Chronic condition diagnosis is associated with minimal disruption to the gut microbiome

Melissa Solem

ABSTRACT

The gut microbiome consists of the microbial cells living in the digestive tract. While the effects of specific chronic conditions on the composition of the gut microbiome is an area of active research, the impact of any chronic condition diagnosis has not yet been examined. We hypothesized that diagnosis with a chronic condition would be associated with a significant reduction in gut microbiome diversity. Using a previously published dataset, we compared the composition of fecal samples obtained from healthy subjects and subjects with a chronic condition. Although relative abundance of various bacterial taxa remained relatively constant across disease statuses, those of Proteobacteria and Actinobacteria were significantly reduced in subjects with a chronic condition. No significant differences in alpha or beta diversity were observed between disease statuses. Our results indicate that there is no reduction in gut microbial diversity common to all chronic conditions. This conclusion indicates that studies examining the way in which a specific chronic condition, or a group of related conditions, impacts the microbiome would likely yield the most insight for clinical or translational applications.

INTRODUCTION

Chronic conditions and their prevalence: Chronic conditions significantly impact an individual's daily living for a duration of at least one year and/or require ongoing medical treatments (Gregory & Coleman-Jensen, 2017). 2014 estimates indicate that 60% and 42% of Americans have one or multiple chronic conditions, respectively (Buttorff et al., 2017). Common chronic conditions include hypertension, coronary heart disease, hepatitis, stoke, cancer, asthma, diabetes, arthritis, and many others (Gregory & Coleman-Jensen, 2017). Currently, most studies comparing individuals with any one or more chronic conditions and those with no chronic conditions investigate financial costs and quality of life (Heyworth et al., 2009; Paez et al., 2009); very few studies compare clinical data between healthy and general disease states, limiting our understanding of sweeping, medically-significant differences between diseased and healthy individuals.

The microbiome and chronic conditions: The microbiome is defined as the genomic content of the trillions of microbial cells living within or on the human body; its composition is unique to each individual (Ursell et al., 2012). The microbiome has been implicated in several physiologically important processes, including metabolic and immune functions (Shreiner et al., 2015). The gut microbiome is the body's most diverse microbiota; its constitution varies greatly between healthy adults due to the influence of the host's genome, diet, social conditions, and other factors (Goodrich et al., 2014; Vangay et al., 2018; Wu et al., 2011).

Relative to healthy controls, changes in the microbiome's composition (dysbiosis) are implicated in a variety of chronic conditions, including diabetes, Alzheimer's disease, rheumatoid arthritis,

and Chron's disease (Cheng et al., 2022; Gevers et al., 2014; Lambeth et al., 2015; Ling et al., 2020). Although these conditions' effects on the gut microbiome vary, a general trend of reduced alpha diversity has been observed in patients of different chronic conditions including type II diabetes, Parkinson's disease, and Alzheimer's disease, relative to healthy controls (Li et al., 2020; Ling et al., 2020; Petrov et al., 2017). Additionally, studies of inflammatory bowel disease, generalized anxiety disorder, and obesity indicate that the gut microbiomes of patients with the chronic condition of interest more closely associate with other patients than with healthy controls (Imhann et al., 2018; Jiang et al., 2018; Palmas et al., 2021). Despite these advances in the understanding of gut microbiome alterations in specific chronic conditions, the diversity and structure of the gut microbiota have not yet been compared between general disease (i.e., any one or more chronic conditions) and healthy states; by identifying alterations characteristic of the diseased microbiome, we have the potential to better characterize the healthy gut microbiome and understand how it is modified in the context of disease.

Experimental purpose and design: This study aims to identify alterations of the gut microbiome in patients with one or more chronic conditions, relative to patients with no self-reported chronic conditions. 16S taxonomic data and clinical metadata presented in a previously-published dataset (Huttenhower et al., 2012) will be analyzed, allowing us to examine if patterns of dysbiosis are associated with the presence of any chronic condition. We hypothesize that chronic conditions will associate with a decrease in the alpha diversity of the gut microbiome. We expect the gut microbiome compositions of patients with one or more chronic conditions to more closely associate with other chronic condition patients than with healthy controls. This work will provide a novel comparison of the gut microbiomes of patients with and without any

chronic condition, allowing for an enhanced understanding of how the gut microbiome is altered by chronic disease.

METHODS

Data Collection and OTU Picking: Clinical data and fecal samples were collected, processed, sequenced, and taxonomically profiled as described by the Human Microbiome Project (HMP) (Huttenhower et al., 2012). 16S sequences from the HMP dataset were assigned to an operational taxonomic unit (OTU) identifier and were clustered at a 97% identity threshold, as described (Huttenhower et al., 2012).

Pre-processing: Using QIIME2 (2018.6, Bolyen et al., 2019) we rarefied this OTU table to a depth of 3000. From this rarefied table, Shannon's diversity and Bray-Curtis dissimilarity indices were calculated using QIIME2 (2018.6, Bolyen et al., 2019). Data was loaded into and subset in the statistical package RStudio (2023.6.0.421, Posit team, 2023). Clinical metadata was subset so that samples with chronic condition listed as "NA" were removed. The rarefied OTU table, Shannon's diversity values, and Bray-Curtis dissimilarity values were processed to only contain values corresponding to samples contained in the clinical metadata.

Data Analysis: Data analysis was performed in R (4.3.0 (2023-04-21), R Core Team, 2023) and RStudio (2023.6.0.421, Posit team, 2023). Data was analyzed for normality using the Shapiro-Wilk Normality Test, returning p-values of 0.1853 and 4.352×10^{-5} for samples with and without a chronic condition, respectively. Because one of these values is less than 0.1, the data was analyzed via nonparametric statistical tests. Data was analyzed using Mann-Whitney U tests or

PERMANOVA, with each figure's relevant statistical test and n-values listed in the corresponding legend. The accepted level of significance was $p \leq 0.05$.

The Shannon alpha diversity index values of patients with and without one or more chronic conditions were analyzed using a Mann-Whitney U test. Boxplots were generated using the "ggplot2" package (Wickham, 2016). P-values were added to the figure using Google Drawings.

Taxa summaries were created for patients with and without one or more chronic conditions using the "ggplot2" package (Wickham, 2016). Through inspection, we chose to compare the relative abundances of Proteobacteria and Actinobacteria between these patient populations. For each taxon, the relative abundance values of the groups were analyzed using a Mann-Whitney U test. Bar plots were generated using the "ggplot2" package (Wickham, 2016). P-values were added to figures using Google Drawings.

Weighted UniFrac PCoA metrics (a measure of beta diversity) of the condition status groups were analyzed using the "ape" package (Paradis & Schliep, 2019), which was used to calculate principal coordinate vectors. The "vegan" package (Oksanen et al., 2022) was used to run a PERMANOVA analysis on this data. A PCoA plot was generated using the "ggplot2" package (Wickham, 2016). P-values were added to figures using Google Drawings.

RESULTS

Alpha diversity of the gut microbiome did not significantly differ with chronic condition status. We first examined if changes in microbial species richness within a sample is associated with the presence of one or more chronic conditions. We found that there is no statistically significant difference in alpha diversity (as quantified by Shannon's diversity index) between

individuals with (n = 60) and without (n = 340) a chronic condition (Mann-Whitney U test, p = 0.5421, Fig. 1).

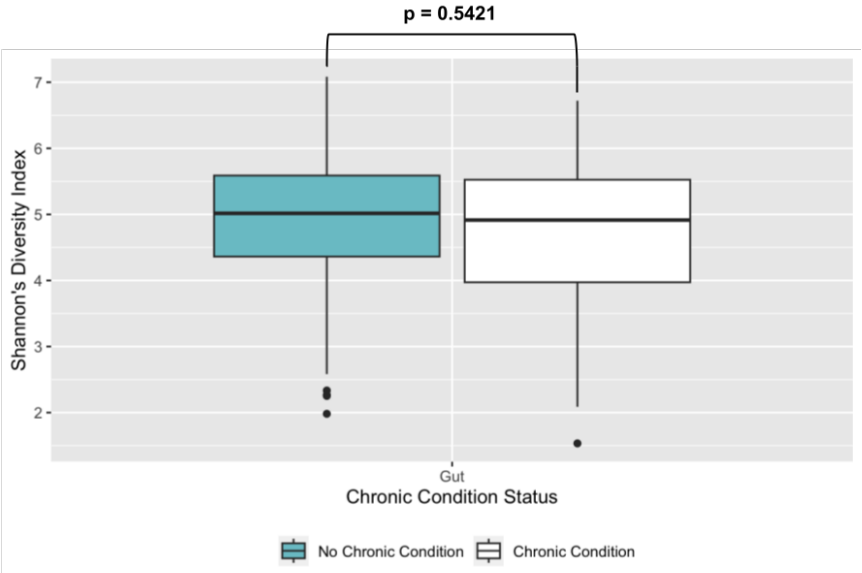
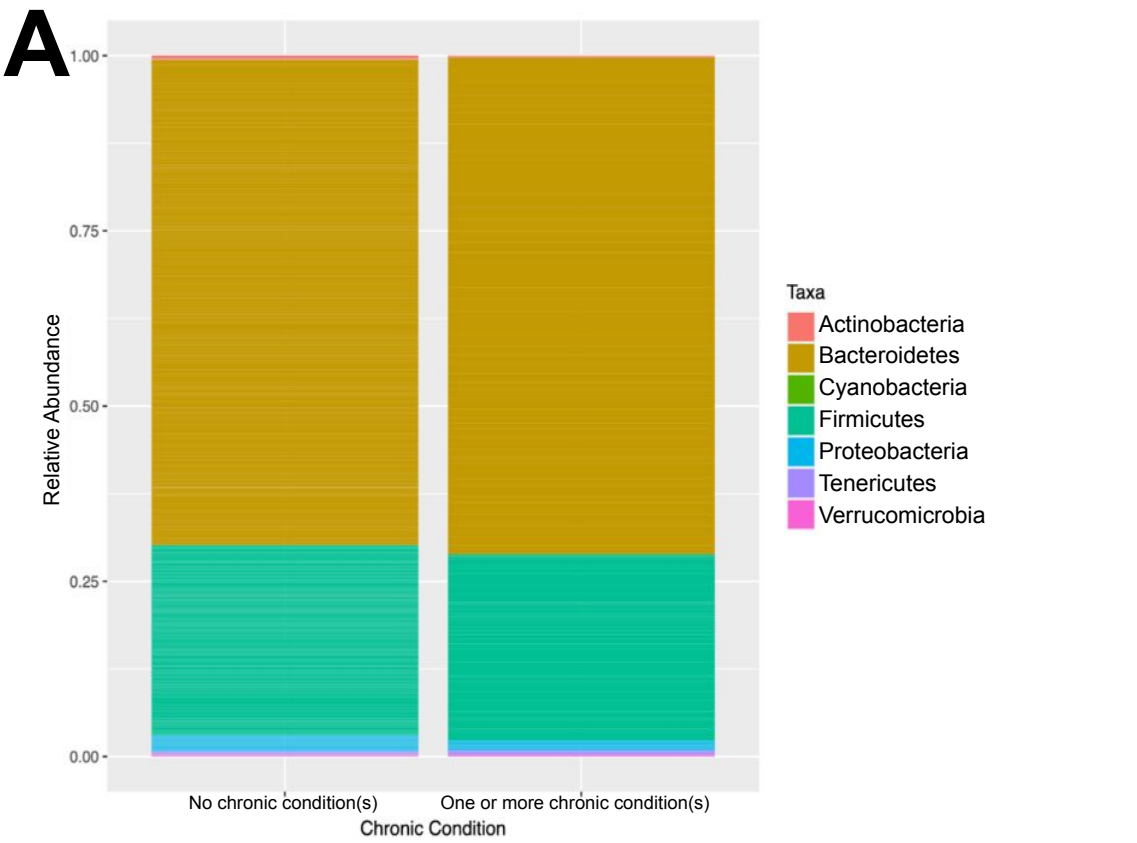


Figure 1: Alpha diversity was not impacted by chronic condition status. No significant difference is observed in alpha diversity of the gut microbiomes of individuals with and without chronic condition(s). Shannon's diversity index of fecal samples obtained from patients with and without a chronic condition (Mann-Whitney U test, n = 340 [no chronic condition], n = 60 [chronic condition], p = 0.5421). Blue and white bars represent patients without and with a chronic condition, respectively. Solid bars across boxplots represent median value, whiskers represent median ± 1.5*Interquartile-range, and dots represent outliers.

Gut microbiome composition did not differ with chronic condition status. We next investigated if gut microbiome taxonomic composition is significantly altered between patients with and without one or more chronic condition(s). We examined the relative abundances of various bacterial phylum of stool samples collected from patients with (n = 60) and without (n = 340) chronic condition(s) (Fig. 2A). Through inspection, we chose to analyze the relative

abundances of Proteobacteria and Actinobacteria between samples derived from these patient populations. We found that the relative abundance of Proteobacteria is significantly higher in patients without a chronic condition than those obtained from patients with a chronic condition (Mann-Whitney U test, n = 340 [no chronic condition], n = 60 [chronic condition], $p < 2.2 \times 10^{-16}$, Fig. 2B). The relative abundance of Actinobacteria is also significantly higher in samples taken from these patient populations (Mann-Whitney U test, n = 340 [no chronic condition], n = 60 [chronic condition], $p < 2.2 \times 10^{-16}$, Fig. 2B).


A
1.00
0.75
0.50
0.25
0.00
Relative Abundance
No chronic condition(s)
One or more chronic condition(s)
Chronic Condition
Taxa
Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Proteobacteria
Tenericutes
Verrucomicrobia

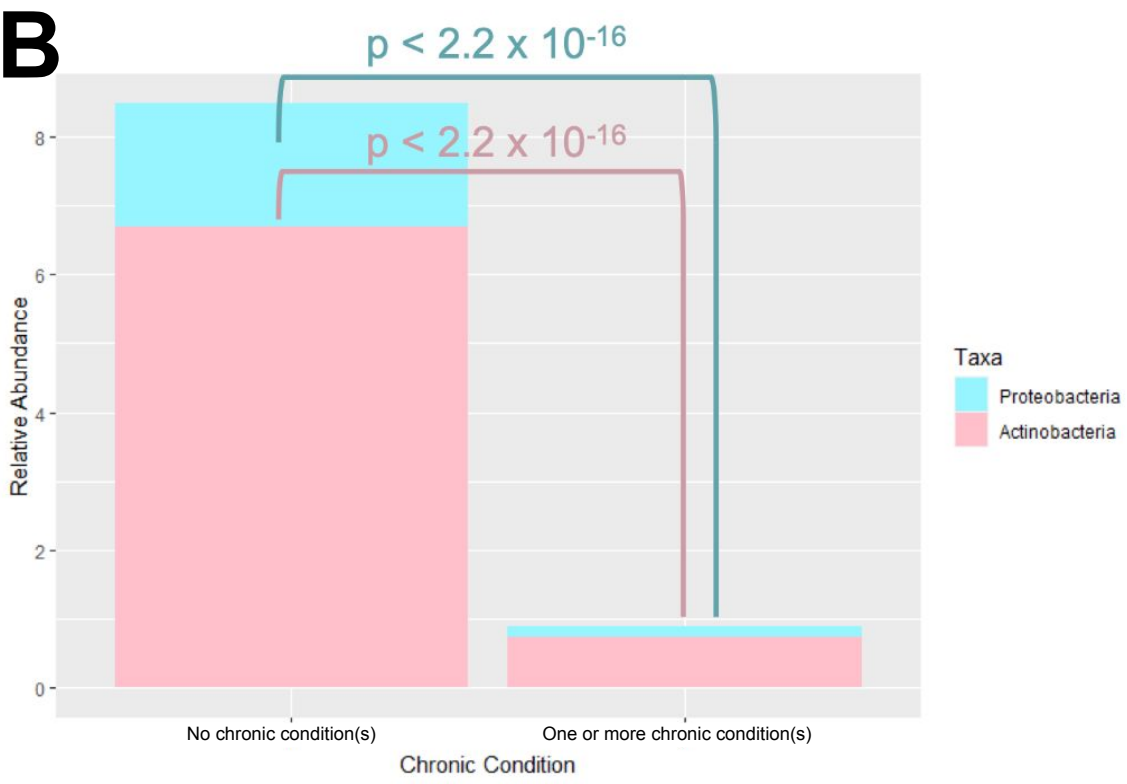

B
p < 2.2 x 10-16
p < 2.2 x 10-16
8
6
4
2
0
Relative Abundance
No chronic condition(s)
One or more chronic condition(s)
Chronic Condition
Taxa
Proteobacteria
Actinobacteria

Figure 2: Relative abundances of Proteobacteria and Actinobacteria significantly decrease with chronic condition(s). (A) Taxa summary comparing the prevalence of bacterial phylum from stool samples derived from patients with and without a chronic condition diagnosis. **(B)** Relative abundances of Proteobacteria ($p < 2.2 \times 10^{-16}$) and Actinobacteria ($p < 2.2 \times 10^{-16}$) were significantly lower in the gut of patients with one or more chronic conditions, relative to patients with no chronic conditions (Mann-Whitney U test, n = 340 [no chronic condition], n = 60 [chronic condition]).

Beta diversity does not significantly differ with chronic condition status. We next compared the diversity between samples of different chronic condition status. To determine the difference in gut microbiome composition between patients with (n = 60) and without (n = 340) one or more chronic condition(s), we analyzed the beta diversity. A PCOA plot was generated using the weighted UniFrac dissimilarity metric (Fig. 3). Only 0.11% of the dissimilarity observed in the gut microbiomes of patients with and without a chronic condition is explained by chronic condition status, indicating that beta diversity is not significantly altered in the presence of a chronic condition (PERMANOVA, $R^2 = 0.0011$, $p = 1.0 \times e^{-12}$, Fig. 3).

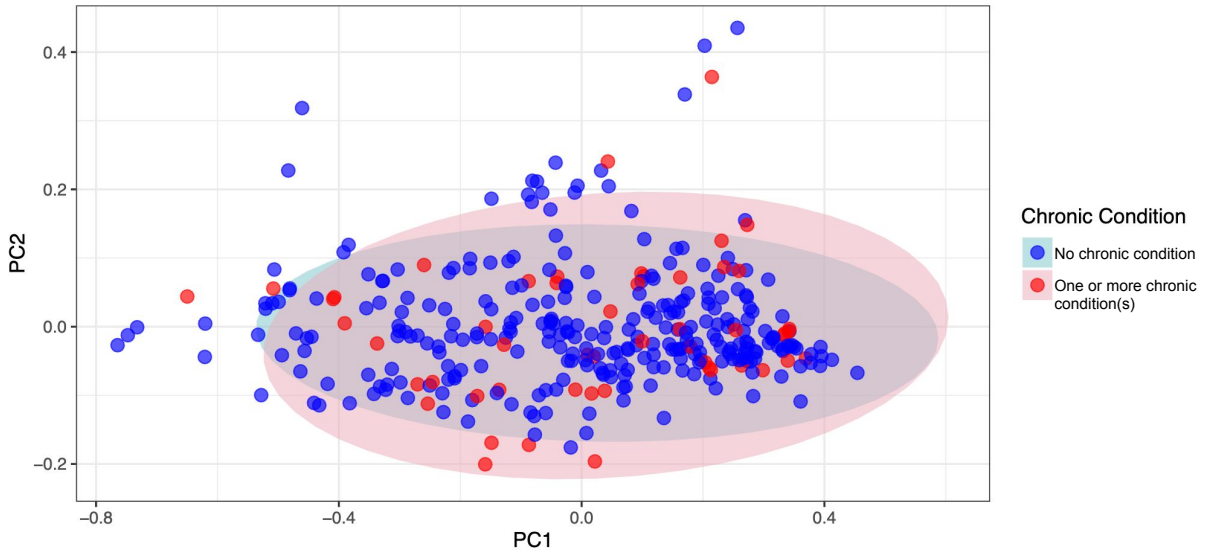


Figure 3: Chronic condition status is not a strong predictor of the dissimilarity observed in the gut microbiome between patients with and without a chronic condition diagnosis. The beta diversity of the gut microbiome is displayed in a weighted UniFrac PCoA grouped by chronic condition status (PERMANOVA, n = 340 [no chronic condition], n = 60 [chronic condition], $R^2 = 0.0011$, $p = 1.0 \times 10^{-12}$). Samples derived from patients without and with a chronic condition are represented by red and blue, respectively. Normal confidence ellipsoids are shown for each group.

DISCUSSION

Multiple studies have concluded that specific chronic conditions, including latent autoimmune diabetes, gastric cancer, Alzheimer's disease, and rheumatoid arthritis, are associated with significant changes to the gut microbiome (Fang et al., 2021; Liu et al., 2022; Ling et al., 2020; Yu et al., 2022). However, the impact of diagnosis of any chronic condition has not yet been examined. Here, we compared fecal samples (Huttenhower et al., 2012) obtained from patients with and without a diagnosis of one or more chronic condition. Our results indicate no significant

differences in the alpha diversity of samples corresponding to these disease statuses (Fig. 1). Although the relative abundances of microbial phylum remained relatively constant between condition statuses (Fig. 2A), those of Proteobacteria and Actinobacteria were significantly reduced in subjects with a chronic condition (Fig. 2B). Only 0.11% of the dissimilarity observed in the gut microbiomes of patients with and without a chronic condition is explained by chronic condition status (Fig. 3), indicating that chronic condition status is not associated with any distinct compositional patterns. Our results indicate that, although changes in relative abundance of specific taxa may be observed, diagnosis with any chronic condition does not result in any significant reductions of gut microbial diversity.

Recent research implicates dysbiosis of the gut microbiome in a myriad of specific chronic conditions; however, prior to our study, the literature lacked an analysis investigating how the gut microbiome in the presence of any chronic condition differs from one of a healthy individual. Our findings provide a novel characterization of the comprehensive impact of a myriad of chronic conditions on the human gut microbiome, relative to “healthy” controls. Our results indicated that relative abundance of Actinobacteria was significantly higher in control samples than those obtained from patients with chronic disease. Interestingly, decreases in Bifidobacteria, a class of the Actinobacteria phylum, have been associated to chronic inflammation of the gut, highlighting a potential role of the phylum in gastric disease (Binda et al., 2018). In samples obtained from patients with a chronic condition, we also observed a decrease in Proteobacteria relative abundance. Surprisingly, increases in gut microbiome Proteobacteria have been associated with several intestinal and extraintestinal diseases, including chronic gut inflammation, heart disease, and asthma (Rizzatti et al., 2017). We therefore conclude that, while Proteobacteria dysbiosis may be characteristic of specific chronic

conditions, it does not serve as a biomarker for all such diseases. As we determined that no other significant differences exist between the healthy and chronic condition gut microbiome, we conclude that gut microbiome research should examine the impacts of a specific chronic condition or a group of related conditions, as these targeted studies would likely yield the most insight for clinical or translational applications.

If repeated, this study could yield more insight if performed with a more detailed dataset. Despite providing a very useful and large set of human microbiome samples, the Human Microbiome Project's dataset (Huttenhower et al., 2012) contained limited patient health history data. The dataset did not specify the types and number of chronic conditions a patient was diagnosed with. The original publication did not specify which conditions were considered chronic in the context of the study; when the study's lead author was contacted to clarify this distinction, no response was provided. These unknowns made it difficult to ascertain the effect of multiple chronic conditions on gut microbial diversity, nor understand the effects of specific chronic conditions.

Additionally, the nature of patient-reported medical histories lends some uncertainty to the accuracy of the information provided. It is possible that patients may not have accurately reported a chronic condition diagnosis due to social stigmas surrounding certain conditions. For example, among HIV-positive injection-drug users, socially desirable response tendency was determined to correlate with the accuracy of self-reported HIV serostatus. This study suggests that social stigmas may discourage HIV-positive individuals from accurately disclosing their disease status (Latkin & Vlahov, 1998). Additionally, some patients may have not received a formal diagnosis of a chronic condition and were therefore falsely grouped with control ("healthy") participants. For example, chronic obstructive pulmonary disease (COPD) is

associated with changes in the gut microbiome (Bowerman et al., 2020), yet it remains underdiagnosed in many communities (Diab et al., 2018). It is possible that other common chronic conditions were not diagnosed in individuals in the study, potentially leading trends to remain unidentified.

Although we recommend that investigations into the impact of chronic conditions on the microbiome center on one condition or a group of related conditions, there remains potential benefits of understanding how the "healthy" microbiome compares to the diseased microbiome. To continue the investigation into the impact of diagnosis of any chronic condition on the gut microbiome, we recommend that three groups are compared: patients with no chronic condition, patients with one chronic condition, and patients with more than one chronic condition. This may provide a more nuanced analysis than was provided in this paper. Researchers could also investigate the impact of chronic conditions on other microbiomes of the body or examine the interaction of chronic condition status and gender on microbial diversity. Through understanding the impact of chronic conditions on the microbiome, therapeutic strategies targeting microbial dysbiosis may be developed to alleviate symptoms associated with these conditions, improving quality of life for millions of patients.

Code Availability: All code used in this project is contained in a GitHub repository (https://github.umn.edu/kram0247/Group10_3004_F23).

Acknowledgements: I would like to thank Natalie Bennett and Dr. Maxwell Kramer for their assistance with code development. I acknowledge Benito Antonio Martínez Ocasio (Bad Bunny) for providing me motivation while coding and writing the manuscript. I acknowledge the

Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resource that contributed to results reported in this work. URL: https://www.msi.umn.edu

REFERENCES

Binda, C., Lopetuso, L. R., Rizzatti, G., Gibiino, G., Cennamo, V., & Gasbarrini, A. (2018). Actinobacteria: A relevant minority for the maintenance of gut homeostasis. *Digestive and Liver Disease*, *50*(5), 421–428. https://doi.org/10.1016/j.dld.2018.02.012

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), Article 8. https://doi.org/10.1038/s41587-019-0209-9

Bowerman, K. L., Rehman, S. F., Vaughan, A., Lachner, N., Budden, K. F., Kim, R. Y., Wood, D. L. A., Gellatly, S. L., Shukla, S. D., Wood, L. G., Yang, I. A., Wark, P. A., Hugenholtz, P., & Hansbro, P. M. (2020). Disease-associated gut microbiome and metabolome changes in patients with chronic obstructive pulmonary disease. *Nature Communications*, *11*(1), Article 1. https://doi.org/10.1038/s41467-020-19701-0

Buttorff, C., Ruder, T., & Bauman, M. (2017). *Multiple Chronic Conditions in the United States*. RAND Corporation. https://www.rand.org/pubs/tools/TL221.html

Cheng, M., Zhao, Y., Cui, Y., Zhong, C., Zha, Y., Li, S., Cao, G., Li, M., Zhang, L., Ning, K., & Han, J. (2022). Stage-specific roles of microbial dysbiosis and metabolic disorders in

rheumatoid arthritis. *Annals of the Rheumatic Diseases*, *81*(12), 1669–1677. https://doi.org/10.1136/ard-2022-222871

Diab, N., Gershon, A. S., Sin, D. D., Tan, W. C., Bourbeau, J., Boulet, L.-P., & Aaron, S. D. (2018). Underdiagnosis and Overdiagnosis of Chronic Obstructive Pulmonary Disease. *American Journal of Respiratory and Critical Care Medicine*, *198*(9), 1130–1139. https://doi.org/10.1164/rccm.201804-0621CI

Fang, Y., Zhang, C., Shi, H., Wei, W., Shang, J., Zheng, R., Yu, L., Wang, P., Yang, J., Deng, X., Zhang, Y., Tang, S., Shi, X., Liu, Y., Yang, H., Yuan, Q., Zhai, R., & Yuan, H. (2021). Characteristics of the Gut Microbiota and Metabolism in Patients With Latent Autoimmune Diabetes in Adults: A Case-Control Study. *Diabetes Care*, *44*(12), 2738–2746. https://doi.org/10.2337/dc20-2975

Gevers, D., Kugathasan, S., Denson, L. A., Vázquez-Baeza, Y., Van Treuren, W., Ren, B., Schwager, E., Knights, D., Song, S. J., Yassour, M., Morgan, X. C., Kostic, A. D., Luo, C., González, A., McDonald, D., Haberman, Y., Walters, T., Baker, S., Rosh, J., … Xavier, R. J. (2014). The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host & Microbe*, *15*(3), 382–392. https://doi.org/10.1016/j.chom.2014.02.005

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J. T., Spector, T. D., Clark, A. G., & Ley, R. E. (2014). Human Genetics Shape the Gut Microbiome. *Cell*, *159*(4), 789–799. https://doi.org/10.1016/j.cell.2014.09.053

Gregory, C. A., & Coleman-Jensen, A. (Eds.). (2017). *Food Insecurity, Chronic Disease, and Health Among Working-Age Adults*. https://doi.org/10.22004/ag.econ.261813

Heyworth, I. T., Hazell, M. L., Linehan, M. F., & Frank, T. L. (2009). How do common chronic conditions affect health-related quality of life? *British Journal of General Practice*, *59*(568), e353–e358. https://doi.org/10.3399/bjgp09X453990

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., Creasy, H. H., Earl, A. M., FitzGerald, M. G., Fulton, R. S., Giglio, M. G., Hallsworth-Pepin, K., Lobos, E. A., Madupu, R., Magrini, V., Martin, J. C., Mitreva, M., Muzny, D. M., Sodergren, E. J., … The Human Microbiome Project Consortium. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*(7402), Article 7402. https://doi.org/10.1038/nature11234

Imhann, F., Vila, A. V., Bonder, M. J., Fu, J., Gevers, D., Visschedijk, M. C., Spekhorst, L. M., Alberts, R., Franke, L., Dullemen, H. M. van, Steege, R. W. F. T., Huttenhower, C., Dijkstra, G., Xavier, R. J., Festen, E. A. M., Wijmenga, C., Zhernakova, A., & Weersma, R. K. (2018). Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. *Gut*, *67*(1), 108–119. https://doi.org/10.1136/gutjnl-2016-312135

Jiang, H., Zhang, X., Yu, Z., Zhang, Z., Deng, M., Zhao, J., & Ruan, B. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *Journal of Psychiatric Research*, *104*, 130–136. https://doi.org/10.1016/j.jpsychires.2018.07.007

Lambeth, S. M., Carson, T., Lowe, J., Ramaraj, T., Leff, J. W., Luo, L., Bell, C. J., & Shah, V. O. (2015). Composition, Diversity and Abundance of Gut Microbiome in Prediabetes and Type 2 Diabetes. *Journal of Diabetes and Obesity*, *2*(3), 1–7. https://doi.org/10.15436/2376-0949.15.031

Latkin, C. A., & Vlahov, D. (1998). Socially desirable response tendency as a correlate of accuracy of self-reported HIV serostatus for HIV seropositive injection drug users. *Addiction*, *93*(8), 1191–1197. https://doi.org/10.1046/j.1360-0443.1998.93811917.x

Li, Q., Chang, Y., Zhang, K., Chen, H., Tao, S., & Zhang, Z. (2020). Implication of the gut microbiome composition of type 2 diabetic patients from northern China. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-62224-3

Ling, Z., Zhu, M., Yan, X., Cheng, Y., Shao, L., Liu, X., Jiang, R., & Wu, S. (2020). Structural and Functional Dysbiosis of Fecal Microbiota in Chinese Patients With Alzheimer's Disease. *Frontiers in Cell and Developmental Biology*, *8*, 634069. https://doi.org/10.3389/fcell.2020.634069

Liu, D., Zhang, R., Chen, S., Sun, B., & Zhang, K. (2022). Analysis of gastric microbiome reveals three distinctive microbial communities associated with the occurrence of gastric cancer. *BMC Microbiology*, *22*(1), 184. https://doi.org/10.1186/s12866-022-02594-y

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., … Weedon, J. (2022). *vegan: Community Ecology Package*. https://CRAN.R-project.org/package=vegan

Paez, K. A., Zhao, L., & Hwang, W. (2009). Rising Out-Of-Pocket Spending For Chronic Conditions: A Ten-Year Trend. *Health Affairs*, *28*(1), 15–25. https://doi.org/10.1377/hlthaff.28.1.15

Palmas, V., Pisanu, S., Madau, V., Casula, E., Deledda, A., Cusano, R., Uva, P., Vascellari, S., Loviselli, A., Manzin, A., & Velluzzi, F. (2021). Gut microbiota markers associated with

obesity and overweight in Italian adults. *Scientific Reports*, *11*(1), Article 1. https://doi.org/10.1038/s41598-021-84928-w

Paradis, E., & Schliep, K. (2019). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*, 526–528. https://doi.org/10.1093/bioinformatics/bty633

Petrov, V. A., Saltykova, I. V., Zhukova, I. A., Alifirova, V. M., Zhukova, N. G., Dorofeeva, Yu. B., Tyakht, A. V., Kovarsky, B. A., Alekseev, D. G., Kostryukova, E. S., Mironova, Yu. S., Izhboldina, O. P., Nikitina, M. A., Perevozchikova, T. V., Fait, E. A., Babenko, V. V., Vakhitova, M. T., Govorun, V. M., & Sazonov, A. E. (2017). Analysis of Gut Microbiota in Patients with Parkinson's Disease. *Bulletin of Experimental Biology and Medicine*, *162*(6), 734–737. https://doi.org/10.1007/s10517-017-3700-7

Posit team. (2023). *RStudio: Integrated Development Environment for R*. Posit Software, PBC. http://www.posit.co/

R Core Team. (2023). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rizzatti, G., Lopetuso, L. R., Gibiino, G., Binda, C., & Gasbarrini, A. (2017). Proteobacteria: A Common Factor in Human Diseases. *BioMed Research International*, *2017*, e9351507. https://doi.org/10.1155/2017/9351507

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. *Current Opinion in Gastroenterology*, *31*(1), 69–75. https://doi.org/10.1097/MOG.0000000000000139

Ursell, L. K., Metcalf, J. L., Parfrey, L. W., & Knight, R. (2012). Defining the Human Microbiome. *Nutrition Reviews*, *70*(Suppl 1), S38–S44. https://doi.org/10.1111/j.1753-4887.2012.00493.x

Vangay, P., Johnson, A. J., Ward, T. L., Al-Ghalith, G. A., Shields-Cutler, R. R., Hillmann, B. M., Lucas, S. K., Beura, L. K., Thompson, E. A., Till, L. M., Batres, R., Paw, B., Pergament, S. L., Saenyakul, P., Xiong, M., Kim, A. D., Kim, G., Masopust, D., Martens, E. C., … Knights, D. (2018). US Immigration Westernizes the Human Gut Microbiome. *Cell*, *175*(4), 962-972.e10. https://doi.org/10.1016/j.cell.2018.10.029

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science (New York, N.Y.)*, *334*(6052), 105–108. https://doi.org/10.1126/science.1208344

Yu, D., Du, J., Pu, X., Zheng, L., Chen, S., Wang, N., Li, J., Chen, S., Pan, S., & Shen, B. (2022). The Gut Microbiome and Metabolites Are Altered and Interrelated in Patients With Rheumatoid Arthritis. *Frontiers in Cellular and Infection Microbiology*, *11*. https://www.frontiersin.org/articles/10.3389/fcimb.2021.763507