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DECREASED GUT MICROBIOME TRYPTOPHAN METABOLISM AND SEROTONERGIC SIGNALING IN PATIENTS WITH PERSISTENT MENTAL HEALTH AND GASTROINTESTINAL SYMPTOMS AFTER COVID-19

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BACKGROUND

An estimated 15%-29% of patients report new gastrointestinal symptoms after COVID-19 while 4% -31% report new depressive symptoms. These symptoms may be secondary to gut microbiome tryptophan metabolism and 5-hydroxytryptamine (5-HT)-based signaling.

METHODS

This study utilized specimens from 2 patient cohorts: (1) fecal samples from patients with acute COVID-19 who participated in a randomized controlled trial testing prebiotic fiber; and (2) blood samples from patients with acute COVID-19. Six months after recovering from COVID-19, both cohorts answered questions related to gastrointestinal symptoms and anxiety or depression. Microbiome composition and function, focusing on tryptophan metabolism-associated pathways, and plasma 5-HT were assessed.

RESULTS

In the first cohort (n=13), gut microbiome L-tryptophan biosynthesis during acute COVID-19 was decreased among those who developed more severe gastrointestinal symptoms (2.0-fold lower log activity comparing those with the most severe gastrointestinal symptoms versus those with no symptoms, $P=0.06$). All tryptophan pathways showed decreased activity among those with more GI symptoms. The same pathways were also decreased in those with the most severe mental health symptoms after COVID-19. In an untargeted analysis, 5 additional metabolic pathways significantly differed based on subsequent development of gastrointestinal symptoms. In the second cohort (n=39), plasma 5-HT concentration at the time of COVID-19 was increased 5.1-fold in those with gastrointestinal symptoms alone compared to those with mental health symptoms alone ($P=0.02$).

CONCLUSIONS

Acute gut microbiome-mediated reduction in 5-HT signaling may contribute to long-term gastrointestinal and mental health symptoms after COVID-19. Future studies should explore modification of 5-HT signaling to reduce post-COVID symptoms.

KEY WORDS

COVID-19, disorders of brain gut interaction, tryptophan, serotonin, microbiome

INTRODUCTION

Gastrointestinal (GI) symptoms are a common sequela of acute COVID-19, with surveys suggesting a 15-29% prevalence of at least 1 GI symptom at 6 months post-infection (1-3).

The development of chronic GI symptoms post-infection is not unique to COVID-19, and post-COVID GI symptoms may be understood using the framework of post-infection irritable bowel syndrome (PI-IBS) (4-7). Approximately 10% of patients meet criteria for IBS 1 year after diagnosis with infectious enteritis (8). The cause of PI-IBS is unknown, but may be related to changes in serotonergic signaling and serotonin (5-hydroxytryptamine; 5-HT) metabolism (9). Anxiety and depression are established risk factors for the development of PI-IBS (4,8), and 5-HT is a key enteric neurotransmitter, with roles in activating peristalsis and secretory functions (10,11).

The gut microbiota influence 5-HT through the production and metabolism of tryptophan, the rate-limiting precursor of 5-HT (12-15). Postprandial 5-HT concentrations have been associated with IBS sub-types (16) and patients with IBS can have decreased nocturnal levels of tryptophan metabolites (17). Furthermore, acute tryptophan depletion induces IBS-C like symptoms and brain patterns on functional MRI in healthy controls, supporting the role of 5-HT as a modulator of the brain-gut-microbiome axis (18). PI-IBS patients have increased mucosal 5-HT containing enterochromaffin cells, and also differ from healthy controls in microbiome composition (19,20).

It is uncertain whether GI symptoms that arise after COVID-19 are similar to the symptoms that arise after PI-IBS from non-COVID infectious causes. There is a mechanistic rationale for considering COVID-19 as a cause of PI-IBS. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus that causes COVID-19 actively replicates within

the GI mucosa (21,22). Acute COVID-19 alters the gut microbiome (23), although it is unclear whether these alterations are caused by the receipt of antibiotics and other medical interventions or by SARS-CoV-2 infection *per se* (24). At the same time, pandemic-related stress and anxiety (25,26)— established risk factors for IBS—are also crucial determinants of who gets long-term gastrointestinal symptoms after COVID-19 (2,3).

Anxiety and depression are relatively common after COVID-19, with the frequency of depression ranging from 4%-31% and anxiety 6%-63% (27,28) A prospective cohort of patients with long COVID found high prevalence of depression (15%) and post-traumatic stress disorder (22%) (29).

This study investigated the hypothesis that alterations in gut microbial tryptophan metabolism during COVID-19 lead to decreased 5-HT signaling and post-COVID gastrointestinal symptoms. We sought to assay both longitudinal fecal microbial samples and plasma samples for 5-HT; however, we did not have access to stool and blood samples from a single cohort. Instead, we combined data from 2 similar COVID-19 cohorts, 1 which provided serial fecal samples and 1 which provided blood samples. Both cohorts answered clinical questions related to gastrointestinal and mental health symptoms at baseline and 6 months after diagnosis of COVID-19.

METHODS

Complete methods appear in the **Methods Supplementary File**.

Cohort 1: The gut microbiome and post-COVID gastrointestinal symptoms

Study population

This was a sub-study conducted within a parent double-blind randomized controlled trial with 1:1:1 assignment of patients to placebo, inulin 16 g/day, or inulin 32 g/day given in 2 divided doses for 7 days (NCT03865706). At the start of the COVID-19 pandemic, the parent trial was paused and patients with moderate severity COVID-19 were randomly assigned the trial intervention. Individuals ≥ 18 years old were included if they reported that they were free from chronic GI symptoms at baseline, and required hospitalization but not intensive care unit-level care for COVID-19 before June 1, 2020, and received broad spectrum antibiotics within the 24 hours before enrollment. Both the parent study and this sub-study were approved by the Columbia University Irving Medical Center (CUIMC) Institutional Review Board (IRB).

Biosamples and sequencing

Patients donated deep rectal swabs immediately prior to the study intervention (Day 0) and subsequently at Days 3, 7, 14, and 30 or until hospital discharge. At the end of the study, samples were sequenced for the V4 hypervariable region of the 16S ribosomal RNA gene using a previously described protocol (30). The PICRUSt2 pipeline was used with 16S data to estimate the abundance of functional pathways including those for tryptophan biosynthesis (31).

Measurement of post-COVID gastrointestinal and mental health symptoms

Six months after COVID-19 diagnosis, subjects were contacted by telephone and asked questions evaluating the Rome IV criteria for IBS. All patients completed the IBS severity scoring system (IBS-SSS) although none met formal IBS criteria (32). The IBS-SSS was selected

rather than competing instruments because it has been highly validated in non-COVID cohorts, and it was used to classify gastrointestinal symptoms as none/mild (IBS-SSS <50, which was approximately the median in the cohort), mild/moderate (IBS-SSS 50 to 200), or moderate/severe (>200, which was the 90th percentile in the cohort). Combined symptoms of anxiety/depression were reported using the EQ-5D-5L (33) as a 4-point Likert scale (no symptoms, mild, moderate, or severe), because none of the respondents were in the most severe level 5 category.

Outcomes and statistical approach

The primary outcome was differences in tryptophan metabolism between patients with different levels of severity of gastrointestinal and/or self-reported anxiety/depression, assessed using PICRUS^t results. Tryptophan metabolism was compared across categories using a repeated-measures mixed model. Unsupervised analyses were also performed for differences in microbiome composition or function across groups; to enhance rigor, features were first tested for false discovery rate (FDR)-adjusted differences ($P < 0.05$) and hits were then re-tested using the repeated-measures mixed model.

Cohort 2: Plasma serotonin and post-COVID gastrointestinal symptoms

Patient population

Individuals >18 years old were included if they had mild-moderate COVID-19 between April and November 2020 and enrolled in a COVID-19 longitudinal cohort. From within this cohort, 40 patients were randomly selected including 20 patients with and 20 patients without gastrointestinal symptoms 6 months after COVID-19 (not matched).

Measurement of 5-HT

Biobanked plasma was retrieved and tested for the absolute concentration of 5-HT (in pg/mL) using liquid chromatography (LC)-mass spectrometry (MS) with a spike-in internal standard (34).

Measurement of post-COVID gastrointestinal and mental health symptoms

Six months after COVID-19, patients were asked via electronic survey to indicate the presence of COVID-related GI symptoms including diarrhea, constipation, abdominal pain, and if present rate symptoms on a scale from 1 to 5 (very mild, mild, moderate, severe, very severe). On the same Likert scale, they were asked to rate “anxiety” or “sadness”.

Outcomes and statistical approach

The primary outcome was 5-HT concentrations, compared in patients with versus without any GI symptoms, in patients with versus without self-reported anxiety or sadness,, and in a factorial manner. The Mann-Whitney U test was used to assess for differences between any 2 groups and the Kruskal-Wallis test for differences between more than 2 groups.

RESULTS

Cohort 1: The gut microbiome in those with and without post-COVID gastrointestinal symptoms

Patient characteristics

Thirteen patients with moderate severity COVID-19 were randomized to placebo, inulin 16 g/day, or inulin 32 g/day. At the time of enrollment, all patients were hospitalized but not intubated and all received broad-spectrum antibiotics within the 24 hours before enrollment. Additional patient characteristics are given in **Table 1**. Six months later, they completed the IBS-SSS with median score of 45 (interquartile range [IQR] 2 to 90). None of the patients met Rome IV criteria for IBS.

Microbiome composition/function

A median of 2 fecal samples per patient were collected over 3 days (IQR 0 to 7 days). On principal coordinates analysis, there was a high degree of stability within individuals over time (i.e., individual patients continued to resemble themselves longitudinally, **Figure 1A**). Samples were then partitioned based on IBS-SSS, assessed 6 months after COVID-19 diagnosis. There were minimal differences between categories based on IBS-SSS in microbial composition (**Figure 1B**) or in microbial function (**Figure 1C**).

Tryptophan metabolism

Tryptophan is the rate-limiting precursor of 5-HT and is metabolized by the gut microbiome. The 6 major metabolic pathways related to tryptophan were designated *a priori* for analysis

(BioCyC ID numbers 6629, TRPSYN, NADSYN, 6505, 5651, and 5655). Of these, only PWY-6629 and TRPSYN-PWY (the 2 major superpathways for L-tryptophan biosynthesis) were represented in >50% of samples and were analyzed. In both pathways, decreased tryptophan biosynthesis was observed among those with increased gastrointestinal symptoms (**Figure 2A**). The patient with the most severe symptoms (IBS-SSS > 200) had predominantly diarrhea, but further sub-typing of symptoms could not be performed because of the limited number of patients within categories.

Our prior work suggested an interaction between gastrointestinal and mental health symptoms (3,35) We therefore next examined gut microbiome tryptophan biosynthesis based on severity of self-reported anxiety/depression. Decreased activity within the tryptophan biosynthesis pathway was correlated with increased severity of mental health symptoms (**Figure 2B**, $P < 0.05$ in both pathways). When gastrointestinal and mental health symptoms were classified in a factorial manner, a similar pattern was seen (**Figure S1**). In an untargeted analysis, there were no differential compositional features based on IBS groups but there were 5 differential metabolic pathways, all related to the tricarboxylic acid (TCA) cycle (**Figure 3**). All of these showed significant differences in tryptophan biosynthesis activity between the groups with the least and the most GI symptoms.

Inulin intervention

Patients received a median 7 doses (IQR 2 to 14 doses) of placebo, low- or high-dose inulin. There were no adverse events related to inulin. Looking across intervention groups, the median fiber intake was 0 g for placebo, 56 g for low dose inulin, and 116 g for high-dose inulin over a median of 3 days. There were no differences between intervention groups in tryptophan

metabolic pathways (**Figure S2**), IBS-SSS (**Figure S3**), or mental health symptoms (data not shown). There were no FDR-adjusted differential features based on intervention group.

Cohort 2: Plasma serotonin in those with and without post-COVID gastrointestinal symptoms

Patient characteristics

Of 1,783 patients in the COVID-19 longitudinal cohort, 749 (42%) completed the 6-month follow-up survey. From these 749 patients, 40 were randomly selected and plasma 5-HT was successfully quantified in 39 of them (28 women and 11 men) using samples banked at the time of COVID-19 diagnosis. Seven patients (18%) were hospitalized for COVID. Median age was 47 years (IQR 39-59 years) (**Supplementary Table 1**).

Post-COVID gastrointestinal symptoms

Forty-nine percent of the cohort had new symptoms self-perceived to be COVID-related. Diarrhea was the most common post-COVID gastrointestinal symptom, reported by 28% of the cohort. Constipation was reported by 10% and abdominal pain by 15%.

5-HT concentration and post-COVID gastrointestinal symptoms

Median plasma 5-HT concentration was 25,023 pg/mL (IQR 11,286 to 37,313 pg/mL) at the time of COVID-19. There was no difference in 5-HT concentrations comparing those with or without any post-COVID gastrointestinal symptoms, or those with or without specific GI symptoms, including diarrhea, constipation, or abdominal pain (**Table 2**).

5-HT concentration and post-COVID mental health symptoms

We next explored whether a correlation existed between 5-HT concentrations and self-reported anxiety or sadness (2,36). Six months after the initial COVID-19 diagnosis, 31% of patients reported sadness and 51% reported anxiety, with 54% reporting either symptom. The median 5-HT concentration was significantly lower among those who reported sadness (19,415 vs 27,637 pg/mL, $P=0.028$), anxiety (19,415 vs 32,553 pg/mL, $P<0.01$), or either symptom (19,158 vs 32,953 pg/mL, $p<0.01$, **Table 2**) compared to those who did not. 5-HT concentration was also lower in 3 patients who reported pre-COVID mental health symptoms compared to those who did not, although this difference was not significant (5,695 vs 26,334 pg/mL, $P=0.07$). Patients were then categorized into 4 groups according to the presence of both post-COVID anxiety or sadness and post-COVID GI symptoms in a factorial manner. Plasma 5-HT concentrations were highest among those without symptoms, lowest among those with anxiety or sadness only, and intermediate among those with mixed gastrointestinal and anxiety or sadness symptoms (Kruskal-Wallis test for a difference between groups $P=0.02$, **Table 2** and **Figure 4**).

DISCUSSION

New gastrointestinal symptoms are common after COVID-19 (3,37,38). The mechanisms underlying these symptoms are unknown, and 1 hypothesis likens the problem to PI-IBS. It has been postulated in PI-IBS that the gut microbiome alteration leads to persistent changes in the host—perhaps mediated through serotonergic signaling—to cause IBS-like symptoms. This study interrogated a 5-HT based explanatory mechanism for post-COVID gastrointestinal

symptoms. Two distinct cohorts were utilized. Within the first cohort, which provided stool samples, there was decreased microbial tryptophan biosynthesis based on subsequent development of GI symptoms. Within the second cohort, which provided blood samples, there was a decrease in plasma 5-HT concentrations associated with the subsequent development of sadness or anxiety, and GI symptoms (but not GI symptoms alone). In both cohorts, there were significant differences in tryptophan biosynthesis or 5-HT concentrations when comparing patients with neither, either, or both GI and mental health symptoms, suggesting an interaction between these symptoms, which is consistent both with prior research and with the established relationship between IBS and mental health (20,39,40). Overall, this study provides preliminary evidence for potential biological links between COVID-19 and long-term disorders of gut-brain interactions such as IBS. We view these results as a first step which may help to delineate areas for future research.

Our study found significant differences in microbial tryptophan biosynthesis—pre-specified as the outcome of interest—comparing those with or without new GI symptoms 6 months after COVID-19. Some of these differences were large in magnitude. Prior studies have also found decreased serum tryptophan metabolites among patients with COVID-19 which may be unsurprising because COVID-19 is an inflammatory state and shunting of tryptophan towards its main metabolite, kynurenine, is a feature of the systemic inflammatory response syndrome (41). In an untargeted analysis of serum metabolites, Thomas *et al.* identified decreased tryptophan production as the most prominent feature distinguishing patients with acute COVID-19 from controls (42). In a different cohort, the ratio of kynurenine to tryptophan was highest among those with acute COVID-19, middling in acutely ill controls, and lowest in healthy controls (43). Using serial samples, Ansone *et al.* found that serum L-tryptophan levels were

depleted during acute COVID-19 and recovered only 47% during 40 days of follow-up (44). Our results suggesting that there may be reduced gut microbial tryptophan biosynthesis in patients with more severe post-COVID GI and anxiety/depression are novel yet mesh with prior studies.

We also found differences based on gastrointestinal symptoms in 5 untargeted microbial metabolic pathways. Four of the 5 differentially expressed pathways were directly related to aerobic respiration through the TCA cycle (i.e., production of ATP); the fifth pathway was related to thiazole biosynthesis of thiamine (vitamin B1), an essential cofactor for the pyruvate dehydrogenase multienzyme complex which oxidizes glucose into the TCA cycle. The TCA cycle is closely associated with serotonin synthesis (45-47) and all of the pathways showed the same directionality (decreased activity among those with more GI symptoms). Liu *et al.* recently reported on 106 patients with COVID-19 and identified 32 metabolic pathways that differed based on post-acute COVID-19 syndrome (primarily fatigue) (48). It is interesting that all of the pathways identified in this study were associated with ATP production, which mediates intestinal injury in animal models (49). This may be a fruitful area for future studies.

Serotonin is produced in intestinal enterochromaffin cells from tryptophan and may be elevated or decreased among patients with post-infection IBS compared to controls (11,50-53). In this study, we did not have blood samples from the same cohort that provided gut microbiome samples so, to address the question of plasma 5-HT concentrations, we gathered blood samples from a separate cohort of patients who similarly completed 6 month follow-up after COVID-19 infection. The majority of bodily 5-HT is synthesized within the intestinal epithelium and is stored within platelets. It is thus conceivable that plasma 5-HT concentrations reflect the local levels produced by EC cells (54,55). Plasma 5-HT concentrations have been reported to be

increased in patients with acute COVID-19 presenting with diarrhea, but there have been no previous studies evaluating the association between 5-HT and chronic post-COVID gastrointestinal symptoms (56).

Our analysis showed that 5-HT concentrations at the time of acute COVID were lower in patients who later reported COVID-related sadness or anxiety; there was evidence of an interaction between sadness/anxiety and GI symptoms, and the lowest 5-HT concentrations were observed in those with COVID-related sadness/anxiety but not GI symptoms. Only 8% of patients reported significant mental health symptoms prior to COVID-19, compared to 54% 6 months after COVID, mirroring other studies which show a large increase in the mental health burden after recovery from COVID (29,57,58). It is unclear whether these new symptoms are due to the virus itself, the sequelae of hospitalization, the toll of other co-existing long-COVID symptoms, or non-medical stressors related to the pandemic. It is likely that mental health symptoms are likely both a cause and an effect of chronic gastrointestinal symptoms post-COVID and the observation that patients with the lowest 5-HT concentrations had highly increased odds of reporting new anxiety or sadness 6 months later supports the role of 5-HT in mediating symptoms post-COVID infection. The efficacy of serotonin modulating medications for treating COVID related depression, anxiety, or IBS should be further investigated.

This study has several key strengths. COVID-19 disease severity and antibiotic administration (in the microbiome cohort) were homogeneous. In prior studies, these 2 factors—disease severity and receipt of antibiotics—have been the main drivers of observed microbiome differences (24,59,60). The study was longitudinal, with follow-up after 6 months and, in the cohort providing fecal samples, gathered serial samples from individual patients. It interrogated

a specific hypothesis, derived from prior literature on post-infection IBS, and tested this hypothesis in multiple ways (i.e., using both stool and blood samples).

The study also has limitations. The sample size was small, especially in the cohort providing fecal samples. 5-HT concentrations were measured at the time of COVID diagnosis and were not obtained postprandially (which is when differences between IBS phenotypes and controls are most apparent), and there may be significant differences in serotonin signaling that become apparent in the months after COVID. The severity of gastrointestinal symptoms in cohort 1 was assessed using the IBS-SSS, but none of the patients met Rome criteria for IBS, and most had mild symptoms. Using the standard IBS-SSS scoring system would have meant that almost all our patients were rated as mild, leaving no groups to compare. Instead, we used the approximate median IBS-SSS score (45) to divide the cohort in half and then selected the 90th percentile of IBS-SSS score (200) to define (relatively) severe symptoms (IBS-SSS >200). Another limitation arises from the fact that 2 different cohorts were initially designed as separate studies, resulting in different methods for assessing self-reported mental health symptoms in both cohorts, as the primary authors did not have input on the questionnaire for cohort 2. Therefore cohort 2 assessed self-reported “sadness” and “anxiety” separately, while cohort 1 assessed combined symptoms of anxiety/depression together. These self-reported symptoms do not necessarily indicate the presence of a clinical psychiatric diagnosis. Further, the specific timing and use of psychotropic medications during the 6 months post-COVID were unknown, which may affect the severity of such symptoms. Last, all the patients were unvaccinated individuals from the first wave of the pandemic and their results may not be generalizable to later waves of patients.

In sum, this prospective study of patients with mild to moderate severity COVID-19 found decreased fecal microbial tryptophan biosynthesis and plasma 5-HT concentrations during acute COVID-19 that associated with gastrointestinal symptoms 6 months later. Grouped symptoms of anxiety/depression interacted with gut microbiome tryptophan biosynthesis and grouped symptoms of anxiety/sadness interacted with plasma 5-HT concentration. These findings are preliminary and derived from a relatively small number of individuals. Nonetheless, they may help pave the way for longitudinal studies that address the role of the brain-gut axis and specifically whether serotonergic signaling mediates long COVID-19 gastrointestinal and mental health-related symptomatology. Additional studies to compare serotonin levels in a larger cohort of patients meeting criteria for IBS, as well as patients who were versus those who were not clinically diagnosed with depression and/or anxiety following COVID-19, rather than relying on self-reported symptoms alone, would provide valuable additional information.

TABLES

Table 1. Cohort 1 characteristics at baseline and during COVID-19 treatment for 13 patients treated with prebiotic inulin for moderate severity COVID-19.

Characteristics	n (proportion)
Age (years, median/IQR)	58 (50-64)
Sex	
Male	6 (46%)
Female	7 (54%)
Race	
White	9 (69%)
Black	3 (23%)
Asian	1 (8%)

Ethnicity	
Hispanic	6 (46%)
Non-Hispanic	7 (54%)
Days hospitalized with COVID-19 (median/IQR)	5 (3-12)
COVID treatments*	
Supplemental oxygen	13 (100%)
Steroids	3 (23%)
Remdesivir	1 (8%)
Antibiotics**	
Any antibiotic	13 (100%)
Ceftriaxone	8 (62%)
Piperacillin-tazobactam	5 (38%)
Doxycycline	3 (23%)
Azithromycin	4 (31%)
Vancomycin	4 (31%)
Discharge on supplemental oxygen	4 (31%)
Psychotropic medications	
Selective serotonin reuptake inhibitor	0 (0%)
Tricyclic antidepressant	0 (0%)
Serotonin- norepinephrine reuptake inhibitor (duloxetine)	1 (8%)

IQR: interquartile relationship

*One subject received both convalescent plasma and ivermectin; a different subject received hydroxychloroquine.

**Antibiotics received within the 24 hours before enrollment. Ten of 13 subjects received multiple antibiotics. In addition to the antibiotics listed, 1 subject received levofloxacin, and another received cephalexin.

Table 2. Cohort 2 plasma 5-HT concentrations at the time of COVID-19, stratified by gastrointestinal and mental health symptoms at 6 months of follow-up.

	n (%)	Median 5-HT concentration in pg/mL (IQR)	P-value*
Post-COVID gastrointestinal symptoms			
Any gastrointestinal symptoms	19 (49%)	24,962 (17,744 - 31,154)	0.91
No gastrointestinal symptoms	20 (51%)	26,199 (6,969 - 93,297)	
Specific gastrointestinal symptoms			
Diarrhea	11 (28%)	24,962 (11,286 - 29,989)	0.47
No diarrhea	28 (72%)	25,158 (11,454 - 93,297)	
Constipation	4 (10%)	23,817 (18,451 - 29,233)	0.93
No constipation	35 (90%)	25,023 (10,806 - 40,918)	
Abdominal pain	6 (15%)	25,127 (19,672 - 28,476)	0.88
No abdominal pain	33 (85%)	25,023 (10,806 - 40,918)	
Post-COVID Sadness or anxiety			
Sadness	12 (31%)	19,415 (5,182 - 26,617)	0.028
No sadness	27 (69%)	27,637 (12,102 - 93,790)	
Anxiety	20 (51%)	19,415 (6,030 - 26,751)	<0.01
No anxiety	19 (49%)	32,553 (21,399 - 93,790)	
Sadness or anxiety	21 (54%)	19,158 (6,365 - 25,292)	<0.01
No sadness or anxiety	18 (46%)	32,953 (22,199 - 93,790)	
Post-COVID gastrointestinal and mental health symptoms			
Neither GI nor mental health symptoms	13 (33%)	32,553 (22,199 - 93,790)	0.02
Mental health but no GI symptoms	7 (18%)	6,365 (4,648 - 25,023)	
GI but no mental health symptoms	5 (13%)	33,354 (31,154 - 40,918)	
Both GI and mental health symptoms	14 (36%)	21,371 (14,442 - 28,210)	

5-HT: serotonin; COVID: coronavirus-19 disease; GI: gastrointestinal; IQR: interquartile range
 *Mann Whitney U test used for comparison of any two groups; Kruskal-Wallis test for comparison of any 3 or more groups.

Figure legends

Figure 1. Cohort 1: Principal coordinates plots of gut microbiome composition and function during acute COVID-19. Principal coordinates plots from 16S sequencing results based on OTU read count from samples gathered during acute COVID-19. (A) Microbial composition, organized by subject (each individual subject has a unique color) at 6 months of follow-up after COVID-19. (B) Microbial composition, organized by gastrointestinal (GI) symptoms at 6 months of follow-up. (C) Microbial function, imputed from 16S sequencing results and organized by GI symptoms at 6 months of follow-up. Gastrointestinal symptoms were classified based on irritable bowel syndrome severity scoring system (IBS-SSS) points.

Figure 2. Cohort 1: Gut microbiome tryptophan metabolism during acute COVID-19, stratified based on gastrointestinal and depression/anxiety symptoms at 6 months of follow-up. Gastrointestinal and depression/anxiety symptoms were classified in 13 subjects 6 months at 6 months of follow-up after COVID-19. Tryptophan metabolism was imputed from 16S sequencing results from longitudinal samples gathered for up to 30 days after the diagnosis of COVID-19. Gastrointestinal symptoms were classified based on irritable bowel syndrome severity scoring system (IBS-SSS) points and depression/anxiety symptoms based on responses to the EQ-5D-5L which characterizes depression/anxiety on a 4 point Likert scale (none, mild, moderate, and severe). *P*-values are for a repeated measures mixed model incorporating between- and within-subjects effects.

Figure 3. Cohort 1: Gut microbiome metabolic pathways that significantly differed during acute COVID-19 based on gastrointestinal symptoms at 6 months of follow-up. Shown are the differential pathways based on gastrointestinal symptoms that were identified in an untargeted analysis of all pathways using 16S sequencing results. Gastrointestinal symptoms were classified based on irritable bowel syndrome severity scoring system (IBS-SSS) points. *P*-values are for a repeated measures mixed model incorporating between- and within-subjects effects.

Figure 4. Cohort 2: Plasma 5-HT concentrations during acute COVID-19, stratified based on gastrointestinal and sadness/anxiety symptoms at 6 months of follow-up. Mann Whitney U *P*-value is shown for a difference between groups.

Supplemental table 1 -<http://links.lww.com/CTG/A871>

Supplementary Figures -<http://links.lww.com/CTG/A872>

Supplemental methods -<http://links.lww.com/CTG/A873>

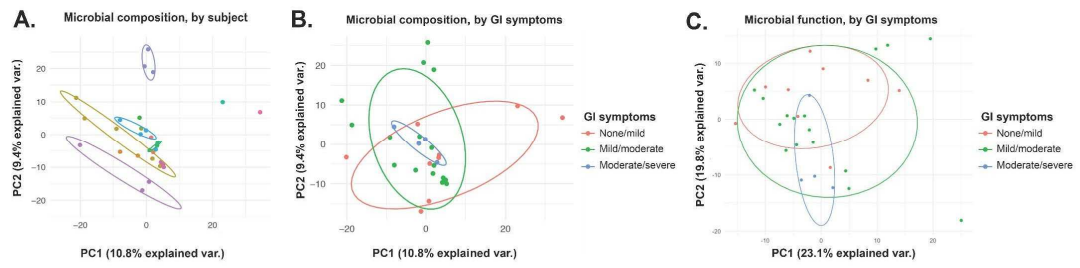
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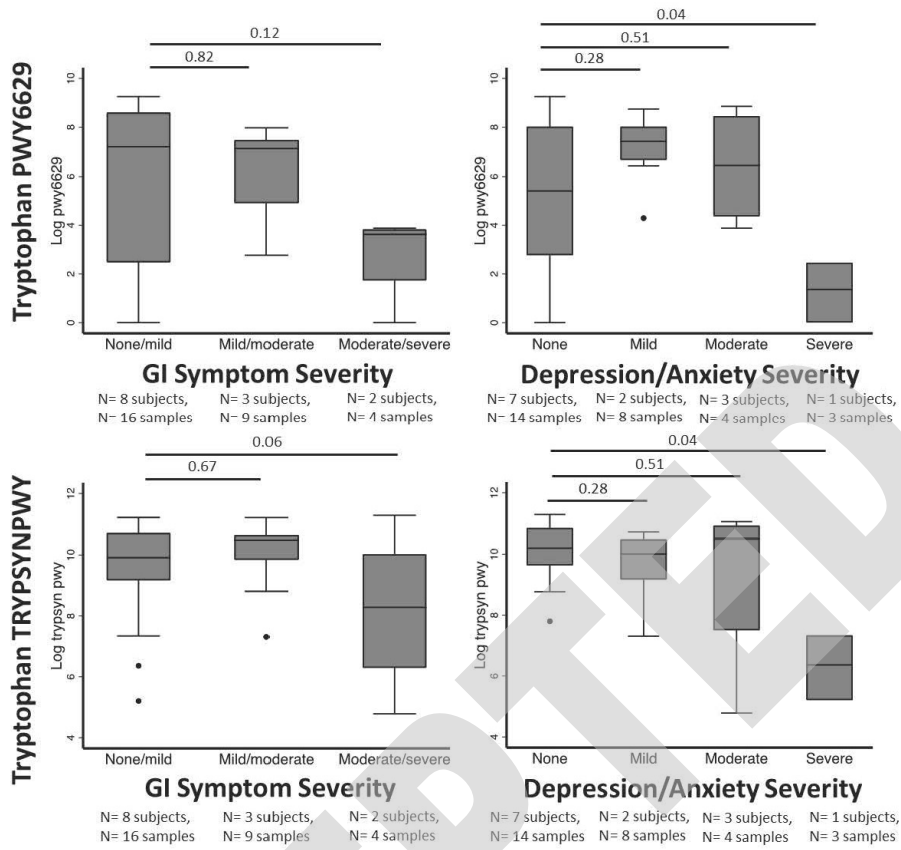
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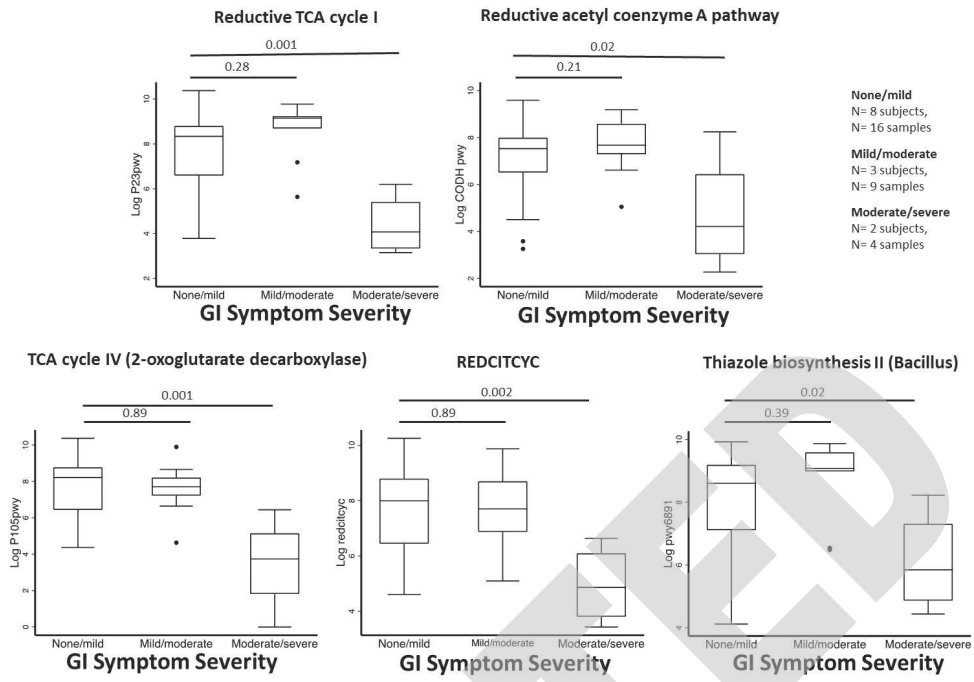
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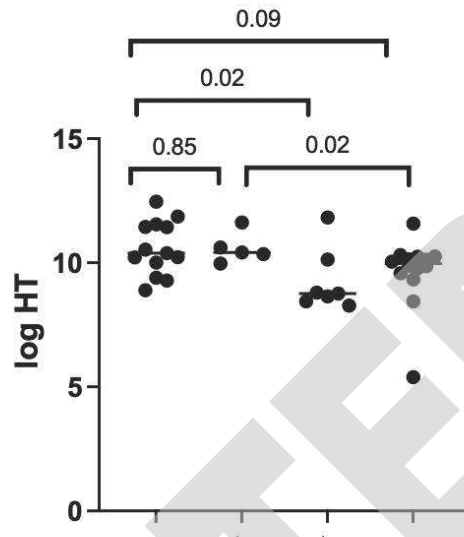
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