nature mental health

Article

https://doi.org/10.1038/s44220-023-00145-6

Association of probable post-traumatic stress disorder with dietary pattern and gut microbiome in a cohort of women

Received: 17 November 2022

Accepted: 19 September 2023

Published online: 19 October 2023

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Post-traumatic stress disorder (PTSD) is a psychiatric condition that may occur in people who have experienced or witnessed traumatic events. The microbiota–gut–brain axis has been suggested to play an important role in mental health. Here we analysed information on trauma exposure and PTSD symptoms with gut microbiome data and dietary information of 191 individuals enrolled in a substudy of an ongoing longitudinal cohort of women. We demonstrated that higher PTSD symptom levels were associated with less adherence to the Mediterranean diet pattern, and this association was also linked to specific PTSD putative protective species such as *Eubacterium eligens*. Moreover, the microbial pathways involved in the biosynthesis of pantothenate and coenzyme A were identified as PTSD putative protective, and these pathways were mainly contributed by PTSD putative protective species such as *Akkermansia muciniphila*. These findings have the potential to inform dietary- or microbiome-based interventions for PTSD prevention or amelioration.

Post-traumatic stress disorder (PTSD) may occur in people who have experienced or witnessed traumatic or terrifying events such as life-threatening accidents, physical or sexual assault, war-related events or natural disaster. It is a major contributor to the global disease burden and is estimated to affect almost 4% of the world's population[1,2]. Numerous studies have shown that PTSD is associated with medical comorbidities, including asthma[3], cardiovascular disease[4,5], chronic pain and inflammation[6,7], obesity[8], type 2 diabetes[9], gastrointestinal disorders[10] and cognitive decline[11]. Although a substantial body of research has greatly improved our knowledge regarding the prevalence, clinical symptoms, and consequences of PTSD over the past decades, much remains to be learned about why some people are more vulnerable to developing PTSD after trauma and the mechanisms driving the relation between PTSD and chronic diseases. The potential role of the gut microbiome in mental health and a range of chronic diseases is increasingly recognized[12,13], although its role in PTSD specifically has yet to be understood. In addition, our understanding of the aetiology of PTSD and the mechanisms underlying its association with physical health remains limited.

On the basis of the literature and our own work, there are at least three reasons to hypothesize an important role for the gut microbiome in PTSD. First, numerous studies have suggested that the gut

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microbiome is linked to the development and function of the central nervous system[14–16]. Indeed, the gut–brain axis, which involves multiple biological systems and bidirectional communication between the gut microbiome and the brain, is crucial to the maintenance of intestinal homeostasis and host health[17,18]. The gut–brain axis provides a mechanism via which the gut microbiome may influence mental health. This possibility was supported most recently by a meta-analysis summarizing evidence documenting gut microbiota perturbations associated with a transdiagnostic pattern with depletion of certain anti-inflammatory butyrate-producing bacteria and enrichment of pro-inflammatory bacteria in patients with depression, bipolar disorder, schizophrenia, and anxiety[19]. Of note, PTSD was not included due to the paucity of studies. Second, the gut microbiome influences both amygdala development and response[20]. PTSD is a disorder of fear; it is well established that dysregulation in brain circuits involved in learned fear, with a central role for the amygdala, is involved in the disorder[21,22]. Recent work suggests amygdala reactivity may drive the relation between PTSD and cardiovascular disease[23] and diabetes[24]. Hence, the gut microbiome may be especially important for PTSD. Third, we recently found that PTSD was associated with a lower improvement in overall diet quality over 20 years in an age-adjusted model, among a cohort of women whose diet quality generally improved over time[25]. Previous studies reported that higher (versus lower) PTSD symptoms were associated with more frequent intake of unhealthy foods, including sugar-sweetened beverages and fast food, among adolescents and young adults[26–28]. Food and nutrition modulates the human gut microbiome in ways that meaningfully affect human health[29]. Comprising trillions of microbes including bacteria, archaea, fungi, and viruses[30], the human gut microbiome plays a vital role in our physiology, metabolism, homeostasis, and immunity[31]. Thus, PTSD may be associated with gut microbiome perturbations through diet.

So far, very few observational human studies have examined the associations between PTSD and the human microbiome[32–35]. The first exploratory study[32] ($n = 30$), in a South African cohort, found that three phyla (Actinobacteria, Lentisphaerae, and Verrucomicrobia) distinguished those with PTSD ($n = 18$) from those who were trauma exposed but did not have a PTSD diagnosis ($n = 12$). This study also found that a lower total relative abundance of these taxa correlated with having more PTSD symptoms. A second study[33], focusing on combat-exposed male cirrhotic veterans ($n = 93$), found that combat-exposed patients with versus without PTSD exhibited greater cognitive impairment, lower microbial diversity, higher pathobionts and lower autochthonous taxa composition and altered gut–brain axis functionality. A third study[34], also in a South African cohort, compared PTSD cases ($n = 79$) to trauma-exposed controls ($n = 58$). The authors identified a consortium of four genera (*Mitsuokella*, *Odoribacter*, *Catenibacterium,* and *Olsenella*), previously associated with periodontal disease, which could distinguish PTSD status with 66.4% accuracy. The relative abundance of this consortium was higher in the PTSD group than in the trauma-exposed controls and correlated positively with PTSD severity as well as with having experienced childhood trauma. Another recent study[35] examined associations between PTSD severity and oral microbiota composition in a cohort of Israeli veterans who participated in the 1982 Lebanon war, finding that decreased levels of bacteria *sp_HMT_914*, *332* and *871* and *Noxia* were correlated with PTSD severity. We emphasize that previous studies share some common limitations, including small sample sizes and 16S ribosomal RNA gene sequencing, which may miss associations with specific microbial species and metabolic pathways (functions). In addition, none of those studies used a no trauma-exposed group, which is critical for distinguishing the effects of trauma versus PTSD.

Our prior work suggested that PTSD may be associated with host health through its relations with diet and the gut microbiome. In this Article, using data from the same cohort, we hypothesized that PTSD symptoms would also be associated with diet quality. By analysing whole-metagenome shotgun sequencing data, we explored the relationship between PTSD and the gut microbiome at both taxonomical and functional levels. Specifically, we used data from two substudies within a large cohort of female registered nurses in the United States (the Nurses' Health Study II: NHS-II) to systematically examine the associations of trauma exposure and PTSD status in 2008 with dietary data and microbiome data collected in 2013. The primary goal of this study is to understand the associations among PTSD symptoms, dietary intake, and the gut microbiome, which may provide the basis for future research examining mechanisms driving the relation of PTSD with chronic disease, as well as suggest whether dietary-based interventions on the gut microbiome may provide an opportunity for the prevention or amelioration of PTSD.

Results

Characteristics of the study population

The NHS-II is a large prospective cohort study of US women with 116,430 registered nurses (25–42 years old) enrolled in 1989 (ref. 36). This analysis draws data from two substudies within the NHS-II: the PTSD substudy[37] conducted in 2008 and the mind–body study (MBS)[38] conducted in 2013. The MBS collected information on multiple subjective psychosocial factors, detailed demographic characteristics, and biospecimens[38]. Information about trauma exposure and PTSD symptoms in MBS participants was collected in 2008 as part of the NHS-II PTSD substudy[37]. Participants provided up to four faecal specimens—a set of faecal specimens collected 48–72 h apart, followed by the collection of a second set about 6 months later (Fig. 1). Microbial DNA was extracted from 809 stool samples collected from 213 participants. Study participants were excluded from the current study if they did not provide information about trauma exposure, PTSD symptoms or stool samples. The final study sample ($n = 191$) comprised 44 participants with probable PTSD (Methods), 119 participants exposed to trauma but without PTSD (trauma no PTSD) and 28 participants without trauma exposure (no trauma). Detailed phenotypic data are reported in Supplementary Table 1.

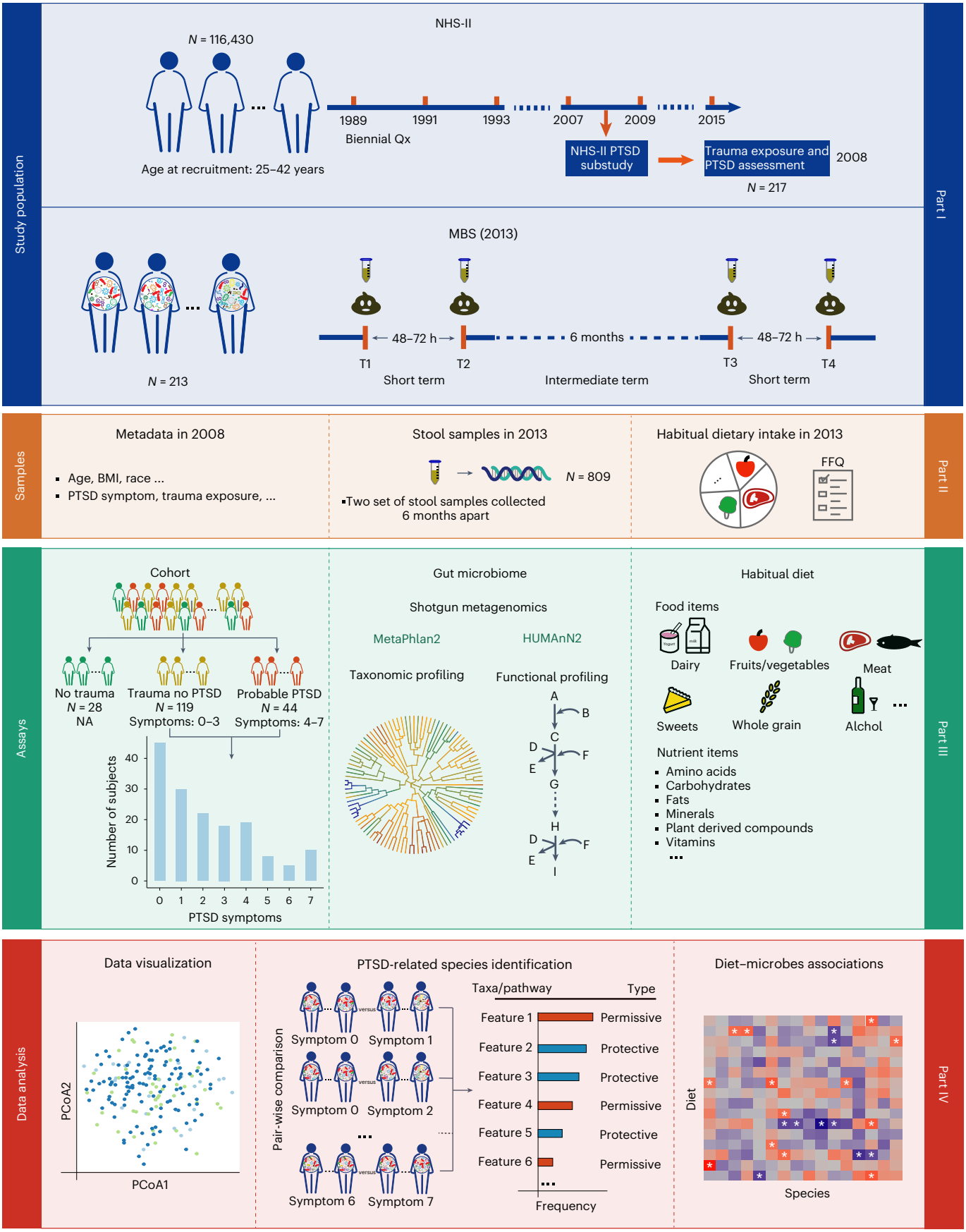
Stability of the gut microbiome over time

As we collected multiple gut microbiome samples from MBS participants over short and intermediate time intervals, we first investigated the stability of the gut microbiome over time. Sequencing data preprocessing was performed by the KneadData quality control pipeline, including removing low-quality reads and host-sequence contamination reads. Metagenomic reads were profiled for taxonomic and functional composition using MetaPhlAn2 (ref. 39) and HUMAnN2 (ref. 40), respectively. We compared the within-individual stability of the taxonomic composition and the functional pathways over short (48–72 h) and intermediate (6 months) periods. A subgroup of 157 MBS participants who provided stool samples at all four sampling timepoints was analysed.

Taxonomic and functional profiling identified 345 microbial species and 424 metabolic pathways (Supplementary Fig. 1). Most of the

Fig. 1 | Conceptual framework of the study. A total of 213 participants from the MBS, nested within the NHS-II cohort, were included to examine the associations of the gut microbiome with diet and PTSD. Each participant provided up to four stool samples, one set of stool samples was collected 48–72 h apart followed by a second set about 6 months later. Phenotypic data were collected through in-person assessment, including personal characteristics, PTSD symptoms, and habitual dietary intake by semiquantitative FFQ data. DNA was extracted from all faecal samples. The taxonomic and functional profilings were performed using MetaPhlAn2 and HUMAnN2, respectively. Qx, self-administered questionnaire covering a variety of lifestyle factors, health outcomes, and other health-related information; T1-T4, sampling time points; NA, not applicable.

https://doi.org/10.1038/s44220-023-00145-6



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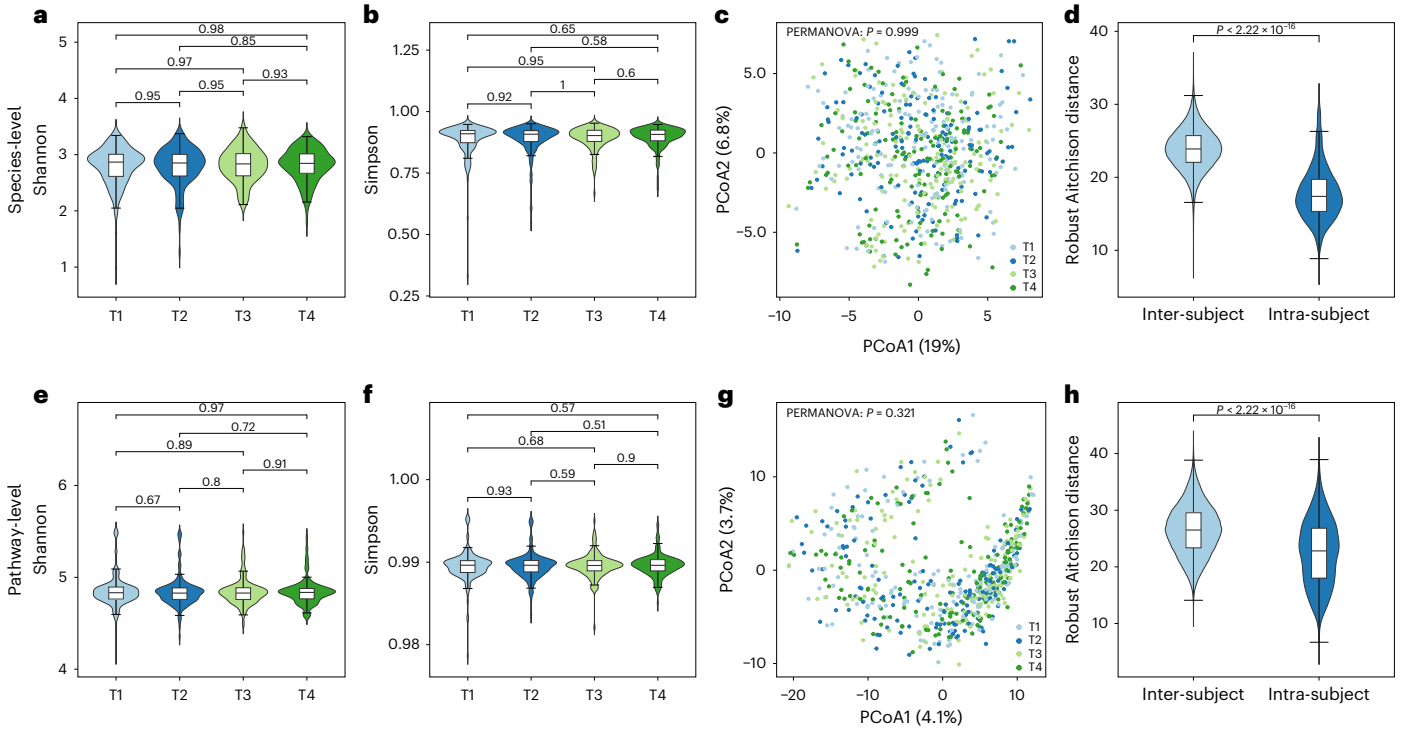

Fig. 2 | The stability of gut microbiome over time in a cohort of adult women. **a**,**b**, Violin box plots of alpha diversity of the taxonomic profiles at the species level using Shannon (**a**) and Simpson (**b**) indices ($N = 628$ samples). **c**, PCoA of samples from different sampling timepoints at the species level based on the robust Aitchison distance. **d**, Box plots for beta diversity of taxonomic profiles using the robust Aitchison distance at the species level ($N = 628$ samples). **e**,**f**, Violin box plots of alpha diversity at the functional pathway level using Shannon (**e**) and Simpson (**f**) indices ($N = 628$ samples). **g**, PCoA of samples from different sampling timepoints at the functional pathway level based on the robust Aitchison distance. **h**, Box plots for beta diversity of functional profiles using the robust Aitchison distance at the functional pathway level ($N = 628$ samples). In **a**, **b**, **e** and **f**, the significant difference was determined by Wilcoxon signed-rank test (two sided). PERMANOVA in **c** and **g** was performed based on the robust Aitchison distance with 9,999 permutations. In **d** and **h**, the significant difference was determined by Mann–Whitney *U*-test (two sided).

microbial species (75.36%) and metabolic pathways (89.62%) were observed at all four timepoints, with only 7.54% of species and 3.30% of functional pathways unique to a single timepoint. Using the Shannon and Simpson indices, we found no significant difference in alpha diversity of taxonomic composition (Fig. 2a,b) or metabolic pathways at different timepoints (Fig. 2e,f). Principal coordinate analysis (PCoA) based on the robust Aitchison distance showed no distinct community structure at the different timepoints (Fig. 2c,g), which was further confirmed by the permutational multivariate analysis of variance (PERMANOVA) using the robust Aitchison distance. We then compared the within-individual stability of taxonomic and functional composition (Fig. 2d,h) based on the robust Aitchison distance, finding that taxonomic and functional composition was more similar within participants over time than that between participants, which is consistent with findings from a previous male adult cohort that used same sampling strategy[41]. This suggests that the human gut microbiome is stable over time intervals of as long as 6 months, and thus a limited number of measurements may be adequate to investigate reliable associations with long-term health. In the subsequent analyses, we treated the data collected at the first sampling timepoint as a discovery set and the data collected at other timepoints as the test set to internally validate the key findings in the discovery set.

PTSD and overall microbiome structure

We next evaluated associations between overall microbiome structure and host factors (for example, PTSD symptom, age, body mass index (BMI), dietary scores, etc; Fig. 3) to identify important confounding factors. Using the Envfit analysis together with the robust Aitchison distance[42], we found several host factors were associated with microbiome structure. For example, BMI, depression, and antidepressants were significantly associated with the overall gut microbial composition at the species level (Fig. 3a). Antidepressants and childhood trauma questionnaire (CTQ) were top factors explaining the variation in metabolic pathways (Fig. 3b). We found no significant association between PTSD symptoms and the overall structure of microbiome.

To characterize gut microbial patterns associated with PTSD case status, we performed a diversity analysis to compare microbiome samples across women reporting no trauma, trauma no PTSD, and probable PTSD (Fig. 4) without adjustment for covariates. Shannon and Simpson indices were used to measure the alpha diversity of gut microbiome samples. We found no significant differences in Shannon and Simpson diversity among the three groups at the species level (Fig. 4a,b). PCoA plots of species-level microbial compositions based on the robust Aitchison distance showed that microbiomes of the three groups were not compositionally distinct ($P = 0.51$, PERMANOVA; Fig. 4c). Similar results were observed for functional pathways (defined manually on the basis of an intuitive understanding of the relationship and function of particular sets of enzymes, Fig. 4e–g). At the species level, the robust Aitchison distance was significantly higher among individuals in the probable PTSD group compared with those reporting no trauma or trauma no PTSD (Fig. 4d). At the pathway level, within-group variation in the probable PTSD group was only significantly higher than the no trauma group (Fig. 4h).

The dominant gut microbial phyla were Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria, with the two phyla Firmicutes and Bacteroidetes representing almost 90% abundance of the gut microbiota. Actinobacteria was significantly lower among women reporting no trauma compared with those reporting trauma but

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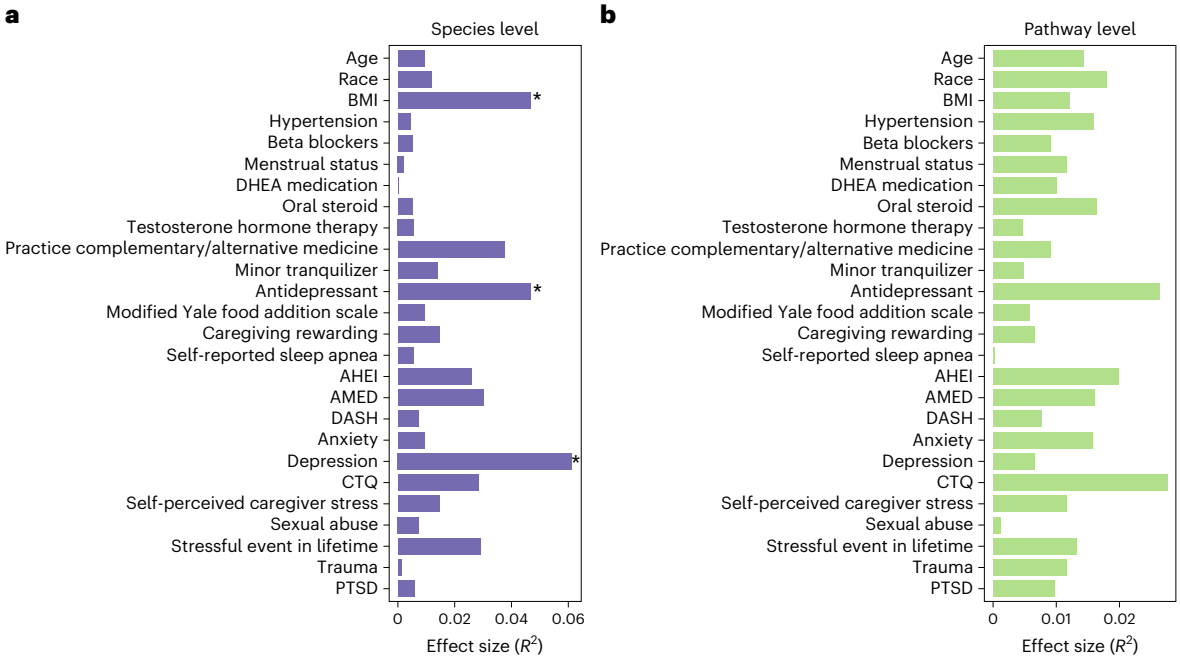

Fig. 3 | Host factors associated with the gut microbiome. a,**b**, The amount of variance (r^2) explained by each host factor in the taxonomic profiles at the species level (**a**) and functional profiles at the metabolic pathway level (**b**) determined by the EnvFit test (two sided) based on the robust Aitchison distance with each covariate included in separate models. The asterisks denote significant covariates (*P* value-adjusted by Benjamini–Hochberg adjustment, adjusted *P* value <0.15). Depression: probable depression; PTSD: no trauma, trauma no PTSD and probable PTSD.

no PTSD (Supplementary Fig. 2a). No significant differences in relative abundances of Firmicutes, Bacteroidetes, Proteobacteria, and Firmicutes to Bacteroidetes ratio in the three groups were observed (Supplementary Fig. 2b–e).

Diet quality is associated with PTSD

Motivated by the bidirectional relationship between diet and host heath[29,43,44], we next assessed the relationship between dietary scores and continuous host factors (for example, a continuous count of PTSD symptoms, age, BMI and so on; Fig. 5). Among the three dietary indices, PTSD symptom score was found to be significantly negatively correlated with the Alternate Mediterranean Diet (AMED) score (Fig. 5a). We further sought to determine the correlation between Mediterranean diet (MedDiet) components with the PTSD symptom score (Fig. 5b). In general, the consumption of plant-based foods was negatively correlated with the PTSD symptom score, while the consumption of red/processed meat was slightly positively correlated with PTSD symptoms (Fig. 5b,c). Moreover, AMED score was significantly associated with age, BMI, and Shannon diversity (species level).

Identification of a PTSD-related gut microbiome signature

To evaluate whether a subset of microbiome features was associated with trauma no PTSD and probable PTSD, we conducted traditional microbiome-wide association studies (MWAS). In our study, only three and four differentially abundant species were identified using analysis of composition of microbiomes[45] (ANCOM) in no trauma versus trauma no PTSD and trauma no PTSD versus probable PTSD comparisons, respectively (Supplementary Fig. 3 and Supplementary Tables 2–4). However, no differences in species abundance were found in the no trauma versus probable PTSD comparison. We next investigated differentially abundant pathways associated with trauma no PTSD and probable PTSD (Supplementary Fig. 4 and Supplementary Tables 2–4). Although we identified several differential abundant microbial species/pathways between no trauma, trauma no PTSD and probable PTSD, no overlapping (always identified from those three comparisons) species or functional pathways were found, making it hard to determine the relationship between PTSD symptoms and the gut microbiome.

To overcome this limitation of the current MWAS approach, we next employed the generalized microbe–phenotype triangulation (GMPT)[46] method to characterize the links between PTSD symptoms and the gut microbiome. We first categorized participants into eight different groups based on the number of PTSD symptoms (range 0–7, Supplementary Fig. 5). Differentially abundant species and functional pathways were then calculated by ANCOM[45] for all 28 pairwise comparisons of the 8 groups. Using this approach, all pairwise differential abundance analyses yielded 36 differentially abundant species (Supplementary Table 5) and 73 functional pathways (Supplementary Table 6). We then calculated Spearman correlation coefficients between the average abundance of differentially abundant microbiome features and PTSD symptoms in different groups. Those differentially abundant microbiome features with positive (or negative) Spearman correlation coefficients were denoted as PTSD putative permissive (or protective) features.

Of the top ten identified microbial species, three species (*Parabacteroides goldsteinii*, *Barnesiella intestinihominis,* and *Paraprevotella* unclassified) were denoted as PTSD putative permissive species, and seven species (*Eubacterium eligens*, *Parabacteroides distasonis*, *Akkermansia muciniphila*, *Bacteroides massiliensis*, *Bifidobacterium longum*, *Dialister invisus,* and *Roseburia inulinivorans*) were labelled as PTSD putative protective species (Table 1). Importantly, two putative protective species (that is, *A. muciniphila* and *P. distasonis*) were previously found to be significantly enriched in resilient mice compared with susceptible mice using a single-trauma PTSD model (foot shock)[47]. Seven and three metabolic pathways were identified as PTSD putative permissive and protective metabolic pathways among the top ten pathways, respectively (Table 1). For example, Pathway(PWY)-6270: isoprene biosynthesis I; PWY-7560: methylerythritol phosphate pathway II; PWY-5667: Cytidine diphosphate (CDP)–diacylglycerol biosynthesis I; PWY0-1319: CDP–diacylglycerol biosynthesis II and PWY-5188: tetrapyrrole biosynthesis I (from glutamate) were classified

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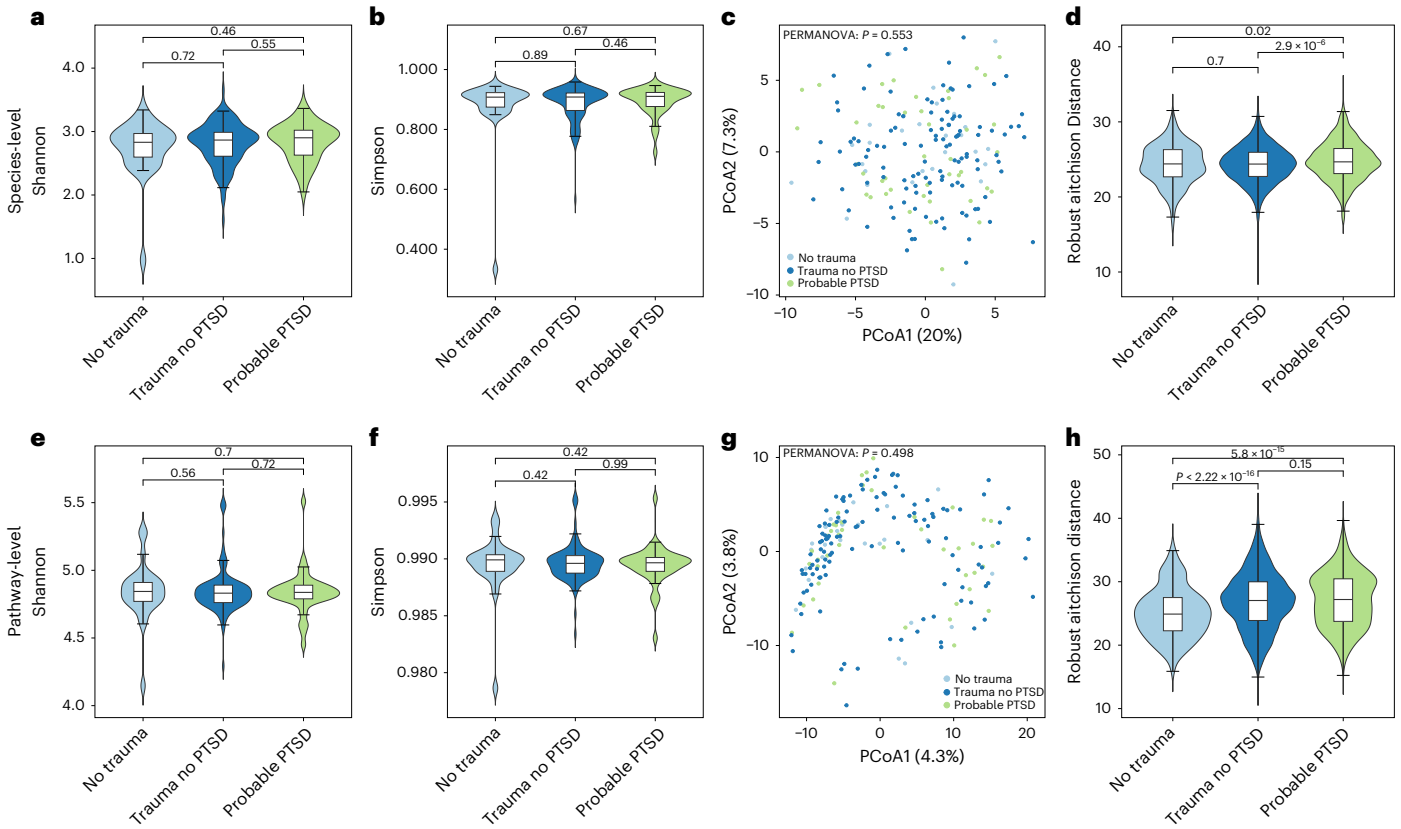

Fig. 4 | Associations between the diversity of the gut microbiome and PTSD status. a,**b**, Violin box plots of alpha diversity of taxonomic profiles at the species level using Shannon (**a**, *N* = 189 samples) and Simpson (**b**, *N* = 189 samples) indices. **c**, PCoA of microbiome samples from different groups at the species level based on the robust Aitchison distance. PERMANOVA was performed based on the robust Aitchison distance with 9,999 permutations. **d**, Violin box plots for beta diversity of taxonomic profiles using the robust Aitchison distance at the species level (*N* = 189 samples). **e,f,** Violin box plots of alpha diversity of functional profiles atthe pathway level using Shannon (**e**, *N* = 189 samples) and Simpson (**f**, *N* = 189 samples) indices. **g**, PCoA of the microbiome samples from different groups at the functional pathway level based on the robust Aitchison distance. PERMANOVA was performed based on the robust Aitchison distance with 9,999 permutations. **h**, Violin box plots for beta diversity of functional profiles using the robust Aitchison distance at the functional pathway level (*N* = 189 samples). The significant difference was determined by Mann–Whitney *U*-tests (two sided).

as PTSD permissive pathways. Notably, we found three PTSD protective metabolic pathways all involved in the pantothenate or coenzyme A (CoA) biosynthesis, including pantothenate and CoA biosynthesis I (PANTOSYN-PWY), CoA biosynthesis I (COA-PWY) and pantothenate and CoA biosynthesis III (PWY-4242). Pantothenate (also known as vitamin B5) is the key precursor for the biosynthesis of CoA, a universal and essential cofactor involved in myriad metabolic reactions, including the synthesis of phospholipids, the synthesis and degradation of fatty acids, and the operation of the tricarboxylic acid cycle[48].

Microbial species associated with various types of food

Given established associations between diet and PTSD symptoms (Fig. 5) and the importance of diet in shaping gut microbiota[49], our next analysis examined the gut microbiome and diet in our sample. For each of the 59 food items, we used Spearman correlation to identify the associated bacterial species. Overall, we found 45 species (with at least 20% prevalence) significantly correlated with at least four food items. Those 45 species are shown in Supplementary Fig. 6. We then assigned the 59 food items to 15 food groups[50], finding the top two microbiome-related food groups (based on the number of significant associations) were vegetables and red/processed meat, followed by fruits and alcohol (Supplementary Fig. 6). After normalizing the data by the number of food items in each group, we found that the fish and alcohol were most frequently associated with microbial species. Food items correlated most strongly with microbial species included fish, raw carrots, wine, oranges, spinach/collard greens cooked, caffeinated coffee, and hot dogs. Taxa linked to foods items mostly included *E. eligens*, *Erysipelotrichaceae bacterium 6145*, *Roseburia* unclassified, *Ruminococcus sp 5139BFAA*, *Eggerthella* unclassified, and *Lachnospiraceae bacterium 5163FAA* (Supplementary Fig. 6). The strongest food–microbe association was *Streptococcus thermophilus* and yoghurt consumption ($\rho = 0.31$). This is concordant with the study performed by Asnicar and colleagues in the Personalised Responses to Dietary Composition Trial, showing enrichment of the probiotic taxa *S. thermophilus* with greater full-fat yoghurt consumption[50].

We then quantified the correlation between microbial species abundances and nutrient intakes. Supplementary Fig. 7 shows a heat map summarizing the Spearman correlation between nutrients and microbial species. Notably, we found that nutrients from carbohydrates, fats, and minerals were most frequently associated with the gut microbiome. Species linked to nutrient items mostly included *Bacteroides caccae*, *R. inulinivorans*, *Roseburia intestinalis*, *Bilophila wadsworthia,* and *Bilophila* unclassified. For example, *B. caccae* was negatively associated with the intake of nutrients from amino acids, fats, and minerals. Taken together, these findings suggest a close association between dietary intake and the gut microbiome.

Associations among PTSD, diet and the gut microbiome

As shown in Supplementary Fig. 6, *E. eligens* is the microbial species associated with most food items, including many negative associations

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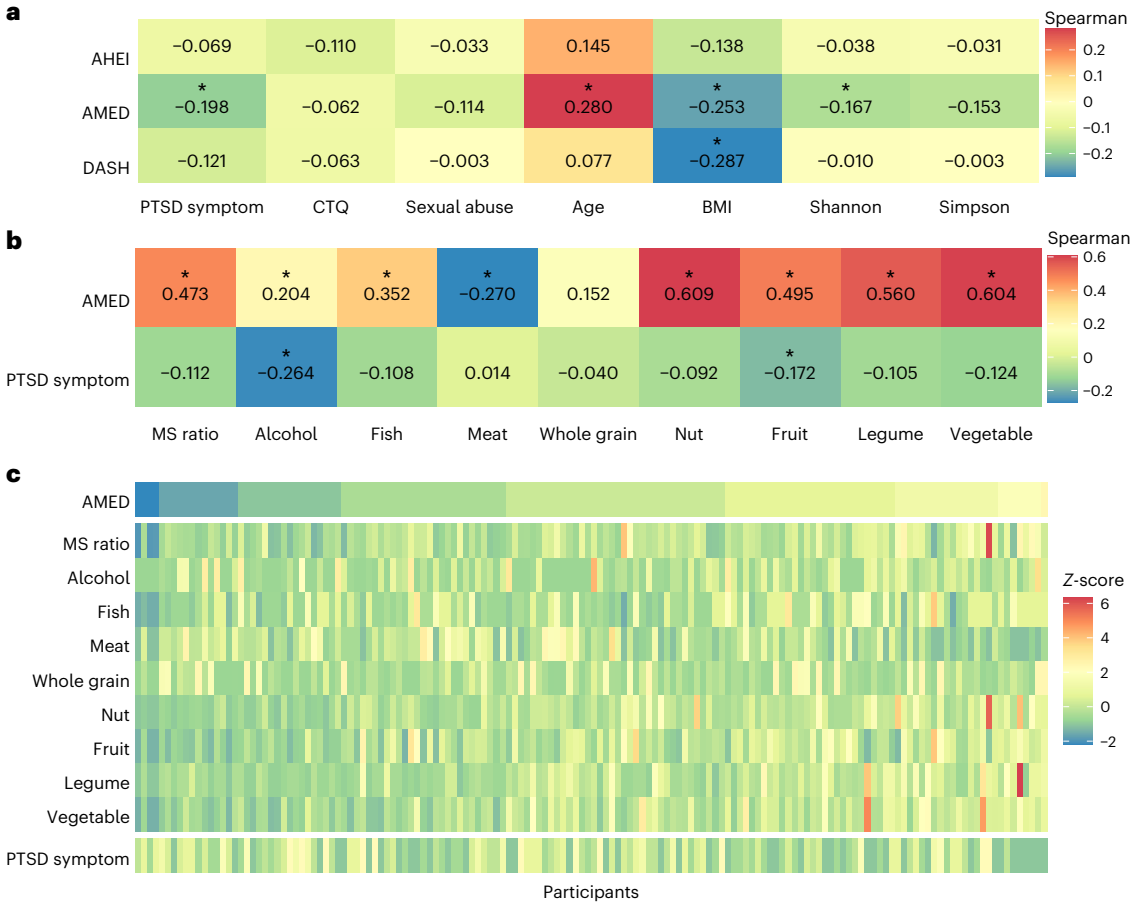

Fig. 5 | Correlation among PTSD score, host factors and microbiome diversity. a, Spearman correlations between PTSD score and other host factors (numerical) including personal characteristics, dietary indices, and microbiome diversity. **b**, Spearman correlations between PTSD score and components of the MedDiet. Significant negative or positive associations are marked with asterisks. **c**, Distributions of adherence to the MedDiet pattern, intake levels of constituent foods, and PTSD symptoms. Samples are ordered by the AMED (from lowest to highest). The horizontal axis represents different participants.

with relatively unhealthy food groups (for example, beverages, sweets and desserts, and red/processed meat). In particular, the abundance of *E. eligens* was negatively associated with the following food items: pie, carbonated beverages with sugar, candy without chocolate, hot dogs, bacon and processed meats. Notably, we also found positive associations between *E. eligens* and a few healthy food groups, such as vegetables (for example, raw carrot, spinach/collard greens cooked, and yellow squash), fruits (for example, orange and banana) and fish.

On the basis of the GMPT method, *E. eligens* was identified as the top PTSD putative protective species (Table 1). To test the consistency of this signature over time, we calculated the Spearman correlation coefficient between the average relative abundance of *E. eligens* and PTSD symptoms at the three other timepoints. The inverse association of *E. eligens* with PTSD symptoms was highly consistent across these timepoints (Supplementary Fig. 8). This link was furthermore confirmed by the fact that *E. eligens* was positively (and negatively) associated with the beneficial (and detrimental) components of the MedDiet (Supplementary Fig. 6). At the functional pathway level, we found that PTSD symptoms were inversely related to several pantothenate and CoA biosynthesis-related functional pathways (that is, PANTOSYN-PWY, COA-PWY and PWY-4242). PTSD putative protective species, such as *A. muciniphila*, largely contributed (contributional diversity, the extent to which multiple taxa contribute to a particular functional pathway) to these pathways (Supplementary Fig. 9 and Supplementary Table 6). Although *E. eligens* was not found to directly contribute to these functional pathways, the Kyoto Encyclopedia of Genes and Genomes database revealed that *E. eligens* is actually involved in pantothenate and CoA biosynthesis (M00120). Furthermore, the associations between the abundance of these functional pathways and PTSD symptoms remain negative over time (Supplementary Fig. 10).

To further investigate the connection among diet, PTSD symptoms and the gut microbiome in an integrated manner when considering the temporal relationship, we applied the mediation analysis[51] to study the impact of PTSD symptoms on diet or the gut microbiome, which revealed 19 mediation linkages (Supplementary Fig. 11 and Supplementary Table 7). The top species presented in these linkages were *Eubacterium siraeum*, *Adlercreutzia equolifaciens*, *Alistipes finegoldii*, *Gordonibacter pamelaeae,* and *Clostridium clostridioforme*. The top food items involved in these linkages were raw carrots, dairy whole milk and carbonated beverages with sugar. For example, we found that raw carrot consumption can mediate the impact of PTSD symptoms on *E. eligens* ($P_{mediation}$ = 0.048; Supplementary Fig. 10a). We also observed that *A. equolifaciens* could mediate the impact of PTSD symptoms on the intake of dairy–cottage/ricotta cheese ($P_{mediation}$ = 0.026; Supplementary Fig. 11b).

Discussion

Our study comprehensively describes associations among probable PTSD, diet, and the gut microbiome with whole metagenome sequencing data in a well-characterized cohort of women. In this study, we comprehensively evaluated the associations of PTSD symptoms with dietary patterns and gut microbiome in 191 women. In our analysis, several species and their functions were identified as PTSD putative protective features, associated with having fewer PTSD symptoms

https://doi.org/10.1038/s44220-023-00145-6

Table 1 | Identification of PTSD-related microbial species and functional pathways

	Features	Spearman	Type	*f*	*W* score	Reference
Species	*Parabacteroides goldsteinii*	0.524	Permissive	9	105.00	MDD[56], Autism[58]
	Eubacterium eligens	−0.452	Protective	6	114.67	Autism[58]
	Parabacteroides distasonis	−0.452	Protective	5	131.60	Seizure[99]
	Akkermansia muciniphila	−0.238	Protective	5	131.40	Seizure[99], GWI[59], AD[100]
	Barnesiella intestinihominis	0.048	Permissive	5	119.00	Autism[58]
	Bacteroides massiliensis	−0.071	Protective	4	128.00	PD[101]
	Paraprevotella unclassified	0.333	Permissive	4	119.75	Depression[102]
	Bifidobacterium longum	−0.262	Protective	4	118.75	Stress[103], SDA[104]
	Dialister invisus	−0.548	Protective	4	109.75	Autism[105]
	Roseburia inulinivorans	−0.048	Protective	4	97.00	Depression[106], AN[107]
Pathways	PWY-6270	0.0234	Permissive	7	298.14	–
	PWY-7560	0.0238	Permissive	7	296.86	–
	PWY-5667	0.310	Permissive	7	290.71	Antidepressant medication[60]
	PWY0-1319	0.310	Permissive	7	290.71	Antidepressant medication[60]
	PWY-5188	0.333	Permissive	6	310.83	–
	PANTOSYN-PWY	−0.476	Protective	6	278.83	Mental health score[62]
	PEPTIDOGLYCANSYN-PWY	0.2143	Permissive	6	267.50	–
	COA-PWY	−0.381	Protective	6	265.33	Neurodegenerative disorders[63]
	PWY-4242	−0.262	Protective	6	245.83	Mental health score[62]
	TRPSYN-PWY	0.143	Permissive	5	299.80	–

Top ten potential permissive and protective species and functional pathways ranked on the basis of frequency (*f*: number of times identified by pairwise differential abundance analysis) and mean *W* score (the higher the *W* score the more significant the differences in abundance levels). We then calculated the Spearman correlation coefficient between the average relative abundance of candidate features and PTSD symptoms in different phenotype groups. Those differentially abundant features with positive (or negative) correlation coefficients were denoted as putative PTSD permissive (or protective) species or pathways. A summary of previous studies that reported the association relationship between the candidate species/pathways and mental disorders is also presented. MDD: major depressive disorder; GWI: Gulf War illness; AD: anxiety and depression; PD: Parkinson's disease; SDA: stress, depression and anxiety behaviours; AN: anorexia nervosa. PWY-6270: isoprene biosynthesis I; PWY-7560: methylerythritol phosphate pathway II; PWY-5667: CDP–diacylglycerol biosynthesis I; PWY0-1319: CDP–diacylglycerol biosynthesis II; PWY-5188: tetrapyrrole biosynthesis I (from glutamate); PANTOSYN-PWY: pantothenate and CoA biosynthesis I; PEPTIDOGLYCANSYN-PWY: peptidoglycan biosynthesis I (meso-diaminopimelate containing); COA-PWY: CoA biosynthesis I; PWY-4242: pantothenate and CoA biosynthesis III; TRPSYN-PWY: L-tryptophan biosynthesis.

following trauma exposure, such as *E. eligens* and *A. muciniphila* species, and functions pertaining to pantothenate and CoA biosynthesis. Our study also linked the number of PTSD symptoms to low MedDiet adherence, particularly in association with low intake of plant-based foods. Notably, we found a significant association of the PTSD putative protective species *E. eligens* with host dietary intake, including being positively (and negatively) associated with the beneficial (and detrimental) components of the MedDiet.

Although growing evidence points to the substantial relationship between the gut microbiota and mental health, only a few studies have examined the association between the human gut microbiota and PTSD, based on 16S rRNA gene sequencing data analysis[32–34]. Consistent with previous studies[32,34], we did not find significant differences between trauma-exposed women with and without PTSD in alpha (Shannon index) and beta (based on the robust Aitchison distance) diversity. Importantly, MWAS has shown great potential to connect microbiome composition with disease states, raising the possibility of microbiome-targeted therapeutics[52]. However, it is important to note that MWAS typically generate a list of implicated microbes as biomarkers of disease, with no clear relevance to their role in disease[52–55]. To better understand the links between the gut microbiome and PTSD symptoms, we applied the GMPT method to identify PTSD-related microbial features at both compositional and functional levels. Interestingly, these identified PTSD-related (putative permissive and protective) species that have been implicated in multiple other psychiatric disorders, including major depressive disorder (for example, *P. goldsteinii*)[56], Alzheimer's disease (for example, *E. eligens*)[57], autism (for example, *E. eligens*)[58] and Golf War illness (for example, *A. muciniphila*)[59]. Specifically, mice with a higher abundance of two protective species (that is, *A. muciniphila* and *P. distasonis*) showed more resilience to foot shock stress[47]. Our analysis also identified some PTSD putative permissive pathways, such as CDP-diacylglycerol biosynthesis. Interestingly, a previous study found that antidepressant medications significantly stimulated CDP-diacylglycerol accumulation in multiple tissues[60], which is consistent with the fact that a large proportion of PTSD participants have a history of antidepressant medication. Pantothenate (also known as vitamin B5) is the key precursor for the biosynthesis of CoA, and the functional pathways of pantothenate and CoA biosynthesis are negatively associated with PTSD. Related to our findings, a previous study reported that a deficiency of pantothenate could lead to neurodegeneration or dementia in Huntington's disease[61]. Moreover, a prior study demonstrated that higher levels of pantothenate from dietary intake led to better mental health[62], which is in keeping with prior findings that defects in CoA biosynthesis lead to neurodegenerative disorders[61,63]. Notably, we demonstrated that PTSD has an inverse association with host dietary habits, especially a healthy Mediterranean-style dietary pattern. As a predominantly plant-based diet, several prospective studies have demonstrated that higher consumption of healthy food components of MedDiet was associated with a decreased risk of depression[64,65]. The directionality between PTSD and diet remains unclear, although previous work in the same cohort as our substudy shows PTSD leads to an altered diet[25]. Other research indicates that people with PTSD are more likely to have anorexia nervosa[66] and binge-eating disorder[67], and thus have more difficulty maintaining a healthy dietary pattern. Moreover, a specific association between MedDiet and a PTSD putative protective species *E. eligens* was found, including positive and inverse associations with healthy (for example, fruits) and unhealthy (for example,

red/processed meat) components of MedDiet, respectively. Related to our findings, a prior study in a large cohort of elderly individuals similarly found a positive association between *E. eligens* and adherence scores to the MedDiet[68]. In particular, *E. eligens*, a short-chain fatty acids (SCFA)-producing species, was previously identified as pectin (a type of soluble fiber that is rich in fruits and vegetables) utilizing Lachnospiraceae species of the human gut[69,70]. SCFAs, a set of microbial metabolites that constitute the major products from bacterial fermentation of dietary fibre in the intestines, are often considered key candidate mediators in the microbiota–gut–brain axis[71]. In addition, individuals with lower MedDiet adherence will probably consume less plant-derived foods with few pectin contents, which may partially explain the MedDiet's role in shaping the level of *E. eligens*.

We acknowledge several limitations in our study. First, some host factors (for example, BMI and depression) were identified as the top factors explaining the variation of the gut microbiome; yet, given the complex nature of host and environmental factors, further study is warranted to assess the extent to which this might be true in other populations and also to test systematically the interactions between a consistent set of host factors and the human gut microbiome. Second, the diagnosis of PTSD is based on the short screening scale for PTSD, which assesse seven symptoms of Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV PTSD. Additional research with a formal clinical diagnosis of PTSD is needed to confirm the findings from this study. Third, we applied compositionality-aware methods to overcome an inherent limitation of comparing relative abundances between samples, a limitation shared by many such molecular epidemiological investigations. The quantitative measurements of absolute abundance of microbial taxa will further help us to better understand the role of the gut microbiome in PTSD. Fourth, gut transit time is an important factor in shaping the gut microbiota composition and activity[72]. Unfortunately, this was not measured in our cohort. Future studies taking into account inter-individual and intra-individual differences in gut transit time will help us to better understand gut microbiome variations in PTSD. Fifth, the functional pathways of the gut microbiome are functional capacity, and they do not necessarily reflect actual translation. Future studies integrating metagenomics with multi-omics data, such as metatranscriptomic, metaproteomic, and metabolomics will further help us disentangle the host–microbiome–PTSD interaction. While we employed mediation analysis to examine the impact of PTSD on diet or the gut microbiome, it is important to note that we cannot distinguish between the following hypotheses due to the observational nature of our study and the temporal relationship between PTSD symptoms and microbiome data: (1) women with PTSD have difficulty maintaining a healthy dietary pattern, and low adherence to the MedDiet further leads to the decrease of gut bacterial species *E. eligens*. The deteriorating effect of PTSD on adherence scores to the MedDiet and the *E. eligens* abundance may result in the decreased level of end products of fermentation of dietary fibres, such as SCFAs. The disrupted synthesis of SCFAs or other metabolites due to either gut microbiota dysbiosis or dietary imbalance may further contribute to the development of chronic disease[73–75]; (2) participants with trauma exposure have difficulty maintaining high adherence to the MedDiet, which in turn leads to greater risk of developing PTSD and changes in the gut microbial community; (3) inherent differences of the gut microbiome in the individual with trauma exposure may contribute to psychological vulnerability to PTSD, and PTSD had a deteriorating effect on adherence scores to the MedDiet or (4) even more complex sceneries comprising PTSD, dietary intake, gut microbiome and other unmeasured covariates. Although it is not possible to determine the causality relationships from this observational study, our data indirectly permit fairly specific speculation and hypothesis generation. Testing these hypotheses in an integrated fashion is critical for the rational design of microbiome–diet-based interventions (for example, probiotics and prebiotic administrations) to prevent or reverse PTSD and other chronic medical conditions. Together, these findings are the first step towards diet–microbiome-based ameliorative or therapeutic strategies in PTSD and chronic disease.

Methods

Ethical statement

The study protocol of MBS was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health. Written informed consent was obtained from all participants.

Study population

The NHS-II is a large and prospective cohort study of 116,429 registered American nurses aged 25–42 years, who enrolled in 1989 (ref. 38). Each participant completed the baseline questionnaire, providing basic demographic information. Subsequently, the cohort has been subject to biennially mailed questionnaires to gather updated data on various lifestyle and health-related factors and ascertain incident diseases. As a substudy nested within the NHS-II, MBS was established in 2013 and invited a total of 688 women (238 from the diet/physical activity validation study). Among those invited, 293 women (42% of those invited) expressed willingness to participate, 54 declined and the remaining did not respond. Consents were returned by 269 women (91% of those willing to participate), and 233 of them (85%) sent back a completed biospecimen kit with at least one eligible stool sample. Additional details on the study design of MBS are provided in our previous study[38].

Sample collection

Participants in the MBS were requested to provide stool samples at multiple timepoints (Fig. 1). Collection kits were dispatched to participants, complete with detailed instructions and shipping labels. Then, the samples were shipped to our biorepository via overnight courier. Details on sample collection can be found in our previous study[38]. Between 2013 and 2014, a total of 213 participants provided up to two pairs of stool samples. Each pair of stool samples was collected from two consecutive bowel movements, approximately 48–72 h apart. The second set of two such samples was collected about 6 months after the initial collection. One hundred ninety-one participants with both PTSD and microbiome data were included. The detailed demographic information for MBS is provided in Supplementary Table 1. Among these 191 participants, 160, 21, 10 and 0 participants provided 4, 3, 2 and 1 stool samples, respectively.

Trauma and PTSD assessment, and covariate measurement

Trauma exposure was evaluated using a modified version of the brief trauma questionnaire, comprising 16 items[76]. The brief trauma questionnaire is widely recognized as a valid and reliable measure of trauma exposure, aligning closely with interview-based measures of trauma exposure[76–78]. It inquired about experiences related to exposure to 15 traumatic events (for example, natural disaster exposure, unwanted sexual contact and physical assault), along with 'a seriously traumatic event not already covered'. The exposure to each event at any timepoint in their life was coded as present or absent. Participants identified which event took place first and which event was their worst or most distressing experience. They also provided their ages during these experiences. The occurrence of PTSD symptoms in participants' lifetimes was measured in 2008 with respect to the most distressing traumatic event using the Breslau seven-item short screening scale for PTSD[79]. This scale assesses the presence or absence of seven PTSD symptoms. On the basis of previous publications[80–82], participants reporting four or more lifetime PTSD symptoms were classified as having a 'probable PTSD diagnosis' as a cutoff score of four has been found to have good concordance (sensitivity of 85% and specificity of 93%) with clinical diagnoses of PTSD[79]. The reliability of self-reported age-of-onset of trauma and PTSD symptoms was found to be excellent in this sample,

with an intraclass correlation coefficient of 0.95. The cohort comprised 44 participants with a probable PTSD diagnosis (probable PTSD), 119 with trauma without a probable PTSD diagnosis (trauma no PTSD) and 28 who reported not experiencing any trauma (no trauma).

We then used a validated standard questionnaire to collect detailed information on demographics and psychological data, such as age, BMI, race, sexual abuse, anxiety, antidepressant use and so on (Supplementary Table 1).

Depression was assessed on the MBS survey using the ten-item Center for Epidemiologic Studies Depression scale, with participants scoring ≥10 classified as having probable depression[83,84]. Participants who reported being ever diagnosed with depression by a doctor or using antidepressant medication on the 2013 NHS questionnaire were also categorized as having probable depression.

BMI was based on self-reported weight from the MBS survey and participants' height at enrollment in the NHS. Participant's level of anxiety was assessed using the Generalized Anxiety Disorder-7 scale, which asked participants to report symptoms of anxiety experienced in the last 2 weeks[85]. Minor tranquilizer use was assessed using self-report medication use from the 2013 NHS questionnaire.

Childhood physical and emotional abuse before age 12 years was assessed using five questions from the physical and emotional abuse subscale of the CTQ. These questions inquired about the frequency of family members: (1) hitting so hard it left bruises, (2) punishing in a way that seemed cruel, (3) hurtful or insulting, (4) screaming and yelling, and (5) punishing with a belt, board, cord or other hard object[86]. Responses, ranging from 0 (never) to 4 (very often) were averaged across the five questions. Sexual abuse was assessed using two questions on unwanted sexual touching and forced or coerced sexual contact by an adult or older child before age 12 years and between 12 and 17 years from the sexual maltreatment scale of the conflict tactics scales[87]. Participants' responses were labeled as follows: 'No, this never happened' (0), 'Yes, this happened once' (1) or 'Yes, this happened more than once' (2). Scores were summed and then grouped into four groups: having experienced none (score of 0), infrequent (score of 1 or 2), moderately frequent (score of 3 or 4) or frequent sexual abuse (score ≥5).

Participants were queried about whether they experienced any of 12 stressful events (for example, job loss or death of someone close) in the past 6 months, with responses summed to create a measure of total stressful events[88]. Participants were also queried about whether they provided regular support to a child or grandchild as well as a disabled or ill spouse, parent or other persons. If they responded affirmatively, they were then asked how stressful they found caregiving (0: not a caregiver; 1: not at all or a little bit stressful and 2: moderately or extremely stressful) and how rewarding they found caregiving (0: not a caregiver; 1: not at all or a little bit rewarding and 2: moderately or extremely rewarding)[38]. From these items, we derived two variables assessing the level of reward from caregiving and perceived stressfulness of caregiving, with lower scores indicating less of each variable.

Food addiction in the last 6 months was assessed with the seven-question modified Yale food addiction scale[38,89]. Participants reporting three or more symptoms were classified as having a food addiction. Participants were considered to practice complementary or alternative medicine if engaged in seven types of complementary or alternative medicine, including yoga, tai-chi, qigong, meditation, mindfulness, acupuncture or other practice. Participants self-reported history of hypertension, sleep apnoea, beta blocker use, dehydroepiandrosterone (DHEA) medication, testosterone hormone therapy, menstrual status and oral steroid use were assessed from the 2013 NHS questionnaire.

Measurement of adherence to specific dietary patterns

In the NHS-II study, food-frequency questionnaires (FFQ) data were collected at baseline and updated every 2–4 years. In 2013, participants reported their habitual dietary intake (ranging from never to ≥6 times per day) of standard portion size (for example, 0.5 cup of strawberries, one banana and 0.5 cup of cooked spinach) over the previous year on each FFQ. We then converted the frequencies and portions of each food item into the average daily intake for each participant. To derive nutrient values, we used the Harvard University Food Composition Database. On the basis of the FFQ data collected in NHS-II, we computed three dietary scores to measure the degree of adherence to the specific dietary patterns: (1) the Alternate Healthy Eating Index 2010 (AHEI)[90]: a score that measures adherence to a diet pattern based on foods and nutrients most predictive of chronic disease risk, (2) the AMED score[91]: an adaption of the original MedDiet score, and (3) Dietary Approaches to Stop Hypertension (DASH) style diet score[92]: a score capturing a dietary pattern proven to reduce hypertension.

The AHEI-2010 included 11 variables: six components for which higher intakes are better (that is, vegetables, fruit, whole grains, nuts and legumes, long-chain fats and polyunsaturated fatty acids), one component for which moderate intake is better (that is, alcohol) and four components that must be limited or avoided (that is, sugar-sweetened drinks and fruit juice, red/processed meat, trans fats and sodium)[90]. Each component of the AHEI-2010 received a score ranging from 0 to 10, and then the overall AHEI-2010 score was calculated by summing each component's score. The AHEI-2010 score can range from 0 (non-adherence) to 110 (perfect adherence), with higher scores representing healthier diet patterns[93].

The AMED score was calculated on the basis of nine components, including vegetables (excluding potatoes), fruits, nuts, whole grains, legumes, fish, the ratio of monounsaturated to saturated fat, red/processed meat and alcohol[91]. The total AMED score ranges from 0 (minimal adherence) to 9 (maximal adherence). The score criteria are the following: intake above the FFQ-specific median intake received 1 point for vegetables, fruits, nuts, whole grains, legumes, fish and ratio of monounsaturated to saturated fat; otherwise, they received 0 points. Red/processed meat consumption below the FFQ-specific median received 1 point; otherwise, 0 points. Consuming alcohol between 5 and 15 g per day for women and 10–25 g per day for men earned 1 point; otherwise 0 points.

DASH includes fruits, vegetables (excluding potatoes), nuts and legumes, low-fat dairy products, whole grains, sodium, sweetened beverages, and red/processed meat[92]. We computed a DASH score for each FFQ. For each of the components, we classified women into quintiles according to their intake ranking. The component score for fruits, vegetables, nuts and legumes, low-fat dairy products, and whole grains is the women's quintile ranking, that is, quintile 1 is assigned 1 point and quintile 5, 5 points. For sodium, red/processed meat and sweetened beverages, low intake is better. The DASH score ranges from 8 to 40. Therefore, the lowest quintile is given a score of 5 points and the highest quintile, 1 point.

DNA extraction and metagenome sequencing

DNA purification from stool aliquots was performed according to standard protocols used in the Human Microbiome Project[94,95]. Construction and sequencing of sample libraries were conducted at Broad Institute. In particular, metagenome libraries were constructed using the Illumina TruSeq or Nextera method with ~180 nt inserts and sequenced on one of the Illumina HiSeq platforms (2500 or 4000) targeting a minimum of ~2 Gnt per sample with 100 nt paired-end reads.

Microbiome taxonomic and functional potential profiling

We performed quality control on the raw metagenomics sequencing data by initially discarding low-quality reads. Subsequently, we removed reads belonging to the human genome by mapping the data to the human reference genome with KneadData[96]. Microbial taxonomic profiling was performed using MetaPhlAn2 (ref. 39), which uses a library of clade-specific markers to provide panmicrobial (bacterial, archaeal, viral, and eukaryotic) profiling. We then performed functional profiling for metagenomes by applying HUMAnN2 (ref. 40), which

maps DNA reads to a customized database of functionally annotated pan-genomes.

Statistical analysis

Microbial diversity measures were calculated at the species and functional pathway levels using the 'vegan' R package. We removed one microbiome example due to the extremely low number of species ($n = 10$) identified from it. Measures of alpha diversity included Shannon and Simpson indices. For beta diversity for community composition and functional capacity, we used the robust Aitchison distance[97] measure, which was also used in the PCoA. The difference in microbiome composition and functional capacity by sampling timepoints and PTSD status were tested with the PERMANOVA using the 'adonis' function in the 'vegan' R package. All PERMANOVA tests were performed with the 9,999 permutations based on the robust Aitchison distance.

To estimate the effect size and significance of each covariate, we applied the 'envfit' function in 'vegan' package. The ordination was performed using PCoA based on the robust Aitchison distance, and the significance value was determined using 9,999 permutations. All *P* values were derived from 'envfit' and then adjusted using the Benjamini–Hochberg method with a target rate of 0.15 for *q* values. In the GMPT method, we first categorized participants with PTSD score information into eight different disease severity groups, then ranked the level of PTSD symptoms from lowest to highest with scores from 0 to 7. For pairwise differential abundance analysis, we used ANCOM, with a Benjamini–Hochberg correction at a 5% level of significance, adjusted for BMI, depression, and antidepressant use. Then, we ranked those differentially abundant species/pathways based on their frequency present in all pairwise comparisons in descending order and then ranked species/pathways on the basis of their *W* score in descending order if they had the same frequency. Finally, for each differentially abundant species/pathway, we calculated the Spearman correlation coefficient between its average abundance and PTSD symptoms. Those differentially abundant species/pathways with positive (or negative) correlation were candidate PTSD permissive and protective features, respectively.

Spearman correlations between diet and bacterial species were computed using the 'corr.test' function from the 'psych' R package. Correlations and *P* values were computed for each pair of diet items and species; *P* values were corrected using the Benjamini–Hochberg procedure, which are reported in the text as *q* values. A bacterial feature needs to be detected in at least 20% of samples to be included. In the heat maps for the correlation matrix, the asterisk indicates that the correlation index for the corresponding species-diet pair is significant at a *q* value <0.05. The mediation analysis was conducted using the mediate function from 'mediation' R package[51,98]. The microbial species that were significantly associated with both food items and PTSD symptoms were identified using Spearman correlations with the Benjamini–Hochberg procedure. All statistical analyses were performed in R (version 4.1.2).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data that support the findings of our study can be accessed through Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health. Data are available (https://sites.google.com/channing.harvard.edu/cohortdocs/) with the permission of Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health.

Code availability

The codes for statistical analyses and visualization are available in the GitHub repository (https://github.com/ShanlinKe/PTSD).

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Acknowledgements

Y.-Y.L. is supported by grants R01AI141529, R01HD093761, RF1AG067744, UH3OD023268, U19AI095219 and U01HL089856 from the National Institutes of Health, USA, as well as a pilot grant from the Biology of Trauma Initiative of Broad Institute, USA. K.C.K., A.R., L.D.K. and A.L.R. are supported by R01MH101269 from the National Institutes of Health, USA. Y.-Y.L. and K.C.K. are supported by the Harvard T.H. Chan School of Public Health Dean's Fund for Scientific Advancement Incubation Award, and the Office of the Assistant Secretary of Defense for Health Affairs, through the Traumatic Brain Injury and Psychological Health Research Program (Focused Program Award) under award no. (W81XWH-22-S-TBIPH2), endorsed by the Department of Defense, USA. S.K. is supported by the Women's Health Interdisciplinary Stress Program of Research (WHISPR) Pilot Grant from the Connors Center at Brigham and Women's Hospital. Research reported in this publication was also supported by the NHSII cohort infrastructure grant U01 CA176726 from National Institutes of Health, USA.

Author contributions

Y.-Y.L. and K.C.K. conceived, designed and obtained funding for the project. S.K. performed all the data analysis. X.-W.W. contributed to the code of diet and nutrition data analysis. A.R., A.L.R. and T.H. contributed to the analysis of PTSD symptoms and phenotypic data. K.C.K., L.D.K., and Y.-Y.L. contributed to the result interpretation. S.K. and Y.-Y.L. wrote the manuscript. X.-W.W., A.R., T.H., A.L.R., F.G., L.D.K. and K.C.K. revised the manuscript. All authors approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s44220-023-00145-6.

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Peer review information *Nature Mental Health* thanks Sahar El Aidy and the other, anonymous reviewers for their contribution to the peer review of this work.

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Last updated by author(s): Sep 16, 2023

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Data collection	No software was used for the data collection.
Data analysis	For the raw metagenomic sequencing data, low-quality reads were discarded and reads belonging to the human genome were removed by mapping the data to the human reference genome with KneadData. Microbial taxonomic profiling and functional profiling were performed using Metaphlan2 (v0.11.1) and HUMAnN2 (2.6.0), respectively. All statistical analysis was performed with R (version 4.1.2). Details are provided in the methods section of the manuscript. The codes for statistical analyses and visualization are available in the GitHub repository (https://github.com/ShanlinKe/PTSD).

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The data that support the findings of our study are available from Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health. Data are available (https://sites.google.com/channing.harvard.edu/cohortdocs/) with the permission of Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	This study population consisted 191 female registered nurses from a sub-study (Mind Body Study) of Nurses' Health study II.
Population characteristics	The mean age of the population was 60.22. Details of the population characteristic can be found in the Results and Methods sections and Table1.
Recruitment	The study population were recruited From the Mind-Body Study (MBS). MBS was established in 2013 to invite individuals to participate if they had a valid email address on file, had participated in the original blood/urine collection done previously with the larger cohort, and had either: (1) participated in the diet/physical activity validation study collecting multiple biospecimens from 2011 to 2012 and were not part of another active ongoing substudy; or (2) given a second blood and urine sample between 2008 and 2011 and completed the 2011 biennial questionnaire. The NHS-II is a large, prospective cohort study of US women comprised of 116,429 registered nurses (25–42 years old) enrolled in 1989.
Ethics oversight	The study protocol was approved by the institutional Review Boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

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Sample size	The study population is preexisting. Women invited to the MBS were participants in NHSII, which is a large, prospective cohort study of US women with 116,429 registered nurses (25–42 years old) enrolled in 1989. Women were invited if they had a valid email address on file, had participated in the original blood/urine collection and had either (a) participated in the diet/physical activity validation study collecting multiple biospecimens from 2011 to 2012 and were not part of another active ongoing substudy or (b) given a second blood and urine sample between 2008 and 2011 and completed the 2011 biennial questionnaire. The Mind-Body study invited a total of 688 women (238 from the diet/physical activity validation study) to participate. Of these, 293 women (42% of invited) responded that they were willing to participate, 54 said they were not willing, and the remaining did not respond. Consents were returned by 269 women (91% of those willing to participate), with 233 (85%) returning a completed biospecimen kit with at least one eligible stool sample. More details can be find from the previous study: Huang et al., The Mind–Body Study: study design and reproducibility and interrelationships of psychosocial factors in the Nurses' Health Study II (2019), 10.1007/s10552-019-01176-0.
Data exclusions	Study participants were excluded from the current study if they did not provide information about trauma exposure, PTSD symptoms or stool samples.
Replication	Participants provided up to 4 fecal specimens–a set of fecal specimens collected 48–72h apart, followed by the collection of a second set about 6 months later. The key findings can be replicated across different time-points. Reproducibility of data and results is ensured by performing internal program and technical review. We ensured the reproducibility of our findings by performing internal validations during the iteration of data analysis. We rerun all the data analysis before the submission. All attempts at replication were successful.
Randomization	Not applicable. This study is not a clinical trial. There are not experimental groups and interventions in this study.
Blinding	Not applicable. This is an observational study without any interventions.

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