diveristy_Metrics.R", respectively.

Indicator analysis in R. To determine if there are indicator taxa associated with age, we used the R packages *dplyr* (31), *phyloseq*, and *indicspecies* (32). QIIME2 data was loaded as a

phyloseq object in R and grouped based on family taxonomic rank using the function "group_by_taxonomy". Afterwards, the function "multipatt" was used with 999 permutations to run the analysis to find indicator species belonging to our two groups "Above" and "Below" as defined in the "Data Filtering" subsection. Only statistically significant taxa ($p < 0.05$) were kept as indicators. Families that were believed to be strong indicators were defined based on their stat value (>0.7) as their corresponding fidelity and/or specificity score would be high (>0.8) (Table 1). The chosen threshold was arbitrarily chosen. Further information regarding the analysis parameters are detailed in "Indicator_Analysis.R".

TABLE. 1 No associations between antidepressant consumption and age groups. Comparison between the patients belonging to different antidepressant consumption and age subgroups reveal that there is no visible association between them. A difference of only 6% is seen when comparing the number of patients "Above" and "Below". y/o - years old.

	Antidepressant Consumption		
	Yes	**No**	**Total**
Above 60 y/o	48 (24%)	155 (76%)	203 (71%)
60 y/o and Below	16 (30%)	67 (70%)	83 (29%)
Total	64 (22%)	222 (78%)	286

Differential abundance of gut microbial families in R. To determine if there are differentially abundant taxa in PD patients who take antidepressants, we used the R packages *tidyverse*, *vegan* (33), *ape* (34), *phyloseq* (34), and *DESeq2* (35) to perform differential abundance analysis. The data was filtered to a depth of 6000 to match the sequencing depth used for QIIME2. Sequences with less than 0.05% relative abundance were removed to retain high abundance taxonomic groups. Only organisms with a statistically significant FDR-correct p-value (< 0.05) are shown. Further information regarding the analysis parameters are detailed in "DESeq.R".

RESULTS

No relationship between antidepressant use and age in PD patients was found. The percentages of patients using antidepressants that are in the "Above" age group (24%) and "Below" (30%) are very similar, with only a 6% difference (Table 1). The same was observed with patients who do not use antidepressants in the "Above" age group (76%) and, "Below" age group (70%) (Table 1). These discrepancies are not significant enough to show a strong influence from either age or antidepressants, which supports the independence of these two factors.

Antidepressant use and age have a significant effect on the gut microbial community diversity of PD patients. To determine if there were differences in microbial communities for PD patients who used antidepressants and PD patients of different age ranges, we conducted weighted and unweighted UniFrac analyses respectively. Using weighted UniFrac analysis, we found a significant difference in microbial community composition between PD patients who use antidepressants and those who do not (Fig. 1A, PERMANOVA: $F_{\text{degress of freedom}}$ = f-value, q = 0.028) but there was no significant difference when using unweighted UniFrac analysis (PERMANOVA: $F_{\text{degress of freedom}}$ = f-value, q = 0.138). Using unweighted UniFrac analysis, we found a significant difference in microbial community composition between PD patients "Above" and "Below" (Fig. 1B, PERMANOVA: $F_{\text{degress of freedom}}$ = f-value, q = 0.013), but there was no significant difference when using weighted UniFrac analysis (Fig. 1B, PERMANOVA: $F_{\text{degress of freedom}}$ = f-value, q = 0.078). Overall, antidepressant use appears to have lowered microbial richness and abundance while increasing age increased microbial richness.

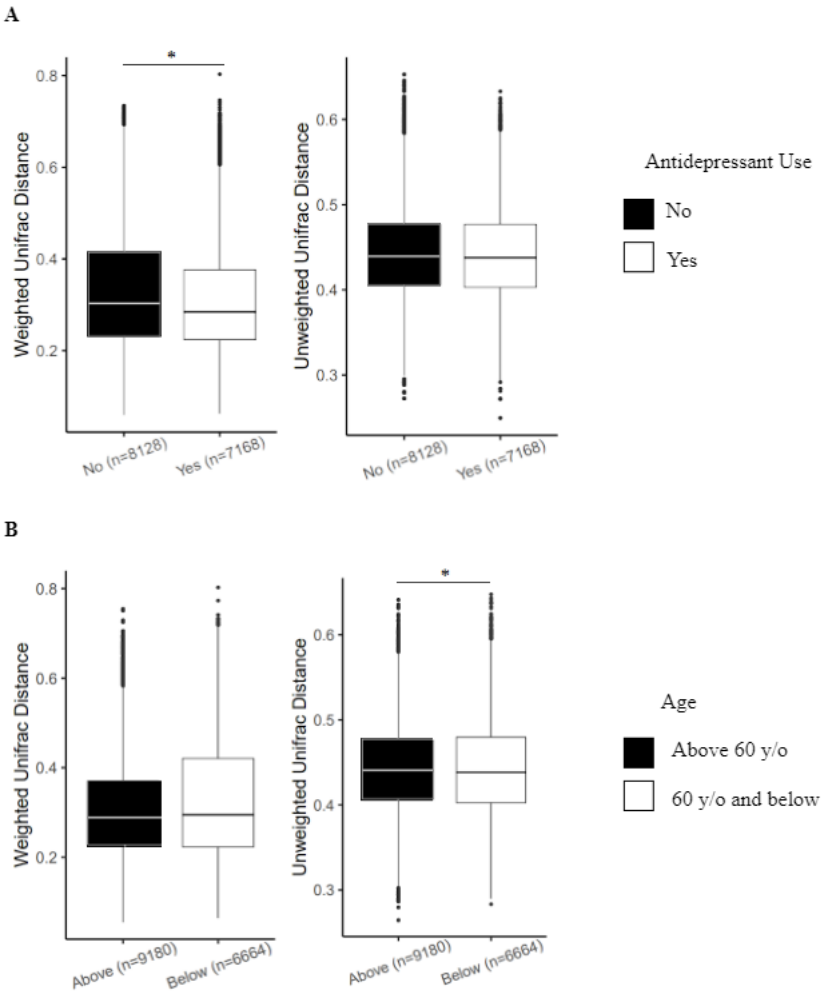

FIG. 1 Beta diversity analyses of PD patients based on (A) antidepressant use and (B) age. Boxes represent interquartile range (IQR), with the middle line representing the mean. Dots represent outliers, and n-values represent the number of connections (i.e. pairs) in each group used in the analysis. Pairwise PERMANOVA determined the FDR-corrected p-values of each analysis, which were considered significant if $q < 0.05$ (*). **(A)** There was a significant difference in microbial community composition for weighted UniFrac (PERMANOVA: $F_{\text{degrees of freedom}}$ = f-value, q = 0.028) but not unweighted UniFrac (PERMANOVA: $F_{\text{degrees of freedom}}$ = f-value, q = 0.138). **(B)** There was a significant difference in microbial community composition for unweighted UniFrac (PERMANOVA: $F_{\text{degrees of freedom}}$ = f-value, q = 0.013) but not unweighted UniFrac (PERMANOVA: $F_{\text{degrees of freedom}}$ = f-value, q = 0.0.078). y/o - years old.

High number of unique taxa between antidepressant use groups and between age groups. To identify the number of unique taxa between PD patients who use antidepressants and the unique taxa between the "Above" and "Below" age group, the presence and absence of specific microbial species in each group were identified and summarised (**Figure 2**). Between antidepressant users and non-users, a 52% overlap in taxa was found, with 32% of the non-overlapping taxa unique to antidepressant users and 16% to non-users (**Figure 2A**). PD patients in the "Above" age group had 13% of unique taxa, while the ones in the "Below" group had 38% of unique taxa (**Figure 2B**). There was a 49% overlap between the unique taxa present for "Above" and "Below" age groups (**Figure 2B**). The findings show that around half of the total species present are unique (i.e. non-overlap) to either antidepressant use groups or age groups.

Strong indicator families were found only among the PD patients in the "Above" age group. We observed that there were eight significant bacterial families associated with the "Above" group and one significant uncultured bacterial family associated with the "Below" group (Table 2). For the "Above" group, *Eubacterium coprostanoligenesm*, *Oscillospiraceae*, *Butyricicoccaceae*, *Anaerovoracaceae*, and *Coriobacteriaceae* were the best indicators for the cohort based on their stat value (> 0.7) which is denoted as the square root of the indicator value. There were no strong indicator families for the "Below" group. The results suggest that there were only indicator families representative of the gut microbial community in PD patients older than 60 years old.

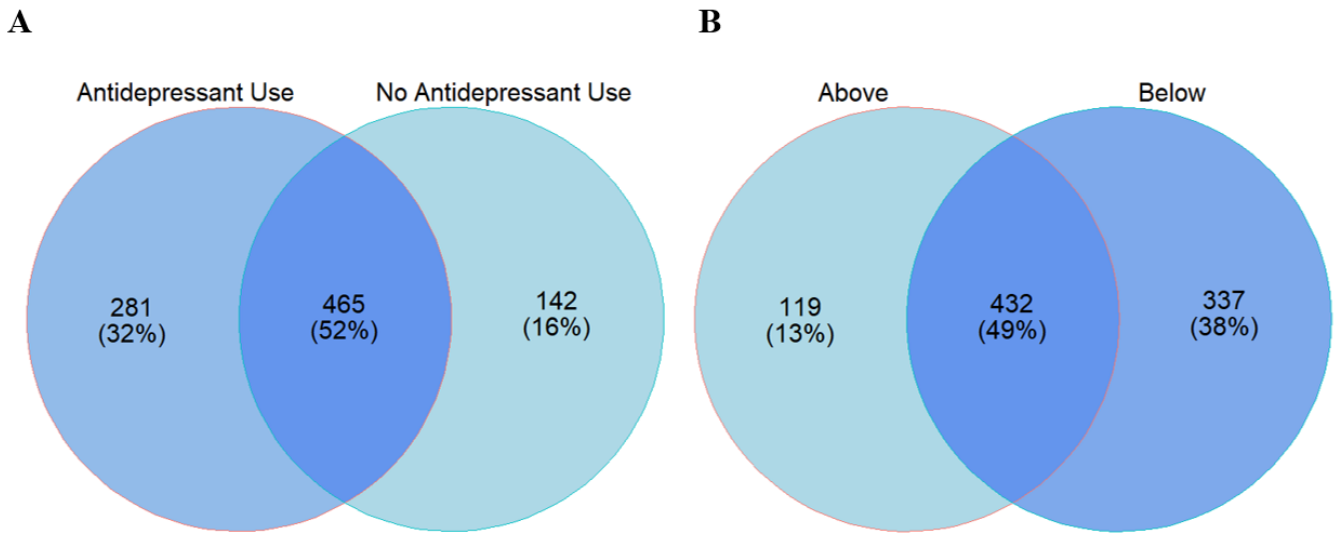

FIG. 2 Unique taxa based on antidepressant use (A) and age group (B). A total of 888 taxa were identified in the analysis. Percentages represent the proportion of total taxa in each group.

TABLE. 2 Indicator species analysis for "Above" group

Species associated with PD filtered "Above" age group (> 60 years of age)				
Family	**Specificity**	**Fidelity**	**p-value**	**stat**
Eubacterium coprostanoligenes	0.5928	0.9722	0.021	0.759
Oscillospiraceae	0.5678	1.0000	0.013	0.753
Butyricicoccaceae	0.5960	0.9236	0.036	0.742
Hungateiclostridiaceae	0.8698	0.2292	0.032	0.446
Anaerovoracaceae	0.6475	0.8333	0.028	0.735
Coriobacteriaceae	0.6516	0.8264	0.040	0.734
Eggerthellaceae	0.7798	0.5694	0.004	0.666
Flavobacteriaceae	1.0000	0.1667	0.003	0.408
Species associated with PD filtered "Below" age group (≤ 60 years of age)				
Uncultured bacterium	0.6878	0.5094	0.025	0.592

Differentially abundant taxa in PD patients filtered for antidepressant use. To determine the difference in the abundance of taxonomic families between PD patients who took antidepressants against those who did not, differential abundance analysis was conducted with R. Through the analysis, we found that the family *Prevotellaceae* was significantly more abundant (p = 0.0006, log2 fold change = -2.640699) in PD patients who did not take

antidepressants (Fig. 3). Our findings show that the usage of antidepressants reduces the abundance of the bacterial family *Prevotellaceae* within PD patients.

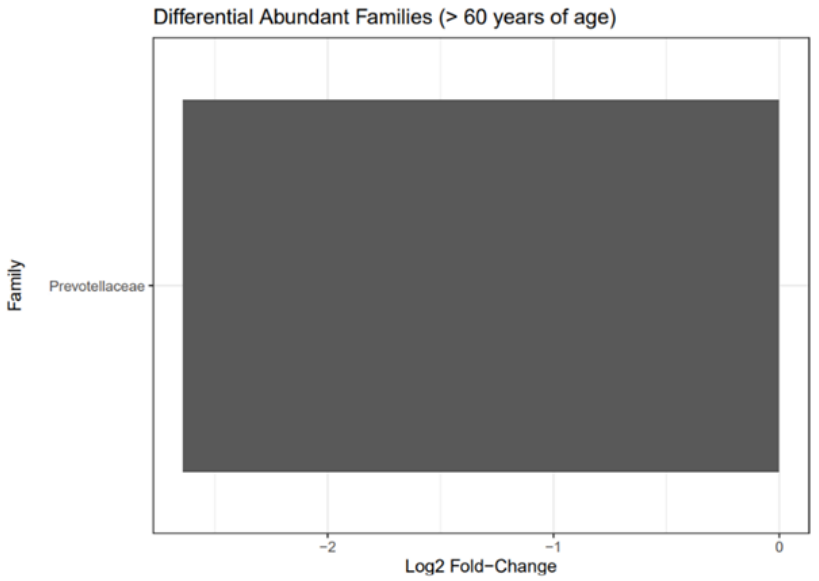

FIG. 3 Differential abundance analysis based on antidepressant usage. Only one bacterial family was significantly abundant in PD patients who did not take antidepressants. *Provotellaceae* was found to be of lower abundance ($p = 0.0006$, $\log_2$ fold change = -2.640699).

DISCUSSION

PD is a complex neurodegenerative disorder marked by a series of mental and behavioural changes (1). As the gut microbiota is an important factor in several neurodegenerative disease including PD, our objective was to determine if age and antidepressant use altered the gut microbial community within PD patients. We found that for antidepressant use, abundance accounted for gut microbial community diversity differences between patients who took antidepressants and those who did not. For age, we found that abundance was not as significant as a factor in determining gut microbial community diversity differences. In both cases, unique taxonomic families associated with PD were identified.

Antidepressant use and patient age had a significant effect on gut microbial diversity of PD patients. Antidepressant use and patient age were chosen as factors to analyze given the impact of antidepressants (15) and age (18, 19) on the gut microbiome (Table 1). However, we found no relationship between the two factors, so further downstream analyses were conducted independently of each other.

Between PD patients using antidepressants and non-users, a significant difference in the gut microbial communities was found using weighted UniFrac analysis. Weighted UniFrac accounts for microbial abundance as well as phylogenetic distance, while unweighted UniFrac, which showed no significant difference, accounts for microbial phylogenetic distance alone. This indicates that abundance (i.e. relative distribution) of microbes plays an important role in determining the difference in diversity between the two communities. In addition, the antidepressant use group appeared to have a lower weighted UniFrac distance compared to non-users, indicating that antidepressant use may reduce the abundance and phylogenetic distance of the gut microbiome in PD patients. Lukić *et al.* (2019) observed an increase in unweighted UniFrac distance, which we do not observe here. A likely reason for this difference is that our data is collected from human patients, whereas Lukić *et al.* (2019) used a mouse model. However, past studies have observed that antidepressants may have antimicrobial properties as well (36), which may explain the reduced abundance observed in our analyses.

Between PD patients “Above” and “Below”, we found a significant difference in their gut microbial communities using unweighted UniFrac analysis. Since unweighted UniFrac accounts for only microbial phylogenetic distance, this may indicate that microbial abundance does not play a role in determining gut microbial diversity in relation to the age of the patient. Moreover, past studies have seen differences in the beta diversity of older individuals,

including the unweighted UniFrac metric (18). Badal *et al.* (2020), also discuss an increase in the uniqueness of the gut microbiome in older individuals. In addition, we observed higher microbial diversity in PD patients above 60 years of age. Overall, our results are consistent with the findings of Badal *et al.* (2020).

Several bacterial families were indicators for gut microbial communities found within PD patients older than 60 years of age. Within PD patients who were over the age of 60, we observed several representative indicator families, but only one weak indicator family representative in patients who were equal to over below the age of 60. Of the indicator families, we found that *Eubacterium coprostanoligenes* (37), *Oscillospiraceae* (38), *Butyricicoccaceae* (6), *Anaerovoracaceae,* and *Coriobacteriaceae* (39-42) were strong indicators for the older cohort. From these families, *Eubacterium coprostanoligenes*, *Butyricicoccaceae*, *Oscillospiraceae*, and *Coriobacteriaceae* were found to be of altered abundance relative to healthy patients based on past research.

Interestingly, *Eubacterium coprostanoligenes* is found to be important in cholesterol homeostasis by decreasing the amount of cholesterol absorbed in the gut through the conversion of cholesterol to coprostanol (37). According to literature, PD patients were found to have generally lower cholesterol levels than the healthy controls (43). Furthermore, lower cholesterol is also linked to advanced or accelerated aging (44). Thus, an increase in the abundance of *Eubacterium coprostanoligenes* is consistent with the microbiota of older PD patients. Additionally, a study exploring the change in the microbiota of idiopathic rapid-eye-movement sleep behaviour disorder patients, a disorder strongly linked with the development of PD, also found an increase in *Eubacterium coprostanoligenes* (37).

Aside from *Eubacterium coprostanoligenes*, the bacterial family *Butyricicoccaceae* was found in lower abundance in PD patients and was linked to inflammatory bowel disease (IBD) (20). In the broader literature, various neurodegenerative diseases including PD, Alzheimer's disease, and multiple sclerosis are often associated with gastrointestinal issues (6). An observation by Romano *et al.* (2021) noted that *Butyricicoccaceae* was responsible for the production of butyrate, an important energy source of intestinal epithelial cells in lowering the risk of gut inflammation (20). Our findings are consistent with the theory as reduced abundance *Butyricicoccaceae* bacteria corresponds to less available butyrate for uptake by intestinal epithelial cells resulting in inflammation.

Lastly, for *Coriobacteriaceae* and *Oscillospiraceae*, they were found to be of greater abundance in PD patients; however, there is little to no past research linking these taxonomic families to PD symptoms.

Overall, our findings suggest that these families have strong implications and associations with PD symptoms, however, differential abundance analysis and relative abundance analysis will need to be conducted in future work as our indicator analysis only confirmed their relevance within our filtered cohort. We did not find these significant differences in abundance when the metadata was separated by age and will require testing different parameters to find these potential taxa.

Antidepressant use lowered the abundance of *Prevotellaceae* bacteria relative to patients who did not use them. Within PD patients who used antidepressants, we observed a significant decrease in the bacterial family *Prevotellaceae* relative to patients who did not take antidepressants. Although our results are consistent with previous research by Lukić *et al.* (2019) in the reduction of specific groups of taxa, we did not observe the same reduced groups. However, the reduction in *Prevotellaceae* was still consistent with other findings.

Several other researchers analyzed the gut microflora in PD patients to find several groups with altered abundance relative to healthy control patients (20, 38). Among these groups, *Prevotellaceae* was found to be less abundant, although it is worth noting that antidepressant usage was not a factor tested in their study. Surprisingly, the researchers found that *Prevotellaceae* was associated with idiopathic rapid eye movement behavioural sleep disorder (iRBD) (38). As sleep disorder is often a common symptom of PD, determining when a patient's abundance of *Prevotellaceae* decreases may be a potential indicator for early development of the disease. Taken together with our results, following studies should observe *Prevotellaceae* and track how its abundance changes in the initial stages of PD development.

In summary, many of our identified taxonomic families were associated with a number of metabolic and cognitive processes. These bacterial families influenced factors including

cholesterol level, sleep disorder, and gastrointestinal problems, which are all relevant issues that may influence the development of PD. Although our indicator analysis did not determine the abundance of the identified families, their prevalence in aging individuals with PD can be tracked in future studies to confirm their relevance. Overall, our findings show several taxonomic groups associated with many of these symptoms that have strong implications.

Limitations There are multiple limitations present in our study. A few significant limitations are carried over from the way the metadata is collected. 16S amplicon sequencing has inherent biases, which may confound the collected sequencing data. Some issues include PCR amplification biases, lower resolution, and lower sensitivity which can misrepresent the microbiota availability (45). As mentioned in Cirstea *et al.* (2020), due to the cross-sectional nature of the collected metadata, causality between factors cannot be established. Furthermore, virtually half of the individuals selected as the controls were the spouses of the patients. While spouses will invariably have more similar commensal microbiota, PD patients with non-familial controls will not, possibly introducing a confounding factor to the diversity analyses. Another issue with the use of spousal controls is the imbalance of sex. PD is more likely to manifest in males (46), making the control cohort more female oriented while the PD patient cohort is more male oriented. Lastly, Cirstea *et al.* (2020), details financial constraints preventing a larger cohort from being analysed, possibly missing out on additional associations.

There are further limitations due to the analyses we have performed. Following up on the reproducibility of the data analyses performed in other studies (7, 9), we confirmed that idiosyncratic choices in the analyses of the same data may produce differing levels of significance. This variability lends to possible changes in future analyses where parameters such as truncation length or sampling depth is changed. Furthermore, despite the filtering of the metadata for the targeted categories (e.g. age, antidepressant use), confounders may still affect the validity of our results. Additional statistical tools such as multivariate models, stratification, or linear regression models can be used to confirm the legitimacy of our analyses (47).

Our analyses are also limited in the low resolution for specific factors. A deeper dive into antidepressant use is restricted by the lack of information on drugs, dosage, and frequency of usage. Each of these factors may have a profound effect on the presence and types of microbiota found in each individual (15). Since the antidepressant metadata is confined to "Yes" and "No", only an examination into whether antidepressants are used can be done. We also understand that depression statuses (e.g. depression score) may have an effect on commensal microbiota, however, due to time constraints, we were not able to tie this additional factor into our analyses (48). There are limitations when looking just at age as well. Choosing to divide and analyse the age cohort into "Above" or "Below" may cause the analysis to miss finer details compared to if age was analysed using multiple age groups (1). Employing additional groups can also improve our ability to analyse early onset PD (below 50 years old). Our decision to work with age compared to age of onset may also change the interpretation of the microbial diversity within the cohort.

Conclusions Our study had several goals to determine the effect of antidepressant usage and age on the gut microbiota of PD patients. We aimed to determine if there is a relationship between age and antidepressant usage in terms of PD status, explore the effects of age and antidepressant usage on the gut microbiome, and conduct indicator taxa and differential abundance analysis to investigate specific taxonomic groups and their relation to PD symptoms. We established that there was no relationship between age and antidepressant usage and treated them as two independent variables. Furthermore, beta diversity analysis revealed that antidepressant use significantly lowered the abundance of gut microbes in PD patients and that patients above 60 years of age had a significantly higher phylogenetic distance of their gut microbes. Upon closer inspection of taxonomic groups within age cohorts, we found several indicator families where *Eubacterium coprostanoligenes*, *Oscillospiraceae*, *Butyricicoccaceae*, and *Coriobacteriaceae* had altered abundance based on past research. Two of these families in particular, *Eubacterium coprostanoligenes* and *Butyricicoccaceae*, were connected with common PD risk factors such as cholesterol

metabolism and gastrointestinal disorders, respectively. Differential abundance analysis also indicated that a reduction in *Prevotellaceae* could be an important indicator for PD development. Consistent to previous findings regarding antidepressant usage and age and their effects on the gut microbiota, our results are consistent in supporting that antidepressant usage and age are relevant factors in determining PD development.

Future Directions As discussed in our limitations section, there were other components of the metadata that were not accounted for in our study due to the reduction of sample size with the use of more filters. Because PD is a complex illness, there are a multitude of other factors that could have caused a change in gut microbial community leading to the condition. Sex was one of these factors since PD is more likely to manifest in males than females (46). In addition, there were many different consumables that were eaten that varied between participants. These confounding variables that were not addressed for this specific analysis could have a stronger influence in establishing a difference in diversity within the gut, relative to age or antidepressant usage.

For our project in particular, there were two main limitations that future studies could address. Although we measured antidepressant usage, we did not factor BDI depression score as a variable, but past research has shown that mood can often influence the gut microbiota. In addition, we separated the metadata based on age 60 as it is the reported average age in PD development according to Parkinson's Canada, however, it is important to acknowledge that PD can occur earlier than 50 y/o (known as Young Onset Parkinson's). For this reason, future research can consider splitting age into multiple cohorts to detect gradual shifts in gut microbial community structure. Alternatively, the recorded age of onset for PD can be a more reliable indicator for this analysis as we may be able to compare specific age ranges in the initial development of PD with similar ages in the control patients to observe shifts in gut microbiota at a defined time point for the disease.

Finally, further analyses could be conducted to determine the reason no significant difference was observed using the unweighted UniFrac metric for antidepressant usage, and no significant difference was observed using weighted UniFrac for age group. While the difference in abundance can be explained for antidepressant usage, it remains unclear why no effect is observed on the phylogenetic distance of gut microbes.

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CONTRIBUTIONS

Each individual on this team was an integral part of this study. The general contributions are as follows. All authors contributed to the background research, planning, and design of this study. M.Y. and J.Z. conducted the analyses in QIIME2, and M.Y. conducted the analyses in R. Abstract and introduction were written by P.T., methods by M.Y., results by M.H., M.Y., and J.Z., study limitations by M.H., future directions by M.Y., and acknowledgements and contributions were written by P.T. The discussion and conclusion portions were co-written by all four authors. All co-authors contributed to the editing of this manuscript.

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