Volume 9:1-14

UJEMI+

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

High-fish intake is associated with later-onset Parkinson's disease and enrichment of short-chain fatty acid-producing microorganisms

Ishana Lodhia[1], Lochlan Breckenridge[1,2], Fares Burwag[1], Renee Lim[1]

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada
[2]Department of Earth, Ocean, and Atmospheric Sciences, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Epidemiological studies suggest that dietary habits can modulate the pathogenesis of Parkinson's disease. As an important source of omega-3 fatty acids, consumption of fish promotes brain health, potentially mediated in part by the microbiome. In this study, we investigated the influence of a high-fish diet on Parkinson's disease onset and whether this is associated with enrichment of microbial taxa that maintain a healthy gut. We hypothesized that later-onset Parkinson's disease is correlated with individuals consuming high dietary fish, and that the putative effect is mediated through enrichment of short-chain fatty acid-producing microorganisms. We used dietary information and taxonomic data obtained from fecal samples of 197 Parkinson's disease patients surveyed by Cirstea *et al*. We observed a significant correlation between high-fish intake and later-onset Parkinson's disease in males. Although we did not find global perturbations in gut microbiota composition due to fish intake and age of onset, short-chain fatty acid-producing microbes, including *Intestinimonas* and *Lachnospiraceae*, were enriched in high-fish-consuming male Parkinson's disease patients relative to low-fish. Further, these microorganisms were identified as indicator species for high-fish intake and later-onset Parkinson's disease. Additionally, we determined that high-fish diets may be associated with short-chain fatty acid metabolic pathways, namely acetate and acetyl-CoA synthesis. Together, our findings suggest that a high-fish diet selects for enrichment of short chain fatty acid-producing microorganisms that may contribute to delaying onset of Parkinson's disease in males by maintaining gut health.

INTRODUCTION

In North America, Parkinson's disease (PD) is recognized as the second most prevalent age-related neurodegenerative illness diagnosed following Alzheimer's disease (1). Although preventative measures to slow disease progression remain largely unknown, several epidemiological studies have established that environmental risk factors have a greater influence over genetic risk factors (2). PD risk is age-related, but age of onset can vary considerably; 4% of PD patients in North America are diagnosed before 50, which means that there are likely environmental and lifestyle factors that influence when disease occurs. Specifically, a spotlight on dietary habits has revealed its dual disease-promoting and therapeutic benefits on the development of PD (2). The Western diet (WD), characterized by

Published Online: September 2023

Citation: Lodhia, Breckenridge, Burwag, Lim. 2023. High-fish intake is associated with later-onset Parkinson's disease and enrichment of short-chain fatty acid-producing microorganisms. UJEMI+ 9:1-14

Editor: Lauren Pugsley, University of British Columbia

Address correspondence to: https://jemi.microbiology.ubc.ca/

processed foods, high salt intake, refined sugar, and omega-6 fatty acids is one of the greatest risk factors for neurodegenerative diseases (2). Conversely, diet can be tuned to confer protective benefits against neurodegenerative diseases as has been observed in PD patients surveyed following the Mediterranean diet (MeDi). Unlike the WD, the MeDi focuses on high daily fiber intake (>25-30 g/day as opposed to <10-15 g/day) and consists of fresh fruits and vegetables, nuts, grains, fish, and no red meat consumption (2).

Epidemiological studies have identified a correlation between the MeDi and reduced PD risk, as well as a later-onset of disease by up to 8.4 years in men with no effect in women (2,3). The focus on fish in the MeDi is of particular interest as it is a source of omega-3 (ω3) polyunsaturated fatty acids (PUFAs), which promote brain health (e.g., synaptic plasticity and reducing inflammation) and is therefore relevant to our study on PD (2,4). In the limited number of studies conducted on gut microbiota alterations with ω3 supplementation, overall, *Faecalibacterium* decreases while *Bacteroidetes* and *Lachnospiraceae* increase (5). The general consensus is that ω3 PUFAs promote gut health by shifting the microbiota composition towards selection of short-chain fatty acid (SCFA) producers (5,6). Of note, the SCFA butyrate maintains proper function of the colonic mucosa and the intestinal barrier (6). The MeDi diet is associated with an increase in the microbial production of SCFAs, while the PD-associated microbiome is characterized by a significant reduction of SCFA-producing bacteria and an increase in gram-negative bacteria, which instead feed on protein and promote neuroinflammation and neurodegeneration (2). However, it is not yet clear how increased fish intake affects the gut microbiome composition, and whether these changes to the gut microbiome may also reduce PD risk.

A study by Cirstea *et al*. (7) investigated the relationship between microbiota composition and gut dysbiosis in PD by assessing the taxonomic differences between PD patients (n=197) and healthy individuals (n=103). Metadata collected on dietary information for the 300 participants includes intake of fish. We used taxonomic data collected from fecal samples of PD patients surveyed in Cirstea *et al.* (7) in addition to the dietary information obtained using the EPIC-Norfolk Food Frequency Questionnaire (8) to ascertain if delayed onset of PD is correlated with a high-fish diet, and to determine which gut microbes are differentially abundant with fish intake and PD onset. Our findings correlated high-fish consumption with increased incidence of later-onset PD in male participants only, which is consistent with the sex-stratified diet differences reported by Metcalfe-Roach *et al.* (3). Although we observed no global changes in alpha and beta diversity, SCFA-producing microbes, namely *Intestinimonas* and *Lachnospiraceae*, were differentially abundant in participants consuming a high-fish diet and are an indicator species of high-fish intake and later-onset PD in males. Our findings support the potential for a high-fish diet to promote the enrichment of gut microbes that may elicit protection against the pathogenesis of Parkinson's disease.

METHODS AND MATERIALS

Dataset collection. The dataset utilized throughout this project was generated from the cross-sectional cohort study conducted by Cirstea *et al.* (7) at the University of British Columbia (UBC). Fecal samples from 300 participants, 197 of which were PD patients from the Pacific Parkinson's Research Centre at UBC, were collected using the OMNIgeneGUT Kit and sequenced to analyze microbiota composition. Cirstea *et al.* (7) used barcoded 515F/806R primers (GTGCCAGCMGCCGCGGTAA/GGACTACHVHHHTWTCTAAT) to amplify the V4 region of the 16s rRNA gene which was followed by sequencing on the Illumina MiSeq platform. Additionally, metadata on dietary habits was collected according to the EPIC-Norfolk Food Frequency Questionnaire.

Kaplan-Meier survival analysis. A sex-stratified survival analysis comparing fish intake with PD age of onset using the R (v4.2.2; 9) packages survminer (v0.4.9; 10) and survival (v3.5-5; 11) provided the proof-of-concept for our research project. Total fish consumption (including both men and women) followed a right-skewed distribution (Fig S1); therefore, we used the median to define our high/low threshold as 27.2 g of fish per day.

Metadata manipulation. A new metadata column ("fish_age_status") indicating high- and low-fish intake and early- and later-onset PD was created for downstream analyses using the Tidyverse (v1.3.2; 12) package in R. We generated four categories: "High Fish, Later-Onset (n = 31)", "High Fish, Early-Onset (n = 18)", "Low Fish, Later-Onset (n = 26)", and "Low Fish, Early-Onset (n = 36)." The high- and low-fish threshold was defined as 27.2 g of daily fish intake as per our population median. Age of onset was relatively normally distributed; therefore, we used the mean of our study set to define the early/later PD onset threshold as 59 years of age (Fig S2). Since our survival analysis indicated a stronger relationship between fish intake and age of onset in the male cohort, we filtered the metadata to only include male participants.

QIIME2 pipeline. 16S rRNA reads from the dataset by Cirstea *et al.* (7) were imported into Quantitative Insights Into Microbial Ecology 2 (QIIME2) (v2021.11; 13) and demultiplexed using the manifest file. Denoising to correct for sequencing errors and clustering into amplicon sequencing variants (ASVs) were performed using the Divisive Amplicon Denoising Algorithm 2 (DADA2) (14). Since the mean Phred quality scores for all base positions were above 30, the reads were not trimmed and retained at a length of 251 nucleotides. The ASV feature table generated underwent taxonomy-based filtering to remove mitochondrial and chloroplast sequences, which was followed by frequency-based filtering to eliminate rare ASVs (<0.005% of total reads) not considered as biological variants. A taxonomic classification of our representative sequences was performed using the 99% SILVA 138 reference database and 515F/806R primers (15, 16). A rooted phylogenetic tree was constructed by aligning ASVs using Multiple Alignment Fast Fourier Transform (MAFFT) and FastTree (17, 18). The ASV feature table, taxonomy table, and rooted phylogenetic tree were exported from QIIME2 to R for further analysis.

Alpha and beta diversity metrics. For the purpose of our downstream analyses, a phyloseq object containing the processed ASV table, taxonomy table, rooted phylogenetic tree, and sample metadata was created in R using the packages Phyloseq (v1.42.0; 19), Tidyverse (12), and Ape (v5.6-2; 20). We generated a rarefied version of the Phyloseq object with a sampling depth of 3500 to optimize ASV saturation and sample retention. To investigate the differences in gut microbial diversity between our four groups ("High Fish, Later-Onset", "High Fish, Early-Onset", "Low Fish, Later-Onset", and "Low Fish, Early-Onset"), we performed and visualized diversity metrics in R using the following packages: Microbiome (v1.20.0; 21), Phyloseq (19), Tidyverse (12), Ape (20), Vegan (v2.6-4; 22), and ggplot2 (v3.4.1; 23). Shannon's diversity index, which considers community abundance and richness, was used to quantify the alpha diversity of each group (24). We visualized these results using a boxplot and determined significance using Kruskal-Wallis pairwise testing ($\alpha = 0.05$). A weighted UniFrac analysis, which considers abundance and phylogenetic relatedness, was used to evaluate beta diversity (25). The results were visualized using a PCoA plot and significance was determined with PERMANOVA ($\alpha = 0.05$).

Differential abundance analysis. To determine if there are differentially enriched microbial taxa in the gut of male PD patients consuming higher quantities of fish, we conducted differential abundance analysis in R using Differential Expression Sequence analysis (DESeq) (v1.38.3; 25). The packages DESeq2 (26), Phyloseq (19), Tidyverse (12), and Ape (20) were used. DESeq2 is a parametric test that models sequence abundance using a negative binomial distribution. The taxonomy data were glommed to the genus level and we applied an abundance filter of 10^{-4}. Since our data were divided into four groups based on fish intake and age of onset, we chose not to use a prevalence filter as this risked eliminating taxa that were mostly present in only one age of onset / fish intake group (Fig S3). Low-fish intake was defined as the reference group. An adjusted p-value of < 0.05 was defined as the statistical significance threshold. The significantly differentially abundant genera were visualized with a barplot and volcano plot using ggplot2 (22) and ggrepel (v0.9.3; 27).

Indicator species analysis. To identify key taxa associated with high-fish intake and later-onset PD, a non-parametric indicator species analysis was performed using the indicspecies

package (v1.7.12; 28) in R. Genus-glommed and rarefied taxonomy data was used to identify indicator genera for each of our four groups ("High Fish, Later-Onset", "High Fish, Early-Onset", "Low Fish, Later-Onset", and "Low Fish, Early-Onset"). Indicator values were generated based on both abundance and prevalence (Indicator Value = 100 * mean relative abundance * mean relative frequency). Data were filtered for p-values < 0.05. Genera that were deemed to indicate biologically irrelevant pairs (ie: high fish, late onset and low fish, early onset) or three of the four groups were excluded from further analysis.

Correlation analysis. Linear models in base R were iteratively applied across 13 out of the 16 genera of interest derived from our differential abundance analysis using age as a response variable and relative abundance, disease status, and their interaction term as explanatory variables. We only included cultured genera and removed any that did not meet log2 fold change threshold of ±1. This was also applied separately to the female participants in our cohort to identify correlations that were exclusively present in males. Controls were also used in this part of the analysis to provide a baseline with which to compare the correlation in Parkinson patients; this would allow us to control for any correlations that exist due to aging. Age was used as a proxy for age of onset for Parkinson's patients given the high degree of correlation between the two variables (Fig S2). This is primarily an artifact of the study design whereby PD patients were specifically recruited following their diagnosis by a particular window of time (approx. 7 years). We controlled for possible confounding variables including kilocalorie intake and BMI. The p-value and coefficient of the interaction term (*Age * Disease*) was captured and visualized using a heatmap (v1.0.12; 29) utilizing kmeans (Euclidean distance) to cluster taxa. Unless otherwise specified, all corrections for multiple hypothesis testing were conducted using a false discovery rate adjustment.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) Version 2.0. Functional annotations of the ASVs obtained from our male PD cohort were inferred using PICRUSt2.0 (20, 30, 31). We particularly focused on the MetaCyc pathway output and determined differentially abundant metabolic pathways using DESeq (25) between high dietary fish and low dietary fish consuming individuals in our population of interest. Low fish intake was defined as the reference group. Pathways with adjusted p-values under 0.05 were retained for further analysis.

RESULTS

A high-fish diet is significantly correlated to later-onset PD in males. To determine if later onset of PD correlates with increased fish consumption, we performed a Kaplan-Meier survival analysis. Using a threshold of 27.2 g of daily fish intake (Fig S1), our analysis revealed that high-fish intake is associated with a delayed age of disease onset relative to individuals consuming a low-fish diet ($p<0.05$) (Fig 1A). The trendlines describing the Parkinson's-free survival between high- and low-fish consumption particularly diverge at 50 years of age in the mixed male-female PD cohort. Since Metcalfe-Roach *et al.* (3) reported that there were dietary sex differences, we sex-stratified the metadata (Fig 1B). This strengthened the association between high-fish intake and increased incidence of later-onset of PD in males ($p<0.05$), while revealing no effect of fish intake on disease onset in females ($p>0.05$). Thus, our study moving forward focused on the male cohort only.

No changes in global gut microbiota composition associated with fish intake and age of onset across male PD patients. To assess if there were any global perturbations in gut microbial community composition due to fish consumption and age of onset, we evaluated alpha and beta diversity for our four groups ("High Fish, Later-Onset", "High Fish, Early-Onset", "Low Fish, Later-Onset", and "Low Fish, Early-Onset"). For alpha diversity, Kruskal-Wallis testing revealed that there were no significant differences between any of the groups for Shannon's diversity index, indicating that all four groups were similar in terms of community richness and abundance (Fig 2A). Similarly, statistical testing using PERMANOVA indicated that none of the four groups significantly differed in terms of beta diversity when characterized using a weighted UniFrac analysis, as seen by the clustering in the PCoA plot (Fig 2B). The lack of global microbiota composition changes thus indicates that any microbial differences in fish intake can be attributed to select taxa.

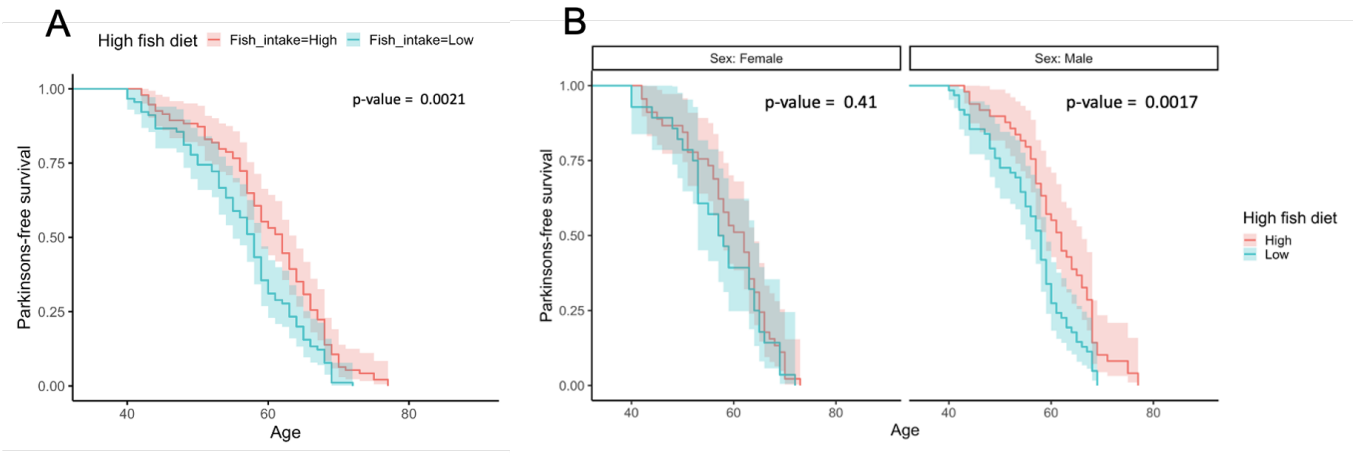

FIG. 1 Kaplan-Meier curves comparing fish intake to disease onset reveal a correlation between high-fish consumption and later-onset PD in males. (A) Mixed male-female PD cohort. (B) Sex-stratified comparison for fish intake versus age of onset. Window indicates a 95% confidence interval. Significance: $p < 0.05$.

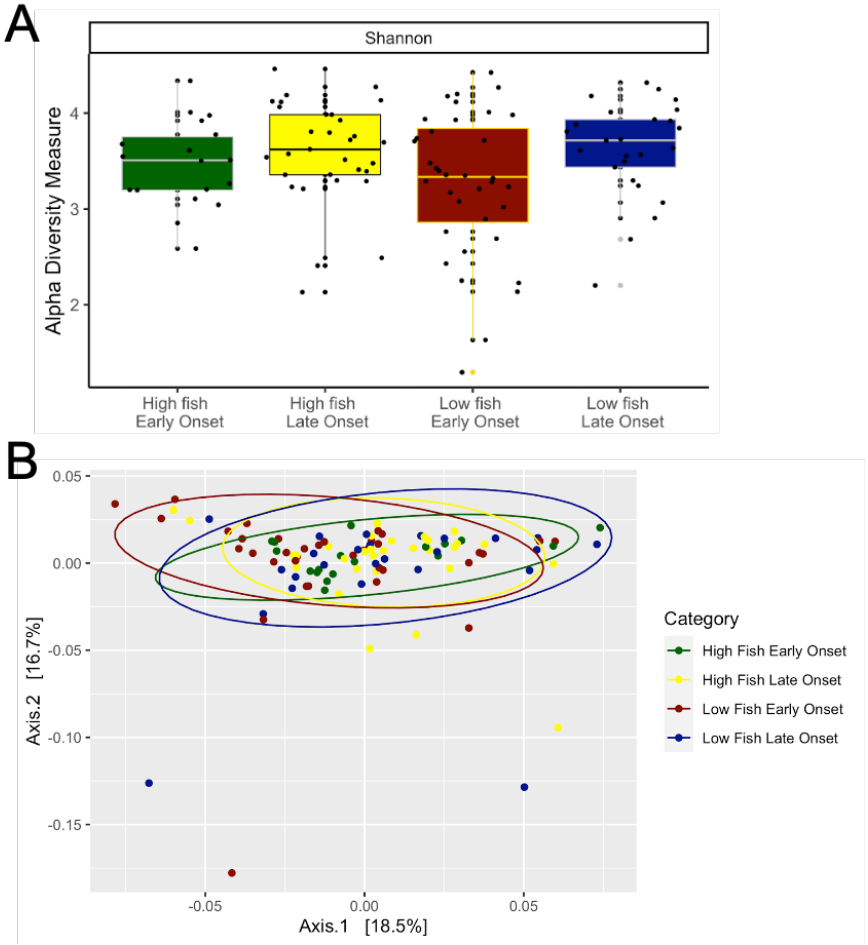

FIG. 2 No significant differences in gut microbial diversity between PD patients of each unique fish intake (high/low) and age of onset (early/later) combination. (A) Shannon's diversity index was used to measure alpha diversity and was visualized with boxplots. Kruskal-Wallis testing yielded insignificant q values at $\alpha = 0.05$. (B) Weighted UniFrac was used to determine beta diversity. The resulting PCoA plot shows no distinct separation of the ellipses (95% confidence interval), suggesting no notable changes in diversity between the groups. This was confirmed with PERMANOVA testing using $\alpha = 0.05$.

High-fish intake reveals enrichment of short-chain fatty acid producers in male PD patients. Differential Expression Sequence (DESeq) analysis was used to identify taxa that were more or less abundant in male PD patients consuming high-fish diets compared to those with low-fish diets. Unrarefied taxonomy data were glommed by genus and analyzed. 16 genera had p values < 0.05 and fold change magnitudes > 2, including six genera that were more abundant and 10 that were less abundant in patients with high-fish intake (Fig 3A, B). Two of these genera were uncultured.

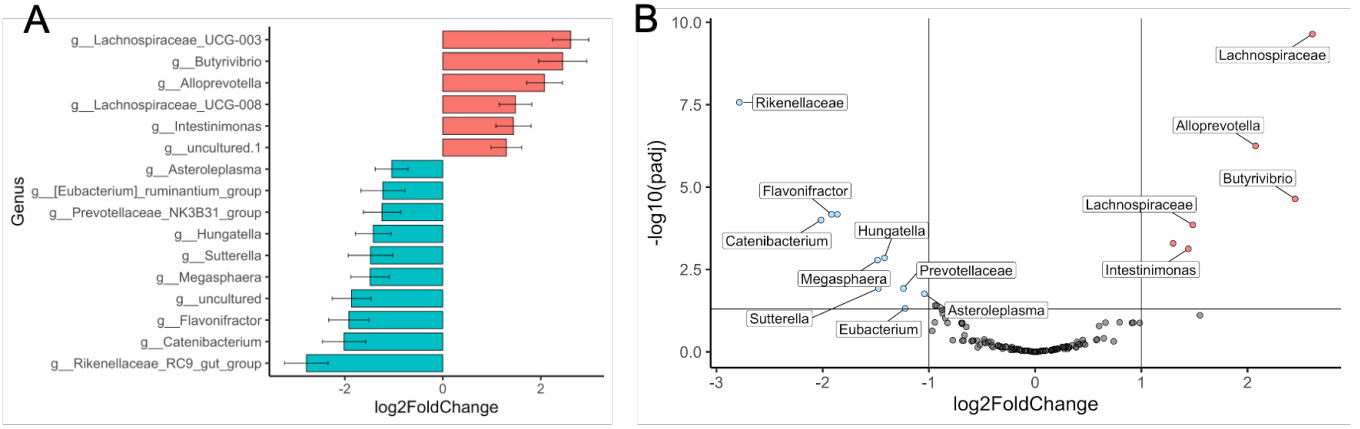

FIG. 3 DESeq analysis reveals changes in abundance of gut microbes in high-fish intake male PD patients. (A) Bar plot indicating genera differentially abundant with high-fish intake compared to low-fish intake patients. **(B)** Volcano plot showing adjusted p-values and fold changes of all genera. Genera in blue indicate lower abundance while red signifies greater abundance in high-fish intake PD patients compared to low-fish intake PD patients. Only genera with > 1 Log_2 fold change are shown. Significance: $p < 0.05$.

***Intestinimonas* is an indicator genus specific to high-fish intake and later-onset PD.** In total, 15 indicator genera for fish intake and age of onset were observed. Six were removed from further analysis as they corresponded to multiple conflicting categories. The nine remaining genera served as marker taxa for fish intake and age of onset in male PD patients (Table 1). Of these, *Intestinimonas*, uncultured *Bacteroidales*, and *Lachnospiraceae* UCG-008 were also observed in DESeq analysis for high-fish intake PD patients (Fig 3). Three genera - *Intestinimonas*, uncultured *Bacteroidales*, and *Lachnospiraceae* NK4B4 - were associated specifically with high-fish, later PD onset patients. No indicator genera were associated with low fish intake, early onset patients.

TABLE. 1 Key genera distinguishing high- vs. low-fish intake and early vs. later age of onset in males identified through indicator species analysis. Three genera corresponded to high-fish and later age of onset in male PD patients. Significance: * < 0.05, ** < 0.01.

Genus	Condition	Observed Indicator Value (IV)	*P*
Paludicola	High Fish, Early Onset	0.423	**
Anaerotruncus	Low Fish, Later Onset	0.333	*
Merdibacter	Low Fish, Later Onset	0.363	*
Intestinimonas	High Fish, Later Onset	0.481	*
Uncultured *Bacteroidales*	High Fish, Later Onset	0.402	**
Lachnospiraceae NK4B4	High Fish, Later Onset	0.341	*
Lachnospiraceae UCG-008	High Fish	0.417	*
Uncultured *Tannerellaceae*	Later Onset	0.583	**
Hydrogenoanaerobacterium	Later Onset	0.432	*

Relative abundance of high-fish enriched genera are not significantly correlated with Parkinson's age of onset when controlling for age correlation. In our cohort of interest (male PD patients), the correlation between age and relative abundance of *Intestinimonas* is more positive relative to control male participants, suggesting that people with later-onset PD have higher levels of *Intestinimonas* than controls of the same age. This

is reflected in a positive interaction term coefficient (0.19) for the male participants in our cohort (Fig 4A). The coefficient in the female cohort is negative and much smaller (-0.098), indicating that this trend is specific to male PD patients, which aligns with the sex-specific delay in disease onset characterized by a high-fish diet in male patients (Fig 1B). However, once corrected for multiple hypothesis testing, all interaction term coefficients had a non-significant p-value (padj > 0.05).

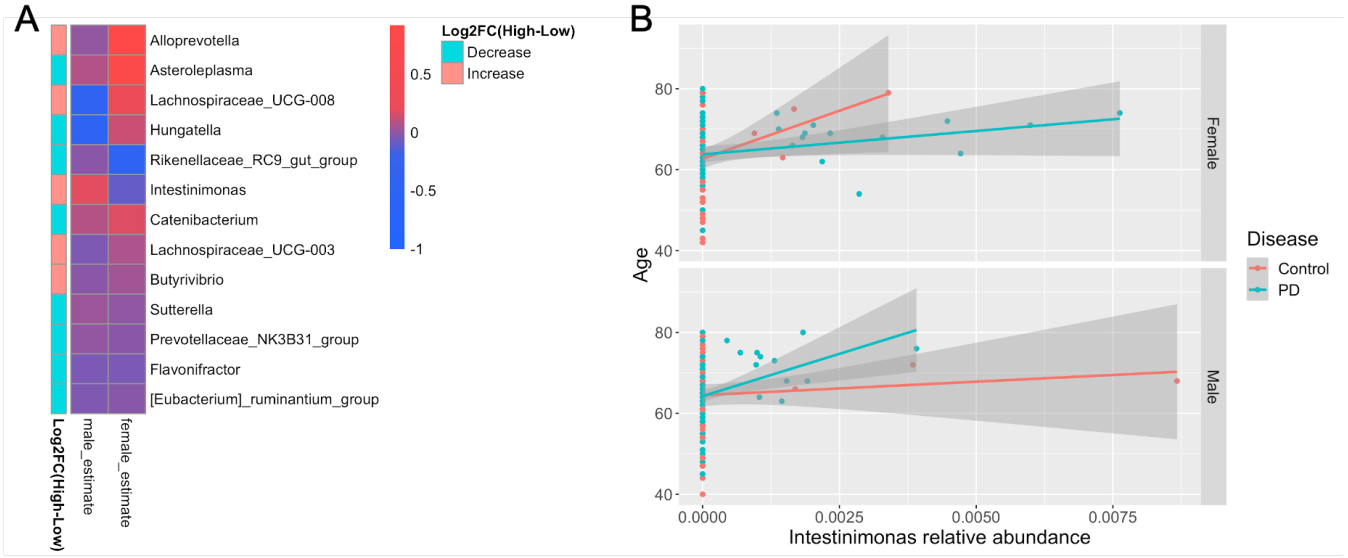

FIG. 4 Correlation between participant age and relative abundance of microbial taxa of interest. (A) Linear modeling across 13 taxa of interest featuring both significantly over-abundant and under-abundant taxa. The interaction term coefficient for disease status and relative abundance is displayed. All taxa expressed interaction terms with non-significant adjusted p-values (>0.05). **(B)** The correlation between relative abundance and participant age for *Intestinimonas*.

High dietary fish intake is potentially associated with increased short chain fatty acid metabolic pathways in male PD patients. Based on inferred MetaCyc pathways, DESeq reveals an overabundance in active pathways involved in the synthesis of SCFAs and SCFA precursors (Fig 5). These include amino-acid derived acetyl Coenzyme A (acetyl-CoA) and propionate through threonine degradation. Additionally, high-fish diets are associated with increased phenylacetate, phenylethylamine, and 4-hydroxyphenylacetate degradation - all which are involved in the production of acetyl-CoA and succinate. High-fish diets are also involved in increased production of bacterial lipopolysaccharide (LPS), peptidoglycan, and aerobactin synthesis. Significantly downregulated pathways include the mevalonate pathway, phosphopantothenate biosynthesis, and formaldehyde assimilation.

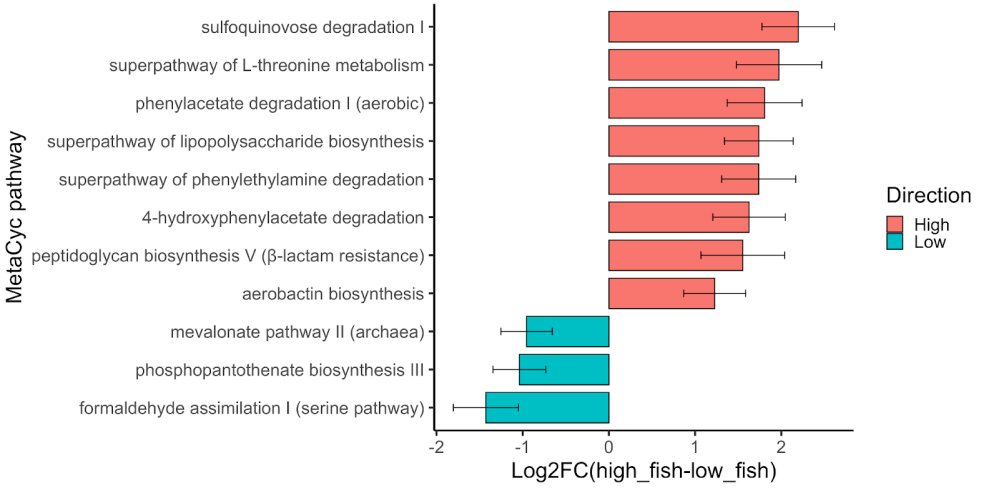

FIG. 5 Differentially abundant PICRUSt-based inferred metabolic pathways between male PD participants consuming high and low dietary fish. Log2 fold change is expressed as high-fish-consuming relative to low-fish-consuming participants. Only MetaCyc pathways that meet a significance level of alpha < 0.05 are displayed.

DISCUSSION

Parkinson's disease (PD) is the second leading age-related neurodegenerative illness in North America and is currently treated with the palliative drug levodopa to replenish dopamine levels (1, 32). However, irreversible motor complications occur in 50% of PD patients due to long-term levodopa use, thus a need for new interventions to treat symptoms and potentially delay disease progression is paramount (33). Epidemiological studies provide evidence that environmental risk factors heavily influence pathogenesis and suggest that diet impacts progression through dual disease-promoting and therapeutic effects (2). The Western diet (WD) in particular is regarded as a prominent risk factor for neurodegenerative disease, while the Mediterranean diet (MeDi) has been shown to confer protective benefits against neurodegenerative decline in PD patients in terms of reduced PD risk and later-onset of disease in men only (2,3).

A key feature of the MeDi is incorporation of fish; a rich source of omega-3 (ω3) polyunsaturated fatty acids (PUFAs) in the form of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which cannot be synthesized de novo in humans and promote overall brain health (e.g., increasing synaptic plasticity and reducing inflammation) (2,4). Studies have shown that ω3 supplementation leads to gut microbiota alterations that result in higher abundances of short-chain fatty acid (SCFA) producing microbes (5,6). SCFAs exert a range of beneficial effects on host health, including anti-inflammatory, immunoregulatory, and neuroprotective activities (34). In this study, we aimed to ascertain if onset of PD is correlated with higher dietary consumption of fish and if SCFA-producing microbes are differentially enriched, potentially eliciting protection against PD development.

Our findings revealed that high-fish intake is indeed associated with later-onset PD, although this effect is only evident in males (Fig 1B). Since fish intake and age of disease onset unsurprisingly did not lead to global dysbiosis as per alpha and beta diversity (Fig. 2), we then looked to see if select microbial taxa were differentially expressed between male PD patients consuming high- versus low-fish (Fig 3). Further, linear models were applied to correlate differentially abundant taxa to participant age, which was used as a proxy for age of onset (Fig 4). Additionally, we conducted an indicator species analysis to assess if any genera were associated with our condition of interest (high-fish, later-onset) (Table 1).

Linear modeling revealed that no taxa identified through DESeq significantly correlated to age of onset (Fig 4A). However, *Intestinimonas* had a stronger correlation with males than females, and was the only genus both more abundant in high-fish-consuming PD patients and an indicator species for high-fish intake and later disease onset (Fig 4B, Fig 3, Table 1). Moreover, we observed a positive correlation with age of onset in male patients, which was not apparent in the female PD cohort (Fig 4B). As an important butyrate producer, *Intestinimonas* promotes anti-inflammatory responses and helps to maintain the healthy gut phenotype (35). *Intestinimonas* also metabolizes lysine as a precursor to butyrate, which may explain the elevated *Intestinimonas* in high-fish PD patients, as fish are rich sources of lysine and other proteins (36). In relation to ω3 PUFAs, a study by Saravi *et al.* (37) assessed if long dietary supplementation with the plant-derived ω3, alpha-linolenic acid (ALA), could restore a youthful enterotype in aging mice. Of interest, high dietary intake with ALA partly re-established hallmarks of a youthful microbial community, including increased abundance of *Intestinimonas*. However, in the context of PD, Aho *et al* (2021) found *Intestinimonas* was negatively correlated with SCFAs (38). Nevertheless, *Intestinimonas* is a promising promoter of gut health in response to high-fish intake, although its influence on PD is still unclear.

We observed higher abundances of *Lachnospiraceae* UCG-003 and UCG-008, and *Butyrivibrio* for male PD patients consuming higher amounts of fish (Fig 3). In healthy middle-aged individuals, Watson *et al*. found supplementation with ω3 PUFAs led to an increased abundance of butyrate-producing *Lachnospiraceae* genera including *Lachnospira* and *Roseburia* (39). The *Lachnospiraceae* family includes a number of species with diverse functions in the maintenance of gut health, and many produce SCFAs (40). In addition, *Lachnospiraceae* are often found to be less abundant in PD patients and are negatively correlated with disease duration (41, 42). This suggests that *Lachnospiraceae* may play a role in delaying or preventing PD. However, *Lachnospiraceae* are also correlated with worse motor symptoms in PD and are implicated in obesity and multiple sclerosis (43, 44). Furthermore, conflicting evidence exists on whether *Butyrivibrio*, another member of the

Lachnospiraceae family, is associated with PD (45, 46, 47). *Lachnospiraceae* appear to have complex influences on neurological health, thus we cannot confirm that greater abundance of *Lachnospiraceae* observed in male PD patients consuming high-fish diets elicits a protective effect against PD development.

Alloprevotella was also enriched in high-fish intake male PD patients and produces SCFAs with negative correlations to inflammation markers in mice with inflammatory bowel disease (Fig 3) (48, 49). We identified *Merdibacter* as an indicator of low fish and later PD onset, but this taxon has not previously been linked to neurological diseases (Table 1) (50). We also found that *Anaerotruncus* indicated patients with low-fish diets and later PD onset (Table 1). Other studies, however, found that *Anaerotruncus* is more abundant in PD and associated with worse motor and nonmotor symptoms (43, 47). Although *Anaerotruncus* appears to be correlated to later PD onset in our study, this does not necessarily mean that *Anaerotruncus* is beneficial.

In our study, *Rikenellaceae* was significantly less abundant in male PD patients consuming a high-fish diet compared to those consuming a low-fish diet (Fig 3). Higher abundance of Rikenellaceae has repeatedly been associated with Parkinson's disease (45, 51, 52). As this bacterium is often correlated with PD, a decrease in *Rikenellaceae* in our high-fish intake male PD patients may indicate improved outcomes. *Sutterella*, *Hungatella*, and *Hydrogenoanaerobacterium* are negatively associated with Montréal Cognitive Assessment (MoCA) scores in PD patients (53). In our study, *Sutterella* and *Hungatella* were less abundant in a high-fish diet, which further suggests that these taxa indicate worse PD outcomes and provides a further link between fish intake and reduced PD severity (Fig 3). However, we found *Hydrogenanaerobacterium* also indicated later PD onset; therefore, it is unclear whether *Hydrogenanaerobacterium* is helpful or harmful to PD progression (Table 1).

Asteroleplasma has previously been found to correlate to PD progression but was suspected to be a false positive result (52). *Paludicola* and *Megasphaera* are more abundant in females with Fibromyalgia, a neurological disorder associated with the gut microbiome, specifically with lower levels of propionate (54). We found *Paludicola* was associated with earlier-onset male PD patients, but specifically in those patients consuming high-fish diets. Meanwhile, *Eubacterium*, a propionate producer, was less abundant in Fibromyalgia patients. *Eubacterium, Flavonifractor*, and *Catenibacterium* were also found to be less abundant in patients with colorectal cancer (55). Our findings showed that these three genera are less abundant in high-fish intake PD patients. If these genera positively influence gut health, then their depletion in high-fish consuming PD patients may be harmful. Further research on the roles of all of these bacteria in gut health may shed light on how the microbiome affects colorectal cancer, Fibromyalgia, and PD progression.

Using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2), marker genes were used to infer key metabolic pathways potentially active in the gut (30). DESeq revealed a significant increase in pathways associated with the production of acetate, propionate, and SCFA precursors such as acetyl-CoA and succinate (Fig 5). Acetate is the most abundant SCFA in the gut, but it can be transformed into butyrate by the enzyme butyryl kinase and butyryl-CoA:acetyl-CoA transferase. Butyrate production in the gut is highly dependent on this cross-feeding, which is mediated by a variety of butyrate-producing bacteria (56). Numerous studies attribute a role for SCFAs in mediating protection against a variety of neurodegenerative diseases, including PD. These mechanisms have the potential to target both the pathophysiology (alpha-synuclein mediated dopaminergic receptor degeneration) and symptomatic presentation of PD. Radioactive tracing has also shown that SCFAs are capable of crossing the blood brain barrier (BBB) following absorption through the gut (57, 58). Within the central nervous system, SCFAs have demonstrated the potential to induce the production of dopamine (59), while inhibiting alpha synuclein-mediated degradation of dopaminergic neurons (60). Additionally, SCFAs are believed to help maintain the integrity of the BBB, and impairment of the BBB has been linked to PD in animal models (61). In the gut, reduced SCFA production in PD patients is associated with impaired colonic peristalsis and reduced intestinal barrier integrity, leading to PD-associated constipation and greater risk of gut colonization by pathobionts (62, 63). This suggests that SCFA supplements are a potential avenue to mitigate PD risk and manage

gut and motor symptoms. In the context of our study, a delayed age of onset identified in high-fish intake male PD patients could be due to a protective role associated with PUFA-driven remodeling of the gut microbiome favoring SCFA-producing taxa.

Other pathways of interest upregulated in our high-fish male PD cohort include phenylethylamine metabolism and lipopolysaccharide biosynthesis. Phenylethylamine is found in dietary sources including chocolate, wine, and nuts and is involved in altering brain chemistry (64). *In vivo* studies have shown that chronic exposure to phenylethylamine is associated with the neurodegeneration of dopaminergic receptors (65). Since phenylethylamine is absorbed by intestinal epithelial cells and readily crosses the blood brain barrier (66, 67), microbial-mediated breakdown of the compound may potentially prevent its accumulation in the central nervous system and delay PD onset. Increased biosynthesis of LPS is not surprising in PD patients given the association to gut inflammation (68); however, it is unexpected for its synthesis to be upregulated in male patients consuming a high-fish diet since SCFA abundance is associated with improved gut epithelial integrity and suppression of inflammatory response.

Limitations The food and nutrition metadata categories, including the 'fish' category, were collected by Cirstea *et al.* (7) through the EPIC-Norfolk Food Frequency Questionnaire. This method of reporting dietary fish intake creates limitations regarding this study as self-reporting through a questionnaire is subject to inaccuracies. Additionally, the questionnaire lacks specificity toward the type of fish consumed, which is of importance given the varying nutritional composition of different categories of fish. In comparison to lean fish (e.g., bass or cod), fatty fish (e.g., salmon or tuna) have higher levels of omega-3 FAs, a factor that is directly related to our investigation into how fish consumption is protective against PD (69). Moreover, the median thresholds we chose for age of onset and fish intake are arbitrary and may not accurately reflect biologically significant divisions. In addition to different thresholds, testing multiple diversity metrics may elucidate differences in microbiome composition between our groups of interest.

Similar to the study conducted on the original dataset by Cirstea *et al.* (7), the observational nature of the data limits this study to drawing correlational, not causal, conclusions. In an attempt to offset the effects of confounding variables, we controlled for kilocalories and BMI in our correlational analysis, but controlling for these two variables still does not eliminate the possibility of other confounding variables influencing our results. A controlled experiment design would be necessary in order to establish causality that could be explained by mechanistic links.

Another limitation of this study that stems from the original dataset is the limited sample size; of the 300-patient cohort, only 111 subjects were males with PD. The relatively small number of male PD patients in this study reduces the statistical power of the various statistical tests we used, potentially resulting in us missing key findings that would be revealed by a larger sample size. In addition, a limited sample size led to the inability to apply a prevalence filter in our DESeq analysis without losing key results that had been validated by our Indicator Species analysis. These limitations illustrate how increasing the sample size would enable a more robust analysis and allow us to be more confident in our results. In addition to a small sample size, DESeq is known to produce false positives; therefore, species identified through our study may not be relevant to PD progression. Future studies could utilize optimized packages for metagenomics or establish more thresholds for false discovery.

Conclusions The aim of our study was to investigate whether a diet rich in fish has any impact on PD onset and if there is a unique gut microbiota composition associated with a high-fish diet that may be involved in eliciting protection against the development of PD. We hypothesized that a high-fish diet is correlated with a delayed onset of PD because consuming high amounts of fish is thought to promote the enrichment of SCFA-producing microbes, which can be protective against neurodegenerative diseases. Our findings indicate that a high-fish diet is indeed associated with protection against PD onset but only in males. Results from our diversity analyses identified no significant alterations in gut microbial composition due to fish intake and age of onset, suggesting that fish consumption does not cause global changes in the gut microbiota to affect PD onset. However, we did observe the enrichment of

SCFA-producing microbes, including *Intestinimonas* and *Lachnospiraceae*, in male PD patients consuming high amounts of dietary fish compared to those consuming lower amounts of fish. Our indicator species analysis also identified these genera as key markers for high-fish intake and later-onset of PD, further establishing their possible role in eliciting protection against PD pathogenesis in males. MetaCyc analysis revealed that a high-fish diet is associated with the enrichment of SCFA metabolic pathways, particularly acetate and acetyl-CoA synthesis, which are believed to confer protection against PD. All together, these findings establish a correlation between a high-fish diet and later onset of PD in males and suggest that protection against PD could be mediated by promotion of SCFA-producing microbes.

Future Directions This study provides proof of concept for further investigation into how dietary fish intake influences protection against PD, potentially mediated by the enrichment of SCFA-producing bacteria. To address one of the major limitations of this study, conducting a similar study with a larger sample size would potentially elucidate more key taxa and allow for verification of the trends we observed in the correlation analysis. Furthermore, collecting metabolomic data from PD patients would enable a better understanding of the metabolic pathways impacted by the presence of SCFA-producing bacteria enriched by a high-fish diet. This would provide further insight into the mechanistic links between fish intake and age of PD onset. After further *in silico* verification of our taxa of interest, *in vivo* studies of these key taxa would be the logical next step in establishing their protective effect against PD-associated gut-inflammation. This could be accomplished through experimental studies on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) murine models of PD involving the introduction of individual key taxa or dietary supplements (e.g., ω3) and then observing PD-related pathologies and obtaining metabolomic data to support mechanistic links (70).

ACKNOWLEDGEMENTS

We would like to thank Avril Metcalfe-Roach for his continuous guidance, support, and feedback over the course of this project. Thank you to Dr. Evelyn Sun and Dr. Melissa Chen for teaching us how to use QIIME and R Studio. We would also like to thank Dr. Mihai Cirstea for providing the metagenomic dataset that we used for this research. We would also like to thank the anonymous reviewer for constructive feedback on this manuscript.

CONTRIBUTIONS

IL manipulated the metadata for downstream analyses, performed diversity metrics, helped write the DESeq code, and contributed to the following manuscript sections: abstract, introduction, methods for dataset, survival analysis, metadata manipulation, and DESeq, results for survival analysis, and discussion. RL contributed to the QIIME2 analysis, diversity metrics, and DESeq analysis, and contributed to the following manuscript sections: methods for QIIME pipeline and diversity metrics, results for diversity metrics, discussion, and references. LB performed QIIME2 analysis, Indicator Species Analysis, contributed to DESeq and correlation analyses, and contributed to the following manuscript sections: methods and results for Indicator Species Analysis and DESeq, and the discussion. FB contributed to the QIIME2 analysis, DESeq analysis, correlation analysis, and PICRUSt2 analysis, and contributed to the following manuscript sections: methods and results for correlation and PICRUSt2-based analysis, results and figures, and discussion. All authors contributed to the conception of the project and troubleshooting of code.

REFERENCES

1. Parkinson's Foundation. Statistics. https://www.parkinson.org/understanding-parkinsons/statistics. Accessed 21 Feb 2023.
2. Jackson A, Forsyth CB, Shaikh M, Voigt RM, Engen PA, Ramirez V, Keshavarzian A. 2019. Diet in Parkinson's disease: Critical role for the microbiome. Front Neurol 10:1245.
3. Metcalfe-Roach A, Yu AC, Golz E, Cirstea M, Sundvick K, Kliger D, Foulger LH, Mackenzie M, Finlay BB, Appel-Cresswell S. 2021. MIND and mediterranean diets associated with later onset of Parkinson's disease. J Mov Disord 36(4):977-984.
4. Bousquet M, Calon F, Cicchetti F. 2011. Impact of omega-3 fatty acids in Parkinson's disease. Ageing Research Reviews 10(4):453-463.
5. Costantini L, Molinari R, Farinon B, Merendino N. 2017. Impact of omega-3 fatty acids on the gut microbiota. Int J Mol Sci 18(12):2645.

6. Fu Y, Wang Y, Gao H, Li DH, Jiang RR, Ge L, Tong C, Xu K. 2021. Associations among dietary omega-3 polyunsaturated fatty acids, the gut microbiota, and intestinal immunity. Mediators Inflamm 2021:2e8879227.
7. 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. J Mov Disord 35(7):1208-1217.
8. 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 Nutr 4:847–858.
9. R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
10. Kassambara A, Kosinski M, Biecek P. 2021. survminer: Drawing Survival Curves using 'ggplot2'. R package version 0.4.9. https://CRAN.R-project.org/package=survminer.
11. Therneau TM, Grambsch P. 2000. Modeling Survival Data: Extending the Cox Model. Springer, New York. R package version 3.5-5. https://CRAN.R-project.org/package=survival.
12. 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. R package version 1.3.2. J Open Source Softw 4:1686.
13. 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 MGI, Lee J, Ley R, Liu YX, 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 JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, and Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857.
14. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583.
15. Bokulich N, Kaehler B, Rideout J, Dillon M, Bolyen E, Knight R, Huttley G, Gregory Caporaso J. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90.
16. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl Acids Res 41:D590-D596.
17. Katoh K, Misawa K, Kuma K-I, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066.
18. Price MN, Dehal PS, Arkin AP. 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490.
19. McMurdie PJ, Holmes S. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. R package version 1.42.0. PLoS ONE 8(4):e61217.
20. Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. R package version 5.6-2. Bioinformatics 35:526-528.
21. Lahti L, Shetty S. 2012-2019. Microbiome R package. R package version 1.20.0 http://microbiome.github.io.
22. Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H,FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J. 2022. vegan: Community Ecology Package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan.
23. Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. R package version 3.4.1. Springer-Verlag, New York. https://ggplot2.tidyverse.org.
24. Shannon CE, Weaver W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana.
25. Lozupone C, Knight R. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71(12):8228–8235.
26. Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. R package version 1.38.3. Genome biol 15:550–550.
27. Slowikowski K, Schep A, Hughes S, Dang TK, Lukauskas S, Irisson JO, Kamvar ZN, Ryan T, Christophe D, Hiroaki Y, Gramme P, Abdol AM, Barret M, Cannoodt R, Krassowski M, Chirico M,

Aphalo P. 2023. ggrepel: Automatically Position Non-Overlapping Text Labels with 'ggplot2'. R package version 0.9.3. https://CRAN.R-project.org/package=ggrepel.
28. Cáceres MD, Legendre P. 2009. Associations between species and groups of sites: indices and statistical inference. R package version 1.7.12. Ecology 90(12):3566–3574.
29. Kolde R. 2019. Pheatmap: Pretty Heatmap. R package version 1.0.12. https://cran.r-project.org/web/packages/pheatmap/index.html.
30. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. 2020. PICRUSt2 for prediction of meta- genome functions. Nat Biotechnol 38:685–688.
31. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes J, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 9:814-821.
32. National Institute on Aging. Parkinson's Disease: Causes, Symptoms, and Treatments. https://www.nia.nih.gov/health/parkinsons-disease. Accessed 17 April 2023.
33. Gandhi KR, Saadabadi A. 2 May 2022. Levodopa (L-Dopa). *In* StatPearls. StatPearls Publishing, Treasure Island, Florida. https://www.ncbi.nlm.nih.gov/books/NBK482140/. Accessed 17 April 2023.
34. Xiong RG, Zhou DD, Wu SX, Huang SY, Saimaiti A, Yang ZJ, Shang A, Zhao CN, Gan RY, Li HB. 2022. Health Benefits and Side Effects of Short-Chain Fatty Acids. Foods 11(18):2893.
35. Bui TP, Shetty SA, Lagkouvardos I, Ritari J, Chamlagain B, Douillard FP, Paulin L, Piironen V, Clavel T, Plugge CM, de Vos WM. 2016. Comparative genomics and physiology of the butyrate-producing bacterium Intestinimonas butyriciproducens. Environ Microbiol Rep 8(6):1024-1037.
36. Bui TPN, Troise AD, Nijsse B, Roviello GN, Fogliano V, de Vos WM. 2020. Intestinimonas-like bacteria are important butyrate producers that utilize Nε-fructosyllysine and lysine in formula-fed infants and adults. JFF 70:103974.
37. Saravi SSS, Bonetti NR, Pugin B, Constancias F, Pasterk L, Gobbato S, Akhmedov A, Liberale L, Luscher TF, Camici GG, Beer JH. 2021. Lifelong dietary omega-3 fatty acid suppresses thrombotic potential through gut microbiota alteration in aged mice. iScience 24(8):102897.
38. Aho VTE, Houser MC, Pereira PAB, Chang J, Rudi K, Paulin L, Hertzberg V, Auvinen P, Tansey MG, Scheperjans F. 2021. Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease. Mol Neurodegener 16(1):6.
39. Watson H, Mitra S, Croden FC, Taylor M, Wood HM, Perry SL, Spencer JA, Quirke P, Toogood GJ, Lawton CL, Dye L, Loadman PM, Hull MA. 2018. A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. Gut 67:1974-1983.
40. Louis P, Flint HJ. 2017. Formation of propionate and butyrate by the human colonic microbiota. Environ Microbiol 19(1):29-41.
41. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, Mutlu E, Shannon KM. 2015. Colonic bacterial composition in Parkinson's disease. Mov Disord 30(10):1351-1360.
42. Gerhardt S, Mohajeri MH. 2018. Changes of Colonic Bacterial Composition in Parkinson's Disease and Other Neurodegenerative Diseases. Nutrients 10(6):708.
43. Heintz-Buschart A, Pandey U, Wicke T, Sixel-Döring F, Janzen A, Sittig-Wiegand E, Trenkwalder C, Oertel WH, Mollenhauer B, Wilmes P. 2018. The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. Mov Disord 33(1):88-98.
44. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. 2020. The Controversial Role of Human Gut Lachnospiraceae. Microorganisms 8(4):573.
45. 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. 2020. Gut Microbiota and Metabolome Alterations Associated with Parkinson's Disease. mSystems 5(5):e00561-20.
46. Tan AH, Chong CW, Lim S-Y, Yap IKS, Teh CSJ, Loke MF, Song S-L, Tan JY, Ang BH, Tan YQ, Kho MT, Bowman J, Mahadeva S, Yong HS, Lang AE. 2021. Gut Microbial Ecosystem in Parkinson Disease: New Clinicobiological Insights from Multi-Omics. Ann Neurol 89:546-559.
47. Li Z, Liang H, Hu Y, Lu L, Zheng C, Fan Y, Wu B, Zou T, Luo X, Zhang X, Zeng Y, Liu Z, Zhou Z, Yue Z, Ren Y, Li Z, Su Q, Xu P. 2023. Gut bacterial profiles in Parkinson's disease: A systematic review. CNS Neurosci Ther 29(1):140-157.
48. Qu W, Yuan X, Zhao J, Zhang Y, Hu J, Wang J, Li J. 2017. Dietary advanced glycation end products modify gut microbial composition and partially increase colon permeability in rats. Mol Nutr Food Res 61(10):1700118.
49. Li AL, Ni WW, Zhang QM, Li Y, Zhang X, Wu HY, Du P, Hou JC, Zhang Y. 2020. Effect of cinnamon essential oil on gut microbiota in the mouse model of dextran sodium sulfate-induced colitis. Microbiol Immunol 64(1):23-32.
50. Nishiwaki H, Hamaguchi T, Ito M, Ishida T, Maeda T, Kashihara K, Tsuboi Y, Ueyama J, Shimamura T, Mori H, Kurokawa K, Katsuno M, Hirayama M, Ohno K. 2020. Rapid-Eye-Movement Sleep Behavior Disorder Exhibits Similar but Distinct Gut Dysbiosis Compared to Parkinson's Disease. bioRxiv https://doi.org/10.1101/2020.08.07.242453.
51. Qian Y, Yang X, Xu S, Wu C, Song Y, Qin N, Chen SD, Xiao Q. 2018. Alteration of the fecal microbiota in Chinese patients with Parkinson's disease. Brain Behav Immun 70:194-202.
52. Aho VTE, Pereira PAB, Voutilainen S, Paulin L, Pekkonen E, Auvinen P, Scheperjans F. 2019. Gut microbiota in Parkinson's disease: Temporal stability and relations to disease progression. EBioMedicine 44:691-707.

53. Ren T, Gao Y, Qiu Y, Jiang S, Zhang Q, Zhang J, Wang L, Zhang Y, Wang L, Nie K. 2020. Gut Microbiota Altered in Mild Cognitive Impairment Compared With Normal Cognition in Sporadic Parkinson's Disease. Front Neurol 11:137.
54. Kim Y, Kim GT, Kang J. 2023. Microbial Composition and Stool Short Chain Fatty Acid Levels in Fibromyalgia. Int J Environ Res Public Health 20(4):3183.
55. Ai D, Pan H, Li X, Gao Y, Liu G, Xia LC. 2019. Identifying Gut Microbiota Associated With Colorectal Cancer Using a Zero-Inflated Lognormal Model. Front Microbiol 10:826.
56. Boets E, Deroover L, Houben E, Vermeulen K, Gomand SV, Delcour JA, Verbeke K. 2015. Quantification of *in Vivo* Colonic Short Chain Fatty Acid Production from Inulin. Nutrients 7(11):8916-29.
57. McNeil NI, Cummings JH, James WP. 1978. Short chain fatty acid absorption by the human large intestine. Gut 19(9):819-22.
58. Mitchell RW, On NH, Del Bigio MR, Miller DW, Hatch GM. 2011. Fatty acid transport protein expression in human brain and potential role in fatty acid transport across human brain microvessel endothelial cells. J Neurochem 117(4):735-46.
59. Silva YP, Bernardi A, Frozza RL. 2020. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Front Endocrinol 11:25.
60. Hou Y, Li X, Liu C, Zhang M, Zhang X, Ge S, Zhao L. 2021. Neuroprotective effects of short-chain fatty acids in MPTP induced mice model of Parkinson's disease. Exp Gerontol 150:111376.
61. Bullich C, Keshavarzian A, Garssen J, Kraneveld A, Perez-Pardo P. 2019. Gut Vibes in parkinson's disease: The microbiota-gut-brain axis. Mov Disord Clin Pract 6:639–651.
62. Al-Bachari S, Naish JH, Parker GJM, Emsley HCA, Parkes LM. 2020. Blood-Brain Barrier Leakage Is Increased in Parkinson's Disease. Front Physiol 11:593026.
63. Li W, Wu X, Hu X, Wang T, Liang S, Duan Y, Jin F, Qin B. 2017. Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. Sci China Life Sci 60(11):1223-1233.
64. Ryu IS, Kim OH, Kim JS, Sohn S, Choe ES, Lim RN, Kim TW, Seo JW, Jang EY. 2021. Effects of β-Phenylethylamine on Psychomotor, Rewarding, and Reinforcing Behaviors and Affective State: The Role of Dopamine D1 Receptors. Int J Mol Sci 22(17):9485.
65. Borah A, Paul R, Mazumder MK, Bhattacharjee N. 2013. Contribution of β-phenethylamine, a component of chocolate and wine, to dopaminergic neurodegeneration: implications for the pathogenesis of Parkinson's disease. Neurosci Bull 29(5):655-60.
66. Fischer W, Neubert RH, Brandsch M. 2010. Transport of phenylethylamine at intestinal epithelial (Caco-2) cells: mechanism and substrate specificity. Eur J Pharm Biopharm 74(2):281-9.
67. McCulloch J, Harper AM. 1977. Phenylethylamine and cerebral blood flow. Possible involvement of phenylethylamine in migraine. Neurology 27(9):817-21.
68. Candelli M, Franza L, Pignataro G, Ojetti V, Covino M, Piccioni A, Gasbarrini A, Franceschi F. 2021. Interaction between Lipopolysaccharide and Gut Microbiota in Inflammatory Bowel Diseases. Int J Mol Sci 22(12):6242.
69. National Institutes of Health. Omega-3 Fatty Acids. https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/. Accessed 17 April 2023.
70. Konnova EA, Swanberg M. 21 Dec 2018. Chapter 5, Animal Models of Parkinson's Disease. *In* Stoker TB, Greenland JC (ed), Parkinson's Disease: Pathogenesis and Clinical Aspects. Codon Publications, Brisbane, Australia.