Full Length Article

Characterization of the gut microbiota among Veterans with unique military-related exposures and high prevalence of chronic health conditions: A United States-Veteran Microbiome Project (US-VMP) study

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ABSTRACT

The gut microbiome is impacted by environmental exposures and has been implicated in many physical and mental health conditions, including anxiety disorders, affective disorders, and trauma- and stressor-related disorders such as posttraumatic stress disorder (PTSD). United States (US) military Veterans are a unique population in that their military-related exposures can have consequences for both physical and mental health, but the gut microbiome of this population has been understudied. In this publication, we describe exposures, health conditions, and medication use of Veterans in the US Veteran Microbiome Project (US-VMP) and examine the associations between these characteristics and the gut microbiota. This cohort included 331 US Veterans seeking healthcare with the Veterans Health Administration who were 83% male with an average age of 47.6 ± 13.4 years. The cohort displayed a high prevalence of PTSD (49.8%) and history of traumatic brain injuries (76.1%), and high current use of prescription medications (74.9%) to treat various acute and chronic conditions. We observed significant associations between the gut microbiota composition and gastroenteritis, peripheral vascular disease (PVD), bipolar disorders, symptoms of severe depression based on the Beck Depression Inventory, stimulant and opioid use disorders, beta-blockers, serotonin and norepinephrine reuptake inhibitor antidepressants, diabetes medications, and proton pump inhibitors. Many of the Veteran characteristics examined were associated with altered relative abundances of specific taxa. We found that PVD and cardiovascular disease were associated with lower microbiota diversity in the gut (i.e., α-diversity), while supplemental vitamin use was associated with higher α-diversity. Our study contributes novel insights as to whether the unique exposures of Veterans in this cohort correlate with gut microbiota characteristics and, in line with previous findings with other population-level studies of the microbiome, confirms associations between numerous health conditions and medications with the gut microbiome.

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1. Introduction

United States (US) military Veterans have exposures that can be different from those experienced by civilians. An unfortunate outcome of war and conflict can be disruptions to physical and mental health, which can result in changes to physiology and lifestyle that sometimes persist for the duration of Veterans’ lives. Many US Veterans have access to healthcare through the Veterans Health Administration (VHA). Electronic medical records (EMRs) within the VHA often contain comprehensive historical information including combat exposures, medical procedures, health conditions, and medication prescriptions. This information can provide valuable insight into health outcomes.

In recent years, evidence has come to light that environmental factors, health conditions, and medications may impact the gut microbiota (Cenit et al., 2017; Dinan and Cryan, 2017; Maier et al., 2018). There is also evidence that the gut microbiota may be associated with mental health disorders such as depression, bipolar disorder, and schizophrenia (Cheung et al., 2019; Nguyen et al., 2018; Fond et al., 2020). Converging lines of evidence suggest that the connection between the gut microbiota and mental health disorders involves the immune system, specifically inflammatory pathways (Lowry et al., 2016). The gut microbiota has also been implicated in numerous inflammatory-related health conditions, including obesity and diabetes mellitus (Turnbaugh et al., 2009a; Sanna et al., 2019; Muruvada et al., 2017). The gut microbiota may also mediate the benefits of certain medications, such as metformin use for managing blood glucose in diabetes (Forslund et al., 2015; Jackson et al., 2018). Recent population-level studies have helped to quantify the relative importance of different correlates of the gut microbiota (Jackson et al., 2018; Falony et al., 2016), but it is unknown how well these results translate to a US Veteran population seeking care at the VHA, with unique environmental exposures, complex health conditions, and a high prevalence of medication use.

The US-VMP (US-Veteran Microbiome Project) was launched over six years ago in order to longitudinally assess the skin, oral, and fecal microbiomes of US Veterans, as well as collect validated measures of physical and mental health (Bremer et al., 2018). Participants were Veterans eligible to receive care within the ECHCS. This study includes data from all of the N = 331 Veterans who entered the study between June 2016 and November 2018, completed baseline measures, and provided a fecal sample that was successfully sequenced (N = 13 were excluded due to poor sequencing depth of the fecal samples).

2. Materials and methods

2.1. Study design

This was a cross-sectional observational study of US military Veterans.

2.2. Study participants

The US-VMP was launched over six years ago in order to longitudinally assess the skin, oral, and fecal microbiomes of US Veterans, as well as collect validated measures of physical and mental health (Bremer et al., 2018). Participants were Veterans eligible to receive care within the ECHCS. This study includes data from all of the N = 331 Veterans who entered the study between June 2016 and November 2018, completed baseline measures, and provided a fecal sample that was successfully sequenced (N = 13 were excluded due to poor sequencing depth of the fecal samples).

2.3. Data collection

Data collection for the US-VMP has been previously described in detail, including the microbiome sample collection (Bremer et al., 2018). Information was collected during in-person visits and from the VA EMR. Height and weight were collected for calculation of body mass index (BMI). Mental health diagnoses (lifetime bipolar disorders, anxiety disorders, and posttraumatic stress disorder (PTSD)) and current substance use disorders were identified via the Structured Clinical Interview for the Diagnostics and Statistical Manual of Mental Disorders (SCID) Version 5 (DSM-5) Axis I Disorders, Research Version, Patient Edition with Psychotic Screen (SCID-5-I/P W/PSY SCREEN) (First et al., 2016). The

### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ACEI | angiotensin-converting enzyme inhibitor |
| ANCOM | analysis of compositions of microbiomes |
| ARB | angiotensin II receptor blocker |
| ATP | adenosine triphosphate |
| BDI | Beck Depression Inventory |
| BMI | body mass index |
| CHF | congestive heart failure |
| CPD | chronic pulmonary disease |
| CVD | cardiovascular disease |
| DSM-5 | Diagnostic and Statistical Manual of Mental Disorders Fifth Edition |
| ECHCS | Eastern Colorado Health Care System |
| EMR | Electronic Medical Record |
| FDR | false discovery rate |
| GI | gastrointestinal |
| IBD | inflammatory bowel disease |
| ICD-9 | International Classification of Diseases, Ninth Revision |
| ICD-10 | International Classification of Diseases, Tenth Revision |
| IL | interleukin |
| IQR | interquartile range |
| ISI | Insomnia Severity Index |
| MDS | multidimensional scaling |
| NSAIDs | nonsteroidal anti-inflammatory drugs |
| OSU TBI-ID | The Ohio State University Traumatic Brain Injury Identification Method |
| PCS | Physical Health Composite Scale |
| PD | phylogenetic diversity |
| PERMANOVA | permutational multivariate analysis of variance |
| PiCrust | Phylogenetic investigation of Communities by reconstruction of unobserved states |
| PI | proton pump inhibitor |
| PTSD | posttraumatic stress disorder |
| PVD | peripheral vascular disease |
| QIME2 | Quantitative Insights Into Microbial Ecology v2 |
| SCFA | short-chain fatty acid |
| SCID | Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders |
| SD | standard deviation |
| SEPP | SATe-enabled phylogenetic placement |
| SF-36 | 36-Item Short Form Health Survey |
| SNRI | serotonin and norepinephrine reuptake inhibitors |
| SSRI | selective serotonin reuptake inhibitor |
| TBI | traumatic brain injury |
| TNFa | tumor necrosis factor alpha |
| US | United States |
| US-VMP | United States-Veteran Microbiome Project |
| VA | Veteran Affairs |
| VHA | Veterans Health Administration |
SCID-5 is a comprehensive semi-structured clinical interview that is a reliable and valid means to acquire information regarding psychiatric disorders (Diagnostic and Statistical Manual of Mental Disorders, 2013). Presence of bipolar disorders was determined by a lifetime diagnosis of bipolar I, bipolar II, or other specified bipolar disorder. Presence of anxiety disorders was determined by a lifetime diagnosis of panic disorder, agoraphobia, social anxiety disorder, specific phobia, generalized anxiety disorder, separation anxiety disorder, other specified anxiety disorder, and anxiety disorder due to another medical condition. PTSD diagnosis required all necessary criteria including exposure to a traumatic event and sufficient symptoms. Presence of substance abuse was determined by a current diagnosis of abuse of cannabis, hallucinogens, inhalants, opioids, sedatives, hypnotics, anxiolytics, stimulants, or tobacco. Presence of alcohol use disorder was determined by a current diagnosis of alcohol use disorder. Depressive symptoms were identified by responses to the Beck Depression Inventory (BDI) (Beck et al., 1961). Additional measures included the Physical Health Composite Score (PCS) from the 36-Item Short Form Health Survey (SF-36) (Brazier et al., 1992) and the Insomnia Severity Index (ISI) (Morin, 1992). History of traumatic brain injury (TBI) was determined by the Ohio State University Traumatic Brain Injury Identification Method (OSU TBI-ID) (Bogner and Corrigan, 2009). For the individuals with data missing (<5%) from some of these surveys, single imputation was performed using fully conditional specification methods via PROC MI in SAS v9.4 (SAS Institute, Cary, NC, USA).

Health conditions were identified via National Health Interview Survey of Chronic Conditions (Kovar and Poe, 1985). In addition to querying the VA EMR (vital status, inpatient hospitalizations, outpatient clinic visits, and fee basis files) diagnoses of the following were identified using International Classification of Diseases, Ninth and Tenth Revision (ICD-9 and ICD-10) codes: cancer; congestive heart failure (CHF); peripheral vascular disease (PVD); cardiovascular disease (CVD); diabetes; liver disease; renal disease; chronic pulmonary disease (CDP); and gastroenteritis (ICD-9 and ICD-10 codes are listed in Appendix A; Table A1). Diagnoses were included in analyses if they were charted within one year prior to each participant’s consent date in order to ensure that they were relatively current, and that the detection of health conditions was not overly biased towards older, long-term users of VA care. Additionally, a one year timeframe is often used for comorbidity assessment from EMRs (Casey et al., 2016; Lund et al., 2021). Medications were extracted from the VA EMR pharmacy prescriptions and refill records, including all medications with adequate supply to cover the two weeks preceding the fecal microbiota sample collection. Topical medications were excluded.

2.4. Classification of exposures into four groups of predictors

We grouped the potential correlates of the gut microbiota into four broad categories of predictors: 1) demographics; 2) health conditions and metrics; 3) medication classes; and, 4) specific medications. Demographics included age, sex, ethnicity (Hispanic versus non-Hispanic), self-described race (Caucasian, African American, or Other), history of homelessness, history of combat exposure, and deployment history. Health conditions and metrics included BMI, severe depressive symptoms as measured by the BDI, lifetime bipolar disorders, lifetime anxiety disorders, lifetime PTSD, history of TBI, current alcohol use disorder, and current substance use disorders (i.e., cannabis, opioids, and stimulants), SF-36 PCS, ISI, cancer, CHF, PVD, CVD, diabetes, liver disease, renal disease, CDP, and gastroenteritis.

We focused our analyses on medications reported for at least 25 individuals in the cohort. Using the Epocrates drug classification system, (Epocrates) medications were grouped broadly by type of condition typically treated (though many medications are used for numerous conditions) and then into classes of medications, each of which include numerous specific types of medications: 1) psychiatric medications, including antidepressants (atypicals, selective serotonin reuptake inhibitors (SSRIs), and serotonin and norepinephrine reuptake inhibitors (SNRIs)), insomnia medications, and other psychiatric medications; 2) cardiovascular medications, including statins, angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin II receptor blockers (ARBs), blood pressure medications, beta-blockers, antithrombotics, and other cardiovascular medications/supplements; 3) pain medications, including nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, muscle relaxers, and non-opioid analgesics; 4) gastrointestinal (GI) medications, including proton pump inhibitors (PPIs), laxatives, and other GI medications; 5) supplemental vitamins, including vitamin D; 6) neurologic seizure medications; 7) pulmonary medications, including bronchodilators; 8) endocrine medications, including diabetes and thyroid medications; and, 9) urogenital medications, including those for sexual function. Specific medications analyzed included the following, which were the most commonly prescribed: trazadone, bupropion, atorvastatin, lisopril, prazosin, gabapentin, metformin, levothyroxine, sildenafil, albuterol, omeprazole, and cholecalciferol.

2.5. Microbiome sample processing

Sample DNA was extracted as previously described from fecal samples using the PowerSoil DNA extraction kit (Cat. No. 12955–4, Qiagen, Valencia, CA, USA). Marker genes in isolated DNA were PCR-amplified using GoTaq Master Mix (Cat. No. M5133, Promega, Madison, WI, USA) as described in Caporaso et al. (2012) PCR products were cleaned and normalized using the SequelPrep Normalization Kit (Cat. No. A1051001, ThermoFisher, Waltham, MA, USA) following manufacturer's instructions. The normalized amplicon pool was sequenced on an Illumina MiSeq. All library preparation and sequencing were conducted at the University of Colorado Boulder BioFrontiers Next-Gen Sequencing core facility.

Sequencing data were processed using the Quantitative Insights Into Microbial Ecology program (QIIME2 v. 2019.10 (Bolyen et al., 2019)). The Deblur (Amir et al., 2017a) algorithm was used to denoise demultiplexed sequences. SATé-enabled phylogenetic placement (SEPP (Jansen et al., 2018)) analysis was performed to remove sequences that were not 75% similar to any record in the tree. Quality-filtered sequences were assigned taxonomic classification based on the silva_128 database (Quast et al., 2013). Samples that were shipped to the research facility and had taxa that are known to “bloom” during shipping were removed as previously described by Amir et al. (2017b). Additionally, taxa that have been identified to “bloom” with increased time spent at room temperature were also considered for removal (Bokulich et al., 2019). Details of how we performed and assessed “deblooming” analysis can be found in the supplemental materials (Appendix B; Fig. B1; Table B1) (Amir et al., 2017b). For α- and β-diversity and taxonomic evaluations, samples were rarefied to a level of 2535 sequences per sample. The α-diversity metrics assessed were observed species, Shannon diversity, and Faith’s phylogenetic diversity (PD). These measures capture different aspects of the microbiota diversity of each sample: observed species represents the richness of different types of microbiota detected, Shannon diversity gives equal weight to evenness and richness, and PD takes into account phylogenetic relatedness. The β-diversity metrics assessed were unweighted and weighted UniFrac. Jaccard and Bray-Curtis distance metrics were used to infer functional information (MetaCyc pathways (Caspi et al., 2014)) using Picrust2 v2.3.0-b (Douglas et al., 2019). These metrics capture the diversity across samples (differences in the microbiota between samples). Weighted UniFrac and Jaccard are quantitative measures (i.e., take into account abundance of the microbes/functional pathways present). Unweighted UniFrac and Jaccard are qualitative (i.e., take into account only presence/absence of the microbes/functional pathways).

2.6. Statistical analyses

We used Pearson’s correlation coefficient to assess the strength of
Table 1
Characteristics of the US-VMP cohort.

| Demographics          | N  |
|-----------------------|----|
| Age (years)           | 47.6 (13.4) |
| Male sex              | 275 (83.1) |
| Hispanic ethnicity    | 53 (16.0) |
| Race                  |     |
| Caucasian             | 62 (18.7) |
| African American      | 218 (65.9) |
| Other                 | 51 (15.4) |
| Number of periods of homelessness | 1.6 (0.1) |
| Periods of homelessness |     |
| Never                 | 192 (58.0) |
| 1-2 times             | 76 (23.0) |
| 3+ times              | 63 (19.0) |
| Deployment history    |     |
| Never                 | 100 (30.2) |
| 1-2 times             | 144 (43.5) |
| 3+ times              | 87 (26.3) |

Health Conditions and Metrics

| BMI category          |     |
|-----------------------|----|
| Underweight           | 1 (0.3) |
| Normal weight         | 90 (27.2) |
| Overweight            | 114 (34.4) |
| Obese                 | 126 (38.1) |
| Severe depressive symptoms (BDI) | 60 (18.1) |
| Bipolar disorders (lifetime) | 17 (5.1) |
| Anxiety disorders (lifetime) | 38 (11.5) |
| Posttraumatic stress disorder (lifetime) | 165 (49.8) |
| Number of traumatic brain injuries | 1.9 (1.8) |
| Prevalence of TBI in cohort | 76.1 |
| SF-36 Physical Health Composite score | 45.2 (10.2) |
| Insomnia Severity Index | 11.9 (7.6) |
| Prevalence of clinical insomnia | 35 |
| Cancer                | 18 (5.4) |
| Congestive heart failure | 8 (2.4) |
| Peripheral vascular disease | 9 (2.7) |
| Cardiovascular disease | 17 (5.1) |
| Diabetes              | 52 (15.7) |
| Liver disease         | 28 (8.5) |
| Renal disease         | 18 (5.4) |
| Chronic pulmonary disease | 49 (14.8) |
| Gastroenteritis       | 41 (12.4) |
| Alcohol use disorder (current) | 40 (12.1) |
| Substance use disorder (current) | 25 (7.6) |
| Cannabis              | 20 (6.0) |
| Opioids               | 5 (1.5) |
| Stimulants            | 20 (6.0) |

Medications

| Current VA medication | 248 (74.9) |

Footnote. Numbers are presented as mean (SD) for continuous measures and prevalence (%) for categorical data. Severity of depressive symptoms was determined from the BDI. Bipolar disorders (lifetime diagnosis of bipolar I, bipolar II, or other specified bipolar disorder), anxiety disorders (lifetime diagnosis of panic disorder, agoraphobia, social anxiety disorder, specific phobia, generalized anxiety disorder, separation anxiety disorder, other specified anxiety disorder, or anxiety disorder due to another medical condition), posttraumatic stress disorder, current alcohol use disorder, and current substance use disorders (i.e., cannabis, opioids, and stimulants) were determined from the SCID-5-I/P W/PSY SCREEN. TBI history was determined by the OSU-TBI-ID. Abbreviations: BDI, Beck Depression Inventory; BMI, body mass index; OSU-TBI-ID, The Ohio State University Traumatic Brain Injury Identification Method; SCID-5-I/P W/PSY SCREEN, Structured Clinical Interview for the Diagnostic and Statistical Manual for Mental Health Disorders Version 5 (DSM-5) Axis I Disorders, Research Version, Patient Edition with Psychotic Screens; SD, standard deviation; SF-36, 36-Item Short Form Health Survey; TBI, traumatic brain injury.

3. Results

Among the 331 Veterans in the US-VMP cohort, over two-thirds experienced at least one deployment, and many of the Veterans (42%) reported at least one period of homelessness (Table 1). There was high prevalence of health conditions with: 1) over 35% having self-reported clinical insomnia; 2) almost 50% having a lifetime diagnosis of PTSD; and, 3) over three-quarters reporting at least one TBI of any severity (mild, moderate or severe). Approximately 12% of Veterans reported current alcohol use disorder and 7.6% reported some type of other current substance use disorder, such as cannabis, opioids, or stimulants.

3.1. The majority of veterans had multiple medication prescriptions

Three-quarters of the US-VMP cohort had at least one current medication prescription noted within the EMR. These individuals had an average of six prescriptions for unique medications (median = 4, IQR = 2–8). Psychiatric medications were the most prevalent class, with antidepressants being the most common sub-class (Table 2); 137 individuals (41.4% of the overall cohort) had an active prescription for antidepressants, most commonly atypical antidepressants (specifically, trazadone or bupropion), SSRI s, or SNRI s. CVD medications were also common (38.1% of the cohort), such as statins, ACEIs/ARBs, beta-blockers, and other blood pressure medications. Almost one-third (32.6%) of the cohort had some type of pain medication, with 13% taking prescription opioids.

We examined the correlation between health conditions and medications (Fig. 1). The overall health metric of the SF-36 PCS correlated negatively with prescription medications (r = −0.25), as would be expected. The most strongly correlated disease-medication pair was diabetes and metformin (r = 0.58). Many individuals with diabetes were also prescribed CVD medications, particularly statins (r = 0.43). CHF correlated highly with beta-blockers (r = 0.47), and CPD with albuterol (r = 0.43).

3.1.1. The gut microbiota taxonomic compositions were correlated with numerous health conditions and medications

We evaluated associations between the overall gut microbiota composition, Veteran characteristics, and medications using two phylogenetic measures of composition that take into account the types of microbiota present or absent in a sample (unweighted UniFrac) and the relative abundance of the microbiota present (weighted UniFrac) (Lozupone et al., 2011). We identified the strongest correlates of the gut microbiome composition by examining each exposure independently using PERMANOVA (Fig. 2) and also through a stepwise constrained ordination approach (Table 3, Fig. 3) that identifies important sets of permutable multivariate analysis of variance (PERMANOVA) using the vegan package (Oksanen et al., 2018) and a stepwise model building tool for constrained ordination methods (ordistep) (Douglas et al., 2019) based on adjusted R² using the UniFrac distance metrics. We applied the same methods to the inferred gut microbiota MetaCyc functional pathways (Caspi et al., 2014), quantifying the pathway abundance using Jaccard and Bray-Curtis distance metrics. In order to determine the strongest correlates of the three measures of the gut microbiota α-diversity, we used a similar model building tool for linear regression models, LEAPS (Miller, 1984). Analysis of Compositions of Microbiomes (ANCOM) (Mandal et al., 2015) was used to assess whether any gut microbiota taxa were differentially abundant with these predictors. For taxonomic analyses, we adjusted for multiple testing using a Benjamini-Hochberg (Benjamini and Hochberg, 1995) False Discovery Rate (FDR) with a two-tailed statistical significance threshold of 0.05, which was likewise used for two-tailed p-values in analyses of α- and β-diversity. Analyses were performed with QIIME2 v. 2019.10 (Bolten et al., 2019), the open source statistical package R v.3.5.1 (R Core Team, 2018) and python v3.6.7 (Van Rossum and Drake, 2009).
Table 2
Medication prescriptions among the US-VMP cohort.

| Medication class | Specific medication | N (%) |
|------------------|---------------------|-------|
| **Psychiatric**  |                     | 331   |
| Antidepressants  |                     | 152 (45.9) |
| Atypical antidepresants |             | 137 (41.4) |
| Trazodone        |                     | 72 (21.8) |
| Bupropion        |                     | 40 (12.1) |
| SSRI antidepressants |                 | 29 (8.8) |
| SNRI antidepressants |                | 58 (17.5) |
| Insomnia         |                     | 37 (11.2) |
| Other            |                     | 25 (7.6) |
| **CVD**          |                     | 33 (10)  |
| Statins          |                     | 126 (38.1) |
| ACEI/ARBs        |                     | 56 (16.9) |
| Atenolol         |                     | 40 (12.1) |
| Lisinopril       |                     | 44 (13.3) |
| Blood pressure   |                     | 25 (7.6) |
| Prazosin         |                     | 54 (16.3) |
| Beta-blockers    |                     | 26 (7.9) |
| Antithrombotics  |                     | 34 (10.3) |
| Other            |                     | 29 (8.8) |
| **Gastrointestinal (GI)** |               | 41 (12.4) |
| NSAIDs           |                     | 108 (32.6) |
| Opioids          |                     | 43 (13) |
| Muscle relaxers  |                     | 43 (13) |
| Non-opioid analgesic |                | 39 (11.8) |
| **Pain management**  |                  | 30 (9.1) |
| NSAIIDs          |                     | 30 (9.1) |
| Opioids          |                     | 30 (9.1) |
| Muscle relaxers  |                     | 30 (9.1) |
| **Supplemental vitamins**  |          | 83 (25.1) |
| Vitamin D        |                     | 50 (15.1) |
| Cholecalciferol  |                     | 38 (11.5) |
| Vitamins - Other |                     | 50 (15.1) |
| **Neurologic**   |                     | 59 (17.8) |
| Seizures         |                     | 50 (15.1) |
| Gabapentin       |                     | 44 (13.3) |
| **Pulmonary**    |                     | 59 (17.8) |
| Bronchodilators  |                     | 50 (15.1) |
| Albuterol        |                     | 50 (15.1) |
| **Endocrine**    |                     | 52 (15.7) |
| Diabetes         |                     | 52 (15.7) |
| Metformin        |                     | 26 (7.9) |
| **Urology**      |                     | 24 (7.3) |
| Erectile function|                     | 24 (7.3) |
| Sildenafil       |                     | 28 (8.5) |
| **Other medications**  |              | 79 (23.9) |

Footnote: Medications ordered for Veterans covering the period within two weeks of their gut microbiota sample collection. Medications are grouped into broad disease categories that reflect their primary use, and then further grouped into general classes that include numerous specific medications. When medication classes were dominated by one specific medication (>80%), we report the numbers for the specific medication rather than the class and perform subsequent analyses at the specific medication level. Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CVD, cardiovascular disease; GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drugs; PPI, proton pump inhibitors; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; US-VMP, United States Veteran Microbiome Project.

In the stepwise constrained ordination analysis, data were examined for both unweighted and weighted UniFrac in four groups: 1) demographics; 2) health conditions and metrics; 3) medication classes; and, 4) specific medications. In the demographics group, we found that age (P = 0.01) correlated most strongly with the unweighted UniFrac distance metric (Table 3, Fig. 3A). Race (P = 0.01) and sex (P = 0.002) correlated most strongly with the weighted UniFrac distance metric (Table 3, Fig. 3B). Among the health conditions and metrics, we found that PVD (P = 0.02), bipolar disorders (P = 0.04), stimulant use disorder (P = 0.05), gastroenteritis (P = 0.05), and severe depressive symptoms (based on BDI; P = 0.05) correlated with unweighted UniFrac (Table 3, Fig. 3A). Bipolar (P = 0.03) and stimulant use (P = 0.02) disorders also correlated significantly with weighted UniFrac, as did opioid use disorder (P = 0.05) (Table 3, Fig. 3B). Among medication classes and specific medications, beta-blockers (P = 0.01), SNRI antidepressants (P = 0.01), and diabetes medications (P = 0.03) correlated with unweighted UniFrac (Table 3, Fig. 3A). Other GI medications (P = 0.01), PPIs (P = 0.05) and omeprazole, which is a PPI (P = 0.03), correlated with weighted UniFrac (Table 3, Fig. 3B). We were able to identify specific taxa that differed between groups for many of the Veteran demographic characteristics and health metrics/conditions examined, including deployments, severe depressive symptoms, bipolar disorders, anxiety disorders, clinical insomnia, CHF, PVD, diabetes, gastroenteritis, opioid use disorder, and stimulant use disorder (Fig. 4). Likewise, both classes of and specific medications were associated with significant shifts in some taxa, including diabetes medications, SNRI antidepressants, statins, beta-blockers, muscle relaxers, PPIs, other GI medications (which included digestive enzymes and heart burn medications, most commonly ranitidine), other supplemental vitamins, seizure medications, bronchodilators, metformin, omeprazole, and gabapentin.

3.1.2. The overall gut microbiota functional composition was correlated with numerous health conditions and medications

We used a similar approach in order to identify the strongest correlates of the gut microbiome functional pathways (Table 4; Fig. 5), using Jaccard and Bray-Curtis distance metrics based on functional information inferred using Phylogentic investigation of Communities by reconstruction of unobserved states v2 (Picrust2) (Douglas et al., 2020), which are comparable to unweighted and weighted UniFrac, respectively. Sex (P = 0.002), CHF (P = 0.03), muscle relaxers (P = 0.01), and omeprazole (P = 0.02) (a PPI) were associated with differences in the types of functional pathways present or absent (Jaccard distance metric). Sex (P = 0.002) and omeprazole (P = 0.006) were likewise associated with the abundance of functional pathways (Bray-Curtis distance metric), as were race (P = 0.04), SF-36 PCS (P = 0.02), and other supplemental vitamins (P = 0.002). As shown in Fig. 5, samples from individuals with CHF or taking muscle relaxers are clustered together with functions related to the TCA cycle.

3.1.3. The gut microbiota α-diversity metrics were correlated with numerous health conditions and medications

We also examined α-diversity of the gut microbiota taxa (Appendix C; Table C2). Most of the correlates of α-diversity showed an inverse relationship, including CVD (P = 0.04), PVD (P = 0.04), trazadone (P = 0.04), and albuterol (P = 0.02). However, this was not always the case; other supplemental vitamins (P = 0.03) (including all vitamins except for Vitamin D) and other CVD medications/-supplements (P = 0.03) (e.g., aspirin, fish oil, etc.) were associated with higher α-diversity.

4. Discussion

In this study of Veterans with military-related exposures and associated health conditions, we found a number of associations with the microbial taxonomic composition of the gut, including bipolar disorders, severe depressive symptoms based on the BDI, PVD, gastroenteritis, opioid and stimulant use disorders, beta-blockers, SNRI antidepressants, diabetes medications, and GI medications including PPIs, and omeprazole, which is a specific type of PPI. Gut microbiota functional composition was associated with overall perceived physical health (SF-36 PCS), CHF, use of muscle relaxers, omeprazole, and supplemental vitamin use. Military service-related characteristics and conditions, such as...
deployment history, combat exposure, PTSD, and number of TBIs, were not among the strongest correlates of overall gut microbiota metrics, such as $\alpha$- or $\beta$-diversity. However, there were several notable differences in taxonomic relative abundance associated with deployments, as well as with many of the health-related metrics and disorders that are highly prevalent among Veterans, including insomnia, bipolar and anxiety disorders, and depressive symptoms.

We observed that age, race, and sex correlated with the gut microbiota composition, as has been observed in many other population studies, including the Human Microbiome Project (Jackson et al., 2018; Falony et al., 2016; Human Microbiome Project, 2012). Mental health conditions were highly prevalent among the US-VMP cohort, and we found that bipolar disorders were associated with bacterial community structure. Previous findings on the community composition of the gut microbiota and bipolar disorders are mixed, specifically, increased Actinobacteria (Painold et al., 2019), increased Flavonifractor (Coello et al., 2019), and inconsistent associations with Faecalibacterium (Coello et al., 2019; Evans et al., 2017). Our study found that Bacteroides was increased in individuals with bipolar disorders, but this is somewhat difficult to interpret as this is a very common member of the human gut microbiota with wide-ranging correlations. Some prior studies separated bipolar disorders into the clinical subtypes of I and II (Coello et al., 2019; Evans et al., 2017; Aizawa et al., 2016), or differentiated bipolar individuals based on severity of current symptoms (Painold et al., 2019) or pharmacological intervention (Flowers et al., 2017, 2019), which we did not. Additionally, we found decreased Ruminococcus 1 in participants that suffered from severe depressive symptoms based on the BDI. One study that recruited 115 participants experiencing bipolar disorders reported a lower abundance of the family Ruminococcaceae compared to control participants (Evans et al., 2017), though the consistency of this result is difficult to assess as this is a broad and diverse family.

We showed an increase in Blautia genus of the Firmicutes phylum in individuals with anxiety disorders as determined by the SCID-5-I/P W/ PSY SCREEN (First et al., 2016). Gacias et al. showed that Blautia was associated with increases in anxiety-like behavior (social avoidance) in mice (Gacias et al., 2016). Blautia has also been correlated with obesity and type 2 diabetes. (Ozato et al., 2019; Egshatyan et al., 2015). However, other work has suggested that Blautia may have protective effects in some contexts (Wong et al., 2016; Jenq et al., 2015), and a recent study found an inverse association in men between Blautia abundance and anxiety (Taylor et al., 2019) measured by the Depression, Anxiety, and
exposure to traumatic stressors and subsequent diagnoses of mental health conditions (e.g., PTSD) to the development of future physical health conditions (e.g., CVD) (Dyball et al., 2019). Increased permeability of the GI lumen, commonly referred to as “leaky gut”, which also has been shown to lead to exaggerated inflammation (Camilleri, 2019), has been identified in those suffering from affective, GI (e.g., inflammatory bowel disorders [IBD]), and cardiovascular disorders (Ait-Belgnaoui et al., 2012; Bischoff et al., 2014; Novakovic et al., 2020). Similarly, it is common for those GI and cardiovascular disorders to have comorbid affective disorders (Wilmes et al., 2021; Cohen et al., 2015). Given the high rates of inflammatory disorders among Veterans (Tanil-lian et al., 2008; Breland et al., 2017), this is an important population within which to study the microbiota-gut-brain axis and physical and mental health. We predominantly focused on lifetime diagnoses of mental health conditions because we were not adequately powered to examine current mental health conditions, or to compare short-term versus long-term conditions. However, this is an important area for future research, as there is some evidence that prolonged exposure to mental health conditions can lead to enhanced immunological and inflammatory changes (Dickerson et al., 2016; Goldsmith et al., 2016).

Number of deployments showed a positive relationship with Prevotella 6 and Collinsella and a negative relationship with Bifidobacterium. A deployment is a temporary relocation of a military service member, typically to an international location, and can vary in length from three to fifteen months and does not necessarily include combat. Relocation inherently involves the exposure to different environmental microbes (Barberán et al., 2015), changes in diet (Turnbaugh et al., 2009b), novel living quarters (Lax et al., 2014), and novel cohabitants (Song et al., 2013), all of which have been shown to influence the gut microbiome. Prevotella is a highly diverse genus with numerous clades, many of which are typically underrepresented in Westernized countries (Tett et al., 2019). Veterans with multiple deployments may have increased exposure to these types of microbes that are less common among Western populations, which could explain this finding. Collinsella has been associated with low dietary fiber intake and high circulating insulin (Gomez-Arango et al., 2018). There is high prevalence of insulin resistance and diabetes among Veterans (Liu et al., 2017), and the US-VMP cohort generally has a homogeneous, Western-style diet, low in dietary fiber, and high in animal foods, refined carbohydrates, and added sugars (Flowers et al., 2017). A reduction in dietary fiber intake could result in a general switch in the gut away from short-chain fatty acid (SCFA) production and toward more lactate fermentation processes (Pylkas et al., 2005). Bifidobacterium has many known probiotic strains and has been largely considered a genus comprised of beneficial microbes (Azad et al., 2018). Bifidobacterium species are generally known for their ability to catabolize complex carbohydrates and produce the necessary substrates for the Bif Shunt metabolic pathway (O’Callaghan and van Sinderen, 2016). The Bif Shunt pathway breaks down phosphates to generate adenosine triphosphate (ATP) and produces SCFAs as end products (O’Callaghan and van Sinderen, 2016). SCFAs are important signaling molecules, and they can influence systemic inflammation by reducing inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) and increasing anti-inflammatory cytokines such as IL-10 (Vinolo et al., 2011). However, they have also been associated with obesity and may reflect an increased capacity to harvest energy from food (Gentile and Weir, 2018).

Our results show that the most common medications among Veterans in the US-VMP were comparable to adults in the US as a whole, antidepressants and CVD-related medications, including lipid-lowering medications, beta-blockers, and ACEIs (Hales et al., 2019). The prevalence of antidepressants and other mental health medication prescriptions was higher in the US-VMP than in the US generally, which is likely due to recruitment of Veterans that were actively seeking healthcare from a VHA medical center. It is important to note that some of the commonly prescribed medications in this cohort were classified as treatment for a specific condition based on the classification system from Epocrates.
but that in this population, it is likely that certain medications were being used off-label to address other conditions (Leslie et al., 2009). For example, 18% of the cohort was prescribed “seizure medications,” but drugs within this class of neurological medications (e.g., gabapentin) are often used in the VHA at a lower dosage as mood stabilizers or for pain control (Ketter et al., 2003). Another example is prazosin, which in Fig. 1 was classified as a blood pressure lowering medication, but is frequently used among Veterans to treat PTSD-related nightmares (Raskind et al., 2018).

We found that PPIs, particularly omeprazole, as well as other GI medications (which included digestive enzymes and heart burn medications, most commonly ranitidine) showed a strong correlation with overall gut microbiota composition and functional pathways. This is not surprising, as these medications target the gut function. PPIs increase the gut pH, which alters the types of microbes that can survive and grow (Duncan et al., 2009). This finding is consistent with prior literature showing that these commonly prescribed medications are associated with alterations in the gut microbiota, such as lower microbial diversity and increases in potentially commensal microbes, specifically the genera of Enterococcus, Streptococcus, Staphylococcus, and the potentially pathogenic species Escherichia coli. We likewise saw an association between increased Streptococcus and PPIs (Imhann et al., 2016; Jackson et al., 2016). These alterations in the gut microbiota composition are likely not entirely benign, as use of PPIs is also associated with an increased risk of enteric infections, including C. difficile (Janarthanan et al., 2012), which carries a high mortality risk (Oake et al., 2010) and is known to respond well to restoration of a healthy microbiota composition through fecal microbiota transplant therapy (Youngster et al., 2014). Moreover, these
Table 4
Variables most correlated with the gut microbiota functional pathways inferred using PiCrust2.

| Distance metric | Exposure | Variable                        | P     |
|-----------------|----------|---------------------------------|-------|
| Jaccard         | Demographics | Sex                              | 0.002 |
|                 | Health conditions/metrics | Congestive heart failure          | 0.026 |
|                 | Medication classes | Muscle relaxers                   | 0.008 |
|                 | Specific medications | Omeprazole                        | 0.016 |
| Bray-Curtis     | Demographics | Sex                              | 0.002 |
|                 | Health conditions/metrics | SF-36 PCS                         | 0.022 |
|                 | Medication classes | Other supplemental vitamins        | 0.002 |
|                 | Specific medications | Omeprazole                        | 0.006 |

Footnote. β-diversity metrics Jaccard and Bray-Curtis were utilized with PiCrust2 to infer functional pathways most correlated to variables. Variables were grouped into four categories: 1) demographics; 2) health conditions; 3) medication classes; and, 4) specific medications. Abbreviations: PiCrust2, Phylogenetic investigation of Communities by reconstruction of unobserved states v2; SF-36 PCS, 36-Item Short Form Health Survey Physical Health Composite Score.

Findings are particularly relevant in light of recent wide-spread PPI de-implementation strategies, based on findings suggesting that many PPI prescriptions are inappropriate (Orielio et al., 2021).

In some cases, it was difficult to isolate whether a condition or its treatment was more strongly associated with the gut microbiota. For example, we saw that PVD, SNRIs, and beta-blockers were associated with differences in the gut microbiota composition. There were only nine individuals with PVD in the cohort; almost half of them were on beta-blockers, and one-third were on SNRIs, making it difficult to tease apart these effects. This is an important area for further research as PVD showed some of the most pervasive associations with gut microbiota characteristics, including with α-diversity, overall gut microbiota community composition and relative abundance of Faecalibacterium. In a recent study of atherosclerotic CVD, disease status was more strongly associated with the gut microbiota than CVD medication (Jie et al., 2017). However, the relationships among disease, medications, and the gut microbiota are complex, and the efficacy of some medications may be dependent on interactions with the gut microbiota. This is thought to be the case with metformin, the most frequently prescribed medication for type 2 diabetes (Vallianou et al., 2019). Our results confirm those of other studies that diabetes medications are associated with alterations in the overall gut microbiota (Jackson et al., 2018; Vallianou et al., 2019).

This study has some notable limitations and strengths. The participants were mainly recruited through the VA medical facilities in Eastern Colorado, and do not fully represent the Veteran population as a whole, particularly younger, healthier Veterans who are less likely to regularly use VA health services. Fecal samples were collected in-person for some of the participants, while other participants collected samples at home and shipped the samples to the study center. Sample transit time (time between when the sample was collected and when the sample was placed into an ultra-low temperature freezer at the research facility) was variable and conditions before and during shipment may have varied among samples. We tried to address this issue using previously applied deblooming methods, but it is likely impossible to fully correct for this issue. The samples were sequenced based on the 16S rRNA gene, which limits our ability to accurately classify species-level taxonomy or to understand gut microbiota functions. We examined gut microbiota functional pathways that were inferred from taxonomy, but these analyses are preliminary in nature since microbiota functions would be more accurately assessed through shotgun metagenomic sequencing of samples, which we hope to do in the future. A record of a medication prescription in the VA EMR is indicative of a provider order, but it does not necessarily mean the Veteran has taken the medication. However, medication adherence levels tend to be better among those getting care at the VA than outside the VA (Gaffney et al., 2020). The medications recorded in the VA EMR may underestimate the true prevalence of medication use among these individuals since Veterans may get some of their medications over-the-counter, such as those for heartburn. It is difficult to isolate the effects of medication from the effects of health conditions, and medications may have interaction effects, which are difficult to estimate without larger sample sizes. It is also inherently challenging to classify medications into their predominant therapeutic indications, as numerous medications can be used to treat different conditions (Raskind et al., 2018).

We chose to use Epocrates classification system, which is one of the most commonly used sources of drug information by physicians that does not require a subscription (Apidi et al., 2017). This study has unique strengths as well, including the validated measures of health conditions and substance use disorders and the comprehensive information about demographies, health conditions, metrics, medication classes, and specific medications. The color shows whether there is a direct (red) or inverse (blue) relationship between the exposure and taxonomic relative abundance determined by ANCOM. Abbreviations: ANCOM, analysis of composition of microbiomes; BDI, Beck Depression Inventory; FDR, false discovery rate; SNRI, serotonin and norepinephrine reuptake inhibitor; UCG, unclassified genus. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
medical conditions and medications available through the VA EMRs. As the recruited population in the US-VMP increases over time, we will be better powered to more thoroughly characterize the associations between individual characteristics (i.e., current vs lifetime psychological conditions) and the gut microbiota of US Veterans.

5. Conclusions

In this study, we leveraged the extensive information collected in the VA EMR in order to examine the relationships of the gut microbiota to demographic factors, common health-related conditions, military-specific exposures, and medications. This work will help to inform decisions about which types of information are important to collect and consider in microbiome studies, and it adds to the growing body of literature supporting associations between gut microbiota features and numerous health conditions, particularly those that tend to be characterized by underlying inflammation, such as gastroenteritis and affective disorders.

Data availability

The VHA has rules about data security that aim to protect the privacy of Veteran health information. Thus, the personal health data used for this study cannot be made publicly available. Please contact the authors for further information or collaboration.
measures of bacterial community structure. We examined of principal coordinates of the weighted UniFrac distance matrix were used as a means to objectively determine how deblooming analysis impacted of this analysis are shown in Figure B1.

continuous variable of transit days (number of days between when the sample was collected by the participant and when the sample was placed into the total of had been determined to have arti

Footnote: CHF, congestive heart failure; ICD, International Classi

Appendix B. Supplemental Methods

Bloom analysis for US Veteran Microbiome Project (VMP)

Our sampling procedures allowed participants to provide samples in one of two ways: 1) participants could provide samples during their in-person visit; or 2) participants could take a sampling kit to their home to collect their sample and ship the sample back to the research facility. Although the participants were provided with an ice pack and instructed to ship the sample back to the research facility with the ice pack, adherence to this protocol was not always followed. Therefore, samples shipped back to the research facility had the potential to be above freezing temperature for extended periods of time. In contrast, samples collected by participants that elected to provide their sample during the in-person visit were immediately frozen and stored at –80 °C.

Amir et al. were the first to report on the phenomena of “blooming”, where certain taxa that are not obligate anaerobic bacteria can bloom in fecal samples if not quickly refrigerated or frozen (Amir et al., 2017b). Their publication provided detailed explanations, files, and code to remove taxa that had been determined to have artificially “bloomed” in the American Gut Project data. We followed these methods to perform deblooming of our data. A total of N = 95 samples was provided during in-person visits, whereas a total of N = 236 samples were shipped. The R² values from constrained analysis of principal coordinates of the weighted UniFrac distance matrix were used as a means to objectively determine how deblooming analysis impacted measures of bacterial community structure. We examined R² values of both the dichotomous variable of shipped (yes, N = 236; no, N = 95) and the continuous variable of transit days (number of days between when the sample was collected by the participant and when the sample was placed into the –80 °C freezer at the research facility; mean ± SD (2.94 ± 3.82 days)). We performed this analysis on the data before deblooming analysis, after deblooming analysis of all samples (N = 331), and after deblooming analysis of only samples shipped back to the research facility (N = 236). The results of this analysis are shown in Figure B1.

The R² values from the constrained analysis of principal coordinates is a measure of the variance explained by the factor “shipping” or “transit time” on the bacterial community composition. Ideally, after deblooming, these variables would no longer explain any variation in the gut microbiota
composition (i.e., $R^2 = 0$). While this was not the case, the $R^2$ values were substantially reduced by performing the deblooming analysis on only samples shipped back to the research facility relative to no deblooming or deblooming all samples, supporting that this method helped to correct for the effects of sample transit conditions.

Additionally, Bokulich et al. showed that the taxa that artificially bloom in fecal samples exposed to room temperature conditions for extended periods of time are overrepresented by members of the Enterobacteriaceae family (Bokulich et al., 2019). For this reason, we also examined all the sub-operational taxonomic unit (sOTUs) in our dataset belonging to the Enterobacteriaceae family for significant correlation with transit days to ensure that our initial deblooming analysis did not overlook other taxa that may have bloomed. We found 21 sOTUs in our dataset belonging to the Enterobacteriaceae family. The results for the correlations run for each sOTU against transit days can be found in Supplemental Table B1.

There was only 1 sOTU from the Enterobacteriaceae family that was significantly correlated with transit days (sOTU: 4c8288bfbd76958c0c094d87b97650f8; Genus: Escherichia-Shigella; Species: Unknown). This sOTU was also identified by Amir et al. (Amir et al., 2017b) and therefore was already removed from the dataset during the initial deblooming analysis. Consequently, in this report we have used data derived from deblooming of only the samples shipped back to the research facility.

Figure B.1. Bar plots of the $R^2$ values from constrained analysis of principal coordinates of the weighted UniFrac distance matrix for the shipped samples. The analysis was performed on the data before deblooming analysis, after deblooming analysis on all samples, and after deblooming analysis on only samples shipped to the research facility.

Table B.1 Table of correlation results of sOTUs belonging to the family of Enterobacteriaceae against transit days.

| sOTU | Genus          | Species              | R-values | p-value | Significance |
|------|----------------|----------------------|----------|---------|--------------|
| 031562526f6d4e8f099af6cb0f82f | Enterobacter | 0.058 | 0.29 |
| 054e27bdbe3e903f007599c4b2f26 | Providencia | 0.03 | 0.59 |
| 1aee97f7c5f5926f6e941107987b2b | Citrobacter | 0.056 | 0.31 |
| 26d8b09767dca9c841c62951e67b58 | Raoultella | 0.0095 | 0.86 |
| 2c9ba6e577f31128bba81f72487d26 | Morganella | 0.021 | 0.7 |
| 3be2d36c2d2da7c5821c722729e4 | Proteus | 0.016 | 0.78 |
| 411b521b1f6e28d0d1285180570b | Escherichia-Shigella | 0.068 | 0.22 |
| 4e32888bf779589c948bb797650f8 | Escherichia-Shigella | 0.17 | 0.0014 |
| 57f3e01e3ee8b9090c43524a27eb | Citrobacter | 0.093 | 0.092 |
| 583e4c15443c2d1f52d490a554f8 | Klebsiella | 0.035 | 0.52 |
| 64ee88f0e180565c700248d90a | Morganella | 0.021 | 0.7 |
| 83182c1e91333b26f3a3e5805267c | Raoultella | 0.03 | 0.59 |
| 9b517b1a5250f6b8a8abc36e0076b | Klebsiella | 0.017 | 0.75 |
| a1e3c3e1b2516b330a15e2b732a637 | Enterobacter | 0.0091 | 0.87 |
| a2d443c9793f39ed55f206150376715 | Unclassified | 0.043 | 0.44 |
| be0112008f48bca86f528284ec0c8c | Cronobacter | 0.039 | 0.48 |
| 2b80e430b4506c88ede22c2626d8848 | Hafnia-Oblumobacterium | 0.0092 | 0.87 |
| c4b958876c30c5643216e60f88c80 | Morganella | 0.049 | 0.37 |
| e42382a2f14199045507e316746262 | Rosenbergia | 0.0084 | 0.88 |
| ec9ed9b965c13cd3f6190b3632904c | Unclassified | 0.021 | 0.71 |
| fc58660c6565201a434d85b30951f | Serratia | -0.069 | 0.21 |
### Table C.1

Table C.1 Functional pathway labels and pathway details from Fig. 5.

| Pathway label | Pathway details/webpage link |
|---------------|-----------------------------|
| GLYOXDBG | Superpathway of glycol metabolism and degradation |
| GLYCOL-TCA/GLYOX-BYPASS | Superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass |
| P105 | TCA cycle IV (2-oxoglutarate dehydrogenase) |
| 1269 | CMP-3-deoxy-D-manno-octulosonate biosynthesis |
| 2942 | L-Lysine biosynthesis III |
| 5245 | Superpathway of L-methionine biosynthesis (by sulphydrylation) |
| 5918 | Superpathway of b-hem b biosynthesis from glutamate |
| 6737 | Starch degradation V |
| 7392 | Taxadiene biosynthesis (engineered) |
| 4FS-7 | Phosphatidylglycerol biosynthesis I (plasticid) |
| REDCITCYC | TCA cycle VI (Helicobacter) |
| COMPLETE-ARO | Superpathway of aromatic amino acid biosynthesis |
| FASYN-ELONG | Fatty acid elongation – saturated |
| NAGLIPASYN | Lipid IVA biosynthesis (E. coli) |
| PHOSLIPSYN | Superpathway of phospholipid biosynthesis I (bacteria) |
| UDPNAGSYN | UDP-N-acetyl-D-glucosamine biosynthesis I |

### Table C.2

Model selection results for linear regressions of α-diversity. Three measures of α-diversity (Observed species, Shannon diversity, and Faith's Phylogenetic Diversity (PD)) were modeled as a function of predictors from four groups of exposures: 1) demographics; 2) health conditions and metrics; 3) medication classes; and 4) specific diseases and medications showing a significant association with α-diversity show an inverse relationship, but there are some exceptions. For example, vitamins and “other CVD medications,” which includes aspirin and fish oil, are associated with higher α-diversity. The beta estimates correspond to 1 standard deviation change in each metric of α-diversity.

| Predictor group | α-diversity metric | Selected covariates | Estimate | 95% CI | P |
|-----------------|--------------------|---------------------|----------|--------|---|
| Demographics    | Observed species   | Non-significant: Race |
| Tobacco PD      | Shannon            | Non-significant: Race |
| Health conditions & metrics | Observed species | CVD | Non-significant: BMI, Bipolar disorders, Severe depressive symptoms (BDI), Gastroenteritis, Cannabis use disorder |
| Shannon         | PVD                | Non-significant: BMI, Bipolar disorders, Severe depressive symptoms (BDI), Gastroenteritis, Cannabis use disorder |
| Faith's PD      | CVD                | Non-significant: BMI, Bipolar disorders, Severe depressive symptoms (BDI), SF-36 PCS, Cancer, PVD, Gastroenteritis, Cannabis use disorder |
| Medication classes | Observed species | Other Vitamins | 0.37 | (0.05, 0.71) | 0.028 |
| Shannon         | Bronchodilators    | Non-significant: Antithrombotics, Beta blockers, Other CVD medications, Bronchodilators, NSAIDs, SSRI, Other Psychiatric medications, Sexual function medications, Vitamin D |
| Othersupplemental vitamins | 0.38 | (0.05, 0.71) | 0.025 |
| Faith's PD      | Other CVD medications | 0.38 | (0.03, 0.73) | 0.031 |
| Specific medications | Observed species | Trazadone | Non-significant: Albuterol, Bupropion, Sildenafil, Cholecalciferol |
| Shannon         | Albuterol          | Non-significant: Antithrombotics, Beta blockers, Bronchodilators, Non-opioid analgesics, NSAIDs, SSRI, Other Psychiatric medications, Sexual function medications, Other supplemental vitamins, Vitamin D |
| Faith's PD      | Non-significant: Bupropion, Trazadone, Sildenafil, Cholecalciferol |

Footnote. Abbreviations: BDI, Beck Depression Inventory; BMI, body mass index; CVD, cardiovascular disease, NSAIDs, nonsteroidal anti-inflammatory drugs; SF-36 PCS, 36-item Short Form Health Survey Physical Health Composite Score; PD, phylogenetic diversity; PVD, peripheral vascular disease; SSRI, selective serotonin re-uptake inhibitor.

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