Volume 8:1-13

UJEMI+

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Increased body mass is associated with decreased gut microbiome diversity in Parkinson's Disease patients

Josie Setiawan, Novia Chan, Clarisse Echavez, Angela Mathews

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Parkinson's Disease is a common neurodegenerative disorder that results in both motor and non-motor symptoms. The subtle nature of early Parkinson's Disease symptoms is often misinterpreted as normal signs of aging, and a lack of definitive tests to confirm Parkinson's Disease contribute to diagnosis delays. Because gastrointestinal symptoms are often observed years before motor symptoms, previous studies have focused on the relationship between changes in the gut microbiota composition and the pathophysiology of Parkinson's Disease. Previous findings have yielded conflicting results regarding effects of various lifestyle factors on gut microbiota of Parkinson's patients. To investigate contradictions observed from previous studies, our study aims to determine effects of body mass index (BMI) levels, alcohol consumption, and meat consumption on the gut microbiota of Parkinson's patients. Our results indicated that gut microbial diversity decreased in overweight and obese Parkinson's patients compared to healthy Parkinson's patients. There was no relationship between alcohol or meat consumption and gut microbiome composition of Parkinson's patients. The Sutterallaceae and Veillonellaceae taxonomic families have been previously associated with obesity and diseases that have implications to the gut microbiota. Unique family taxa analysis determined that the Sutterellaceae family was more abundant in Parkinson's Disease patients of the obese BMI grouping. Similarly, indicator taxa analysis determined two families were significantly associated to the obese BMI grouping, one of which being the Veillonellaceae family. These results indicate that changes in body mass may affect gut microbial composition and present relevant taxa to be further investigated for understanding the effect of body mass on Parkinson's Disease.

INTRODUCTION

Parkinson's Disease (PD) is a chronic, progressive neurodegenerative disease that affects both motor and non-motor features and affects more than 10 million people globally (1).

The motor and non-motor symptoms of PD are attributed to the loss of striatal dopaminergic neurons and non-dopaminergic neurons, respectively (1). The effect of PD on dopaminergic neurons in the brain leads to decreased dopamine levels and motor impairments such as tremors, rigidity, balance difficulties, and loss of spontaneous movement (2). PD is now considered a multi-systemic disease that affects both the central and peripheral nervous systems, resulting in several non-motor symptoms including gastroparesis and constipation (2). Early PD symptoms present in very subtle ways, and non-motor symptoms are often misinterpreted as normal signs of aging, thus delaying diagnosis (1). Since there are no definitive tests to confirm the presence of PD, current diagnoses require a review of the patient's history, assessment of symptoms, and the ruling out of alternative diagnoses (1).

Published Online: September 2022

Citation: Setiawan J, Chan N, Echavez C, Mathews A. 2022. Increased body mass is associated with decreased gut microbiome diversity in Parkinson's Disease patients. UJEMI+ 8:1-13

Editor: Gara Dexter, University of British Columbia

Copyright: © 2022 Undergraduate Journal of Experimental Microbiology and Immunology.

All Rights Reserved.

Address correspondence to: Josie Setiawan, josie.setiawan@gmail.com

The gastrointestinal symptoms of PD are often observed years before the onset of motor symptoms; thus, the relationship between changes in the gut microbiota composition and the pathophysiology of PD is an area that warrants further research (2, 3). The gut microbiota consists of microbes that contribute to host health and are involved in processes such as dietary fibre breakdown, vitamin production, regulation of metabolism, and modulation of neurological function (3). It has been shown that the relationship between microbial diversity and stool consistency appears to be modified by PD (3). Similarly, constipation and reduced bowel movement frequency are known risk factors of PD development (3). Therefore, exploring the connections between the microbiota and gut function in PD may provide a better understanding regarding the etiology and pathophysiology of the disease (3).

To provide insight into how the microbiota contributes to gastrointestinal disturbances observed in PD patients, Cirstea *et al*. assessed the associations between the microbiota composition, stool consistency, constipation, and systemic microbial metabolites in PD (3). The study was performed on 300 PD patients and controls who provided fecal samples for microbiota sequencing and serum for untargeted metabolomics (3). The study's dataset includes information on motor and nonmotor PD symptoms, medications, diet, and demographics (3). A study of this dataset by Dutra *et al*. investigated the effects of alcohol consumption and increasing body mass index (BMI) status on the gut microbiota of PD patients (4). They reported no correlation between alcohol consumption levels, gut microbiome composition, and PD progression, as well as no association between BMI, gut microbiome composition, and PD status (4).

However, several studies suggest a link between obesity and the human gut microbiome (5). Microbial diversity in the human gut has been confirmed to correlate with BMI levels, and it was found that the microbial communities in the gut differed significantly between obese or overweight, normal, and underweight individuals (5). Compositional changes in the gut microbiome have been reported to be linked to obesity in multiple studies, specifically for overweight and obese individuals with BMIs of 25.0-29.9 and 30+ respectively (6, 7).

Previous studies exploring the link between BMI and PD have yielded contradictory results. One study claimed that high BMI was a potential risk factor for PD (8). However, a separate study did not find any significant associations between BMI and the risk of developing PD (9). Another study that focused on BMI, diabetes, and PD first found that having diabetes was linked to a higher risk of PD development (10). As diabetes is frequently associated with higher BMI status, researchers assumed that this would also lead to the positive correlation of BMI and risk of PD (10). Contrary to this belief, however, researchers found that lower BMI was linked to a higher risk of PD (10). While there have been numerous studies on the relationship between BMI and PD, conflicting findings shed light on the need for further studies to be done to address the contrasting results.

Similar to BMI, past findings on the link between alcohol and meat consumption, the gut microbiome, and PD have yielded inconsistent findings. In contrast to Dutra *et al*., who reported no correlation between alcohol consumption and the gut microbiota, it has been suggested that alcohol intake can directly alter its composition. Lee *et al*. reported greater microbial diversity in a group that consumed alcohol compared to the control group (11). Regarding alcohol and PD, one study showed no link between alcohol consumption and the risk of developing PD (12), whereas another suggested that moderate alcohol consumption may protect against disease progression (13). In relation to meat consumption, a study comparing the gut microbial diversity of vegetarian versus non-vegetarian adults reported an increase in the ratio of highly bile-tolerant organisms such as *Bacteroidetes* and a decrease in the level of *Firmicutes* in non-vegetarians (14). Fernandez *et al*. demonstrated that frequent consumption of red meat has been linked to the incidence and progression of PD, as it is associated with the accumulation of α-synuclein in the enteric nervous system (15). Similarly, another study reported that the high iron content of heme-rich red meat may contribute to earlier progression of PD (16). However, a separate cohort study reported an inverse relationship between processed meat and sausage consumption and PD occurrence, suggesting that meat consumption may exert a potential neuroprotective effect on PD onset (17). Due to the conflicting results of previous findings, further studies are required to establish the link between alcohol and meat consumption and PD.

Based on literature that suggests that each of these three factors may alter the composition of the gut microbiota, we hypothesize that differences in gut microbial diversity will be observed between PD patients with differing BMIs, alcohol intake, and meat consumption in comparison to the non-PD control group. We aim to confirm previous findings and investigate the contradictions regarding each lifestyle factor, by analyzing the metadata from Cirstea et al. (3). Understanding connections between BMI, alcohol consumption, and meat consumption and PD will help to determine which of these lifestyle factors cause significant differences in the gut microbial communities of PD patients.

METHODS AND MATERIALS

Sampling and metadata processing. This study was conducted using the original dataset by Cirstea *et al*., containing 16S rRNA sequences from 197 adult individuals with PD and 103 controls of the same age range (40 to 77 years old) (3). Data was also collected on various factors such as medications, diet, and demographics (3), although the focus of this study was alcohol and meat consumption, and BMI in particular. Dietary information was collected using the EPIC-Norfolk Food Frequency Questionnaire (FFQ) (18) and processed by the FFQ EPIC Tool for Analysis (FETA) into unitary food values reported as average daily intake (19).

The 300 human participants provided fecal samples that underwent DNA extraction, amplification of the 16S rRNA gene, and Illumina sequencing to get raw sequence data (3). Cirstea *et al*. provided the demultiplexed raw sequence data, which was imported into the Quantitative Insights Into Microbial Ecology 2 (QIIME 2) next-generation bioinformatics platform (20). The sequences were quality controlled using the Divisive Amplicon Denoising Algorithm (DADA2) tool (21) to determine amplicon sequence variants (ASVs). Since all sequences were of reasonable to high quality, a truncation length of 251 nucleotides was selected, which was the maximum read length.

Metadata grouping and filtering. Before proceeding with further analysis, the original metadata was sorted and grouped for the purpose of this study. Samples that were missing data for our three selected lifestyle factors of interest were removed and subjects were grouped into categories based on predetermined numerical ranges for each lifestyle factor. For BMI, subjects were grouped into underweight, healthy, overweight and obese categories (TABLE S1) based on the Centers for Disease Control and Prevention (CDC) interpretations of BMI scores (22). For alcohol consumption, the World Health Organization (WHO) definition of a standard drink as 10 grams (23) was used to define low, moderate, and high groupings (TABLE S2). Finally, for meat consumption, National Health Service (NHS) recommendations as well as Canadian daily meat consumption statistics (24, 25) were considered for the grouping of subjects into low, moderate, and high consumption (TABLE S3).

The data was then filtered using QIIME 2 to produce separate tables containing either PD patients or control subjects only. Taxonomy-based filtering of mitochondria and chloroplast sequences was also performed to exclude archaea and eukarya domains.

Taxonomy and diversity metrics in QIIME 2. Alpha rarefaction curves were generated for each filtered table and a sampling depth of 10232 was chosen, as this captured the maximum number of features while retaining a reasonable number of samples in each category, to adequately represent sample richness (Figure S1). The underweight category was disregarded in further analyses as it contained too few samples for both PD and control.

Once rarefaction depth was defined, the same sampling depth was used to run core metrics. To calculate these core metrics, Multiple Alignment using Fast Fourier Transform (MAFFT) was used to align ASVs and relate features to one another (26). Representative sequences in the feature tables were also used to generate a tree and classify taxonomy to microbial organisms found in the samples via several QIIME 2 plugins (27-31). The resulting rooted phylogenetic tree and taxa barplots were used for further taxonomic analysis on RStudio. From these core metrics, alpha and beta diversity analyses were performed between categories of each lifestyle factor for both PD and control filtered datasets. For alpha

diversity, Faith's phylogenetic diversity (32) and Pielou's evenness (33) plots were generated. For beta diversity, Unweighted Unifrac (34) and Bray Curtis dissimilarity (35) plots were generated but were not included in this manuscript as they did not yield significant results. Data outputs from QIIME 2 were exported into RStudio for further visualization and analyses.

Visualization of QIIME2 outputs on R. tidyverse, qiime2R, and ggplot2 (36-38) R packages were used to import QIIME 2 data outputs and sorted metadata into R. According to the code in the Supplemental R Script, alpha diversity metrics plots of Faith's phylogenetic diversity (32) and Pielou's evenness (33) were re-generated on R to visualize microbial community richness and microbial species evenness within microbial communities of healthy, overweight, and obese BMI groupings in PD patients.

Unique taxa analysis on Excel. 90 taxonomic family groups in PD patients were identified from the QIIME 2 taxa bar plot. Samples were sorted according to healthy and obese BMI groupings. Within each of the healthy and obese BMI groupings, relative abundances for each taxonomic family were calculated. To identify the taxonomic families that are unique to the healthy BMI group, all families had a relative abundance of 0 in the obese BMI group were counted. For identifying taxonomic families that are unique to the obese BMI group, all families had a relative abundance of 0 in the healthy BMI group were counted. The numbers of taxonomic families unique to the healthy BMI group, unique to the obese BMI group, and present in both healthy and obese BMI groups were then used to generate a Venn diagram. The relative abundance percentages for the set of taxonomic families unique to the healthy BMI grouping and the set of taxonomic families unique to the obese BMI grouping were subsequently used to generate two separate pie charts.

Differential abundance analysis on R. According to the Supplemental R Script, differential abundance analysis on healthy and obese BMI groupings of PD patients was performed using R and the following R packages: tidyverse, qiime2R, ggplot2, vegan, ape, phyloseq, and DESeq2 (36-42). Following import of QIIME 2 outputs and sorted metadata, a phyloseq object was created and the dataset was rarefied based on sampling depth determined from the QIIME 2 alpha rarefaction curve. Relative abundances for each taxonomic family were calculated and only families that were more abundant than 0.05% were considered for analysis. The phyloseq object was converted into a DESeq object with the healthy BMI grouping set as a reference. Differentially abundant microbes were extracted from the DESeq object and an alpha significance level of 0.05 was then set to detect significantly abundant taxa among healthy and obese BMI groupings of PD patients.

Indicator taxa analysis on R. According to the Supplemental R Script, indicator taxa analysis on the healthy, overweight, and obese BMI groupings of PD patients was performed using R and the following R packages: tidyverse, qiime2R, ggplot, phyloseq, and indicspecies (36-38, 41, 43). QIIME 2 outputs and sorted metadata were imported into R to create a phyloseq object. A family taxonomy table was created and indicator values for each taxonomic family were calculated. Significant taxonomic family indicators of healthy, overweight, and obese BMI groupings of PD patients were generated as an output.

Script and sorted metadata availability. QIIME 2 data processing code can be found in the Supplemental QIIME 2 Script and R data analyses code can be found in the Supplemental R Script.

RESULTS

Greater BMI in PD patients significantly decreases gut microbial phylogenetic diversity but does not affect abundance of microbial species. Alpha diversity metrics on alcohol consumption, meat consumption, and BMI were determined for both PD and control individuals. Regarding alcohol consumption, the only significant difference in gut microbial community richness was between moderate and high groupings of control individuals for Faith's phylogenetic diversity ($q = 0.01$) (TABLE 1). There was no significant difference in gut microbial evenness between alcohol groupings for both PD and control individuals

Table 1. Faith's Phylogenetic Diversity between BMI groupings was the only significant alpha diversity metric in PD patients. Faith's phylogenetic diversity and Pielou's evenness were determined using R for both PD and control individuals. Both richness and evenness of gut microbial communities were analyzed for each grouping of BMI, alcohol consumption, and meat consumption. * = q < 0.05.

Group 1	Group 2	Faith's Phylogenetic Diversity		Pielou's Evenness	
		PD q-value	Control q-value	PD q-value	Control q-value
BMI					
Healthy	Obese	0.02*	0.62	0.72	0.84
	Overweight	0.02*	0.36	0.72	0.61
Obese	Overweight	0.32	0.62	0.72	0.61
Alcohol Consumption					
High	None	0.30	0.11	0.32	0.21
	Moderate	0.68	0.01*	0.75	0.31
No	Moderate	0.29	0.90	0.32	0.26
Meat Consumption					
High	Low	0.36	0.21	0.96	0.82
	Moderate	0.36	0.35	0.95	0.82
Low	Moderate	0.80	0.17	0.95	0.82

(TABLE 1). There was also no significant difference in phylogenetic diversity or evenness of gut microbial communities between meat consumption categories in both PD and control individuals (TABLE 1). R generated boxplots of Faith's phylogenetic diversity and Pielou's evenness revealed that as BMI increases, microbial phylogenetic diversity decreases, specifically with healthy PD individuals having significantly greater microbial community biodiversity compared to overweight or obese PD individuals (FIGURE 1A). This indicates that there are more diverse microorganisms in gut microbial community of healthy PD individuals compared to overweight and obese PD individuals. No significant differences were observed in microbial community evenness between BMI groupings for both PD and control individuals (TABLE 1, FIGURE 1B). Only PD patients of overweight or obese and healthy BMI groupings showed significant gut microbial phylogenetic diversity differences between each other (q = 0.02) (TABLE 1).

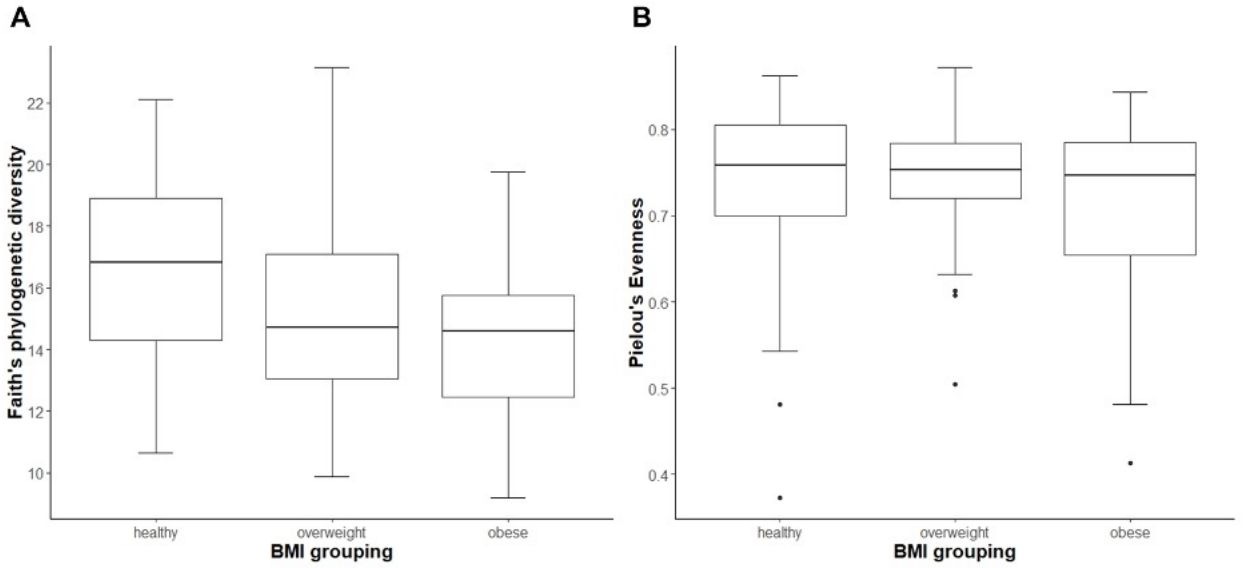

FIG. 1 Significant decrease in Faith's phylogenetic diversity and no significant differences in Pielou's evenness observed with increasing BMI in PD patients. (A) Alpha diversity analyses on microbial community richness (Faith's phylogenetic diversity) conducted on R across healthy, overweight, and obese BMI groupings in PD patients. Median displayed in the boxplot. Error bars indicate the 95% confidence intervals. (B) Alpha diversity analyses on microbial species evenness (Pielou's Evenness) conducted on R within microbial communities of healthy, overweight, and obese BMI groupings in PD patients. Median displayed in the boxplot. Error bars indicate the 95% confidence intervals.

Less unique microbial families identified in obese PD patients compared to healthy but only two differentially abundant families were observed. To conduct an analysis of the unique microbial taxa at the family level between healthy and obese PD patients, a Venn diagram was created using Excel based on the relative abundance of taxonomic families in healthy and obese BMI groups (FIGURE 2). 73 families of microbes were shared among PD patients with healthy and obese BMI. PD patients within the healthy BMI group were observed to have 13 unique family taxa, compared to PD patients within the obese BMI group, who were only found to have 4 unique families of microbes. For PD patients within the healthy BMI grouping, the most abundant families were Hafniaceae, Succinivibrionaceae, Brachyspiraceae, Campylobacteraceae, and Oscillospiraceae with less abundant families including Pseudomonadaceae, Leptotrichiaceae, Gemellaceae, Coriobacteriaceae, and Sporomusaceae. The most abundant family taxa among PD patients within the obese BMI grouping were Comamonadaceae, Heliobacteraceae, and Caulobacteraceae, with a prominent unclassified family being the least abundant of the four families within this group (FIGURE 2).

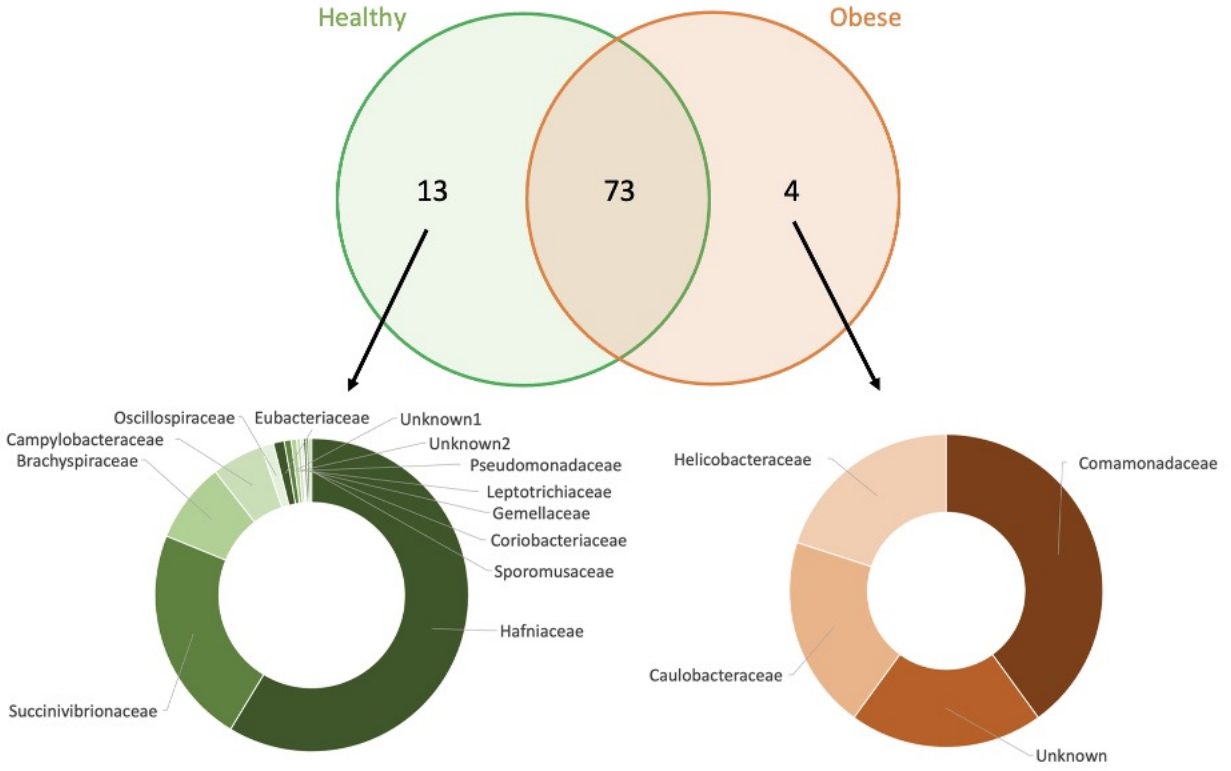

FIG. 2 Unique family taxa analysis on PD patients reveals 13 and 4 unique families of microbes with healthy and obese BMI groupings, respectively. Relative abundance of microbial families within each unique family is calculated using Excel and is depicted with respect to the total number of unique taxa in each group.

To analyze the abundance of family taxa between the healthy and obese BMI groupings, differential abundance analyses were performed on R (FIGURE S2). Two differentially abundant families were found. It was observed that the Sutterelllaceae family is twice as abundant in the obese BMI grouping relative to the healthy BMI grouping, represented by a $\log_2$ fold change of ~1. A $\log_2$ fold change approaching -2 was observed for the Clostridia family, indicating that this family is almost 4 times less abundant in the obese BMI grouping relative to the healthy BMI grouping (FIGURE S2).

11 and 2 significant microbial families indicated in the healthy and obese BMI groupings, respectively. To study the significant family taxonomies between the healthy overweight, and obese BMI groupings of indicator taxa analysis on PD patients of healthy, overweight, and obese BMI groupings was conducted using R. At the family level of taxonomy, 11 family taxa were revealed to be significantly associated with the healthy BMI

grouping on PD patients (TABLE 2). With the obese BMI grouping of PD patients, 2 unique taxa were revealed to be significantly associated: the Veillonellceae family and a family that has yet to be cultured. Studying the A values, the Victivallaceae family has been observed to be the most unique to the healthy BMI grouping of PD patients, while the uncultured family is most unique to the obese BMI grouping. Reviewing the B values, the Christensenellaceae family is the most abundant in the healthy BMI grouping, while the Veillonellaceae family is most abundant in the obese BMI grouping. As the stat values take uniqueness and abundance into account, the Clostridia family is the most significantly associated with the healthy BMI grouping of PD patients, while the Veillonellaceae family is found to be more significantly associated to the obese BMI group of PD patients than the uncultured family (TABLE 2).

Table 2. Indicator family taxa analysis on PD patients of healthy, overweight, and obese BMI groupings reveals 2 significantly associated families of the obese BMI grouping. Indicator taxa analysis was conducted on the family taxonomy level using R. A indicates how unique the family is to the BMI grouping. B indicates how abundant the family is within the microbial community of the BMI grouping. The stat value indicates how associated the family is to the BMI grouping, ** = $p < 0.01$, * = $p < 0.05$.

BMI grouping	Species (According to family taxonomy)	A	B	stat	p-value
Healthy	Clostridia_UCG-014	0.6220	0.8429	0.724	0.005**
	Christensenellaceae	0.5154	0.9857	0.713	0.005**
	Clostridia_vadinBB60_group	0.5448	0.8429	0.678	0.005**
	UCG-010	0.5729	0.7571	0.659	0.005**
	Anaerovoracaceae	0.4702	0.9143	0.656	0.025*
	Oscillospiraceae	0.4049	1.000	0.636	0.015*
	RF39	0.5187	0.6143	0.564	0.005**
	Victivallaceae	0.6410	0.4429	0.533	0.005**
	Izemoplasmatales	0.5226	0.5000	0.511	0.025*
	NA	0.5001	0.5000	0.500	0.040*
	DTU014	0.5455	0.2571	0.375	0.045*
Obese	Veillonellaceae	0.4947	0.8286	0.640	0.015*
	uncultured	0.8266	0.1143	0.307	0.015*

DISCUSSION

Microbial diversity with alcohol and meat consumption. With previous studies revealing conflicting findings relating to the association between gut microbial diversity and alcohol and meat consumption (4, 11, 13, 15, 17) our study aimed to investigate these factors to see if we could confirm findings from literature.

A previous UJEMI study found that there was no difference in the gut microbiome composition between PD patients with varying alcohol consumption levels (4), however, it has been suggested that alcohol consumption can directly alter microbial composition (11). Our findings confirmed that differences in microbial diversity between the defined alcohol consumption groupings were not significant in PD patients, using both Faith's phylogenetic diversity and Pielou's evenness alpha diversity metrics (TABLE 1). Interestingly, there were significant differences found in control subjects between high and moderate alcohol consumption groupings with Faith's phylogenetic diversity (TABLE 1). One study found that moderate alcohol consumption had a protective effect against mortality, compared to those who never drank alcohol as well as those who consumed above the median number of drinks per day (13). This effect appeared to be more prominent in the control subjects relative to PD subjects (13). This suggests that moderate alcohol consumption could be a determining factor for differences in microbial composition in individuals without PD. However, since significant differences were only observed in control subjects between "high" and "moderate" but not "moderate" and "none" (TABLE 1), it is difficult to make definitive claims regarding alcohol consumption and its effect on the gut microbiome in PD patients.

For meat consumption, one study suggested a link between meat consumption and PD progression (15), while another suggested that meat has a neuroprotective effect on PD onset (17). Despite these previous findings, our study observed no significant differences in microbial diversity between "low", "moderate", and "high" consumption categories in both PD and control subjects, using the two alpha diversity metrics generated (TABLE 1). This

may be due to the types of meat that these studies looked at, as most investigated red meat or processed meat. In addition, the definitions for meat consumption that we used to group subjects into categories may have also influenced the results obtained and findings may differ with other studies that used different criteria. Since no significant microbial differences were found in PD patients between the various alcohol and meat consumption categories, further taxonomic analysis was not performed on these categories.

Relationship between BMI, microbial diversity, and PD. Although we hypothesized that differences in gut microbial diversity would be observed in PD patients between categories for all three lifestyle factors, BMI was the only one that showed significant differences in PD patients. Specifically, there were significant differences in microbial diversity between "healthy" and "overweight" as well as "healthy" and "obese" categories using Faith's phylogenetic diversity (TABLE 1). Microbial diversity also appeared to decrease with increased BMI, with the "obese" category showing the least diversity (FIGURE1A). These results align with several studies in literature that found decreased microbial diversity related to obesity and diabetes (44, 45). However, a previous UJEMI study found no statistically significant differences in gut microbiome composition between BMI groupings in PD patients (4). Dutra *et al.*'s study used the same criteria for grouping subjects into BMI categories except analyzed the relationship between BMI and gut microbiome composition using beta diversity analyses (4). Our study focused primarily on alpha diversity analyses, which is what led us to find significant differences in microbial diversity between BMI categories.

Many studies have examined the relationship between the gut microbiota and obesity, but few studies have investigated the role of BMI specifically in PD patients (8, 44-47). Our study found that the control group did not show a significant decrease in microbial diversity in the "overweight" and "obese" categories, whereas the PD group did (TABLE 1). This aligns with a study indicating that high-fat diet-induced obesity can contribute to PD progression and influence the gut microbiota (46). Another study highlighted that although obesity may not play a role in PD pathogenesis, it may be associated with a higher risk of developing PD (9). These findings, in combination with our results, suggest that PD patients possess significantly different gut microbiome compositions compared to the controls and that the interplay between PD and obesity may be a driving factor for this altered microbiota. Despite the evidence pointing towards obesity contributing to PD through decreased microbial diversity, it is important to distinguish causation versus correlation. It is yet unclear whether PD is a consequence of obesity or vice versa, as opposed to a correlation between the two. Several studies have investigated the causal role of microbiome alterations in the development of obesity as well as PD (8, 44, 47) but the relationship between all three is still under further investigation.

While there were significant differences in Faith's phylogenetic diversity between BMI categories in PD patients, there were no significant differences observed for Pielou's evenness (TABLE 1, FIGURE 1B). Faith's phylogenetic diversity measures community richness while incorporating phylogenetic relationships whereas Pielou's evenness considers the uniformity of each species in the microbial community, indicating if species in a sample have the same abundance (48, 49). The absence of significant differences in microbial evenness observed between BMI categories (FIGURE 1B) suggests that diversity is likely driven by phylogenetic distance. Therefore, investigating specific taxonomic and phylogenetic differences in gut microbial composition between BMI categories in PD patients may give further insight into the role that obesity plays in PD onset.

Taxonomic differences in PD patients between healthy and obese BMI groupings. Following unique taxa analysis, our findings identified 13 family gut taxa unique to the healthy BMI grouping and 4 family taxa unique to the obese BMI grouping of PD patients (FIGURE 2). Of the families unique to the obese BMI grouping of PD patients, there are the Comamonadaceae, Caulobacteraceae, and Helicobacteraceae families. In a study on relative bacterial abundances in saliva samples, Comamonadaceae was found to have a greater relative abundance in saliva samples of the obesity group (50), as has been observed in our findings with the lack of Comamonadaceae in the healthy BMI grouping and the presence of Comamonadaceae in the obese BMI grouping. Comamonadaceae abundance has been found to be significantly reduced in PD patients (51), but this is contrary to our observations in PD

patients of the obese BMI grouping. This might suggest that low Comamonadaceae abundance may only be associated with PD patient groupings of lower BMIs. Caulobacteraceae abundance has been associated with increased food intake and decreased satiety which are both factors that have been related to obesity (52). This correlates with our observations of Caulobacteraceae being unique to the obese BMI grouping. Caulobacteraceae has no apparent associations with PD. As unique taxa analysis only considers relative abundance of taxonomic families present in samples, the absence of Caulobacteraceae and PD relationship in current literature may suggest that our findings of Caulobacteraceae in the obese BMI group to be insignificant. As a member of the Helicobacteraceae family, *Helicobacter pylori* gut infections have been observed to be positively correlated with obesity (53). *H. pylori* is reported to have an effect on the development of obesity and obesity is reported to have an influence on the risk of *H. pylori* infections (53). Our findings of Helicobacteraceae being unique to the obese BMI grouping coincides with this frequently observed relationship between *H. pylori* infections and obesity. *H. pylori* infections have also been reported in several studies to be associated with increased risk of PD and to further contribute to PD pathogenesis (53). These results strengthen the reliability of our finding of Helicobacteraceae presence in the obese BMI group of PD patients, and suggests that the impact Helicobacteraceae has on increasing BMI and PD risk may be significant.

Through conducting differential abundance analysis, the $\log_2$ fold change in abundance of 1 indicates a two times greater abundance of Sutterellaceae in PD patients of the obese than the healthy BMI groupings (FIGURE S2). This increase is fairly low, which may suggest that Sutterellaceae is not significantly associated with PD. Sutterellaceae abundance has been noticed to be lower in multiple sclerosis patients, however, no link has been observed to PD patients (53), which may align to the faint differential abundance results observed in our study.

To determine significant taxonomic families associated with healthy and obese BMI groupings, indicator family taxa analysis was conducted. Based on the high value of uniqueness to and abundance within the obese BMI grouping, Veillonellaceae has been indicated to be the most significantly associated family to the obese BMI grouping of PD patients (TABLE 2). Veillonellaceae has previously been observed to be significantly abundant in a obesity group in contrast to a control group (54), which potentially relates to the significant association between Veillonellaceae and the obese BMI grouping observed in our study. Higher Veillonellaceae abundance has been implicated in PD (55-57) which further suggests that Veillonellaceae presence and abundance in the obese BMI grouping is positively correlated with PD.

Limitations This was a study based on retrospective data, which limits our access to patient data across various stages of PD. In addition, we had a limited sample size of 197 patients with PD, which was reduced to 183 after removing subjects lacking data. To adequately represent sample richness, another 49 subjects were discarded due to the chosen sampling depth of 10232. Therefore, our results may not be representative of all PD patients. Including a larger number of patients could provide a more robust verification of our results, or perhaps reveal a greater role of alcohol and/or meat consumption in the gut microbial diversity of PD patients. There is also the limitation in that there was an unequal number of subjects in the PD versus Control groups, as well as the number of patients within the healthy, obese, and overweight BMI groupings, which limits our ability to make an accurate comparison between these groups. Additionally, we acknowledge that the reliance on BMI does not take into account muscle mass, body composition, as well as sex or race-based differences; thus, incorporating additional metrics such as the waist-to-height ratio may prove helpful in estimating patients' body fat for future prospective studies.

Conclusions The aim of this study was to determine the effects of BMI, alcohol consumption, and meat consumption on gut microbial diversity in PD patients. We hypothesized that differences would be observed between different categories for each of the three chosen lifestyle factors, but significant results were only found between BMI groupings in PD patients. In particular, BMI showed significant alpha diversity results in microbial phylogenetic diversity between healthy and obese, and healthy and overweight BMI

groupings of PD patients, but not in microbial community evenness, indicating that the decreased microbial diversity observed with increased BMI is driven by phylogenetic distance and taxonomic differences. We found that alcohol and meat consumption did not have significant differences in microbial phylogenetic diversity or evenness for patients with PD, and that microbial diversity and BMI are inversely linked. It was also found that the Veillonellaceae family was most significantly associated with PD patients within the obese BMI grouping. These findings highlight the effect that body mass has on gut microbial composition and its significance in Parkinson's Disease. The decreased microbial diversity in overweight and obese PD patients and the specific taxonomic families found to be associated with these patients may offer further insight into the connection between obesity, disease, and the gut microbiome.

Future Directions To further explore the role of BMI on both the gut microbiome of PD patients and age of disease onset, future studies could study the differences in microbial diversity of PD patients with differing BMI and compare the age of onset of PD across patients in different BMI groups. Additionally, to account for sex- or race-based differences contributing to BMI status, future studies should stratify patients according to these factors when conducting analyses of microbial diversity and age of disease onset for PD patients. By accomplishing this analysis, results could potentially demonstrate any existing effects of BMI-related microbial differences on PD onset.

Additional studies could also explore the clinical and therapeutic applications of this research by using fecal analysis to detect PD biomarkers in patients with varying stages of PD. The application of fecal analysis in the early detection of PD would provide a non-invasive method of assessing pathogenic microbial families, associated gut dysbiosis and interactions between the gut microbiome and the immune system contributing to the onset of PD. Patient fecal microbial diversity analysis profiles can be compared to the microbial families most significantly associated with PD from this study to provide insight regarding the application of fecal analysis as a diagnostic indicator of PD. Early diagnosis of PD can enable better and more effective treatment through early intervention to reduce disease progression and limit long-term effects on patient quality of life. Generation of a comprehensive biomarker atlas of PD is therefore critical for early intervention and to further understand the complexities of this devastating illness.

For Faith's Phylogenetic Diversity, we did not compare the PD group to the control group within the same BMI ratings due to lack of time and resources. It would be interesting to see if the effects caused by body mass on gut microbiome composition are also shown in the non-PD population. Future studies could also examine the involvement of specific microbial families such as Helicobacteraceae and Veillonellaceae, in the onset of PD. This could be accomplished by quantifying the abundance of these particular families across PD patients in this dataset and other publicly available datasets, according to age of PD onset. With more insight on these particular microbial families, analysis of gut microbiomes can be directed at these microbes and allow for earlier PD diagnosis and further understanding of a patient's PD progression. These studies could also help inform the development of therapeutic probiotics and fecal transplantation to alter the gut microbiome of PD patients as part of early intervention.

ACKNOWLEDGEMENTS

This study would not be possible without the funding, space, and resources provided by the University of British Columbia and Department of Microbiology and Immunology. We are grateful for the invaluable support and guidance the MICB 447 teaching team has given us. We would like to thank Dr. Evelyn Sun and Zakhar Krekhno for teaching this course and providing us with the knowledge required to carry out this project. We would also like to thank our teaching assistants Divya Kriti and Emily Adamczyk for their guidance in tutorials and support with data analysis. Special recognition goes to Dr. Evelyn Sun and Divya who partook in weekly meetings with us to help guide the development of our project. They provided support with analysis troubleshooting, manuscript revisions, and editing, which we are extremely grateful for. Their resourceful insight, encouragement, and feedback

throughout the course were much appreciated. We would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

CONTRIBUTIONS

All individuals of the team were integral to the completion of this draft manuscript and participated in the planning, research, and analysis for this project. All members contributed to data analysis in QIIME 2 while N.C. was responsible for data analysis in R, including differential abundance and indicator taxa analyses. J.S. was responsible for tabulating QIIME 2 results and making the Venn Diagram. The abstract and introduction was written by C.E and materials and methods by J.S. and N.C. Results and discussion sections were divided equally between all team members. A.M. was responsible for the study limitations, conclusion, and future directions. All members were involved in the editing of this manuscript. Co-authorship should be considered equal for all 4 authors.

REFERENCES

1. **DeMaagd G, Philip A.** 2015. Parkinson's Disease and Its Management. *P T* **40**:504–532.
2. **Romano S, Savva GM, Bedarf JR, Charles IG, Hildebrand F, Narbad A.** 2021. Meta-analysis of the gut microbiome of parkinson's disease patients suggests alterations linked to intestinal inflammation. *npj Parkinsons Dis* **7**:1–13.
3. **Cirstea MS, Yu AC, Golz E, Sundvick K, Kliger D, Radisavljevic N, Foulger LH, Mackenzie M, Huan T, Finlay BB, Appel-Cresswell S**. 2020. Microbiota composition and metabolism are associated with gut function in parkinson's disease. *Mov Disord* **35**:1208–1217.
4. **Dutra J, Fung M, Ling M, Zhi RL**. 2021. The effects of alcohol consumption and increased body mass on the gut microbiota of Parkinson's Disease patients. *UJEMI* **26**.
5. **Brandwein M, Katz I, Katz A, Kohen R**. 2019. Beyond the gut: Skin microbiome compositional changes are associated with BMI. *Human Microbiome Journal* **13**:100063.
6. **Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI**. 2005. Obesity alters gut microbial ecology. *PNAS* **102**:11070–11075.
7. **Ley RE, Turnbaugh PJ, Klein S, Gordon JI**. 2006. Human gut microbes associated with obesity. *Nature* **444**:1022–1023.
8. **Chen J, Guan Z, Wang L, Song G, Ma B, Wang Y.** 2014. Meta-Analysis: Overweight, Obesity, and Parkinson's Disease. *Int J Endocrinol* **2014**.
9. **Chen H, Zhang SM, Schwarzschild MA, Hernán MA, Willett WC, Ascherio A.** 2004. Obesity and the Risk of Parkinson's Disease. *American Journal of Epidemiology* **159**:547–555.
10. **Jeong S-M, Han K, Kim D, Rhee SY, Jang W, Shin DW**. 2020. Body Mass Index, Diabetes, and the Risk of Parkinson's Disease. *Mov Disord* **35**:236–244.
11. **Lee E, Lee J-E**. 2021. Impact of drinking alcohol on gut microbiota: Recent perspectives on ethanol and alcoholic beverage. *Curr Opin Food Sci* **37**:91–97.
12. **Peters S, Gallo V, Vineis P, Middleton LT, Forsgren L, Sacerdote C, Sieri S, Kyrozis A, Chirlaque MD, Zamora-Ros R, Hansson O, Petersson J, Katzke V, Kühn T, Mokoroa O, Masala G, Ardanaz E, Panico S, Bergmann MM, Key TJ, Weiderpass E, Ferrari P, Vermeulen R**. 2020. Alcohol consumption and risk of parkinson's disease: Data from a large prospective European cohort. *Mov Disord* **35**:1258–1263.
13. **Paul KC, Chuang YH, Shih IF, Keener A, Bordelon Y, Bronstein JM, Ritz B**. 2019. The association between lifestyle factors and parkinson's disease progression and mortality. *Mov Disord* **34**:58–66.
14. **Bamola VD, Ghosh A, Kapardar RK, Lal B, Cheema S, Sarma P, Chaudhry R**. 2017. Gut microbial diversity in health and disease: Experience of healthy Indian subjects, and colon carcinoma and inflammatory bowel disease patients. *Microb Ecol Health Dis* **28**:1322447.
15. **Fernandez RD, Campos JS dos P, Santos T de OCG.** 2021. Nutritional status and food consumption of patients with Parkinson disease. *Arq Neuropsiquiatr* **79**:676–681.
16. **Mischley LK, Lau RC, Bennett RD**. 2017. Role of diet and nutritional supplements in parkinson's disease progression. *Oxid Med Cell Longev* **2017**:1–9.
17. **Sääksjärvi K, Knekt P, Lundqvist A, Männistö S, Heliövaara M, Rissanen H, Järvinen R**. 2012. A cohort study on diet and the risk of parkinson's disease: The role of food groups and Diet Quality. *Br J Nutr* **109**:329–337.
18. **Mulligan AA, Luben RN, Bhaniani A, Parry-Smith DJ, O'Connor L, Khawaja AP, Forouhi NG, Khaw K-T.** 2014. A new tool for converting food frequency questionnaire data into nutrient and food group values: Feta research methods and availability. *BMJ Open* **4.**
19. **Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R, Oakes S, Khaw KT, Wareham N, Day NE**. 2001. Nutritional methods in the European Prospective Investigation of cancer in Norfolk. *Public Health Nutrition* **4**:847–858.
20. **Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann**

B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MG, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CH, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**:852–857.

21. **Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP.** 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**:581-583.
22. **Centers for Disease Control and Prevention.** 2021. Healthy Weight, Nutrition, and Pysical Activity: About Adult BMI
23. **Kalinowski A, Humphreys K**. 2016. Governmental standard drink definitions and low-risk alcohol consumption guidelines in 37 countries. *Addiction* **111**:1293–1298.
24. **National Health Service.** 2021. Food types: Meat in your diet.
25. **Canada Beef.** 2022. Understanding Beef: Health & Nutrition.
26. **Katoh K, Misawa K, Kuma K, Miyata T.** 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res*. **30**:3059-3066.
27. **Rognes T, Flouri T, Nichols B, Quince C, Mahé F.** 2016. VSEARCH: a versatile open source tool for metagenomics. *Peer* **4**:e2584.
28. **Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J.** 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**:90.
29. **Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL.** 2009. Blast+: architecture and applications. *BMC bioinformatics* **10**(1):421.
30. **Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D, Brucher M, Perrot M, Duchesnay E.** 2011. Scikit-learn: machine learning in python. *Journal of machine learning research* **12**(Oct):2825-2830.
31. **Price MN, Dehal PS, Arkin AP.** 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS ONE* **5**:e9490.
32. **Faith DP**. 1992. Conservation evaluation and phylogenetic diversity. *Biol Cons* **61**:1-10.
33. **Pielou E**. 1966. The measurement of diversity in different types of biological collections. *J. Theor. Biol.* **13**:131-144.
34. **Lozupone C, Knight R**. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol.* **71**:8228-8235.
35. **Sorenson, T.** 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content. *Kongelige Danske Videnskabernes Selskab* **5.1-34**:4-7.
36. **Wickham H, Averick M, Bryan J, Chang W, D'Agostino McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Lin Pedersen T, Miller E, Milton Bache S, Müller K, Ooms J, Robinson D, Paige Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H.** 2019. Welcome to the Tidyverse. *J Open Source Softw* **4**:1686.
37. **Bisanz JE.** 2018. qiime2R: Importing QIIME2 artifacts and associated data into R sessions. R Package version 0.99. https://github.com/jbisanz/qiime2R.
38. **Wickham H.** 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.
39. **Jari Oksanen F, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos PM, Stevens HH, Szoecs E, Wagner H.** 2019. vegan:Community Ecology Package. R Package version 2.5-6. https://CRAN.R-project.org/package=vegan. {Code and/or software.}
40. **Paradis E, Schliep K.** 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**:526-528.
41. **McMurdie PJ, Holmes S.** 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**:e61217.
42. **Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:550.
43. **De Caceres M, Legendre P.** 2009. Associations between species and groups of sites: indices and statistical inference. http://sites.google.com/site/miqueldecaceres/.
44. **Bäckhed F**. 2009. Changes in intestinal microflora in obesity: Cause or consequence? *Journal of Pediatric Gastroenterology & Nutrition* **48**.
45. **Barlow GM, Yu A, Mathur R**. 2015. Role of the gut microbiome in obesity and diabetes mellitus. *Nutrition in Clinical Practice* **30**:787–797.
46. **Fang X**. 2015. Potential role of gut microbiota and tissue barriers in parkinson's disease and amyotrophic lateral sclerosis. *International Journal of Neuroscience* **126**:771–776.

47. **Zhao L**. 2013. The gut microbiota and obesity: From correlation to causality. *Nature Reviews Microbiology* **11**:639–647.
48. **Chao A, Chiu CH, Hsieh TC, Davis T, Nipperess DA, Faith DP**. 2014. Rarefaction and extrapolation of phylogenetic diversity. *Methods in Ecology and Evolution* **6**:380–388.
49. **Wu T, Shen M, Yu Q, Chen Y, Chen X, Yang J, Huang L, Guo X, Xie J**. 2021. Cyclocarya Paliurus polysaccharide improves metabolic function of gut microbiota by regulating short-chain fatty acids and gut microbiota composition. *Food Research International* **141**:110119.
50. **Vascellari S, Palmas V, Melis M, Pisanu S, Cusano R, Uva P, Perra D, Madau V, Sarchioto M, Oppo V, Simola N, Morelli M, Santoru ML, Atzori L, Melis M, Cossu G, Manzin A.** Gut Microbiota and Metabolome Alterations Associated with Parkinson's Disease. *mSystems* **5**:e00561-20.
51. **Wu Y, Chi X, Zhang Q, Chen F, Deng X.** 2018. Characterization of the salivary microbiome in people with obesity. *PeerJ* **6**:e4458.
52. **Baradaran A, Dehghanbanadaki H, Naderpour S, Pirkashani LM, Rajabi A, Rashti R, Riahifar S, Moradi Y.** 2021. The association between Helicobacter pylori and obesity: a systematic review and meta-analysis of case–control studies. *Clinical Diabetes and Endocrinology* **7**:15.
53. **Shen X, Yang H, Wu Y, Zhang D, Jiang H.** 2017. Meta-analysis: Association of Helicobacter pylori infection with Parkinson's diseases. *Helicobacter* **22**:e12398.
54. **Gerhardt S, Mohajeri MH.** 2018. Changes of Colonic Bacterial Composition in Parkinson's Disease and Other Neurodegenerative Diseases. *Nutrients* **10**:708.
55. **Duan M, Wang Y, Zhang Q, Zou R, Guo M, Zheng H.** 2021. Characteristics of gut microbiota in people with obesity. *PLoS One* **16**:e0255446.
56. **Vascellari S, Palmas V, Melis M, Pisanu S, Cusano R, Uva P, Perra D, Madau V, Sarchioto M, Oppo V, Simola N, Morelli M, Santoru ML, Atzori L, Melis M, Cossu G, Manzin A.** Gut Microbiota and Metabolome Alterations Associated with Parkinson's Disease. *mSystems* **5**:e00561-20.
57. **Pietrucci D, Teofani A, Unida V, Cerroni R, Biocca S, Stefani A, Desideri A.** 2020. Can Gut Microbiota Be a Good Predictor for Parkinson's Disease? A Machine Learning Approach. *Brain Sci* **10**:242.