Gut microbial community structure in patients with chronic pancreatitis before and after total pancreatectomy with islet autotransplantation.

A Thesis SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA BY:

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Background

The gut microbial community is hypothesized to play multiple roles in both normal homeostasis and disease states of the intestinal tract [1, 2]. Recent advances in the field of metagenomics have allowed study of associations between microbiota and chronic diseases such as obesity, metabolic syndrome, diabetes, and others [3-7]. This has led to increasing interest in studying potential roles of human intestinal microbiota in disease. Changes in gut microbial community structure have been demonstrated in settings of autoimmune disease [4], diabetes and metabolic syndrome [3, 5, 6], obesity, and following bariatric surgery [7-10]. Microbiota changes have been associated with certain functional disorders of the GI tract [11, 12]. There is also evidence that exocrine pancreatic function directly affects the microbiome [13], and shifts in gut microbial composition have been documented in patients undergoing pancreaticoduodenectomy for pancreatic cancer [14]. An emerging body of literature also describes the effects of opioid use on gut microbiota [15, 16].

Metagenomics studies analyze data based on abundance of operational taxonomic units (OTUs), within-sample diversity (alpha diversity), and between-sample diversity (beta diversity). Abundance of taxa is expressed as mean relative or absolute percent abundance, and significance of an OTU in explaining differences between groups of samples is defined by linear discriminant analysis of effect size (LEfSE) [17, 18]. Alpha diversity is a measure of richness and evenness of the microbial community within an individual sample, and is generally considered a positive trait. Shannon's index is one metric for estimating alpha diversity [19, 20]; a normal value is between 2-4, with higher values indicating more diversity. Beta diversity compares differences between two or more groups of microbial communities [21, 22].

Chronic pancreatitis (CP) and recurrent acute pancreatitis (RAP) are characterized by abdominal pain, frequent hospitalizations, nutritional deficiencies and weight loss, and reduced quality of life in both adults and children [23-27], all of which can be difficult to manage medically [27-31]. Medical therapy consists of symptom management and in some cases endoscopic intervention but many patients require surgery for longer-term

symptom relief [31, 32]. When maximal medical and endoscopic treatments fail, total pancreatectomy with islet autotransplantation (TPIAT) may be considered to remove the source of pancreatitis pain (via total pancreatectomy) while also attempting to mitigate the effects of resultant pancreatogenic diabetes mellitus by returning the patients' own islets to them, obviating the need for chronic immunosuppression [24, 33-37]. TPIAT involves a total pancreatectomy and partial duodenectomy, following which the pancreas is transported to an islet processing lab. While the islets are being prepared, the GI tract is reconstructed, commonly with a Roux-en-Y hepaticojejunostomy and duodenojejunostomy. The islets return to the operating room and are infused into the portal vein, and then engraft in the sinusoids of the liver. This complex surgical procedure comes with the potential for complications, including islet graft loss or attrition, which leaves approximately 60-70% of patients needing at least some daily insulin to manage a mild form of diabetes [35]. After pancreatectomy, patients are exocrine insufficient by definition and functional problems of the GI tract are becoming increasingly recognized. The symptoms of GI dysfunction can persist even after initial recovery from surgery and weaning off opioids, with many patients reporting persistent nausea, vomiting, and decreased appetite. Impaired GI motility is also present in up to 40% of patients post-TPIAT [38].

Before undergoing TPIAT, patients with CP often use opioids, are subject to progressive exocrine insufficiency, have dietary alterations, undergo repeated antibiotic exposure, and have small intestinal bacterial overgrowth (SIBO) [39-42], all of which are potentially associated with intestinal dysbiosis [1, 13, 15, 16, 41, 43]. Despite this, the relationship between gut microbial composition and symptoms of CP remains largely unexplored. Data describing the gut microbial community in individuals with chronic pancreatitis is limited, with only three studies in adults and one in children [43-46]. The recent study by Wang et al. compared intestinal microbiota of 30 children with chronic pancreatitis to 35 healthy controls and found significantly reduced alpha diversity among the CP patients but did not include information on GI symptomatology or opioid use, and excluded patients who used antibiotics within three months of the study [43]. Jandhyala et al. compared intestinal microbiota of 30 patients with chronic pancreatitis and 10 healthy

controls and also found decreased alpha diversity as well as an increase in the ratio of major phyla Firmicutes to Bacteroidetes in CP patients. However, they excluded CP participants with IBS-type symptoms and did not examine opioid usage [45]. Finally, Gorovits et al. and Savitskaia et al. both examined the gut microbial composition of adult patients with chronic pancreatitis but did not compare these with healthy controls [47, 48]. We hypothesized that the intestinal microbiota of patients with CP differ from those of normal controls, and that these differences are clinically meaningful. Our first aim was therefore to compare gut microbes of CP patients with those of healthy controls, and to associate clinical characteristics of CP including GI symptomatology with microbial community structure.

There is evidence that bariatric surgery and other surgical procedures, such as pancreaticoduodenectomy, alter the intestinal microbiome [8, 14]. Furthermore, changes in gut microbiota may contribute to certain metabolic and functional disorders of the GI tract commonly observed after surgery and in non-surgical populations [8, 10, 11]. Changes in composition of gut microflora after TPIAT, however, have never been characterized. Given the changes in anatomy, the removal of the pancreas as a source of systemic inflammation, and the complexities of post-operative care including antibiotic exposure, dietary changes, iatrogenic pancreatic exocrine insufficiency, and post-pancreatectomy diabetes, we hypothesized that gut microbial communities would change post-TPIAT and that these changes would be associated with clinical outcomes such as the severity of symptoms of gut dysfunction. Therefore, an exploratory aim of this study was to characterize, for the first time, shifts in the gut microbial community that occur after patients with CP undergo TPIAT.

Because many disease states have been hypothesized to coexist with alterations of intestinal microbiota, interventional studies have attempted to modulate the gut microenvironment. This has typically been done with probiotics [49-53], but treatment of SIBO with antibiotics and fecal microbial transplant (FMT) are other therapeutic modulations of the intestinal milieu potentially applicable to CP/TPIAT patients. Our institution has extensive experience with FMT as therapy for *C. difficile* colitis [54-56],

and has successfully developed an antibiotic protocol to establish human microbiota-associated mice for pre-clinical modeling of human diseases associated with microbiome changes and potential therapeutic effects of FMT [56]. With more data, mouse modeling of the human CP microbiome could be a path toward testing these novel therapeutic interventions in a CP-specific setting.

In summary, the hypothesis driving the current study was that patients with CP have significant and clinically meaningful differences in gut microbial composition compared with healthy controls. Our primary aim was to compare the gut microbiota of patients with CP being considered for TPIAT with that of healthy controls, and to associate these differences with symptoms of gut dysfunction. Our secondary hypothesis was that the microbiome changes significantly after patients with CP undergo TPIAT. To this end, we aimed to compare participants' gut microbial structure from pre- to post-TPIAT, and to explore whether these changes are associated with GI symptomatology. We believe that data generated from this study will provide an evidence base for developing interventions targeting gut microbiota for CP and TPIAT patients.

Methods

Study design

This was a prospective observational study of intestinal microbiota in patients with chronic pancreatitis or recurrent acute pancreatitis before and after TPIAT. Microbial data from healthy stool donors was used for comparison.

Recruitment

Adult and teenaged (15 years of age and older) patients with chronic pancreatitis or recurrent acute pancreatitis being considered for TPIAT were approached at the time of initial consultation at our institution for participation in the study. The age criterion for CP patients was chosen to maximize recruitment, though evidence suggests that the infant gut microbiome stabilizes by three years of age [57], so age differences outside the first three years of life are unlikely to lead to meaningful differences in microbiome characteristics. Our institution's definitions of chronic and recurrent acute pancreatitis,

along with factors that determine candidacy for TPIAT, have been published previously [35]. Baseline characteristics including demographics, medical history, pancreatitis history, opioid use, and antibiotic use within the three months before stool sample collection were recorded. All patients provided informed consent to participate (or parental consent with patient assent as appropriate), and the study was approved by the University of Minnesota Institutional Review Board (IRB).

Healthy controls for comparison were recruited from the University of Minnesota stool donor program [58]. These donors are screened for a variety of viral, bacterial, and parasitic enteric pathogens and meet rigorous inclusion and exclusion criteria previously described and in accordance with the Investigational New Drug Application 15071 sponsored by the University of Minnesota Microbiota Therapeutics Program. All stool donor activities, which include administration of questionnaires, physical exams, and laboratory testing are approved by the University of Minnesota IRB separate from the current study. Healthy donors in this program have no history of chronic medical conditions, are not taking medications, have never undergone abdominal surgery, and have not taken antibiotics for at least three years before donation.

Fecal sample collection and processing

All patients referred to the University of Minnesota under consideration for TPIAT are asked to provide a stool sample for fecal elastase quantification, to assess pancreatic exocrine function. Participants were asked to collect stool for study purposes at the same time as their clinical lab sample. Stool samples for the study were kept at -20°C until study staff were able to transfer them to -80°C for storage, where they stayed until DNA extraction was performed.

DNA was extracted from 250 mg thawed stool using the DNeasy PowerSoil DNA isolation kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions using the QIAcube inhibitor removal technology (IRT) protocol. DNA concentration was quantified using Qubit 4 Fluorometer (Thermo-Fisher Scientific, Inc.,

Waltham, MA). Extracted DNA was stored at -20°C until sequencing. DNA extraction methods were the same for healthy donor samples.

Amplicon sequencing

The V4 hypervariable region of the 16S rRNA gene was amplified using methods previously described by our group [59]. Pair-ended sequencing at read length of 300 nucleotides was done on the Illumina MiSeq platform (Illumina, San Diego, CA) by the University of Minnesota Genomics Center (Minneapolis, MN). Healthy donors' stool DNA was sequenced using the same methods, with each sample run in triplicate.

Bioinformatics

Sequence data were processed and analyzed using mothur software (ver. 1.41.1) [60]. Operational taxonomic units were defined at 99% sequence similarity, and taxonomic classifications were made against the Ribosomal Database Project version 14 release [61]. Compositional data were non-normalized. For statistical comparisons, samples were rarefied to the same number of sequence reads in all samples by random subsample [62].

Clinical metadata

We collected clinical metadata thought to affect the microbiome, including recent opioid use (within three months), recent antibiotic use (within three months), diet (regular or tube feeds), smoking status (never smoker or current/prior smoker, including the use of cannabis for medical or recreational purposes), alcohol use, and presence of diabetes.

Association of microbiome characteristics with symptoms of gut dysfunction

To associate characteristics of the microbiome with clinical symptoms of gut dysfunction, the Irritable Bowel Syndrome Symptom Severity Score (IBS-SSS) survey was administered to all participants at the time of stool sample collection ±3 days. The IBS-SSS is a clinically validated questionnaire for assessing symptoms of gut dysfunction, including abdominal pain and bloating, constipation/diarrhea, bowel movement frequency, overall patient satisfaction with their bowel habits, and symptom interference

with daily life [12, 63]. The survey is scored from 0 – 500 points, with 0 representing no symptoms and 500 representing symptoms that are severe, disruptive, and constant.

Technique of total pancreatectomy with islet auto-transplantation

Surgical technique of TPIAT has been described previously by our group in adults [35, 64] and children [36]. For the current study, TPIAT was performed by a single surgeon (SC) in patients under 18 years of age, and by three primary surgeons (VAK, GJB, TLP) in patients 18 years of age and over. Briefly, pancreatectomy is performed with pylorus preservation along with 1-2 cm of the duodenum (D1). The pancreas is dissected with attention to maintaining its blood supply until the time of removal, after which it is immediately placed in cold preservation solution and transported to the islet isolation lab. While the pancreas is processed, the gastrointestinal tract is reconstructed, most often with a Roux-en-Y duodenojejunostomy and choledochojejunostomy. Some of our patients are referred after undergoing a Whipple or other pancreas drainage procedure, and in these cases, reconstruction is tailored to the patient's pre-existing anatomy.

Post-operative management

For the participants who provided post-TPIAT stool samples, these were collected between 4-8 weeks after surgery, in the same fashion as pre-TPIAT samples. The IBS-SSS survey was administered again within three days of post-TPIAT sample collection.

All patients undergoing TPIAT receive broad-spectrum antibiotics within 30 minutes before incision, and are re-dosed as appropriate depending on the length of time in the operating room. The pancreas preservation solution and the final islet product are routinely sent for gram stain and culture, and return positive for about 60% of patients based on previously published data [65]. For 48 hours post-operatively, while cultures are pending, patients receive prophylactic broad-spectrum antibiotics. If cultures return positive the antibiotic regimen is tailored to sensitivity results. Antibiotics are occasionally administered for other reasons as clinically appropriate, such as for surgical site or urinary tract infections. All perioperative antibiotic use was recorded for study participants.

All patients undergoing TPIAT are given an enteral feeding tube at the time of surgery. A majority will continue on at least some amount of tube feedings for 4-6 weeks postoperatively, with or without supplemental oral intake. For the purpose of the study, tube feeding regimens were noted at the time of post-operative stool sample collection.

A majority of patients referred for TPIAT use opioid pain medications chronically. They are slowly weaned off opioids as clinically appropriate following surgery. All opioid use at the time of post-operative stool sample collection was recorded for study participants.

Total pancreatectomy results in type 3c diabetes mellitus, and islet auto-transplantation is undertaken to lessen the effects of post-surgical diabetes without the need for immunosuppression. All TPIAT patients at our institution are managed with insulin during the initial islet engraftment phase (0-12 weeks following surgery) to maintain tight glucose control between 80 and 125-140 mg/dL. Therefore, all participants in the study were on insulin (without additional oral hypoglycemic medications) at the time of post-operative stool sample collection.

Statistical analysis

Alpha and beta diversity were calculated using mothur software [60]. Alpha diversity was estimated with Shannon's diversity index. Beta diversity was evaluated with Bray-Curtis dissimilarity matrices, and ordination of Bray-Curtis distances was done using principal component analysis (PCA). Spearman's rank correlation was used to identify genera related to ordination position, and differences in composition among groups were evaluated with non-parametric analysis of similarity (ANOSIM). Linear discriminant analysis of effect size (LEfSE) was used to characterize differences in operational taxonomic units (OTU) between groups. A p-value of 0.05 was considered statistically significant, however Bonferroni's correction for multiple comparisons was used when appropriate. Quantitative data is expressed as mean ± standard deviation, and analyzed using the t-test for normal distributions, and one-way analysis of variance for multiple groups. Associations between clinical metadata from participants with chronic

pancreatitis and Shannon's index were determined by both simple and multiple linear regression. Regression models, along with descriptive statistics, were analyzed using SAS software (Version 3.8, ©2018 SAS Institute Inc., Cary, NC).

Results

Baseline demographics

Table 1 provides a summary of the baseline clinical characteristics of chronic pancreatitis patients (n = 20) and healthy stool donors (n = 6) included in the study.

Patients with chronic pancreatitis have a distinct gut microbial composition compared with healthy controls

Abundance of the genus *Parabacteroides* was significantly higher in patients with CP (LEfSE $P = 0.04$), whereas abundance of genus *Faecalibacterium* was higher in healthy controls (HC) (LEfSE $P = 0.03$) (Figure 1). Alpha diversity was significantly lower in patients with CP (mean Shannon index 3.57) than in HC (mean Shannon index 4.20) (mean difference -0.62, standard error [SE] 0.14, $P < 0.001$) Among patients with CP, alpha diversity of those who had used antibiotics within three months of stool sample collection (mean Shannon index 3.51) did not differ significantly from those who had not (mean Shannon index 3.77) (mean difference 0.26, SE 0.16, $P = 0.1$). Those who had used opioids within three months of stool sample collection (mean Shannon index 3.65) also did not differ significantly from those who had not (mean Shannon index 3.76) (mean difference 0.12, SE 0.20, $P = 0.6$). Principal component analysis (PCA) for comparison between the CP and HC microbial community structure found the differences driven by *Parabacteroides* in CP (P(corr.) < 0.001) and *Faecalibacterium* in HC (P(corr.) = 0.01), and ANOSIM demonstrated that beta diversity differed significantly between CP and HC ($R^2 = 0.26$, $P < 0.001$, Figure 2).

Association between clinical symptoms of gut dysfunction and alpha diversity among patients with chronic pancreatitis

IBS-SSS was significantly associated with alpha diversity (as measured by Shannon index) in participants with CP, so that participants who reported worse functional GI

symptoms (higher IBS-SSS) had, on average, lower Shannon index (less richness and evenness of the gut microbial community) (Pearson's correlation r = -0.62, $P = 0.006$, Figure 3). However, among candidate predictors of IBS-SSS (Table 2), recent use of opioids, patient sex, and the 4 categories combining opioid use and sex were also significantly associated with a significantly higher IBS-SSS; after adjusting for these characteristics, the association between Shannon index and IBS-SSS was no longer statistically significant ($P = 0.15$). The effects of opioid use differed significantly between men and women, so that women with recent opioid use had the highest survey scores on average, while men without recent opioid use had the lowest survey scores (Kruskal-Wallis test $P = 0.007$, Figure 4).

Association between alpha diversity and baseline characteristics among patients with chronic pancreatitis

There were no statistically significant predictors of the Shannon index among selected baseline characteristics of CP patients, including antibiotic use within three months of sample collection, opioid use within three months, or exocrine pancreatic insufficiency, defined as fecal elastase < 150 ($P \geq 0.05$ for all simple associations, Supplemental Table 1).

Total pancreatectomy with islet autotransplantation leads to significant shifts in the gut microbial composition of patients with chronic pancreatitis

Participants who provided stool samples post-TPIAT (n = 5) had significantly higher mean relative abundance of the genera *Bacteroides* and *Clostridium_XIVa* compared with pre-TPIAT (LEfSE $P = 0.009$ and 0.04, respectively, Figure 5). However, the difference in *Clostridium_XIVa* appeared to be driven by a single patient who had a large increase in the abundance of this genus after TPIAT (Patient 15, Figure 5b). Beta diversity was significantly different between HC and post-TPIAT patients ($R^2 = 0.77$, $P < 0.001$), and between CP and post-TPIAT patients ($R^2 = 0.37$, $P = 0.01$) (threshold for statistical significance after Bonferroni corrections: $P = 0.02$). The increase in relative abundance of *Bacteroides* appeared to drive the shift in gut microbial composition from pre- to post-TPIAT on PCA (Spearman's P(corr.) = 0.002, Figure 6).

Despite these changes, the alpha diversity of gut microbes (Shannon index) did not change significantly from pre- to post-TPIAT (mean change -0.36, SE 0.17, $P = 0.1$). Also, post-TPIAT Shannon index was not significantly associated with post-TPIAT IBS-SSS survey results (mean change in survey score +230 points for each 1 point increase in Shannon index, SE 364 points; $P = 0.6$), however post-TPIAT survey results were only available for 4 patients.

Discussion

Changes in gut microbial community structure have been proposed to contribute to disease in humans, but to date there is limited data on its characteristics in patients with chronic pancreatitis or the association of intestinal dysbiosis with clinical features of chronic pancreatitis. Patients with chronic pancreatitis have recurrent opioid and antibiotic exposure, exocrine insufficiency, and chronic inflammation, all of which could contribute to microbiome changes. The present study identifies features that distinguish the gut microbial community of patients with chronic pancreatitis from that of healthy controls, and shows that these differences may be clinically meaningful. Also, for the first time we have explored changes in the gut microflora composition that occur when patients with chronic pancreatitis undergo TPIAT.

Gut microbes of patients with chronic pancreatitis differ significantly from those of healthy controls. First, patients with CP have significantly lower alpha diversity than HC. We did not find a significant difference in alpha diversity among patients with CP based on clinical factors such as recent antibiotic use, opioid use, or exocrine pancreatic insufficiency (EPI). In their study of 30 patients with CP (16 without diabetes and 14 with diabetes) and 10 HC, Jandhyala et al [45] described decreased alpha diversity among the participants with CP, after specifically excluding patients with recent antibiotic use. Among these CP patients, those with diabetes had a lower alpha diversity than those without, but the authors did not include an analysis of other clinical characteristics associated with alpha diversity. Wang et al [43] also found decreased alpha diversity in their group of 30 children with chronic pancreatitis compared with healthy controls after

excluding patients with recent antibiotic or probiotic exposure; they also did not include data regarding opioid use or exocrine pancreatic insufficiency in their analyses. Although our results agree with these previously published studies that alpha diversity is lower among patients with CP, it remains difficult to determine why. We did not find significant associations with antibiotic use, opioid use, or exocrine insufficiency, however patients with CP are medically complex making it difficult to quantify these measures from a small sample size.

In our study, a second difference between the gut microenvironments of patients with CP and those of HC was the relative abundance of genera *Parabacteroides* and *Faecalibacterium*. *Parabacteroides* was significantly increased in patients with CP, while *Faecalibacterium* was more abundant in HC. Jandhyala et al found a decrease in *Faecalibacterium* in patients with CP compared to their HC, though it did not reach statistical significance, and Wang et al reported a significantly decreased abundance of *Faecalibacterium* in CP relative to HC. Previous evidence indicates that *Faecalibacterium* is a beneficial commensal organism that produces butyrate (a source of nutrition for colonocytes) and other short-chain fatty acids via fermentation [14]. Members of *Faecalibacterium* also have anti-inflammatory effects in the GI tract [66, 67]. The consistent findings of lower relative abundance of this genus across studies in CP patients may provide some basis for the hypothesis that modulating the gut microenvironment to promote enrichment of *Faecalibacterium* could be beneficial in CP. As for the increased relative abundance of *Parabacteroides*, this finding has not previously been reported in other studies of gut microbes in CP patients.

Participants in our study who reported worse functional GI symptoms on the IBS symptom severity score (IBS-SSS) survey had significantly less alpha diversity of their gut microbial communities. However, further analysis found that survey results were also significantly associated with sex differences and opioid use. Women who used opioids within three months of stool sample collection reported the worst symptoms, and when taking these factors into account, the association with Shannon index was no longer statistically significant. Our survey tool may have provoked more highly rated symptoms

among women, as it was developed as a tool for evaluating irritable bowel syndrome, which is known to be more prevalent among women, who tend to report worse symptoms, and are more likely to be refractory to treatment [68-70]. As for opioid use, Parkman et al [71] found significant associations between reported functional abdominal pain symptom severity scores and opioid use, though no significant association with objective measures such as gastric emptying scintography or water load test in the same group of patients. These findings indicate that it may be difficult to separate sex differences and the side effects of opioids from other modifiable risk factors (such as decreased alpha diversity of the microbiome) as major causes of functional abdominal symptoms in patients with chronic pancreatitis. It might be beneficial, in the future, to consider evaluating gut microbial community changes using a more objective test of abdominal pain or dysmotility.

Although other authors have previously reported changes in the gut microenvironment that take place after certain surgical procedures, the effects of TPIAT on gut microbial community structure have never been described. We were able to obtain post-TPIAT stool samples from five patients for comparison with their pre-operative samples to generate preliminary data for use in developing future observational and interventional studies of post-TPIAT intestinal dysbiosis. As expected, TPIAT resulted in a significant change in the composition of gut microflora, compared with both CP and HC samples. TPIAT patients undergo anatomic and functional changes to their GI tracts, in addition to broad-spectrum antibiotic exposure, several weeks on tube feedings, and fluctuations in use of opioid and other pain medications. Given these circumstances, it is not surprising to see a shift in their gut microbes. We observed a higher relative abundance of the genera *Clostridium_XIVa* and *Bacteroides* in our post-TPIAT patients, compared with pre-TPIAT. Interestingly, this is similar to some, but not all, findings from other authors who have published data on post-surgical gut microbial changes. Rogers et al described microbiome changes in 50 patients after pancreaticoduodenectomy (PD; Whipple's procedure), and compared them to healthy controls [14]. They found, similar to our results, an increase in the relative abundance of *Bacteroides* at the genus level after PD.

However, they also reported an increased abundance of the genus *Parabacteroides* after PD, which is similar to our findings in the CP group prior to TPIAT.

Although our post-TPIAT patients' gut microbial community structure remained statistically different from that of healthy controls, the increase in abundance of *Bacteroides* is encouraging. Kelly et al [54] found that increased relative abundance of *Bacteroides* was associated with both healthy stool donors and those receiving fecal microbiota transplant (FMT) from healthy donors for successful treatment of recurrent *C. difficile* infections. *Bacteroides* is generally considered a beneficial commensal organism that reduces inflammatory peptide levels in the GI tract and synthesizes essential nutrients such as vitamin K [54, 72-74]. To establish and validate the clinical relevance of our findings in this small group of patients, other studies of the post-TPIAT gut microenvironment are needed, preferably including robust clinical metadata and associations with objective measurements of clinically relevant symptoms, as discussed above. For example, due to our small sample size, it is still unclear whether alpha diversity increases or decreases after TPIAT, and whether changes in relative abundance of certain genera drive meaningful improvements in clinical symptomatology.

In conclusion, the present study provides more data on the characteristics of the gut microbial environment in patients with chronic pancreatitis, and explores the association of clinical symptoms with those characteristics. Our group of chronic pancreatitis patients had decreased amounts of beneficial commensal organisms such as *Faecalibacterium,* and lower alpha diversity compared with healthy controls. Among chronic pancreatitis patients who were surveyed for functional symptoms of the GI tract, those with lower alpha diversity tended to report worse symptoms, although symptoms also differed depending on sex and opioid use. Lastly, we have performed a preliminary analysis of changes in the gut microenvironment that take place when patients with chronic pancreatitis undergo TPIAT, and found that these patients have significant shifts in their gut microenvironment toward greater abundance of healthy commensal gut bacteria. Whether our findings can be applied to improving clinical outcomes for patients with

chronic pancreatitis and TPIAT merits further studies with greater numbers of participants.

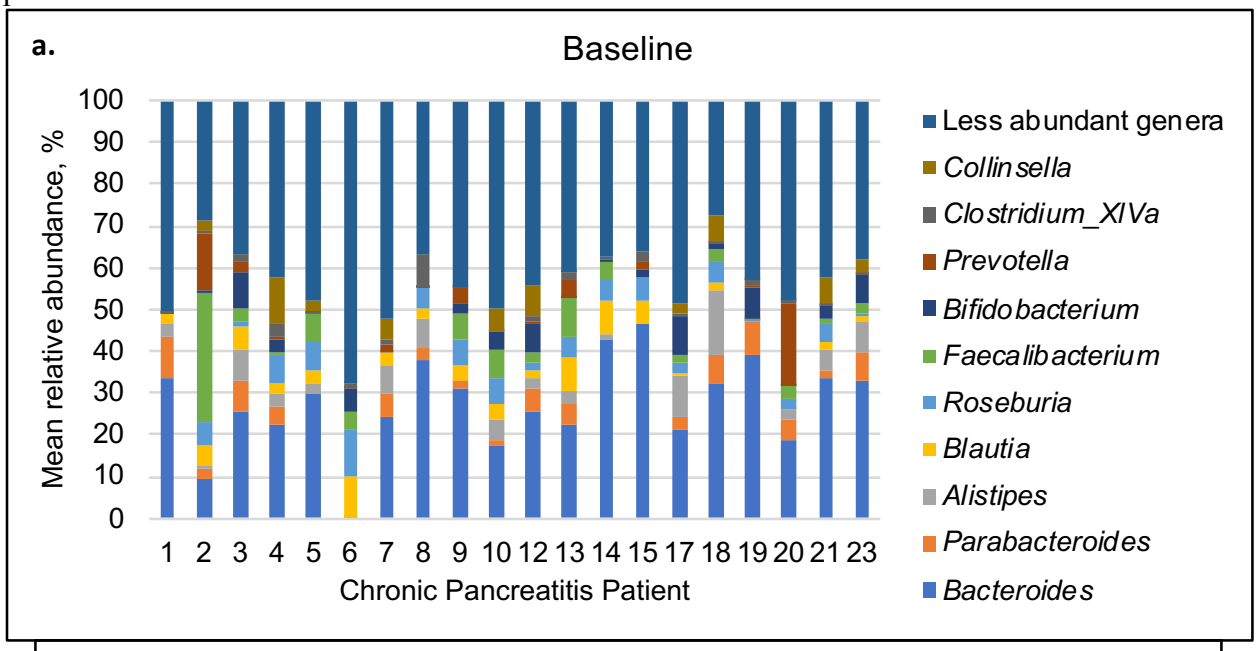
Table 1. Baseline demographics of participants with chronic pancreatitis and healthy controls.

Variable	Chronic Pancreatitis	Healthy Controls
Total number	20	6
Age in years, mean (SD)	35.7 (13.6)	35.8 (19)
Sex, female, n (%)	9 (45)	2 (33)
BMI, mean (SD)	26.5 (4.4)	22.4 (2.2)
Years of pancreatitis, mean (SD)	9.3 (4.6)	--
Pancreatitis etiology, n (%)		--
-Alcohol	1 (5)	
-Obstructive	3 (15)	
-Hereditary	8 (40)	
-Idiopathic	8 (40)	
Prior pancreas surgery, n (%)	5 (25)	--
Fecal elastase <150, n (%)	3 (15)	--
History of smoking, n (%)	6 (30)	--
Diabetes (pre-TPIAT), n (%)	1 (5)	--
Opioid use within 3 months of sample collection, n (%)	16 (80)	--
Antibiotic use within 3 months of sample collection, n (%)	7 (35)	--

Table 2. Baseline clinical characteristics influence reported IBS-type symptoms among patients with chronic pancreatitis. Univariate analysis of candidate predictors of symptoms of GI dysfunction and their associations with the IBS-symptom severity score (IBS-SSS) in patients with chronic pancreatitis, tested with ANOVA for categorical predictors and simple linear regression for continuous variables. Bold font indicates statistically significant *P*-value.

	Mean (*mean change per unit) survey score	Standard error	*P*-value
Shannon index	-308.2*	98.3	**0.006**
Years of pancreatitis pain	-2.0*	6.8	0.8
Opioid use within 3 months of sample collection			**0.02**
No (n = 4)	160.0	62.5	
Yes (n =14)	313.6	27.7	
Etiology of pancreatitis			0.4
Alcohol (n = 1)	280.0	--	
Hereditary (n = 7)	255.0	48.1	
Idiopathic (n = 7)	252.9	47.5	
Obstructive (n = 3)	398.3	52.0	
Age in years	1.4*	2.3	0.6
Sex			**0.03**
Female (n = 8)	348.8	39.2	
Male (n = 10)	224.0	34.1	
Any smoking history			0.4
No (n = 12)	262.9	41.1	
Yes (n = 6)	312.5	30.2	
Prior pancreas surgery			0.8
No (n = 14)	275.7	34.7	
Yes (n = 4)	292.5	57.5	
Fecal elastase			0.7
<150 (n = 2)	247.5	32.5	
>150 (n = 14)	288.2	36.7	
Antibiotic use within 3 months of sample collection			0.4
No (n = 11)	258.2	40.8	
Yes (n = 7)	312.9	39.0	
Sex-by-opioid use categories			**0.007**
Female, recent opioids (n = 6)	391.7	29.3	
Male, recent opioids (n = 8)	255.0	29.9	
Female, no recent opioids (n = 2)	220.0	90.0	
Male, no recent opioids (n = 2)	100.0	90.0	

Figure 1. Relative abundance of gut microflora at the genus level differs between chronic pancreatitis patients and healthy controls. (a) Mean relative abundance at the genus level for each individual patient with chronic pancreatitis. (b) Cumulative mean relative abundance at the genus level for healthy controls and chronic pancreatitis patients.



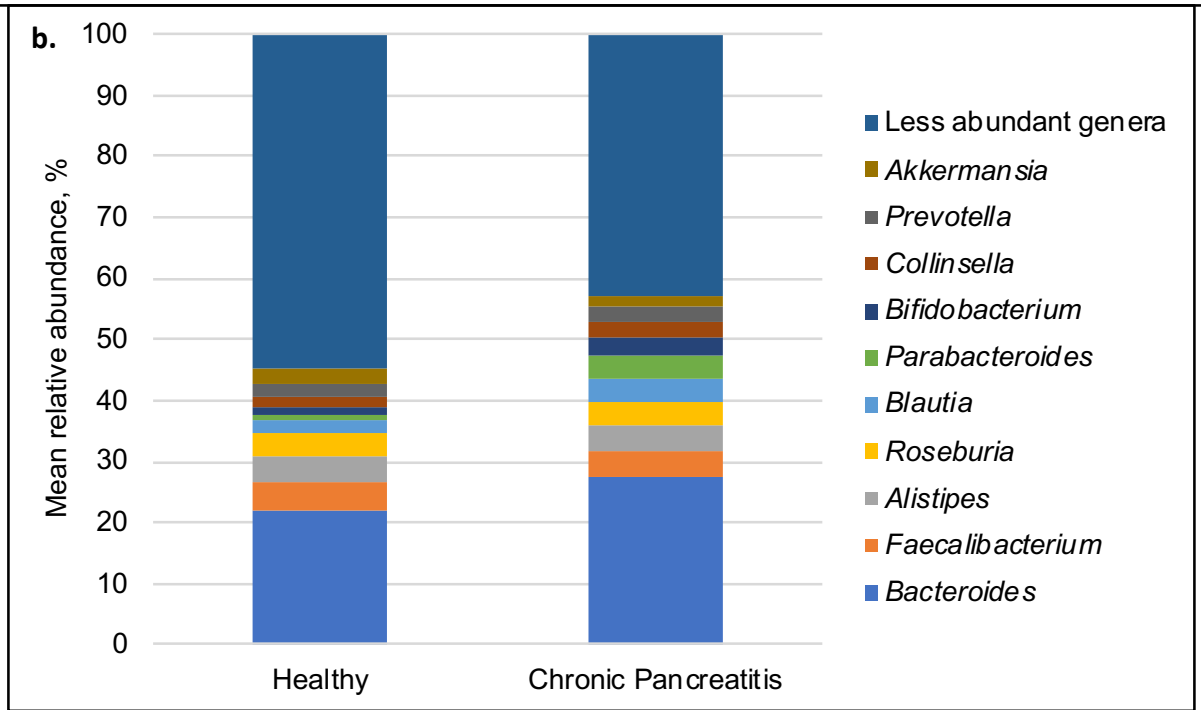


Figure 2. Beta diversity of gut microflora differs between patients with chronic pancreatitis and healthy controls. Principal component analysis of chronic pancreatitis patients (blue) and healthy controls (orange) demonstrates significant separation of the two groups in the first two principle components. $R^2 = 0.26$, ANOSIM $P < 0.001$. *Parabacteroides* (P(corr.) < 0.001) and *Faecalibacterium* (P(corr.) = 0.01) significant by Spearman's rank correlation.

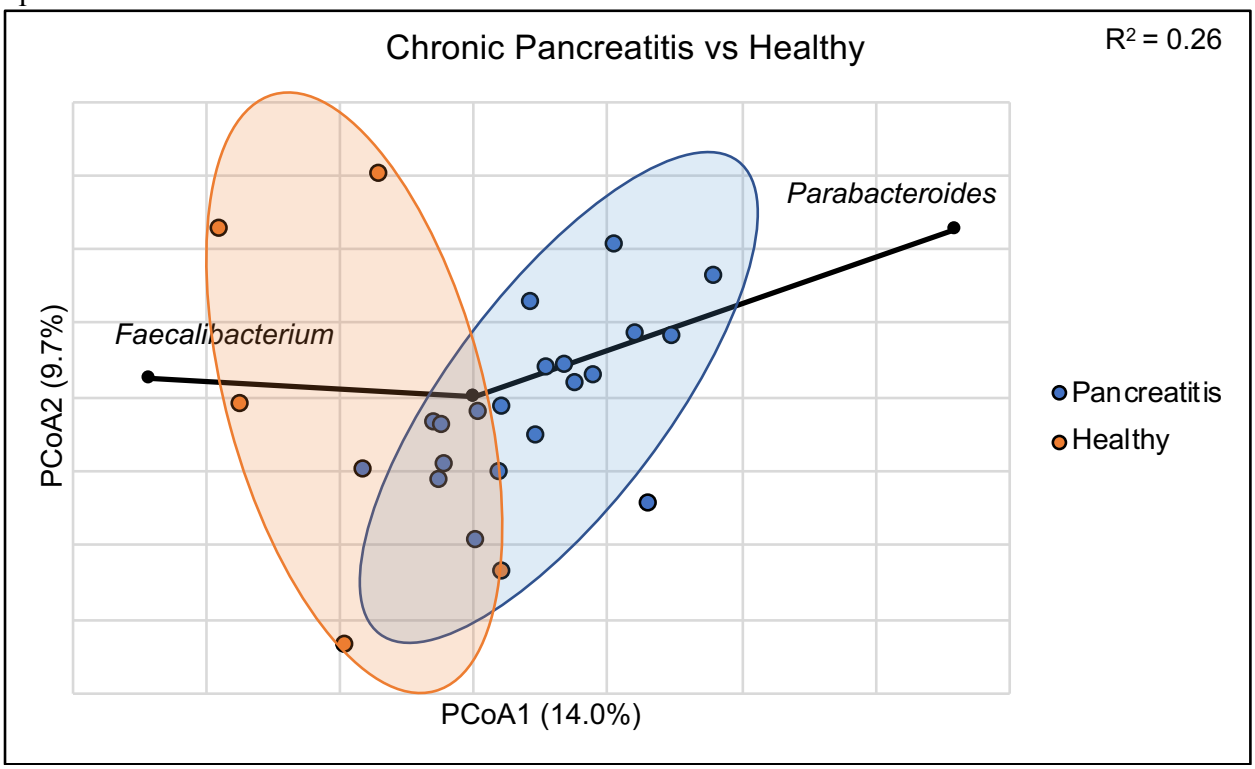


Figure 3. Alpha diversity, measured by the Shannon index, is associated with IBS-SSS survey results in patients with chronic pancreatitis. A higher score on the survey indicates worse, more frequent symptoms reported by the patient. For each 1.0 increase in the Shannon index, the survey score drops 308 points on average (standard error 98.2). Pearson's correlation r = -0.62; *P* = 0.006.

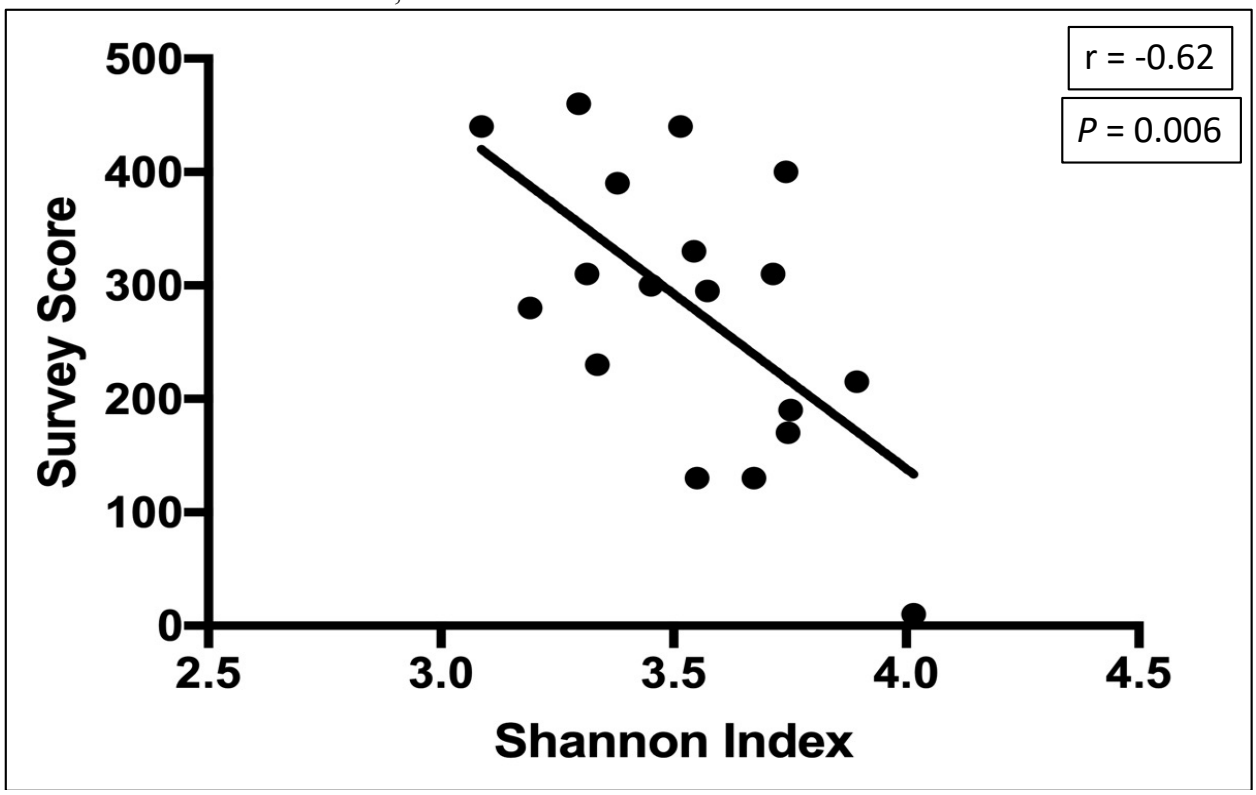


Figure 4. Analysis of covariance for the association of Shannon index with IBS-SSS by patient sex and opioid use within three months of stool sample collection. Pink circles and pink line represent female participants who were positive for opioid use. Blue circles and blue line represent male participants who were positive for opioid use. Purple and teal squares represent females and males, respectively, who did not have opioid use. IBS-SSS differed significantly between the groups (Kruskal-Wallis $P = 0.007$).

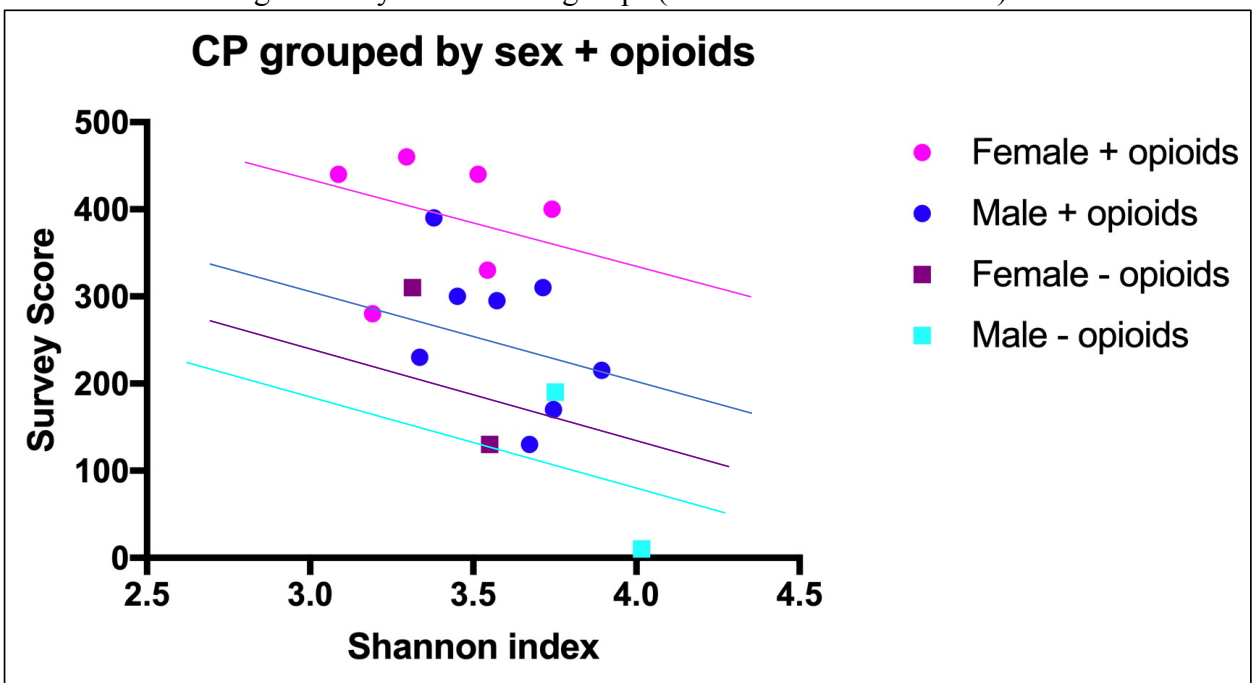


Figure 5. Relative abundance of gut microflora at the genus level differs between TPIAT recipients, chronic pancreatitis patients, and healthy controls. (a) Cumulative mean relative abundance at the genus level for healthy controls (n = 6), patients with chronic pancreatitis (n = 20), and patients after TPIAT (n = 5). (b) Mean relative abundance at the genus level for 5 patients with chronic pancreatitis before ("preop") and 6 weeks after TPIAT (6 wk post).

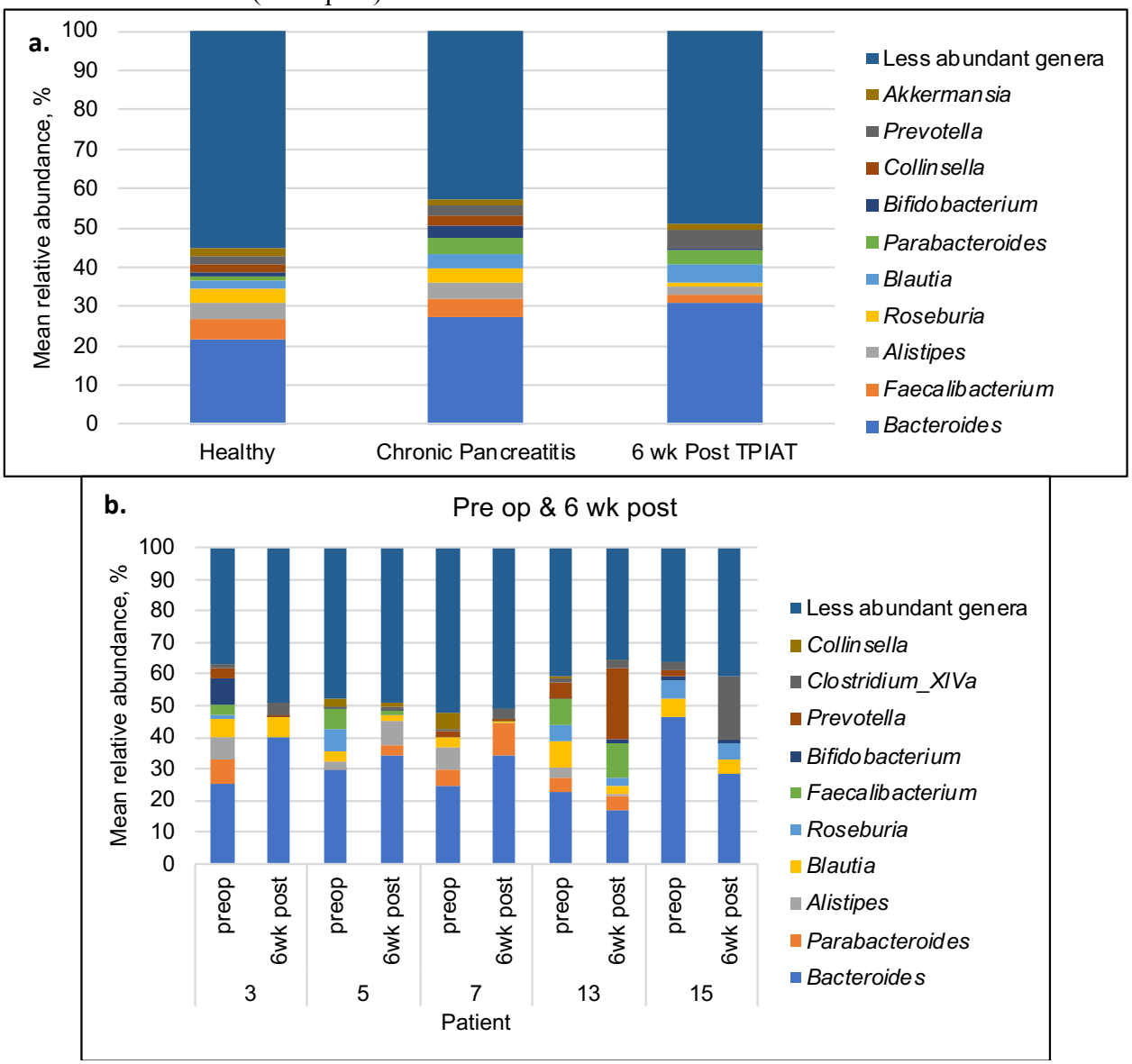
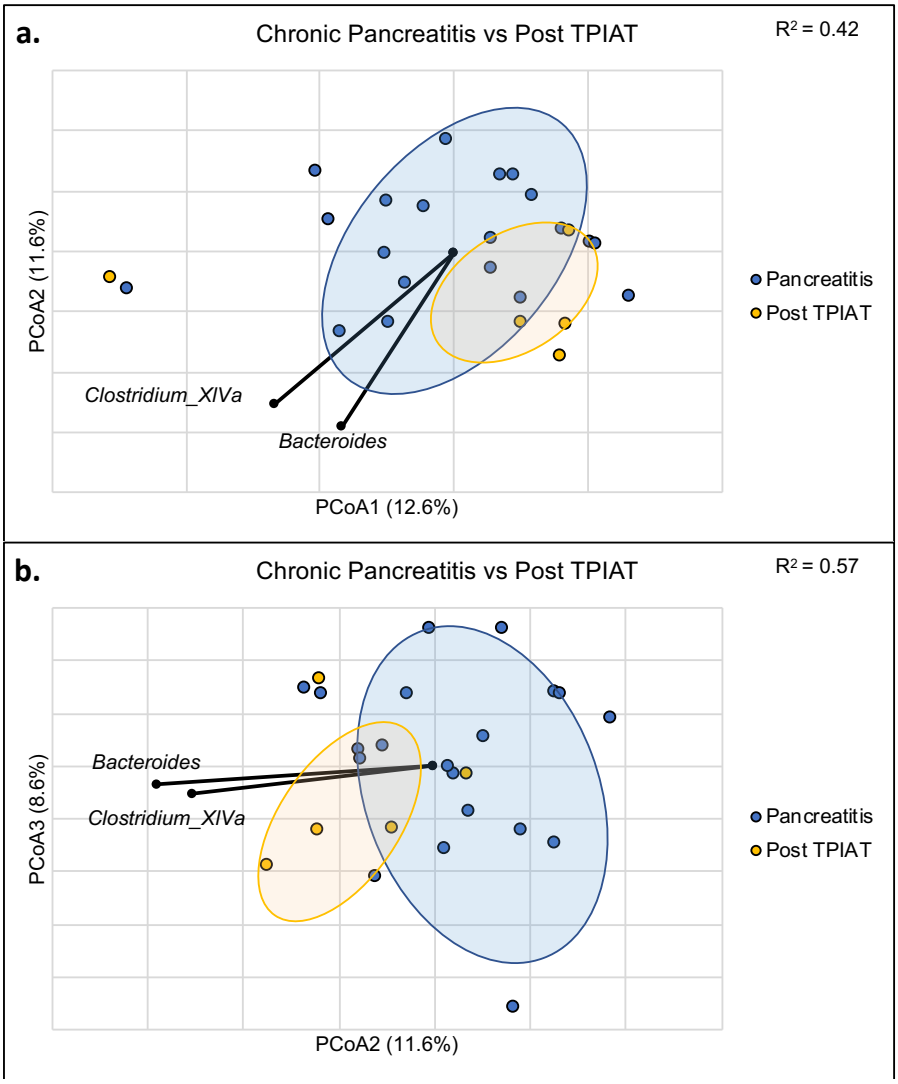


Figure 6. Beta diversity of the gut microenvironment differs between patients with chronic pancreatitis and TPIAT recipients. (a) Principal component analysis of chronic pancreatitis (blue) and post-TPIAT (yellow) for axis 1 vs axis 2, $R^2 = 0.42$. (b) Principal component analysis of pancreatitis and post-TPIAT for axis 2 vs axis 3, $R^2 = 0.57$. ANOSIM between the two groups: $P = 0.008$. *Bacteroides* (P(corr.) = 0.002) and *Clostridium_XIVa* (P(corr.) = 0.01) significant by Spearman's rank correlation.



Supplemental Table 1. Candidate clinical predictors of alpha diversity among patients with chronic pancreatitis and their univariate associations with the Shannon index.

Clinical characteristic	Mean (*mean change per unit) Shannon Index	Standard error	*P*-value
Years of pancreatitis pain	0.012*	0.012	0.4
Opioid use within 3 months of sample collection			0.5
No (n = 4)	3.65	0.15	
Yes (n = 16)	3.55	0.061	
Age	-0.0044*	0.0042	0.3
Sex			0.05
Female (n = 9)	3.45	0.082	
Male (n = 11)	3.67	0.065	
Any smoking history			0.3
No (n = 14)	3.61	0.060	
Yes (n = 6)	3.48	0.12	
Prior pancreas surgery			0.6
No (n = 16)	3.56	0.069	
Yes (n = 4)	3.64	0.053	
Fecal elastase < 150			0.5
Antibiotic use within 3 months of sample collection			0.3
No (n = 13)	3.62	0.072	
Yes (n = 7)	3.48	0.085	

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