The Microbiome in Posttraumatic Stress Disorder and Trauma-Exposed Controls: An Exploratory Study

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ABSTRACT

Objective: Inadequate immunoregulation and elevated inflammation may be risk factors for posttraumatic stress disorder (PTSD), and microbial inputs are important determinants of immunoregulation; however, the association between the gut microbiota and PTSD is unknown. This study investigated the gut microbiome in a South African sample of PTSD-affected individuals and trauma-exposed (TE) controls to identify potential differences in microbial diversity or microbial community structure.

Methods: The Clinician-Administered PTSD Scale for DSM-5 was used to diagnose PTSD according to *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* criteria. Microbial DNA was extracted from stool samples obtained from 18 individuals with PTSD and 12 TE control participants. Bacterial 16S ribosomal RNA gene V3/V4 amplicons were generated and sequenced. Microbial community structure, α-diversity, and β-diversity were analyzed; random forest analysis was used to identify associations between bacterial taxa and PTSD.

Results: There were no differences between PTSD and TE control groups in α- or β-diversity measures (e.g., α-diversity: Shannon index, $t = 0.386$, $p = .70$; β-diversity, on the basis of analysis of similarities: Bray-Curtis test statistic = –0.033, $p = .70$); however, random forest analysis highlighted three phyla as important to distinguish PTSD status: Actinobacteria, Lentisphaerae, and Verrucomicrobia. Decreased total abundance of these taxa was associated with higher Clinician-Administered PTSD Scale scores ($r = –0.387$, $p = .035$).

Conclusions: In this exploratory study, measures of overall microbial diversity were similar among individuals with PTSD and TE controls; however, decreased total abundance of Actinobacteria, Lentisphaerae, and Verrucomicrobia was associated with PTSD status.

Key words: childhood trauma, C-reactive protein, immunoregulation, inflammation, microbiome, posttraumatic stress disorder.

INTRODUCTION

Violence and trauma are highly prevalent in South Africa (SA), with approximately 75% of the population experiencing at least one traumatic event and more than half experiencing multiple traumatic events within their lifetime (1,2). Interpersonal violence (e.g., physical and sexual assault, intimate partner violence) is the leading cause of injury in SA, a country where the homicide rate is seven times higher than the global average (3). The extent and severity of trauma exposure in SA have been found to contribute significantly to the overall burden of disease

BMI = body mass index, **CAPS-5** = Clinician-Administered Posttraumatic Stress Disorder Scale for DSM-5, **CRP** = C-reactive protein, **CTQ** = Childhood Trauma Questionnaire, **DSM-5** = Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, **IBD** = inflammatory bowel disease, **IBS** = irritable bowel syndrome, **IgA** = immunoglobulin A, **IL** = interleukin, **IQR** = interquartile range, **LEC-5** = Life Events Checklist for DSM-5, **MDD** = major depressive disorder, **OTUs** = operational taxonomic units, **PTSD** = posttraumatic stress disorder, **QIIME** = Quantitative Insights into Microbial Ecology, **SA** = South Africa, **TE** = trauma-exposed, **Treg** = regulatory T cell, **VSURF** = variable selection using random forests

SDC Supplemental Content

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(4). Within the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) (5), posttraumatic stress disorder (PTSD) is classified as a trauma- and stress-related disorder that is characterized by the presence of symptoms in four diagnostic clusters (intrusion, avoidance, negative alterations in cognitions and mood, and alterations in arousal and reactivity) that significantly impair psychosocial functioning (5). Symptoms can persist for years after a traumatic event (6) and negatively affect quality of life (7,8). The prevalence of PTSD in SA has been found to range between 2.3% and 19.9% (9,10). In light of the substantial health and economic burden imposed by PTSD, research into the pathophysiology of the disease is imperative to both gain new insights into factors that contribute to the disease and develop novel strategies for prevention and treatment.

Recent research has focused on the role of exaggerated inflammatory responses in the pathogenesis of PTSD. To this end, a subset of $CD4^+$ T cells, the regulatory T cells (Tregs), have been found to be altered in PTSD-affected individuals (11,12). Tregs play an important role in defense against inappropriate inflammatory responses, such as those observed in autoimmunity, allergy, and asthma (13). Reduced levels of Treg cells have been observed after exposure of human participants to a laboratory stressor (14) and in male and female refugees with chronic PTSD relative to healthy controls (11,12). Furthermore, reduced frequency of Tregs is associated with autoimmune diseases such as thyroiditis, inflammatory bowel disease (IBD), and rheumatoid arthritis (13), conditions for which individuals with PTSD show increased risk (15). Consistent with these findings, genome-wide association studies in PTSD cohorts revealed an association with *ANKRD55* (16), a gene associated with several autoimmune and inflammatory disorders, including multiple sclerosis (17,18), Type 2 diabetes mellitus (19), celiac disease (20), and rheumatoid arthritis (21). In addition, Jergović et al. (22,23) observed an altered Treg phenotype in male combat veterans with PTSD compared with healthy controls. PTSD has also been found to result in up-regulation of interleukin (IL) 6 and proinflammatory cytokines, including interferon-γ, IL-1β, and tumor necrosis factor (24–26). Elevated levels of C-reactive protein (CRP), a clinically used marker of inflammation, have also been observed in individuals with PTSD (27–29). Preexisting elevated CRP levels (30), or elevated IL-6 measured within 24 hours after trauma (31), have been found to predict postdeployment Clinician-Administered PTSD Scale (CAPS) scores in war zone–deployed Marines or a diagnosis of PTSD in children 6 months after trauma, respectively.

An important factor determining immunoregulation, indicated by a balanced expansion of effector T-cell populations and Tregs, is the human microbiome (32–34). The human microbiota comprises all the microorganisms (archaea, bacteria, eukaryotes, fungi, and viruses) harbored by the human body, and the complete catalog of these microbial symbionts and their genes constitutes the human microbiome (35). Research suggests that microbial inputs are essential for maintaining homeostasis and optimum health (36), controlling blood-brain barrier permeability (37), and regulating central nervous system (CNS) function (38). A complex, bidirectional system of communication exists between the gut microbiome, the gut, and the CNS (39). Data from animal studies indicate that environmental and gut microbial species elicit a significant impact on cognitive function, memory, and fundamental patterns of behavior, such as social interaction and stress coping (40–43). In addition, stress can influence the composition of the gut microbiota, and the bidirectional communication between microbiota and the CNS in turn influences stress reactivity (40,41,44). Alterations in microbiota have been shown to modulate plasticity-related (45–47), serotonergic (40,45,48,49), and GABAergic (50–52) signaling systems in the CNS. Dysregulation of the gut microbiome (dysbiosis) therefore may influence risk for developing a disease, including stress- or trauma-related disorders (40,41,44).

Gut microbiota have also been found to play a role in programming of the hypothalamic-pituitary-adrenal axis (one of the key regulators of the stress response system) (38,53), with implications for stress-related disorders, including PTSD. Dysregulation of the hypothalamic-pituitary-adrenal axis may contribute to the pathophysiology of PTSD (54). Glucocorticoid-mediated immunosuppression may result in the reduction of inflammatory responses in the short term, but in the long term, it can also lead to an imbalance in the homeostasis between pathobionts (resident microbes with pathogenic potential (55,56)), gut microbiota, and the mucosa. Indeed, glucocorticoids induce the expansion of pathobionts, such as *Helicobacter* species, a gram-negative bacterium, shown to enhance chronic inflammatory diseases (57). Mice exposed to psychological stressors exhibit expansion of *Helicobacter* species, as determined by absolute abundance, evaluated using real-time quantitative polymerase chain reaction (58), or relative abundance (40,41), and this effect can be prevented by the administration of a glucocorticoid receptor antagonist (58). *Helicobacter* species induce colitis in IL-$10^{-/-}$ mice, which lack adequate immunoregulation, an effect that may be due to overactivation of host immune defenses (59–61). Immunization with a heat-killed preparation of an immunoregulatory bacterium that increases Treg and anti-inflammatory cytokines, such as IL-10 and transforming growth factor β (62), has recently been shown to prevent stress-induced increases in a PTSD-like syndrome in mice (40,41), suggesting that the balance of proinflammatory and anti-inflammatory or immunoregulatory microbial inputs could contribute to the risk of developing a PTSD-like syndrome.

The present study investigated the gut microbiome profiles of a relatively homogeneous group of individuals of a unique South African mixed ancestry population.[1] Individuals with a diagnosis of PTSD were compared with individuals who were exposed to a traumatic event but did not develop PTSD, to identify microbial signatures associated with PTSD.

METHODS

Clinical and Metabolic Measures

All research participants provided written informed consent to take part in the study after the study procedures were explained in detail. The Health Research Ethics Committee 2 of Stellenbosch University approved the study. Participants were recruited through purposive sampling using various avenues including referrals from general and psychiatric hospitals and community clinics from Cape Town and surrounding areas as well as through print, radio, and Web advertisements. Samples included in this study were collected from August 2014 to February 2015.

[1]In South Africa, recognized population groups include Black African, Coloured, Indian or Asian, White, and Other. Coloured people, included in this study, constitute the largest population group in the Western Cape (63).

On the basis of the MINI International Neuropsychiatric Interview, version 6.0 (64), participants were excluded if they had bipolar or psychotic disorders or an alcohol or drug use disorder within the past 6 months. Other exclusionary criteria included a neurological disorder, a diagnosis of metabolic syndrome, diarrhea within the past week, any antibiotic use 4 weeks before stool sampling, or a diagnosis of IBD, celiac disease, or irritable bowel syndrome. Written informed consent was obtained from all study participants. The study sample consisted of 18 PTSD and 12 trauma-exposed (TE) control participants of South African mixed ancestry matched for age, sex, time since index trauma, and the number of traumatic event exposures. Demographic and clinical data were collected using structured demographic and medical history questionnaires designed for the SHARED ROOTS parent study. PTSD diagnosis was based solely on CAPS for DSM-5 (CAPS-5) severity scores, with those with a score of 23 or higher being placed in the PTSD cohort. Current PTSD diagnosis and symptom severity were determined using the CAPS-5 (65). The CAPS-5 is a structured diagnostic interview used to diagnose PTSD on the basis of the DSM-5 criteria and is the criterion standard PTSD interview assessment. Plasma CRP concentration was measured as a marker associated with inflammation. CRP assays were used to report CRP concentrations higher than 3.0 mg/dl; high-sensitivity CRP assays were used to report CRP concentrations lower than or equal to 3.0 mg/dl. In 27 cases where these values were obtained using both assays, the values were highly correlated (Pearson correlation, $r = 0.996$; $p < .001$).

Adverse early life experience is an important determinant of risk for PTSD (66,67). Consequently, we estimated adverse early life experience using the Childhood Trauma Questionnaire (CTQ). The CTQ (68) was used to screen for a history of child abuse and neglect. The CTQ consists of 28 self-report items used to calculate a total childhood trauma score by adding scores obtained on five trauma subscales (physical abuse, sexual abuse, emotional abuse, physical neglect, and emotional neglect). Furthermore, prenatal maternal stress, compared with a low-stress control condition, has been shown to affect the infant microbiota measured at 7, 14, 28, 80, and 110 days of age in infants, suggesting important effects of adverse early life experience on the gut microbiome (69).

Microbiome Analyses

Microbial DNA was extracted from 1.4 ml of stabilized stool (stool specimen homogenized in stool DNA-stabilizing buffer) using the PSP Spin Stool DNA Plus Kit (STRATEC Molecular, Birkenfeld, Germany) according to the manufacturer's protocol 2 ("Isolation of total DNA from 1.4 ml-stabilized stool homogenate with enrichment of bacterial DNA"). The 16S ribosomal RNA gene amplicons were generated for the V3 and V4 regions of the 16S ribosomal RNA bacterial gene, which were recommended by Klindworth et al. (70). Illumina adapter overhang nucleotide sequences were added to the gene-specific sequences. The full-length primer sequences targeting this region were 341 forward primer (5′-CCTACGGGNGGCWGCAG-3′) and 785 reverse primer (5′-GACTACHVGGGTATCTAATCC-3′).

Libraries were prepared using the 16S Metagenomic Sequencing Library Preparation Kit from Illumina, according to the manufacturer's instructions. Libraries were sequenced using multiplexed Illumina HiSeq paired-end 100-base-pair sequencing according to the manufacturer's instructions. Base calling was performed and FASTQ sequence reads were generated using Illumina Casava Pipeline 1.8.2. Initial quality assessment was based on data passing the Illumina Chastity filter. Subsequently, reads containing adaptors and/or PhiX control signal were removed. The second quality assessment was based on the remaining reads using the FASTQC quality control tool version 0.10.0.

Taxonomy From DNA Sequences

The operational taxonomic unit (OTU) table was prepared using Quantitative Insights into Microbial Ecology (QIIME) version 1.9 (71). Forward reads were demultiplexed using default parameters, with a minimum quality score threshold set to 25. Following this step, 1,738,164 of 1,959,124 HiSeq reads passed quality control. These reads were assigned to OTUs using the closed-reference OTU picking method with Greengenes 97% reference database (August 13) (72).

Microbial Diversity Analysis

In microbiome studies, two commonly used measures of species diversity are α-diversity (assessing diversity within a sample) and β-diversity (assessing differences between samples, with greater β-diversity indicating greater dissimilarity). α- and β-diversities were analyzed using rarefied data, which corrects for differential sequencing depth among samples, using QIIME (71).

Comparison of Taxa Abundances

OTUs of the rarefied data set were collapsed by taxonomic assignment and compared using QIIME (71). In addition, we used the R package variable selection using random forests (VSURF) (73) for feature selection on the 37 phyla. This method uses random forests, which are an ensemble approach from machine learning that ranks the importance of features in terms of their ability to classify a variable of interest while taking into account the complex interrelationships of the features (74). The VSURF function provides two sets of results: the "interpretation" subset of important variables that may include some redundancy and the "prediction" subset that aims to eliminate redundancy while maintaining predictive accuracy. We then used marginal plots, partial plots, and the *find.interactions* function of the R package randomForestSRC (75) to interpret the findings of the variable selection process. In addition, to interpret the findings, we also used Pearson correlation to evaluate the relationship between the random forest model outcomes and CAPs scores, CTQ scores, and other variables of potential interest.

For additional details of the clinical population and detailed methods for microbiome analysis, see Supplemental Methods (Supplemental Digital Content 1, http://links.lww.com/PSYMED/A411).

RESULTS

Clinical and Biological Measures

A summary of key demographic and clinical data of the study participants is found in Table 1. Median CAPS-5 total scores were 33.5 for the PTSD group and 3.5 for the TE group (Table 1; $p < .001$; ranges, 23–48 for PTSD and 0–20 for TE). A difference in CTQ scores, with higher scores in the PTSD group, approached statistical significance (Table 1; $p = .068$). The type of traumatic event most frequently endorsed as the index trauma by the overall group was assault with a weapon ($n = 7$; 23.3%), followed by the sudden unexpected death of someone close to them ($n = 6$; 20.0%), physical assault ($n = 5$; 16.6%), and sexual assault ($n = 4$; 13.3%). Assault with a weapon ($n = 4$; 22.2%) and physical assault ($n = 4$; 22.2%) were the index traumas most frequently identified by those with PTSD, followed by the sudden unexpected death of someone close to them ($n = 3$; 16.7%) and sexual assault ($n = 3$; 16.7%); the index traumas most often experienced by individuals in the control group were assault with a weapon ($n = 3$; 25%) and the sudden unexpected death of someone close to them ($n = 3$; 25%). Six (33.3%) of those with PTSD were receiving concomitant psychotropic medications (3 [16.7%] were on amitriptyline [5–25 mg] for sleep or neuralgia, 1 (5.6%) was on zolpidem [5 mg] for sleep, and 2 (11.1%) were on fluoxetine [20 mg] and citalopram [20 mg], respectively, for depression). Three individuals with PTSD

TABLE 1. Clinical and Demographic Variables of the Study Participants

Clinical/Demographic Variable	PTSD Participants (n = 18)	TE Controls (n = 12)	p
Age, M (SD), y	42.0 (12.6)	38.7 (11.7)	.52
Female, *n* (%)	14 (77.8)	7 (58.3)	.26
CAPS-5 total score, median (IQR)	33.5 (30.0–36.7)	3.5 (0–9.5)	<.001
Time since index trauma, median (IQR), mo	126 (39–231)	48 (24–126)	.18
No. different types of traumatic experiences on LEC-5, M (SD)	6.3 (1.9)	5.9 (3.9)	.75
CTQ total score, median (IQR)	54 (36.5–81.5)	38 (32.0–44.0)	.068
MINI current MDD, *n* (%)	2 (11.1)	0 (0)	.50
MINI lifetime MDD, *n* (%)	12 (66.7)	6 (50.0)	.46
MINI current comorbid anxiety disorder(s), *n* (%)	6 (33.3)	1 (8.3)	.19
Current psychiatric medication, *n* (%)	6 (33.3)	0 (0)	.057
Psychiatric medication current/lifetime, *n* (%)	7 (38.9)	3 (25.0)	.43
Cigarette smoking (previous 6 mo), *n* (%)	9 (50.0)	5 (41.7)	.87
Alcohol use (previous 6 mo), *n* (%)	9 (50.0)	7 (58.3)	.78
Lifetime history of illicit substance use, *n* (%)	3 (25.0)	5 (41.7)	.21
CRP[a], median (IQR)	1.4 (0.6–2.8)	2.0 (1.0 – 6.2)	.11
BMI, M (SD), kg/m^2	28.5 (7.0)	28.6 (9.8)	.96
Waist circumference, M (SD), cm	90.7 (14.4)	87.4 (16.3)	.57
Systolic blood pressure, M (SD), mm Hg	120.3 (12.6)	131.5 (14.0)	.035*
Diastolic blood pressure, M (SD), mm Hg	75.2 (7.47)	83.7 (9.4)	.016*
Triglycerides, M (SD), mM	0.9 (0.3)	1.0 (0.3)	.30
HDL cholesterol, M (SD), mM	1.5 (0.4)	1.6 (0.5)	.45
Fasting glucose, median (IQR), mM	5.0 (4.7–5.3)	5.0 (4.8–5.2)	>.99

PTSD = posttraumatic stress disorder; TE = trauma-exposed; M (SD) = mean (standard deviation); CAPS-5 = Clinician-Administered Posttraumatic Stress Disorder Scale for DSM-5; IQR = interquartile range; LEC-5 = Life Events Checklist for DSM-5; CTQ = Childhood Trauma Questionnaire; MINI = MINI International Neuropsychiatric Interview, version 6.0; MDD = major depressive disorder; CRP = C-reactive protein; BMI = body mass index; HDL = high-density lipoprotein.

Continuous data were summarized as M (SD), if approximately normally distributed, and as medians and IQRs if nonnormally distributed. Differences between normally and nonnormally distributed data were assessed using Student's *t*-tests and Mann-Whitney *U* tests, respectively. Categorical data were summarized as counts and percentages, and differences between groups were assessed using χ^2 or Fisher exact tests, where appropriate.

*$p < .05$, Student's *t*-test.

[a] CRP >3.0 mg/l based on CRP assay; CRP ≤3.0 mg/l based on hsCRP assay.

(16.7%) and one control (8.3%) were on treatment of hypertension, and one control participant (8.3%) was receiving treatment for hypercholesterolemia. The only variables that differed significantly between the PTSD participants and the TE controls were mean systolic and diastolic blood pressures, with mean systolic and diastolic blood pressures being lower in the PTSD participants (Table 1).

Gut Microbiome Analyses

Of 1,959,124 reads resulting from sequencing, a total of 1,738,164 (88.7%) passed the QC filters applied to remove low-quality reads. Of those reads, 1,690,568 (97.3%) aligned to the Greengenes database (August 13) with at least 97% similarity level. A total of 37 phyla were detected in the resulting data set. The top 2 phyla observed in all participants were Firmicutes and Bacteroidetes, followed by Proteobacteria and Actinobacteria, as expected. Figure 1 shows the 10 most abundant phyla identified in the PTSD participants and TE controls. No significant differences in relative abundances of individual taxa in the PTSD participants and TE controls were observed (Fig. 1; Supplemental Digital Content 1, Fig. S1, http://links.lww.com/PSYMED/A411; Kruskal-Wallis tests with Bonferroni correction).

There were also no significant differences in α-diversity (Supplemental Digital Content 1, Fig. S2, http://links.lww.com/PSYMED/A411; Chao 1 (t = 0.832, p = .41), observed species (t = 0.760, p = .45), phylogenetic diversity (t = 0.510, p = .61), Shannon entropy (t = 0.386, p = .70; using data rarefied at 30,000 reads), or β-diversity (Supplemental Digital Content 1, Figs. S3 and S4, http://links.lww.com/PSYMED/A411) between the PTSD participants and the TE controls. Specifically, analysis of similarities revealed that there were no differences between the PTSD group and the TE control group using the Bray-Curtis distance metric (test statistic = –0.033, p = .70), weighted UniFrac distance metric (test statistic = –0.016, p = .56), or unweighted UniFrac distance metric (test statistic = –0.013, p = .52).

We created a biplot, projecting phylum level taxonomic information onto a PCoA plot, to determine which taxa drive sample distributions in PCoA space. Relative abundance of Cyanobacteria was found to be a major determinant of differences in bacterial community structure among samples (Fig. 2). All Cyanobacteria belonged to order Gastranaerophilales (YS2/4C0d2), which has recently been defined as an order within the class of nonphotosynthetic Melainabacteria (76); however, Di Rienzi et al. (77) and Hug et al. (78) propose Melainabacteria as a separate phylum. Biplot analysis at the genus level revealed no clear condition-specific patterns (Supplemental Digital Content 1, Fig. S5, http://links.lww.com/PSYMED/A411).

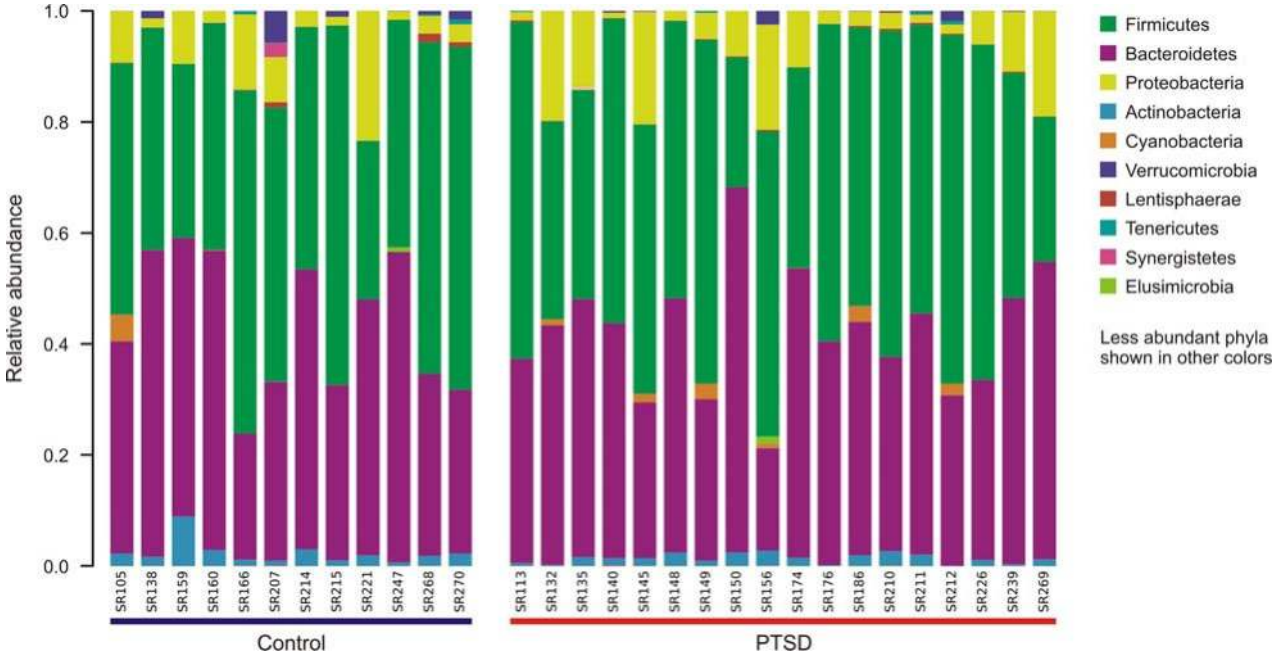

FIGURE 1. Stacked bar chart indicating the relative abundances of the 10 most abundant phyla detected in the gut microbiomes of PTSD participants and TE controls. PTSD = posttraumatic stress disorder; TE = trauma-exposed. Color image is available only in online version (www.psychosomaticmedicine.org).

We used weighted gene coexpression network analysis to investigate potential differences in microbial co-occurrence networks at the OTU level. To summarize the profiles of co-occurrence modules, we calculated the eigenvalue, which provides a mathematically optimal way of summarizing the co-occurrence patterns of all OTUs belonging to each module. To identify functional microbial communities or modules that were correlated with clinical traits, we used correlation tests to relate each eigenvalue to the clinical traits. Zhang and Horvath (79) recommend selecting a soft thresholding power that satisfies a scale-free fit of $R^2 > 0.8$ and a slope approximately equal to –1. As such, we selected $\beta = 5$ ($R^2 = 0.88$, slope = –1.95) for construction of our adjacency matrix. The weighted gene coexpression network analysis generated 22 different modules that were each arbitrarily assigned a unique color label. The bacterial dendrogram and module assignments are shown in Figure S6 (Supplemental Digital Content 1, http://links.lww.com/PSYMED/A411). The most highly connected nodes defined by scaled connectivity were dominated mainly by either the Bacteroidetes phylum or the Firmicutes phylum, with either Lentisphaerae or Proteobacteria also showing the most connectivity for two modules (Supplemental Digital Content 1, Table S1, http://links.lww.com/PSYMED/A411). Module concept and descriptive statistics can be seen in Table S1 (Supplemental Digital Content 1, http://links.lww.com/PSYMED/A411). The cluster coefficient is a measure of localized network density or "cliquishness" within the modules. Network density is a measure of the total amount of possible connections that exist within a given module. Network centrality is a measure of how much one individual node dominates the module's connectedness. The p value was generated from the Student's t-test comparison between the eigenvalues of the PTSD participants and TE controls using Bonferroni correction. Finally, the most connected node phylum was determined by the scaled connectivity coefficient. Analysis of the module eigenvalues determined that there were no differences between PTSD participants and TE controls for any of the 22 modules identified. In addition, none of the traits of the participants that were regressed against the modules were significant when using Bonferroni correction.

The functional potential of microbial communities was investigated using phylogenetic investigation of communities by reconstruction of unobserved states (Supplemental Digital Content 1, Fig. S7, http://links.lww.com/PSYMED/A411) (80). This analysis defined 328 functional groups; of these, 293 (89%) had positive values, whereas 35 (11%) had zero values for all samples. There were no significant differences between the PTSD participants and the TE controls for any functional groups using Kruskal-Wallis one-way analysis of variance and Bonferroni correction to test for relative increases or relative decreases. Although the Kruskal-Wallis test does not explicitly account for compositionality, it can be useful for probing for obvious changes in metagenomics. A total of 5.5% of functional groups showed a greater than 50% relative increase in the PTSD participants compared with the TE controls, whereas a total of 2.4% of functional groups showed a greater than 50% relative decrease in the PTSD participants compared with the TE controls. The greatest percent increase (910%) was observed for photosynthesis—antenna proteins, which include proteins associated with the phycobilisome in Cyanobacteria and red algae—and may reflect increases in Cyanobacteria of the order Gastranaerophilales (YS2/4C0d2) in a subset of PTSD participants. Statistical comparison of this functional group in the PTSD participants and TE controls revealed no significant difference ($t = 3.86$; $p = 0.050$; Bonferroni-adjusted $p = 1.0$).

Random Forest/VSURF

We used the R package VSURF (73) for feature selection on the 37 phyla. Random forest analysis identified three phyla (Actinobacteria,

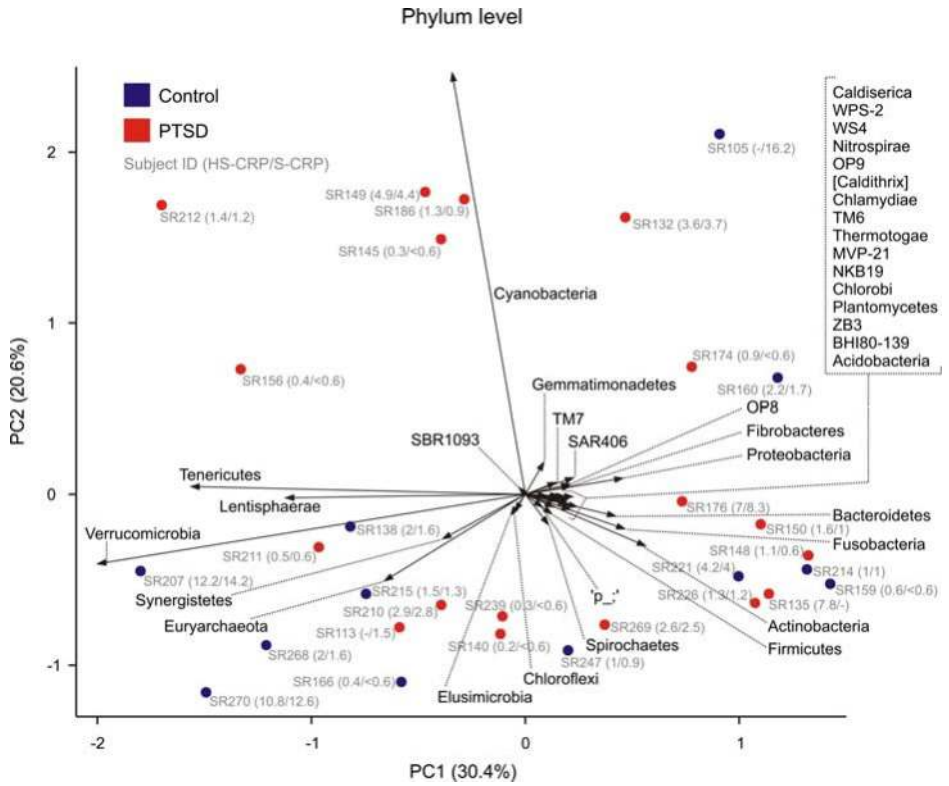

FIGURE 2. Biplot illustrating phylum-level gut microbial community analysis (closed symbols) and composition analysis (vectors) using PCA of center log ratio–transformed and standardized data from 18 PTSD participants (red symbols) and 12 TE controls (blue symbols). The distance between symbols approximates the dissimilarity of their microbial communities, as measured by Euclidean distance. PCA axes 1 and 2 explain 30.4% and 20.6% of the variation, respectively. Vectors point in the direction of the greatest increase of values for the corresponding phylum across all PTSD and TE control participants; 37 different phyla were detected. The angle between arrows indicates approximated correlation (>90° indicates a negative correlation). Gray text indicates participant identification number and plasma CRP concentrations for individual participants as measured using HS-CRP (CRP ≤3.0 mg/l) or S-CRP (CRP >3.0 mg/l) assays. Relative percent abundances of Cyanobacteria for individual PTSD participants were as follows: SR186, 2.87%; SR149, 2.78%; SR212, 2.14%; SR145, 1.67%; SR132, 1.23%; SR156, 0.23%; SR174, 0.06%; SR176, 0.01%; SR211, 0.01%; SR210, 0.0036%; and SR135, 0.0035%. Relative percent abundances of Cyanobacteria for individual TE control participants were as follows: SR105, 4.65%; SR160, 0.11%; SR207, 0.027%; SR138, 0.014%; SR215, 0.0034%; and SR214, 0.0037%. Cyanobacteria were undetectable in all other participants in the filtered data set. Operational taxonomic units associated with the orders Haptophyceae, Streptophyta, Gloebacterales, Pseudanabaenales, and Synechococcales belonging to the Cyanobacterium phylum were observed in fewer than 20% of participants. PCA = principal component analysis; PTSD = posttraumatic stress disorder; TE = trauma-exposed; CRP = C-reactive protein; HS-CRP, high-sensitive CRP; S-CRP = sensitive CRP. Color image is available only in online version (www.psychosomaticmedicine.org).

Lentisphaerae, and Verrucomicrobia) as highly important to classify PTSD versus TE controls. Of these, Lentisphaerae and Verrucomicrobia were correlated, and the more parsimonious set of results included only Actinobacteria and Verrucomicrobia. As per the random forest feature selection method that we used, we refer to this first set of three phyla as the interpretation set of results and the subset of Actinobacteria and Verrucomicrobia as the prediction set. The Cohen *d* effect size for the interpretation model [Actinobacteria, Lentisphaerae, Verrucomicrobia] was 0.894; the Cohen *d* effect size for the prediction model [Actinobacteria, Verrucomicrobia] was 0.864. Thus, both models yielded large effect sizes in terms of the taxonomic differences between individuals with PTSD and TE controls. The interpretation step of the variable selection function had an error rate of 30.7%; the prediction step had an error rate of 26.4%. The marginal plots, which show the unadjusted relationship between the predicted probability of PTSD and abundance of these taxa (Fig. 3), and the partial plots (Supplemental Digital Content 1, Fig. S8, http://links.lww.com/PSYMED/A411), which show the adjusted relationship, were similar. For all three phyla, the general relationship was that higher abundance corresponded with a lower probability of PTSD. Similarly, lower total abundance of these taxa corresponded with higher PTSD CAPS score (Fig. 4; Pearson $r = –0.387$; $p = .035$). There was no evidence of strong two-way interactions among the phyla. The prediction subset of results showed similar patterns (Supplemental Digital Content 1, Fig. S9, http://links.lww.com/PSYMED/A411).

The relative abundance of [Actinobacteria, Verrucomicrobia] was also associated with childhood trauma scores (CTQ, total score), with higher CTQ scores associated with lower total relative abundance (Supplemental Digital Content 1, Fig. S10, http://links.lww.com/PSYMED/A411; Pearson $r = –0.375$; $p = .041$). There

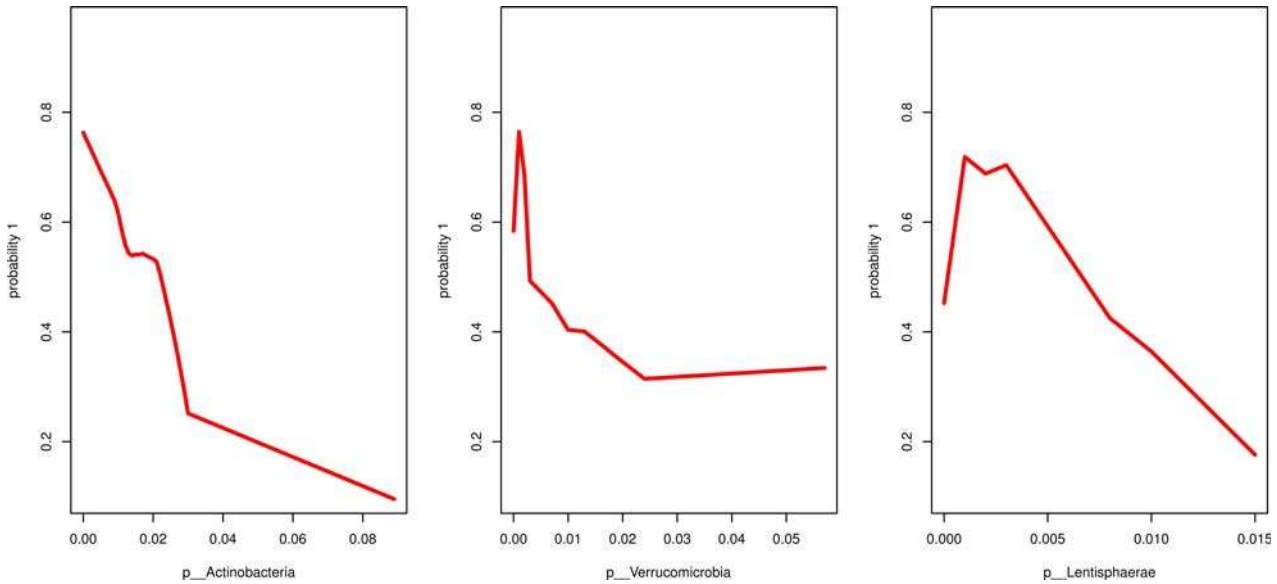

FIGURE 3. Marginal plots of the random forests predicted values for the estimated probability of PTSD from the random forests versus the relative abundance of the three phyla identified as important for distinguishing PTSD status. PTSD = posttraumatic stress disorder. Color image is available only in online version (www.psychosomaticmedicine.org).

was, however, no difference in CTQ scores in PTSD participants versus controls (Table 1; $p = .068$).

DISCUSSION

The present exploratory study evaluated microbial diversity and community structure in 18 PTSD participants and 12 TE controls. We found no significant differences in microbial community diversity or predicted functional capacity between the PTSD participants and the TE controls. However, random forest analysis highlighted three phyla, Actinobacteria, Lentisphaerae, and Verrucomicrobia, as important to distinguish PTSD participants relative to TE controls. Decreased total abundance of these phyla was associated with higher PTSD CAPS scores. These results are interesting in part because they are consistent with predictions on the basis of theoretical grounds related to the hygiene hypothesis or the "old friends" hypothesis (32,80–89) and studies using an animal model of PTSD (40,41), leading to the hypothesis that decreased exposure to Actinobacteria and other anti-inflammatory/immunoregulatory "old friends" leads to increased vulnerability to PTSD (40). In this exploratory clinical

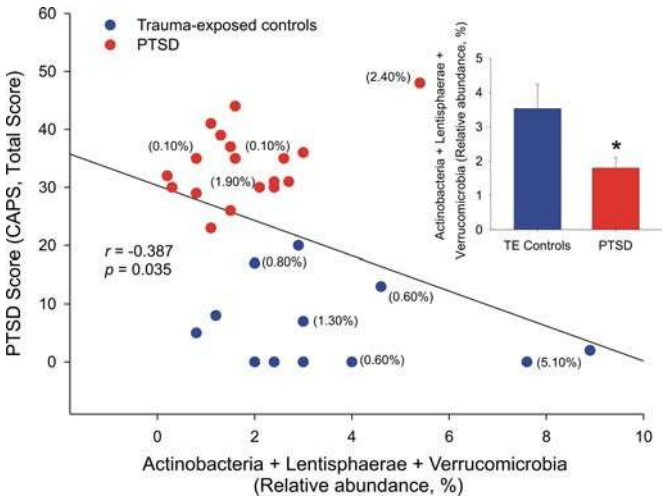

FIGURE 4. Relationship between the random forests interpretation model, relative abundance of [Actinobacteria, Lentisphaerae, Verrucomicrobia] and PTSD scores (CAPS total score). PTSD was negatively correlated with the relative abundance of Actinobacteria, Lentisphaerae, and Verrucomicrobia phyla. In other words, PTSD diagnosis was associated with a decreased abundance of these phyla (Pearson $r = –0.387$; $p = .035$). Percentages in parentheses indicate the percent relative abundance of *Akkermansia*; *Akkermansia* was below the threshold of detection for all other participants. Sample sizes: PTSD participants, $n = 18$; TE controls, $n = 12$. *$p < .05$, Student's *t*-test. PTSD = posttraumatic stress disorder; CAPS = Clinician-Administered Posttraumatic Stress Disorder Scale for DSM-5; TE = trauma-exposed. Color image is available only in online version (www.psychosomaticmedicine.org).

study, the machine learning analysis is consistent with this hypothesis, with decreases in relative abundance of Actinobacteria, Lentisphaerae, and Verrucomicrobia in those with PTSD.

The Verrucomicrobia phylum was strongly represented by a single genus, *Akkermansia*, of which there is one species, *muciniphila* (on average, in participants with Verrucomicrobia, 61.9% of Verrucomicrobia belonged to the genus *Akkermansia*). *A. muciniphila* is thought to be potently anti-inflammatory in humans and induces Treg cells (90). *A. muciniphila* has been reported to be reduced in a number of diseases or conditions associated with a failure of immunoregulation and/or increased inflammation, including Type 1 and Type 2 diabetes, obesity, inflammation and metabolic disorders during obesity, IBD, appendicitis, atopic diseases, autism, and aging (for references, see Ref. (91)). The Actinobacteria phylum was strongly represented by the *Collinsella* genus (*Collinsella* represented 54.2% of Actinobacteria, expressed as mean percent of Actinobacteria among those participants with detectable Actinobacteria). Decreased relative abundance of Actinobacteria also has been described in individuals with major depressive disorder (92), whereas a recent study demonstrates a negative association between relative abundance of Actinobacteria and stress-induced increases in gut permeability (93). Conversely, a recent analysis of semi-supercentenarians (105–109 years of age), in comparison to adults, elderly individuals, and centenarians, found a higher prevalence of *Akkermansia* (Verrucomicrobia) as well as *Eggerthella* and *Bifidobacterium* (prominent genera of gut commensals belonging to the Actinobacterium phylum) in semi-supercentenarians (94). These data identify a clear hypothetical framework that can be investigated in future studies. It will be important to both replicate these findings in such studies and to investigate the effects of the Actinobacteria, Lentisphaerae, and Verrucomicrobia phyla more in-depth.

These data are consistent with a previous clinical study of maternal prenatal stress, in which the relative abundances of Actinobacteria (including a consortium of Actinomycetaceae, *Bifidobacterium*, *Collinsella*, and *Eggerthella*) were low in infants whose mothers had experienced high cumulative stress, and the relative abundance of *Akkermansia* declined dramatically in the group with high cumulative stress after the first month and remained low thereafter (69). It's possible that in the present study, maternal prenatal stress or adverse early life experience induced alterations in the relative abundances of Actinobacteria, Lentisphaerae, and Verrucomicrobia that persisted until the time of adult trauma and, subsequently, led to the development and persistence of PTSD symptoms. Alternative explanations for these findings include current use of psychiatric medications by 6 (33.3%) of the 18 PTSD participants, and the possibility that altered autonomic nervous system function (expected in some individuals with a history of early adversity (95) may result in an altered environment for the gut microbes (96).

It is possible that elevated inflammation at the time of trauma exposure is critical for determining PTSD outcomes (30,31). In support of the hypothesis that elevated inflammation before or immediately after trauma is an important factor in determining the development of PTSD symptoms after trauma exposure, preexisting elevated CRP levels (30) or elevated IL-6 measured within 24 hours after trauma (31) has been found to predict subsequent PTSD symptoms. Studies in rodents are consistent with this hypothesis because individual differences in stimulated IL-6 release before psychosocial stress predict subsequent vulnerability to anxiety and depression-like behavioral responses (97), and immunizations with an immunoregulatory bacterium that prevent stress-induced exaggeration of IL-6 release prevent the development of a PTSD-like syndrome (40,41).

Individual differences in the host immune response may play an important role in vulnerability to PTSD symptoms after trauma exposure. Consistent with this hypothesis, studies in rats show that glucocorticoids decrease IgA (which normally inhibits bacterial adherence to intestinal epithelial cells), increase bacterial adherence over two-fold, and increase bacterial translocation to mesenteric lymph nodes (98). Decreased immunoregulation, as evidenced by decreased frequency of Treg cells, or altered Treg function, may lead to overactive host immune defenses, increased gut permeability, colitis, and exaggerated PTSD symptoms after trauma exposure (11,12,14,22,23). On the basis of the current study, decreases in the relative abundances of Actinobacteria, Lentisphaerae, and Verrucomicrobia (including the prevalent human commensal, *A. muciniphila*) may contribute to decreased immunoregulation in PTSD.

Limitations

There are a number of important limitations of this study that deserve mention. The PTSD and TE control participants were comparable with regard to a number of clinical end points, including age, sex, time since index trauma, number of different types of traumatic experiences, current and lifetime depressive symptoms and comorbid anxiety disorders, smoking and alcohol use in the previous 6 months, lifetime history of illicit substance abuse, and symptoms of metabolic syndrome. That being said, some members of the TE group reported subthreshold symptoms of PTSD, as well as clinically significant symptoms associated with other current psychiatric conditions. There were no differences in plasma CRP concentrations between the PTSD and TE control participants. Although elevated plasma concentrations of CRP, a biomarker of inflammation, have been observed in individuals with PTSD (27–29), symptoms not specifically evaluated with respect to CRP concentrations in the present study, such as reexperiencing symptoms, may account for this association (27). Previous studies have found that 23.0% of individuals in a community sample have elevated CRP (>3 mg/l) (28); a similar percentage of the participants in the present study was found to have elevated plasma CRP concentrations (8/30 participants overall [26.7%], 4/18 PTSD participants [22.2%], and 4/12 TE controls [33.3%]). In contrast to previous studies with larger sample sizes, we were not able to detect increases in plasma CRP concentrations in the PTSD participants, although previous studies did not use TE controls as a comparison group (28,29). Indeed, trauma exposure per se might be a factor that contributed to elevated plasma CRP concentrations in individuals with PTSD in previous studies, because trauma exposure is associated with elevated CRP on the basis of a transdiagnostic meta-analysis (99).

There are a number of other limitations of the current work. First, the study was of a cross-sectional nature. A longitudinal design will be required to determine the potential for causal effects of certain microbial profiles. Second, the sample sizes in this exploratory study were relatively small; consequently, the power of the study was not optimal. To guide the design of future studies, we estimated power by Monte Carlo simulation as implemented in the

HMP package in R, using the Dirichlet-multinomial parameters estimated from the data from this study. We performed 20,000 iterations for a range of read depths (10–100 thousands in increments of 10 thousands) and sample sizes (15–60 in increments of 15). Using these parameters, future studies would have greater than 98% power to detect a difference between groups with 60 samples per group and using a Bonferroni correction for 20 comparisons ($\alpha = .0025$; four clinical functional outcomes and five biological signatures). Third, some in the TE group exhibited posttraumatic symptoms and/or met the criteria for other psychiatric conditions (e.g., major depressive disorder). Fourth, index traumas differed between study participants, and there was also a wide range of time since index trauma. Fifth, an additional potential limitation is that the CAPS-5 is a relatively new measure and guidance regarding criteria for case ascertainment or symptom severity cut points is not widely available. Sixth, inclusion of functional metabolomics would inform potential mechanisms involved in any differences in microbial community structure or diversity. Lastly, future studies should include non–TE controls, in addition to TE controls, to explore the relationships among trauma exposure, the microbiota, and PTSD symptoms.

CONCLUSIONS

In this report, we tested the hypothesis of an association between the gut microbiome and PTSD. In our exploratory study, we were unable to detect differences in microbial community structure, or α- or β-diversity measures; however, random forest analysis identified a biological signature of vulnerability to developing PTSD, specifically decreases in relative abundance of a consortium of three phyla with notable immunoregulation-promoting capabilities, including Actinobacteria, previously associated with lower stress-induced increases in gut permeability in humans. These phyla are biologically plausible in terms of their effects on PTSD, and they could form the a priori hypotheses for larger longitudinal studies, in which we could further evaluate both the combined and individual associations between these phyla and PTSD. Future studies addressing the limitations of this exploratory study will be required to validate these findings.

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