



Degree project

Parkinson's and Microbiota – General factors and possible implications on health and disease

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Abstract

Parkinson's disease is one of the leading neurodegenerative diseases that affect elderly people around the globe. In recent years, Parkinson's disease has been connected to gut microbiome and associations have been made among several novel species of bacteria and the development and severity of Parkinson's disease. The aim of this study was to identify general characteristics of the gut microbiome among incident Parkinson's disease cases and controls and by this broaden our current understanding of the topic. Statistical analyses were performed in a FINRISK 2002 population-based cohort of over 7000 people, out of which 105 incident Parkinson's disease cases were identified and further analysed. α -diversities among the case/control groups did not differ significantly, but there seemed to be gender-based differences in the β -dissimilarity matrix between Parkinson's disease cases and healthy controls. Additionally, a total of ten bacterial species were associated with Parkinson's disease by the generalized linear model with nominal p-values <0.05 , including for example *E. hallii*, *C. comes*, *A. muciniphila*, *E. eligens* and *P. bivia*. In conclusion, the gender-based variations in β -diversities and results from the regression analysis suggests that the gut microbiome may in fact be associated with the development of Parkinson's disease.

Abbreviations

BMI	Body mass index
CNS	Central nervous system
FMT	Fecal microbial transplantation
GBA	Gut-brain axis
GI	Gastrointestinal
GLM	Generalized linear model
HC	Healthy control
PCR	Polymerase chain reaction
PD	Parkinson's disease
SCFA	Short chain fatty acid
SD	Standard deviation
THL	The Finnish Institute for Health and Welfare
UPDRS	Unified Parkinson's Disease Rating Scale
WMS	Whole metagenome sequencing

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Introduction

The human gut microbiota is a unique collection of microorganisms in the digestive tracts of individuals. It consists mainly of bacteria, some eukaryotes, viruses and some archaea. They by and large live in symbiosis, affecting the human health via multiple pathways (Figure 1). Some of the pathways extend beyond the gastrointestinal (GI) tract and connect other organs, such as the kidneys, liver and brain to the gut microbiome. The gut-brain axis (GBA) is an example of this type of bidirectional communication pathway that connects central nervous system (CNS) to gut microbiome (Pröbstel et al., 2020). Recent evidence suggest that gut microbiome-derived metabolites can affect the emotional and cognitive centers of brain and hence contribute to neurodegenerative disease onset, such as Parkinson's disease (PD) (Carabotti et al., 2015; Meng et al., 2020; Schaeffer et al., 2020). Additionally, the GBA has been connected to the development of neurodevelopmental and neuropsychiatric conditions in germ-free animal models (Mayer et al., 2015). Although the functions of the GBA are beginning to be understood, it is still unclear to what extent these metabolites will have clinical usage in prevention and treatment of diseases in the future.

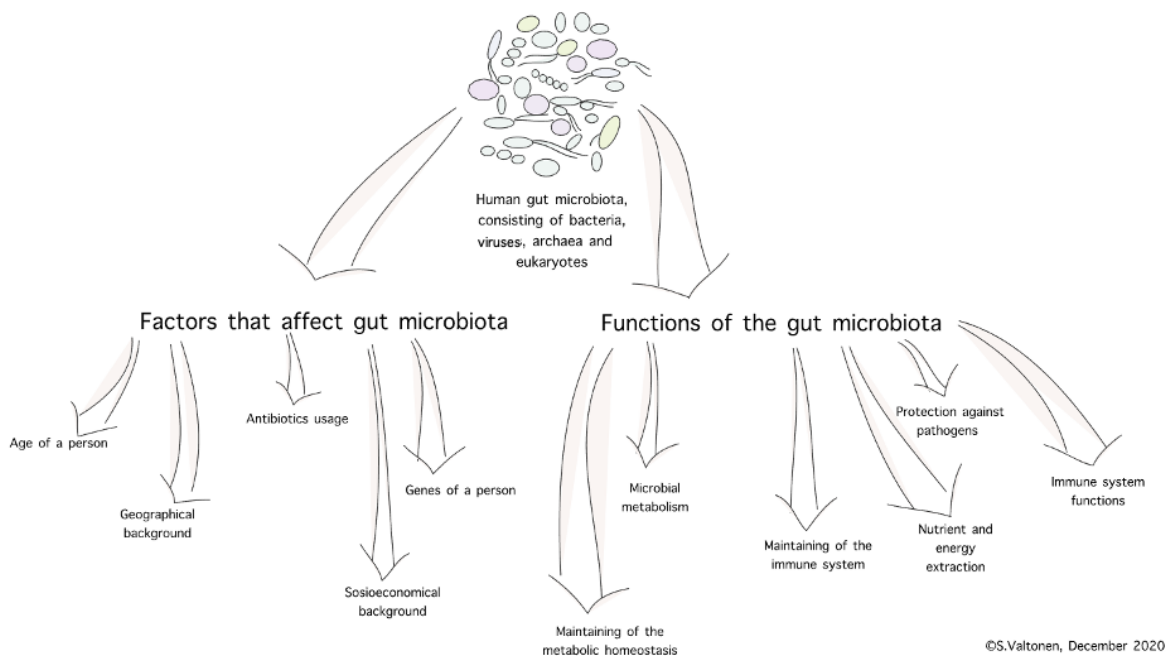


Figure 1. Some of the most important functions of the human gut microbiota and factors that have an effect on the gut microbiome composition. Multiple factors can disrupt the homeostasis of the gut microbiota, such as antibiotic usage, diet via socioeconomic- and geographic background (Clemente et al., 2012; Glowacki & Martens, 2020; Lozupone et al., 2012).

Generally, the gut microbiome does not change substantially during adult life. Alterations of the gut microbiome (dysbiosis) can occur due to diet variations, disease onset and environmental factors such as antibiotic usage and other medications (Clemente et al., 2012). As an example, a common type 2 diabetes medication, metformin, has been associated with gut microbiota alterations as it increases the proportion of bacteria producing short chain fatty acids (SCFAs) (Vila et al., 2020). As the dysbiosis occurs, the metabolites being able to enter into the blood circulation may also alter (Figure 2.) (Velmurugan et al., 2020). The translocation of metabolites via peripheral blood circulation can affect the GBA via, for example, microbial signals, such as neuroactive metabolites, quorum-sensing molecules and SCFAs (Mayer et al., 2015).

Parkinson's disease is an example of a neurodegenerative disease that has been associated with gut dysbiosis (Baldini et al., 2020). PD is connected to both environmental and genetic factors, and

it can consist of both motor and nonmotor symptoms (Elfil et al., 2020). The non-motor symptoms have been connected to α -synuclein aggregation not only in the brain, but also in the GI tract (Skjærbæk et al., 2021). These symptoms include, for example, bloating and dysphagia. Aggregation in both the gut and brain provides evidence of the connection between GBA and PD (Skjærbæk et al., 2021). Additionally, correlations between constipation and PD onset have been known for several years (Elfil et al., 2020). Fasano et al. (2015) have estimated that up to 60% of Parkinson's disease patients suffered from constipation, which can occur several decades before the actual diagnosis.

Notably, connections between BMI, age and PD have also been a topic of interest in past years, and microbial abundances have in fact been hypothesized to shift in PD cases, illustrating the complexity of PD. Several bacterial families, such as *Bifidobacteriaceae*, *Christensenellaceae*, *Lactobacillaceae* and *Pasteurellaceae* have been associated with PD (Appendix, Table 2). However, the exact mechanisms behind these connections remain rather unclear. Changes in the gut microbiota have been connected to PD also in gender-dependent alterations. Baldini et al. (2020) discovered that *Paraprevotella* abundances can increase significantly in female PD patients when compared to male PD patients whereas there was no significant sex-dependent decrease/increase of abundances in healthy controls.

A way of illustrating the gut microbiome composition in microbiome research consists of different diversity measures, such as α - and β -diversities. The α -diversity can be used to assess the species richness in a single sample whereas the β -diversity assesses the between-sample variation of microbes (Walters & Martiny, 2020). Altered β -diversities have been associated with PD in currently available literature indicating that there are in fact differences among the microbial variation of PD cases and controls (Chiang & Ling, 2019). Generally, most of the current studies have not, however, reported any significant α -diversity variations.

Although new information about the gut microbiomes' connection to PD is constantly arising, larger cohorts with longitudinal study methods are warranted to provide new insights into the research. To date the main challenges in PD related microbiome research stem from the small PD cohorts and from the lack of prospective studies. Follow-up sampling is a lengthy process that demands a lot of effort, costs, and time. To solve this problem, several current publications (Appendix, Table 1) in the given field are cross-sectional, meaning that the gut microbiome of healthy controls (HC) are compared with PD cases at a specific timepoint.

As a result of a long tradition in research, the governmental actors in Finland have succeeded in collecting large, population-based cohorts such as the FINRISK 2002, which surveyed the nationwide health and disease risk factor situation. By these prospective population cohorts, information about the incident PD cases can be obtained instead of just analysing the prevalent PD cases. One clinical potential of understanding the microbiota is the use of bacteria in bacteriotherapy, in which probiotics (beneficial bacteria) could be used as a treatment of PD patients via fecal microbial transplantation (FMT). As a result of these population-level cohorts, one of the first clinical studies for bacteriotherapy is in operation in Finland, where 48 PD patients have been selected to 1 year follow-up, a proof of concept study to investigate the implications of fecal microbial transplantation (Yle, 2021). However, before reaching the full clinical potential within the given topic, further studies are warranted.

Research question and aim

This study intended to summarize which general factors of the gut microbiota are associated with Parkinson's disease. To do so, the FINRISK 2002 database was used to explore potential relationships between cases of incident Parkinson's disease and microbiota data in these patients. The aim of this study was to improve our understanding of the importance of gut microbiome for human health and create a basis for future studies on the topic.

Materials and Methods

Study sample

The FINRISK 2002 survey (n=7231) represents a prospective population cohort study that has been conducted to obtain information about the health of the Finnish population (Borodulin et al., 2018). The FINRISK health surveys have been conducted between 1972-2012, with a new independent sampling conducted every 5 years. Participants for the study were 25-74-year old (mean=49,5 years) Finnish people, from five geographic regions in Finland. Metagenomic results were obtained through untargeted shallow shotgun sequencing (Illumina Hi-Seq 400) from the stool samples that individuals took at home in 2002 according to the given instructions. The microbiome sequencing was executed in the University of California San Diego (2017) after the stool samples had been stored at -20°C in Finland (Salosensaari et al., 2020). Borodulin et al. (2018) have explained the FINRISK study methods in detail.

Ethical considerations

The FINRISK 2002 study has been approved by Joint Municipal Board for the Hospital District of Helsinki and Uusimaa Ethics Committee for Epidemiology and Public Health, Statement #87/2001, Dnro 558/E3/2001, final acceptance 2001-12-19. The study was conducted following the ethical principles of the Declaration of Helsinki and all analysis data sets were anonymized. Conclusions from this study will have no impact on the treatment of the persons who donated their data. The basic FINRISK study cohort data can be requested through the Biobank of the Finnish Institute for Health and Welfare (THL), Finland (<https://thl.fi/en/web/thl-biobank/for-researchers/application-process>). The access to the metagenomic data is still limited.

Exclusion of observations

The exclusion criteria for these analyses of the FINRISK 2002 data included the following parameters: (1) Pregnant women (n=40), (2) recent (within 6 months) antibiotics usage (n=907), (3) prevalent Parkinson's disease (n=167) and (4) prevalent cancer (n=256). By these criteria, the initial sample size (n=7231) was reduced to 5933 samples in total. The full gastrointestinal biodiversity data consisted of three different taxonomic subcategories including species-, genus- and phylum-level data.

Descriptive statistics

All statistical analyses were performed using R v.3.6.0 (R Foundation for Statistical Computing, 2017) and executed at the bacterial genus-level if not mentioned otherwise. As a first step, the baseline characteristics of the incident PD cases were compared with healthy controls. Mean values and standard deviations (SD) were compared by using 2-sample t-tests for continuous variables and χ^2 -tests for categorical variables. The microbiome core data was obtained by the exclusion criteria of 0.001% in detection of the genome for bacterium and 0.5% in prevalence at this stage in order to minimize possible rare microbes as false positives and hence affecting the results. The core data was then used for the general trends plotting and for comparing the general features of the gut microbiome composition among incident PD cases and controls. Although the data was not completely normally distributed (as an example, see Appendix, Figure 1), e.g. t-tests and linear regression models are rather robust to deviations from normality when sample sizes are large (Lumley et al., 2002).

General structure of the taxa and α -diversity

The bacterial α -diversity (within-sample diversity) was assessed through Shannon's and Simpson's diversity indexes and the results were additionally confirmed with Chao1 richness estimate which further highlights the rarest species. These univariate indexes were calculated by the 2-sample t-tests and p-values used to assess the differences between PD cases and controls. Multivariate linear regression model was then used to test the association of α -diversity with PD,

using baseline age and gender as covariates for both Shannon's and Simpson's diversity indexes as well as for the Chao1 richness estimate. R package *Microbiome* (Lahti & Shetty, 2019) was used for the α -diversity calculations.

β -diversity and regression analysis

The Bray-Curtis dissimilarity matrix was used to assess the β -diversity (between-sample variation) of the species-level data. The resulting matrix of β -dissimilarities was used to analyse the dissimilarity of PD cases and controls of the FINRISK 2002 cohort. The Bray-Curtis dissimilarity was assessed by the packages *Vegan* (Oksanen et al., 2020) and *Phyloseq* (McMurdie & Holmes, 2013) in R. As for the regression analysis, generalized linear model (GLM) was used to analyse the relationship between the abundances of bacterial species and PD cases. To do so, the core criteria was altered to 0.1% detection of the genome for bacterium and 10 % prevalence. Core data was used to exclude the rarest species which are possibly false positives in the data. Baseline age and gender were used as covariates for this multivariate analysis.

Results

To begin with, the mean values and percentages of the FINRISK 2002 participants were calculated for incident PD cases and controls (Table 1). Statistically significant differences were observed for the variable's hypertension ($p= 0.0046$), diabetes ($p=0.0033$) and baseline age ($p<0.001$) between PD cases and controls.

A total of 2019 different taxa among six different taxonomic ranks was observed in the data consisting of 5933 samples. The core data was used to observe differences in the gut microbiome composition among PD and healthy controls. Figure 2. shows the general taxa composition of gut microbiome for these two groups. By far, the most abundant genus in both groups was *Bacteroidetes*, which seemed to have slightly decreased abundance in PD subgroup when compared with control. Additionally, the *Eubacterium* genus was increased in PD cases in comparison with controls, although the graph does not assess the statistical significance. In total, both PD cases and controls seem to share the same general composition of the gut microbiome composition with some deviations.

Table 1. Baseline characteristics of the FINRISK 2002 participants after the exclusion criteria were applied. Means and standard deviations (SD) were calculated for continuous variables whereas variables that are categorical were presented as percentages for incident PD cases and controls. BMI indicates body mass index.

Variable	Incident PD n=105	Control n=5828	p-value	Test
Women, N (%)	51 (48.6%)	3091 (53.0%)	0.418	(2)
Men, N (%)	54 (51.4%)	2737 (47.0%)	(for sex)	(2)
Hypertension, N (%)	66 (62.6%)	2823 (48.4%)	0.0046	(2)
Smoker, N (%)	22 (21.0%)	1386 (23.8%)	0.560	(2)
Coronary heart disease, N (%)	14 (13.3%)	563 (9.6%)	0.275	(2)
Diabetes, N (%)	27 (25.7%)	867 (14.9%)	0.0033	(2)
Baseline age (mean, SD)	57.6 (9.6)	49.0 (12.8)	<0.001	(1)
BMI (mean, SD)	28.0 (4.7)	27.0 (4.4)	0.142	(1)

(1) = t-test

(2) = χ^2 -test

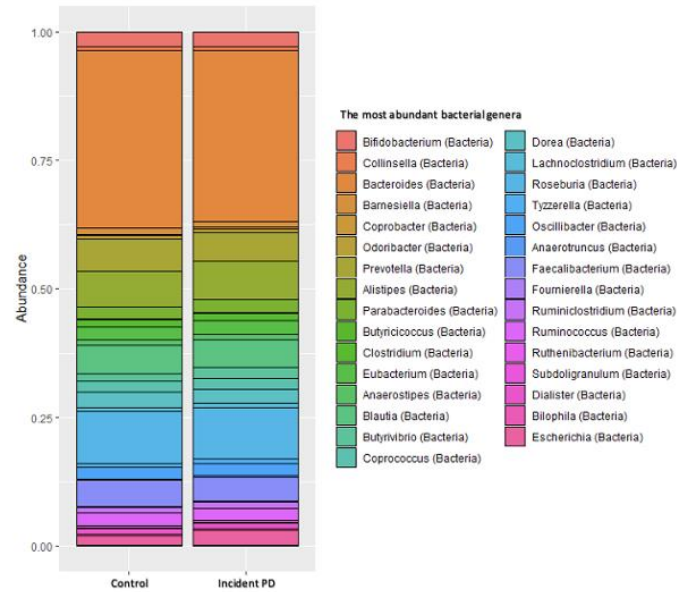


Figure 2. The most abundant bacterial genera (detection=0.001, prevalence=0.5) being detectable in PD/Control samples in the FINRISK 2002 dataset.

No significant differences were found for α -diversities among PD cases and controls. Shannon's diversity index p-values were all above the significance level (species $p=0.62$, genus $p=0.63$, phylum $p=0.73$) and Simpson's followed the same trend (species $p=0.68$, genus $p=0.71$, phylum $p=0.84$). As for the Chao1, the p-values were also above the cut-off criteria of 0.05 (species $p=0.44$, genus $p=0.46$, phylum $p=0.87$). Additionally, the multivariate tests did not yield any statistically significant results when age and gender were used as covariates (Shannon $p=0.29$, Simpson $p=0.42$, Chao1 $p=0.20$). As for the α -diversity distribution among females and males, there seems to be slight differences although this study could not confirm these results by any statistical significances (Figure 3).

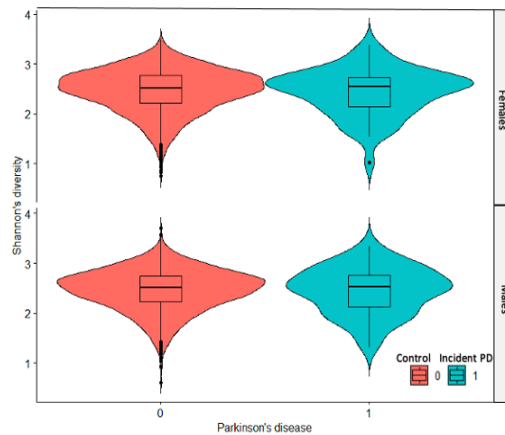


Figure 3. The α -diversity distribution among incident PD patients ($n=105$) and controls ($n=5828$) in the FINRISK 2002 dataset. The violin plots represent Shannon's diversity and illustrate also the shape of the distribution. Red colour corresponds to controls and blue to PD cases.

The β -diversity of the subgroups PD and controls was assessed through the Bray-Curtis β -dissimilarity matrix (Figure 4). Although the distributions seemed to share the same general trends, it could be observed that the Bray-Curtis β -dissimilarity seemed to be slightly higher for females when compared between the two genders. Hence, this study suggests that there might be differences in β -dissimilarities among different genders although additional studies are warranted to confirm these results.

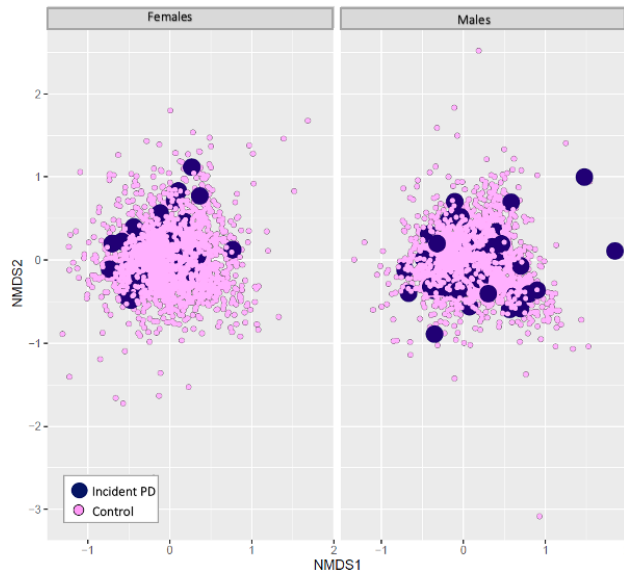


Figure 4. β -dissimilarity (Bray-Curtis) matrix among the incident PD cases and controls in species-level bacterial data.

As a last step, GLM was used to interpret associations between PD and the compositional bacterial taxa. Regression analyses provide information about the relationship among the independent and dependent variables and hence, in this case could be used to find associations between incident PD cases and bacterial abundances. Generalized linear model is a more flexible method when compared to the usual linear regression as it does not expect any normal distribution of the variables and overall allows more flexible modelling. By the GLM, ten bacterial species reached the nominal significance of $p < 0.05$ (Table 2.) and on genus-level, the most significant taxa included *Ruminococcus*, *Cellulomonas*, *Akkermansia*, *Faecalibacterium*, *Lactobacillus* and *Eubacterium* in the given order.

Table 2. The results of the generalized linear model, in which bacterial species and their associations to PD were analysed. Only the species that were found to be associated with PD are shown in the table with nominal p-value of < 0.05 .

Species	P value
<i>E. hallii</i>	0.0025
<i>B. cellulosilyticus</i>	0.0147
<i>C. fastidiosus</i>	0.0222
<i>C. carbonis</i>	0.0304
<i>A. muciniphila</i>	0.0336
<i>E. eligens</i>	0.0370
<i>P. goldsteinii</i>	0.0380
<i>F. prausnitzii</i>	0.0381
<i>C. comes</i>	0.0403
<i>P. bivia</i>	0.0450

Discussion

Main findings

As a main finding, this study suggests that there could be gender-dependent differences between the incident PD cases and controls that are detectable in the Bray-Curtis β -dissimilarity matrix. Additionally, correlation between hypertension, diabetes and age were observed among PD cases when compared with controls. Overall, in consensus with previous publications (Appendix, Tables 1&2), we suggest that the gut microbiome composition may be associated with Parkinson's disease and hence should be studied at a broader range to create a basis for potential clinical applications.

Data

Generally, there is a lack of prospective studies of the gut microbiome's effect on PD and this study could, in fact, only identify two previous publications that were conducted with follow-up of ca. 2 years (Appendix, table 1, publications 19 & 21). Furthermore, differences among research conditions, such as sample collection, cohort sizes and geographical locations affect the composition of gut microorganisms and thus cannot be ignored in analyses (Glowacki & Martens, 2020). Hence, all factors that could have affected the normal gut microbiome were excluded from this study. Antibiotics usage has been reported to be linked with alterations in the host metabolism and reduction in the species richness in gut, hence being able to affect the overall results (Ramirez et al., 2020; Zhang & Chen, 2019). Additionally, Ternáka et al. (2020) have reported on positive correlation between PD prevalence and the usage of certain antibiotics such as penicillinase resistant penicillin and narrow spectrum penicillin. Furthermore, as the gut microbiome composition for pregnant women and cancer patients are still topics of ongoing research and interest, the prevalent cancer patients and pregnant women were excluded from the data as well as prevalent PD cases.

Differences in sampling and sequencing methods can also affect the microbiome results (Thursby & Juge, 2017). In the current literature, the most samples are by far taken as stool samples and 16S ribosomal sequencing used as a sequencing method (Appendix, Table 1, Sample(s)). The FINRISK 2002 data has been sequenced by shallow shotgun metagenomic sequencing (Illumina Hi-Seq 400) whereas nearly all available publications regarding the topic up to date are sequenced by 16S rRNA sequencing. Although the shallow shotgun metagenomic sequencing is rather cost-effective and it provides in most cases more accurate results than 16s rRNA sequencing, it can also result to unevenness and systematic biases (Aird et al., 2011). These types of alterations in the system can make the sequencing results confounded and hence have an effect on the overall results. Generally, the shallow sequencing provides more specific and effective sequencing method for metagenomic data even if deep whole-metagenome shotgun (WMS) sequencing would be the ideal option, although not affordable in most cases (Hillmann et al., 2018).

Notably, Mihaila and colleagues (2019) identified changes of several new bacterial taxa in saliva of PD patients and in fact, The Human Microbiome Project Consortium (2012) has identified much larger changes in the α -diversities of saliva compared with the same diversity within stool samples. Hence, other sampling areas (such as saliva & blood) could be used in the future to obtain results at even more boarder scale. This could partly be an explanation for the novel findings for Mihaila et al. (2019) as their study was the only one conducted on saliva changes.

Parkinson's disease and gut microbiota – general factors

The most abundant bacterial genera in the gut of healthy individuals include two major phyla; *Bacteroidetes* and *Firmicutes* (Sonnenburg & Bäckhed, 2016). The results obtained from the composition analysis (Figure 2.) supports this ratio, as the major genera observed were *Bacteroides*, *Prevotella* and *Roseburia* for both PD cases and controls. Furthermore, all of the main genera presented in the composition analysis have been reported in previous studies to be associated with PD (Appendix, Tables 1&2). Generally, the two groups (PD/controls) seemed to share the same general composition of the gut microbiome with some slight alterations.

The abundance of *Bacteroidetes* seemed to be slightly decreased in PD cases whereas the *Eubacterium* abundances could be identified to be increased in PD when compared with controls. However, these results are rather preliminary and should be further confirmed with statistical analyses before drawing any final conclusions.

Furthermore, it is rather noteworthy to point out that the composition analysis was executed with core data, out of which the rarest species were removed to keep this study applicable. This might have affected the results of this analysis, if those removed species would be the most essential ones regarding PD. Although the analyses by just using the core genera is slightly more effective,

by excluding microorganisms, essential metabolic pathways can be overlooked in follow-up studies and the most significant microbes associated with PD can be ignored. Hence, in the following research efforts, more attention needs to be paid to the identification of individual species that can affect the prevalence and/or severity of PD. Additionally, it is important to keep in mind that as the FINRISK 2002 sequencing results have been compared against National Center for Biotechnology Information RefSeq database (version 82; May 8, 2017), some microbes and their genes can still be unrecognized yet until the reference database updates to include these microbes. Hence, further prospective studies and analyses are needed to assess the overall picture of microbiomes association with PD.

α - and β -diversities

The α -diversity results obtained by this study are mostly in agreement with the currently available literature as no significant differences in the α -diversities between PD cases and controls were found (Figure 3.). Only a few studies (Keshavarzian et al., 2015; Qian et al., 2018 (a)) have reported increased α -diversities among PD cases and just one (Petrov et al., 2017) publication reported on decreased α -diversity among PD cases. By far, most available publications have not found any significant differences in the species richness (Gerhardt & Mohajeri, 2018). Although no significant differences in α -diversities could be found between PD and control groups in the current study, it is worth mentioning that the α -diversities tended to have slightly different distributions among males and females (Figure 3.), although statistical significance could not be observed. Simpson's diversity index and Chao1 richness estimate was used in addition to Shannon's aiming to identify more rare taxa, but none of these results were statistically significant.

In the currently available literature several studies have reported on alterations in the β -diversities between PD cases and controls (Bedarf et al., 2017; Heinz-Buschart et al., 2018; Hill-Burns et al., 2017; Li et al., 2017; Lin et al., 2018; Petrov et al., 2017; Scheperjans et al., 2015; Qian et al., 2017 (a)). The Bray-Curtis β -dissimilarity seemed to differ among females and males in the current study (Figure 4.), although the results could not be confirmed statistically at this stage due to lack of time. Interestingly, males have been previously reported to be at greater risk for developing PD although the results of this study could not identify any between-sample variation for males (Cerri et al., 2019). Instead, the variation seemed to be significant for females. Baldini et al., 2020 has highlighted some gender-dependent alterations for PD cases, such as decreased *Paraprevotella* abundances for females. Hence, one potentially interesting aspect regarding future work could be the gender-dependent changes in gut microbiome and its effect on PD.

Alterations in the gut microbiome

Generalized linear model (GLM) is a method of finding associations between, e.g., the relative abundances of taxa and the nonlinear variables of interest – in this case, PD. GLM allows the use of covariates, such as baseline age and gender to be used for this specific analysis (Lu et al., 2019). As the reported nominal p-values from the GLM are <0.05 for ten species, follow-up analyses are needed to confirm the best testing method and to see the assumption that GLM works in this case and is the best method to be applied. Hence, instead of concluding that the bacterial species and genera stated above are associated with PD, it is more accurate to say that there could be association between these microorganisms, but further analyses are needed.

Generally, the most prevalent and cited bacterial families being associated with PD include *Lachnospiraceae*, *Bifidobacteriaceae*, *Verrucomicrobiaceae*, *Ruminococcaceae* and *Streptococcaceae* (Appendix, Figure 3.) and the results obtained from the GLM in the current study are in line with these earlier results (Appendix, Tables 1&2). In the species-level dataset the results included several bacterial species that have been connected with PD in previous publications, including *E. hallii*, *C. comes*, *A. muciniphila*, *E. eligens* and *P. bivia* (Bedarf et al. 2017; Hill-Burns et al., 2018). Out of these five species, *E. hallii*, *C. comes* and *E. eligens* belong to the *Lachnospiraceae* family. Bedarf et al. (2017) have associated *E. hallii* and *E. eligens* with the PD symptom severity on

Unified Parkinson's disease rating scale (UPDRS III) and in another independent study decreased abundances of *C. comes* have been linked to Crohn's disease (Gevers et al., 2014). Generally, several publications have reported on alterations in the *Lachnospiraceae* family and its connection to PD (Appendix, Figure 2).

Additionally, increased abundances of *A. muciniphila* have been connected to PD as *A. muciniphila* have been shown to be over-represented in PD faeces in comparison with healthy controls (Bedarf et al., 2017; Haikal et al., 2019; Hill-Burns et al., 2018; Li et al., 2020). Lastly, out of the bacterial species found to be associated with PD, Wallen et al. (2021) have connected increased levels of *P. bivia* to PD. Additionally, other independent publications have associated the *Bacteroidaceae* family to PD, although mostly in decreased abundances (Lin et al., 2019; Minato et al., 2017; Petrov et al., 2017). This work could not find other species in current literature reported to be associated with PD.

As a last noteworthy point to bring up is the omission of other microorganisms than bacteria from the data. This means that the focus of the given study was kept solely on bacteria, which could have resulted in the elimination of other important microorganisms present in the human gut. Although bacteria currently seems to be the most studied taxa and yield to several novel findings, other microorganisms should be further analyzed as well. As an example, Mihaila et al. (2019) have reported on findings of phage and yeast in PD cases in addition to bacteria, such as increased levels of *Candida albicans*, *Candida dubliniensis*, and *Saccharomyces cerevisiae*. Additionally, decreased virus abundances have been linked to PD (Bedarf et al., 2017). Although the bacterial composition in the gut exceeds greatly the number of archaea being present, especially methane-producing archaea have also been found to be enriched in controls in a study conducted by Romano et al. in 2021. Hence, in the future, larger scale of microorganisms should be studied to get a better understanding of the overall microbial composition that can affect the PD.

Contribution to society

Parkinson's disease is one of the most serious neurovegetative diseases worldwide and new clinical treatments are continuously warranted (Mihala et al., 2018). Deeper understanding of the gut microflora can open new clinical applications to the treatment of the disease. Hence, to the best of our understanding, the findings of this study can provide a base to continue future research focusing on the bacterial species highlighted in this study. Additionally, the FINRISK 2002 database includes a wide range of data and as a contribution to society, the current study has collated a great amount of data to continue the research with. Lastly, the resolution of the healthy gut microflora was increased by the current study and helped to deepen our understanding of the overall picture of the impact of the gut microflora.

Ethical aspects

The treatment of the FINRISK 2002 survey participants that donated their data will not be based or altered due to this study. All of the samples and answers to the questionnaires were given voluntarily and with knowledge of the FINRISK 2002 surveys aim (Borodulin et al., 2018). To be sure of this, all participants signed consent that their data can be used for the research regarding chronic diseases and their genetic and environmental risk factors (Borodulin et al., 2018). The papers referred in this study were chosen without prior knowledge of the gender of the authors.

Future plans

As for the future plans, few things are worth highlighting to be focused from here on. To start with, the regression analysis needs to be further adjusted to include competing risk analysis to see whether mortality from other causes earlier in life has been able to mask the incidence of PD. Proportional hazards models such as Cox's model could be one application for this aspect. Additionally, in the ideal future work no taxa would be discriminated, such as in this study, where only bacteria are represented. Other sample collection sites and deep metagenomic sequencing

could also provide more accurate results in the future research efforts. Furthermore, larger population cohorts with better statistical power, and replication in other cohorts are warranted (Appendix, Figure 2). Additionally, in the future, one aspect of interest could be to focus even more to the expression of the genes that gut microorganisms harbour and to analyse their functional ability to secrete bioactive chemicals.

Strengths & limitations

As for the main strength of this study, the sample size of this work is one of the largest in current literature. By far, most of the current studies in the given field are cross-sectional, making this study one of the rarest studies as this work is a prospective study with large sample size. However, even though the sample size is large, all of the samples are taken from Finnish population and hence this study does not represent larger scale of geographical variation. Additionally, unlike for many other studies, sequencing of fecal samples was conducted by shallow shotgun metagenomic sequencing, enabling species-level data to be assessed instead of only genus-level alterations as in 16s rRNA sequencing. As a drawback, possible PCR biases could have affected the sequencing results and hence PCR optimizations are important to be recalled in the following research efforts (Sato et al., 2019). Additionally, it is worth mentioning that the stool samples have been stored at a rather long time (-20°C, 15 years) and this could possibly have had an effect on the taxonomical results. However, other studies that have assessed the same sequencing results have not found any deterioration of the data (Palmu et al., 2020; Ruuskanen et al., 2021).

Conclusions

As the main conclusion of this study, we report that gut microbiome composition may be associated with Parkinson's disease as seen in the gender-based β -dissimilarity matrix (Figure 4.). Other factors affecting PD include hypertension, diabetes and more obviously, age. Gender-based differences in the gut microbiome composition could play significant role in the PD development and further studies of the given topic are warranted. As preliminary results, it can be stated that gender might affect the β -diversity among PD cases and controls and hence should be taken into account in clinical applications. Several bacterial species that this study found to be associated with PD are also presented in published literature, further confirming that these preliminary results are steps towards the right direction.

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Appendix

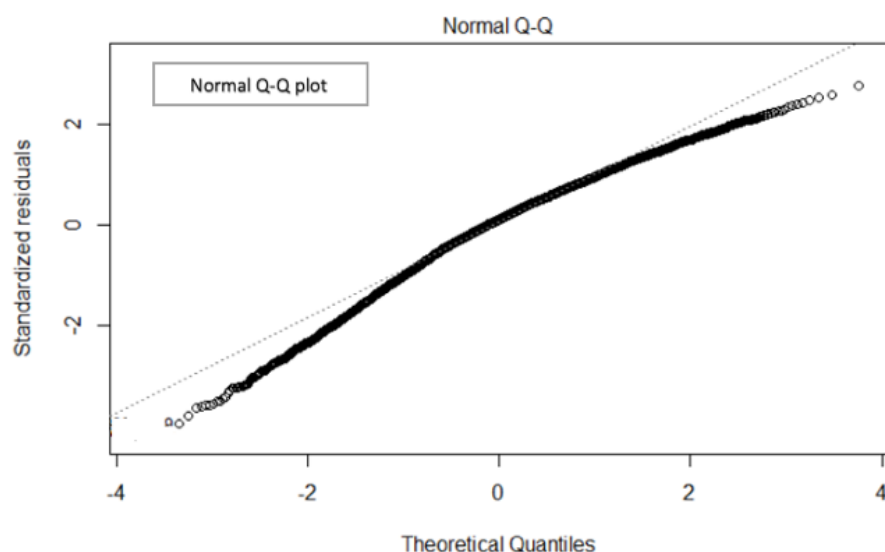


Figure 1. An example of the distribution of the data. The linear model shows the distribution between Parkinson's disease and Shannon's alpha index. No significant outliers were detected, and the data was treated as almost normally distributed.

Table 1. Summary of the latest publications in the field of gut microbiomics that this study could identify.

Publica- tion	Main result(s)	Alteratio n	Associ ation	PD/HC	Sample(s)
1. Barichella et al., 2019	<i>Lactobacillus</i> <i>Lactobacillaceae</i> (F) <i>Roseburia</i> (G), <i>Lachnospiraceae</i> (F) <i>Christensenellaceae</i> (F)	(G), ↑ ↓ ↑	M M NM	237/113 (193 idiopathic PD, 22 PSP, 22 MSA), cross- sectional study	Fecal samples, 16S gene ribosomal RNA sequencing

2.	Bedarf et al., 2017	<i>Akkermansia muciniphila</i> (S), <i>Akkermansia</i> (G)	↑	–	31/28 (all males) (early-stage PD + L-DOPA-naïve), cross-sectional study	Stool samples, shotgun sequencing	
		<i>Alistipes shahii</i> (S), <i>Rikenellaceae</i> (F)	↑				
		<i>Prevotella copri</i> (S), <i>Prevotellaceae</i> (F)	↓				
		<i>Eubacterium bifforme*</i> (S), <i>Eubacteriaceae</i> (F)	↓				
		<i>Clostridium saccharolyticum</i> (S), <i>Clostridiaceae</i> (F)	↓				
3.	Heintz-Buschart et al., 2017	<i>Anaerotruncus spp.</i> (G)	–	M	76/78, cross-sectional study	Stool + nasal wash samples, 16S and 18S ribosomal RNA amplicon sequencing	
		<i>Clostridium XIVa</i>	–	M			
		<i>Lachnospiraceae</i> (F)	–	M			
		<i>Anaerotruncus</i> (G)	–	NM			
		<i>Akkermansia</i> (G)	–	NM			
	<i>OTU_171*</i> , <i>Melainabacteria</i> (G)	↓	–				
4.	Hill-Burns et al., 2018	<i>Bifidobacterium</i> (G), <i>Bifidobacteriaceae</i> (F)	↑	–	197/130, cross-sectional study	Stool samples, 16S gene ribosomal RNA sequencing	
		<i>Lactobacillus</i> (G), <i>Lactobacillaceae</i> (F), <i>Tissierellaceae</i> (F)	↑				
		<i>Akkermansia</i> (G), <i>Verrucomicrobiaceae</i> (F)	↑				
		<i>Christensenellaceae</i> (F)	↑				
		<i>Coprococcus</i> (G), <i>Blauntia</i> (G), <i>Roseburia</i> (G)+unclassified species of <i>Lachnospiraceae</i> (F)	↓				
		<i>Pasteurellaceae</i> (F)	↓				
5.	Hopfner et al., 2017	<i>Barnesiellaceae</i> (F)	↑	–	29/29, cross-sectional study	Stool samples, 16S gene ribosomal RNA sequencing	
		<i>Enterococcaceae</i> (F), <i>Lactobacillaceae</i> (F)	↑				
			↑				
6.	Jin et al., 2019	<i>Bifidobacterium</i> (G), <i>Bifidobacteriaceae</i> (F)	↑	–	72/68 (59 OPD, 13NPD, healthy spouses/family member(s) were used as HC), cross-sectional study	Fecal samples, 16S gene ribosomal RNA sequencing	
		<i>Alistipes</i> (G), <i>Rikenellaceae</i> (F)	↑				
		<i>Catenisphaera</i> (G), <i>Bulleidia</i> (G), <i>Turicibacter</i> (G), <i>Erysipelotrichaceae</i> (F)	↑				S, NM
		<i>Adlercreutzia</i> (G), <i>Coriobacteriaceae</i> (F)	↑				S, NM
		<i>Lactobacillus</i> (G), <i>Lactobacillaceae</i> (F)	↓				S, NM
		<i>Eubacterium</i> (G)	–				+S
		<i>Prevotella</i> (G)	↓				-S, NM
		<i>Lachnospira</i> (G)	–				-S
		<i>Megamonas</i> (G), <i>Megasphaera</i> (G), <i>Veillonellaceae</i> (F)	↑				+S ⁽¹⁾
		<i>Proteus</i> (G), <i>Enterobacteriaceae</i> (F)	↑				

7.	Li et al., 2017	<i>Enterococcus</i> (G),	↑	24/14, cross-sectional study	Fecal samples, 16S gene ribosomal RNA sequencing
		<i>Enterococcaceae</i> (F)			
		<i>Streptococcus</i> (G),	↑		
		<i>Streptococcaceae</i> (F)			
		<i>Ruminococcus</i> (G), <i>Faecalibacterium</i> (G),	↓ +S ⁽²⁾		
<i>Ruminococcaceae</i> (F)					
8.	Li et al., 2020	<i>Blautia</i> (G), <i>Lachnospiraceae</i> (F)	↓	30/30, cross-sectional study	16S gene ribosomal RNA sequencing
		<i>Christensenellaceae</i> (F)	↑		
		<i>Lactobacillus</i> (G), <i>Lactobacillaceae</i> (F)	↑		
		<i>Erysipelatoclostridium</i> (G)	↑		
		<i>Peptoclostridium</i> (G)	↑		
		<i>Alistipes</i> (G), <i>Rikenellaceae</i> (F)	↑		
		<i>Butyricimonas</i> (G), <i>Odoribacteraceae</i> (F)	↑		
		<i>Bifidobacterium</i> (G)	↑		
		<i>Akkermansia</i> (G), <i>Verrucomicrobiaceae</i> (F)	↑		
		<i>Lachnospiraceae</i> (F)	↓ NM		
9.	Lin et al., 2018	<i>Bifidobacteriaceae</i> (F)	↑	75/45, cross- sectional study, ≤5 years of PD (n=44), ≥5 years of PD (n=30)	Fecal samples, 16S gene ribosomal RNA sequencing
		<i>Rikenellaceae</i> (F)	↑ (≥5years of PD)*		
		<i>Deferribacteraceae</i> (F)	↑(≥5years of PD)*		
		<i>Ruminococcaceae</i> (F)	- M		
		<i>Verrucomicrobia</i> (P)	↑		
10.	Lin et al., 2019	<i>Mucispirillum</i> (G), <i>Deferribacteraceae</i> (F)	↑	80/77, cross- sectional study (Further cytokine associations; 120/120)	Fecal samples, 16S gene ribosomal RNA sequencing
		<i>Parabacteroides</i> (G), <i>Porphyromonas</i> (G), <i>Porphyromonadaceae</i> (F)	↑		
		<i>Lactobacillus</i> (G), <i>Lactobacillaceae</i> (F)	↑		
		<i>Bacteroides</i> (G), -	-		
		<i>Bacteroidaceae</i> (F)			
		<i>Prevotella</i> (G), <i>Prevotellaceae</i> (F)	↓ M		
		<i>Rhodococcus</i> (G), <i>Nocardiaceae</i> (F)	↑		
11.	Mihaila et al., 2019	Yeast; <i>Candida albicans</i> , <i>Candida dubliniensis</i> , and <i>Saccharomyces cerevisiae</i>	↑	48/36, cross-sectional study (Early-stage PD)	Saliva samples, next generation sequencing
		<i>Bifidobacterium</i> (G) spp., <i>Bifidobacteriaceae</i> (F)	↑		
		<i>Lactobacillus</i> (G) spp., <i>Lactobacillaceae</i> (F).	↑		
		<i>Coprococcus eutactus</i> (S), <i>Dorea longicatena</i> (S), <i>Blautia glucerasea</i> (S), <i>Lachnospiraceae</i> (F)	↓		
		<i>Bacteroides dorei</i> (S), <i>Bacteroides massiliensis</i> (S), <i>Bacteroidaceae</i> (F)	↓		

12.	Petrov et al., 2017	<i>Stoquefichus massiliensis</i> (S), <i>Erysipelotrichaceae</i> (F)	↓		30/30, cross-sectional study	Fecal samples, 16S gene ribosomal RNA sequencing				
		<i>Bacteroides coprocola</i> (S)	↓							
		<i>Bacteroides plebeus</i> (S)	↓	–						
		<i>Oscillospira</i> (G)	↑							
		<i>Lactobacillus mucosae</i> (S), <i>Lactobacillus</i> (G)	↑							
		<i>Papillibacter cinnamivorans</i> (S), <i>Ruminococcus bromii</i> (S), <i>Ruminococcaceae</i> (F)	↑							
		<i>Catabacter hongkongensis</i> (S), <i>Catabacteriaceae</i> (F)	↑							
		<i>Christensenella minuta</i> (S), <i>Christensenellaceae</i> (F)	↑							
13.	Pietrucci et al., 2019	<i>Lactobacillaceae</i> (F)	↑		80/72, cross-sectional study	Fecal samples, 16S gene ribosomal RNA sequencing				
		<i>Enterobacteriaceae</i> (F)	↑	+S, M						
		<i>Enterococcaceae</i> (F)	↑							
		<i>Lachnospiraceae</i> (F)	↓	+S, M						
14.	Pietrucci et al., 2020	<i>Alcaligenaceae</i> (F)*	↑		472/374, cross-sectional study	3 different machine learning algorithms, SRA database				
		<i>Veillonellaceae</i> (F)*	↓	–						
15.	Qian et al., 2018 (a)	<i>Clostridium IV</i> (G), <i>Clostridium XVIII</i> (G), <i>Anaerotruncus</i> (G), <i>Butyricoccus</i> (G), <i>Clostridiaceae</i> (F)	↑		45/45, cross-sectional study (Healthy spouses were used as HC)	Fecal samples, 16S ribosomal RNA gene sequencing				
		<i>Aquabacterium</i> (G), <i>Comamonadaceae</i> (F)	↑	M ⁽³⁾						
		<i>Sphingomonas</i> (G), <i>Sphingomonadaceae</i> (F)	↑							
		<i>Escherichia/Shigella</i> (G), <i>Enterobacteriaceae</i> (F)	↑	-DD						
		<i>Isoptericola</i> (G), <i>Promicromonosporaceae</i> (F)	↑	+DD						
		<i>Cloacibacterium</i> (G), <i>Flavobacteriaceae</i> (F)	↑	+DD						
16.	Qian et al. 2018 (b)	<i>Enhydrobacter</i> (G), <i>Vibrionaceae</i> (F)	↑		45/45, cross- sectional study (Healthy spouses were used as HC)	Blood samples * 16S ribosomal RNA (rRNA) sequencing				
		<i>Microbacterium</i> (G), <i>Microbacteriaceae</i> (F)	↑							
		<i>Limnobacter</i> (G), <i>Burkholderiaceae</i> (F)	↓							
		<i>Paludibacter</i> (G), <i>Paludibacteraceae</i> (F)	–	+DD						
		<i>Saccharofermentans</i> (G)	–	+DD						
		<i>Janibacter</i> (G), <i>Intrasporangiaceae</i> (F)	–	M						
		<i>Nocardioides</i> (G), <i>Nocardioideaceae</i> (F)	–	M						
		<i>Pseudonocardia</i> (G), <i>Pseudonocardiaceae</i> (F)	–	M						
		17.		<i>Prevotellaceae</i> (F)			↓	M	72/72,	Fecal samples, 16S

	Scheperjans et al., 2015	<i>Enterobacteriaceae</i> (F)	↑	M	cross-sectional study	ribosomal RNA gene sequencing
18.	Weis et al., 2019	<i>Faecalibacterium Ruminococcaceae</i> (F)	(G), ↓	NM	34/25, cross-sectional study	Fecal samples, 16S ribosomal RNA gene sequencing
		<i>Fusicatenibacter Lachnospiraceae</i> (F)	(G), ↓			
		<i>Peptoniphilus Clostridiaceae</i> (F)	(G), ↑			
		<i>Clostridiales family XI</i>	↑			
		<i>Prevotella</i> (G), <i>Prevotellaceae</i> (F)	–	M		
19.	Aho et al., 2019	<i>Puniceicoccaceae</i> (F)*	–		64/64, prospective study, duration of 2.25 years	Stool samples, 16S gene ribosomal RNA sequencing
		<i>Faecalibacterium Ruminococcaceae</i> (F)	(G), ↑		38/34	Sigmoid mucosal biopsies + fecal samples, 16S gene ribosomal RNA sequencing
20.	Keshavarzian et al., 2015	<i>Ralstonia</i> (G), <i>Ralstoniaceae</i> (F)	↑	–		
		<i>Blautia</i> (G), <i>Coprococcus</i> (G), <i>Roseburia</i> (G)	↓			
		<i>Lachnospiraceae</i> (F)				
21.	Minato et al., 2017	<i>Bifidobacterium Bifidobacteriaceae</i> (F)	(G), ↓	M, NM	36PD/28PD prospective study, duration of 2 years	Stool samples, 16S gene ribosomal RNA sequencing
		<i>Bacteroides fragilis Bacteroidaceae</i> (F)	(S), ↓	M, NM		
		<i>Atopobium Coriobacteriaceae</i> (F)	(G), –	M		
		<i>Faecalibacterium Ruminococcaceae</i> (F)	(G), ↓			
		<i>Butyricoccaceae</i> (F)*	↓			

PD/HC corresponds to Parkinson's disease/Healthy Controls

PSP=Progressive Supranuclear Palsy

MSA=Multiple System Atrophy

OPD= >1 year of PD symptoms

NPD=new PD patients

M = Significant association with motor symptoms

NM = Significant association with non-motor symptoms

S = Significant association with disease severity (+yes, -no)

– No data

DD = disease duration (+yes, -no)

*=primary finding of the given study

↑= increased abundance in PD samples when compared with HC

↓ =decreased abundance in PD samples when compared with HC

(1)= Significant association with disease severity in the genus *Megasphaera*

(2)= Significant association with disease severity in the genus *Faecalibacterium*

(3)= Significant neg. association with disease duration in the genus *Escherichia/Shigella*

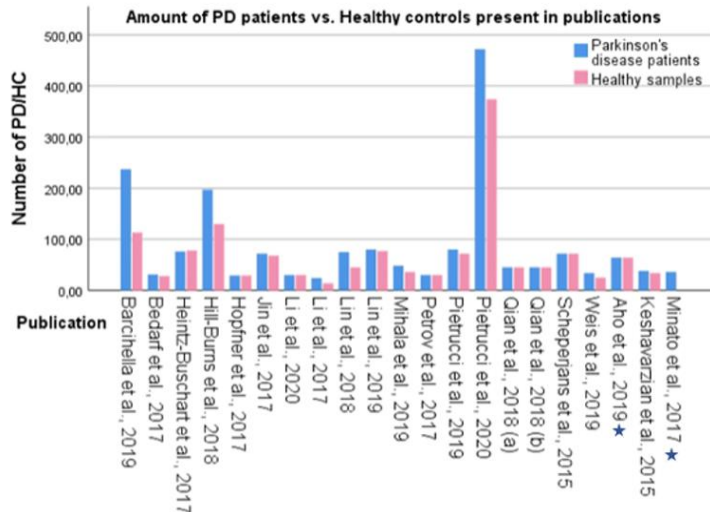


Figure 2. Sample sizes of the currently available publications. The sample sizes of these studies are marked as follows: (a) blue=Parkinson’s disease cohort and (b) pink = healthy controls. The blue stars indicate prospective studies. Publications with no stars are cross-sectional.

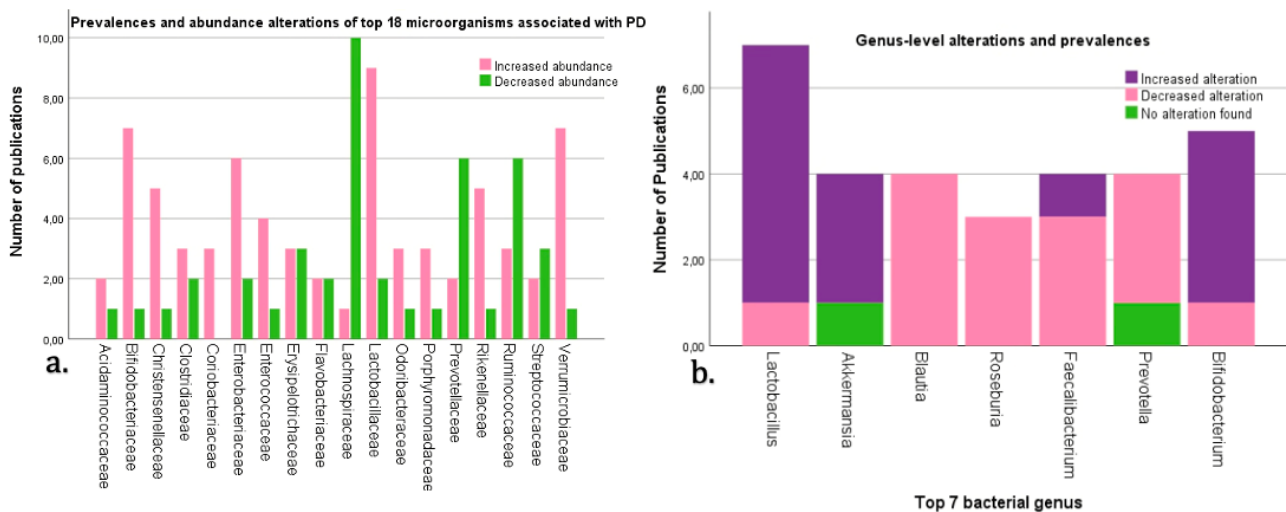


Figure 3. A) The most commonly reported microbes that are associated with PD. These top 18 microorganisms are selected from the current literature this study could identify. Inclusion criteria include that the microorganism have been referred to be associated with PD in at least three independent studies. Several microbes were presented in at least three studies and by highest number *Lactobacillaceae* was reported to be connected to PD by 10 separate studies. B) the corresponding genus-level alterations are presented by the same cut-off criteria.

Table 2. The bacterial families that were found to be altered in PD when compared with age-matched healthy controls. Literature numbers can be seen from Table 1 (Appendix).

Phylum	Family	Alteration in PD	Literature
Firmicutes	<i>Acidaminococcaceae</i>	↓↑	6, 7, 11
Actinobacteria	<i>Actinomycetaceae</i>	↓	14
Proteobacteria	<i>Aeromonadaceae</i>	↓	14
Proteobacteria	<i>Alcaligenaceae</i>	↑	14
Proteobacteria	<i>Alcanivoracaceae</i>	↑	14
Firmicutes	<i>Bacillaceae</i>	↓↑	11
Bacteroidetes	<i>Bacteroidaceae</i>	↓↑	12, 14, 21
Bacteroidetes	<i>Barnesiellaceae</i>	↓↑	5, 14

Actinobacteria	<i>Bifidobacteriaceae</i>	↓↑	1, 4, 8, 9, 11, 12, 14, 22
Proteobacteria	<i>Bradyrhizobiaceae</i>	—	
Proteobacteria	<i>Brucellaceae</i>	↑	11
Proteobacteria	<i>Burkholderiaceae</i>	↓↑	14, 16
Firmicutes	<i>Caldicoprobacteraceae</i>	↑	14
Proteobacteria	<i>Campylobacteraceae</i>	↓	11, 14
Firmicutes	<i>Catabacteriaceae</i>	↑	12
Proteobacteria	<i>Caulobacteraceae</i>	↓	14
Firmicutes	<i>Christensenellaceae</i>	↓↑	1, 4, 6, 8, 12, 14
Firmicutes	<i>Clostridiaceae</i>	↓↑	2, 6, 14, 15, 18
Proteobacteria	<i>Comamonadaceae</i>	↓↑	14, 15
Actinobacteria	<i>Coriobacteriaceae</i>	↑	1, 7, 14
Actinobacteria	<i>Corynebacteriaceae</i>	↓	14
Deferribacteres	<i>Deferribacteraceae</i>	↑	9, 10
Proteobacteria	<i>Desulfovibrionaceae</i>	↓↑	10, 14
Proteobacteria	<i>Enterobacteriaceae</i>	↓↑	1, 6, 7, 11, 13, 14, 15, 16, 17
Firmicutes	<i>Enterococcaceae</i>	↓↑	5, 7, 10, 13, 14
Firmicutes	<i>Eubacteriaceae</i>	↓	2
Firmicutes	<i>Erysipelotrichaceae</i>	↓↑	2, 6, 7, 8, 12, 14
Bacteroidetes	<i>Flavobacteriaceae</i>	↓↑	11, 14, 16
Fusobacteria	<i>Fusobacteriaceae</i>	↓	14
Actinobacteria	<i>Intrasporangiaceae</i>	—	
Firmicutes	<i>Lachnospiraceae</i>	↓↑	1, 4, 6, 7, 9, 12, 13, 14, 18, 20, 22
Firmicutes	<i>Lactobacillaceae</i>	↓↑	1, 4, 5, 6, 8, 10, 11, 12, 13, 14, 22
Fusobacteria	<i>Leptotrichiaceae</i>	—	
Euryarchaeota	<i>Methanobacteriaceae</i>	↓	14
Proteobacteria	<i>Methylobacteriaceae</i>	↑	11, 22
Actinobacteria	<i>Microbacteriaceae</i>	↑	11, 16
Firmicutes	<i>Mogibacteriaceae</i>	↑	14
Proteobacteria	<i>Moraxellaceae</i>	↑	7, 14
Actinobacteria	<i>Nocardiaceae</i>	↑	11
Bacteroidetes	<i>Odoribacteraceae</i>	↑	6, 8, 10, 14
Firmicutes	<i>Oscillospiraceae</i>	—	
Proteobacteria	<i>Oxalobacteraceae</i>	↑	14
Bacteroidetes	<i>Paludibacteraceae</i>	—	
Proteobacteria	<i>Pasteurellaceae</i>	↓↑	4, 14
Bacteroidetes	<i>Paraprevotellaceae</i>	↓	14
Firmicutes	<i>Peptococcaceae</i>	↑	14
Firmicutes	<i>Peptostreptococcaceae</i>	↑	14
Bacteroidetes	<i>Porphyromonadaceae</i>	↓↑	1, 6, 10, 14
Bacteroidetes	<i>Prevotellaceae</i>	↓↑	2, 4, 6, 7, 10, 12, 14, 17
Actinobacteria	<i>Promicromonosporaceae</i>	↑	11, 16
Actinobacteria	<i>Propionibacteriaceae</i>	—	
Proteobacteria	<i>Pseudomonadaceae</i>	↓	14
Actinobacteria	<i>Pseudonocardiaceae</i>	—	
Verrucomicrobia	<i>Puniceicoccaceae</i>	—	
Proteobacteria	<i>Ralstoniaceae</i>	↑	20
Proteobacteria	<i>Rhizobiaceae</i>	↓	11
Proteobacteria	<i>Rhodobacteraceae</i>	↑	14
Bacteroidetes	<i>Rikenellaceae</i>	↓↑	2, 6, 8, 9, 14
Firmicutes	<i>Ruminococcaceae</i>	↓↑	4, 6, 7, 12, 14, 18, 20, 22
Ascomycota	<i>Saccharomycetaceae</i>	↑	11
Firmicutes	<i>Staphylococcaceae</i>	↑	14
Firmicutes	<i>Streptococcaceae</i>	↓↑	6, 7, 11, 14
Proteobacteria	<i>Succinivibrionaceae</i>	↓	14
Synergistetes	<i>Synergistaceae</i>	↓	14
Firmicutes	<i>Tissierellaceae</i>	↓↑	4, 14

Firmicutes	<i>Turicibacteraceae</i>	↑	6, 14
Firmicutes	<i>Veillonellaceae</i>	↓↑	7, 10
Verrucomicrobia	<i>Verrucomicrobiaceae</i>	↓↑	2, 3, 4, 8, 10, 14, 22
Proteobacteria	<i>Vibrionaceae</i>	↑	11, 16
Lentisphaeria	<i>Victivallaceae</i>	↑	14
Proteobacteria	<i>Xanthomonadaceae</i>	↑	14

↑= increased abundance in PD samples when compared with HC

↓ =decreased abundance in PD samples when compared with HC

↑↓= bidirectional alteration, i.e. some genus/species within the family were decreased while others were increased.