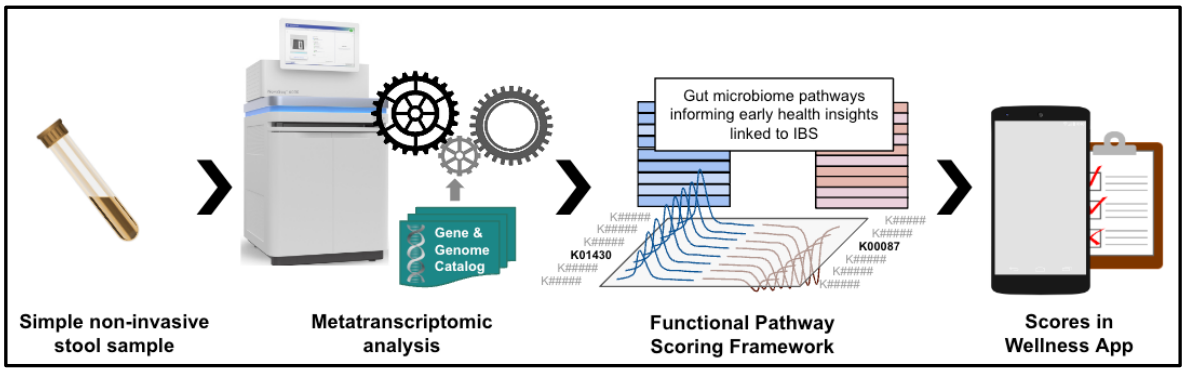
Altered gut microbial functional pathways in people with Irritable Bowel Syndrome enable precision health insights

Eric Patridge[a], Anmol Gorakshakar[1,a], Matthew M. Molusky[1,a], Oyetunji Ogundijo[a], Cristina Julian[a], Lan Hu[a], Grant Antoine[b], Momchilo Vuyisich[b], Robert Wohlman[c], and Guruduth Banavar [*,a]

[1] Equal contribution
[a] Viome Research Institute, Viome Life Sciences Inc., New York City, USA
[b] Viome Research Institute, Viome Life Sciences Inc., Seattle, USA
[c] Washington Gastroenterology (WAGI), Seattle, WA, USA
* Correspondence: guru@viome.com

Graphical Abstract



Abstract: Functional gastrointestinal disorders present diagnostic and therapeutic challenges, and there is a strong need for molecular markers that enable early detection and intervention. Herein, we present an approach to assess an abnormal gut microbiome associated with irritable bowel syndrome using stool-based gut metatranscriptome data from a large adult human population (n = 80,570). We develop a suite of eight gut microbial functional pathway scores, each of which represents the activity of a set of interacting microbial functional features (based on KEGG orthology) relevant to known gut biochemical activities. We use a normative approach within a subpopulation (n = 9,350) to define "Good" and "Not Optimal" activities for these functional pathway scores. We hypothesize that Not Optimal scores are associated with irritable bowel syndrome (IBS) and its subtypes (i.e., IBS-Constipation, IBS-Diarrhea, IBS-Mixed Type). We show that Not Optimal functional pathway scores are associated with higher odds of IBS or its subtypes within an independent cohort (n = 71,220) using both the Rome IV Diagnostic Questionnaire as well as self-reported phenotypes. Rather than waiting to diagnose IBS after symptoms appear, these functional scores can help to provide early health insights into molecular pathways that may lead to IBS. These molecular endpoints could also assist with measuring the efficacy of practical interventions, developing related algorithms, providing personalized nutritional recommendations, diagnostics, and treatments for gastrointestinal disorders like IBS.

Keywords: Stool; Metatranscriptomics; Gut microbiome; Functional pathways; Irritable bowel syndrome; Disorders of gut-brain interactions; Functional gastrointestinal diseases; Precision wellness

1. Introduction

Disorders of gut-brain interactions (DGBIs), previously known as functional gastrointestinal diseases [1], impact nearly 40% of the world's population [2]. According to the 2016 Rome IV criteria, there are more than 33 adult and 20 pediatric DGBIs [3], and there is frequent symptomatic overlap between the DGBI subtypes [4]. Their management is complex because known symptoms are subjective and have both varied presentations [5] and varied treatment strategies [6].

Clinically, DGBIs are situated at the interface of the gut-brain connection. They are often chronic, recurrent, debilitating, and do not have an identifiable underlying pathology [7]. However, DGBIs are affected by factors which are biological, psychological, and sociological, including sources which are environmental or dietary [8]. Common symptoms include altered stool quality and gut transit time, altered motility, hypersensitivity, altered mucosal function, immune disturbances, and altered central nervous system processing [3]. One of the most common DGBIs is Irritable Bowel Syndrome (IBS), affecting nearly 11% of the global population [9]. Symptoms of IBS vary between patients and may include abdominal pain and discomfort related to abnormal bowel habits such as bloating, distention, as well as constipation (for IBS-C), diarrhea (for IBS-D), or symptoms of both (for IBS-M), and the locations and patterns of these may change over time [10]. (It should be noticed that IBS-M is a distinct category and not simply an aggregation of IBS-C and IBS-D.) Further nuances of the symptoms may also distinguish "stool consistency", "gut transit time", and "motility"; for example, with IBS-C it is possible to experience an overall slower gut transit time with increased motility localized upstream of a blockage (i.e., the small intestines), and the associated bowel movement could have either increased or decreased firmness, depending on the stool consistency itself [11,12]. As a result of the range and variability of overlapping symptoms, patients with DGBIs like IBS often present diagnostic and therapeutic challenges for healthcare providers. For many patients, IBS is a diagnosis of exclusion, and this forces patients to pursue a barrage of medical procedures in order to exclude other diagnoses, while experts plead for easier ways to identify and address the classical symptoms [13,14].

Since IBS is associated with an altered microbiome [15], it may be possible to leverage the gut microbiome for health insights and treatment strategies [16]. At the same time, it is important to consider that IBS is also a composite of multiple IBS subtypes (i.e., IBS-Constipation [IBS-C], IBS-Diarrhea [IBS-D], IBS-Mixed Type [IBS-M]), and each subtype may have a distinct microbiome signature [17,18]. Ongoing efforts typically focus on profiling metabolites and/or species from the microbiome as part of mechanistic research focused on identifying the underlying causes of IBS [19]. Despite these efforts and the altered microbiome, there is still no consensus on a set of biomarkers, microbial functions, or species associated with IBS or its subtypes [15,20]. Furthermore, efforts to understand or treat IBS primarily focus on molecular-based diagnostics [21], which are typically reactive measures [22–24], and there remain few options which individuals or providers can use in proactive healthcare [25]. Considering the significant challenges and costs associated with identifying and treating IBS and other DGBIs [14,26,27], there is ample opportunity to augment healthcare services with wellness tools.

While the world waits for diagnostics, mechanistic elucidations, and preventative treatments, one possible step to advance healthcare for DGBIs like IBS is by leveraging the altered gut microbiome for early health insights. Historically, chronic diseases such as IBS or other DGBIs are often linked to altered microbiomes with particular focus on taxonomic representations (i.e., species, genus, phylum, etc.), but in many cases, the active functions of those microbes are far more important to human health than are taxonomic representation [28–30]. Recently, we developed a stool-based test which measures microbial functions (i.e., gene expression) in stool samples and quantifies them using functions defined by the KEGG Orthology [31,32] during high-resolution metatranscriptome sequencing of the gut microbiome (Figure 1). Insights about the gut microbiome are delivered through a series of gut wellness scores, which then inform downstream personalization through our precision wellness application [33] Ultimately, these scores offer valuable early health insights that can contribute to the development of therapeutic interventions to modulate microbial activities, thereby providing a personalized approach to treating DGIBs and promoting improved health outcomes.

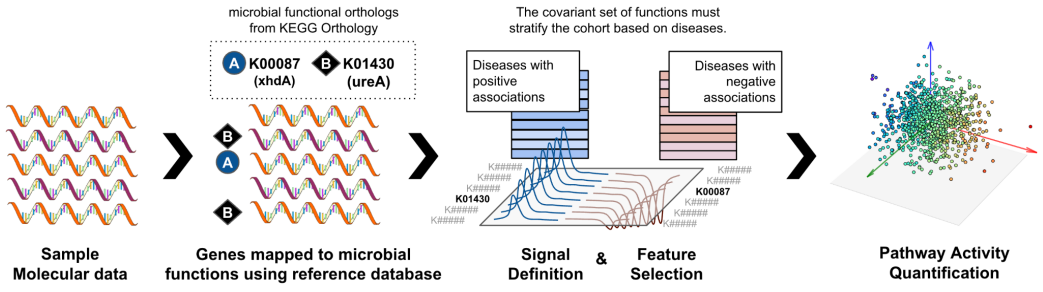


Figure 1. Scoring microbial functions in samples. A suite of scores have been designed for early health insights, and herein, we disclose the *UricAcidProductionPathways* score that includes KEGG identifiers like K00087 and K01430 (Tables 7-8). Signal definition, Feature selection, and Pathway activity quantification are, respectively, Steps 3, 4, and 5 of the Score Development Framework in our earlier publication [34].

Herein, we demonstrate a scalable approach to quantify the transcriptomic activity of microbial functions from the gut metatranscriptome for applications in health and wellness. We present eight gut pathway scores which were designed to provide insights for both gastrointestinal and systemic health issues. Each score was developed using a large adult population (n = 9,350) and a computational method previously demonstrated with saliva [34]. The specific purpose of this manuscript is to validate the ability for these gut pathway scores, designed to assess the transcriptomic levels of microbial functions in stool, to assess altered microbiomes within an independent cohort (n = 71,220). For the current validation, we demonstrate the relationship of the eight gut scores among individuals with IBS and its subtype classifications (IBS-C, IBS-D, and IBS-M), whether cases are defined by self-reported IBS phenotypes or by the Rome IV Diagnostic Questionnaire [35], within an unseen cohort distinct from that used for score development. Each score can be used to identify an altered microbiome, which informs the opportunity to proactively address the altered microbiome.

2. Materials and Methods

Stool samples and questionnaire responses were obtained from a general adult population of Viome customers who were at least 18 years old at the time of sample collection and residing in the USA. All customers consented to their data being used for research purposes, as part of the sign-up process for Viome services. Therefore, no IRB review was required for this data analysis. All study data are de-identified; data analysis team members have no access to personally identifiable information.

Study participants collected their stool samples using Viome's Full Body Intelligence kits, which they received at their homes. Each kit includes a stool collection tube featuring: 1) an integrated scoop to facilitate stool collection, 2) RNA Preservation Buffer (RPB) to maintain the integrity of all RNA molecules during collection and transportation, and 3) sterile glass beads to facilitate sample homogenization. Also included in the kit is a flushable stool sample collection paper, which is placed across the toilet and on which stool is deposited. From the stool, a pea-sized sample is placed inside the collection tube, then vigorously shaken with the RPB to: 1) homogenize the stool, and 2) to expose the sample to RPB. As previously published, the RPB has been clinically validated to preserve RNA for up to 28 days at room temperature, and all molecular biology steps were performed in a CLIA-certified laboratory using our clinically validated methodology [33]. The method includes DNase treatment, non-informative RNA depletion, cDNA synthesis, size selection, and limited cycles of PCR for adding dual unique barcodes to each sample. All samples were sequenced using the Illumina NovaSeq 6000 platform with 2x150 paired-end read chemistry.

When collecting their stool samples, individuals also respond to an extensive questionnaire allowing them to detail their lifestyle, health history, and health conditions. Lifestyle questions relevant to the current study include use of proton-pump inhibitor (PPI) medication, recent use of antibiotics, and use of disease-specific medication (i.e., antidiarrheals or laxatives/stool softeners). Health history is also collected, resulting in hundreds of self-reported phenotypes which include IBS, IBS-C, IBS-D, and IBS-M, as well as all the disease phenotypes listed in Supplementary Table S1. Symptom and condition questions relevant to the current study include abdominal pain, bloating, stool classification according to the Bristol Stool Form Scale (BSFS), and all questions required for the Rome IV Questionnaire [35–38].

Briefly, the BSFS is an ordinal scale of stool consistency ranging from the hardest (Type 1) to the softest (Type 7). Types 1 and 2 are considered to be abnormally hard stools (indicative of decreased gut transit time [37]) while Types 6 and 7 are considered abnormally loose/liquid stools (indicative of increased gut transit time [37]). Type 3, 4 and 5 are therefore generally considered to be the most 'normal' stool form (indicative of average gut transit time [37]).

The Rome IV Diagnostic Questionnaire consists of six questions, beginning with "In the last 3 months, how often did you have pain anywhere in your abdomen?" The answers to these questions are scored and yield a classification of "IBS-C", "IBS-D", "IBS-M", "IBS-U" (for unclassified), or "FALSE" for individuals who do not meet the criteria for any IBS classification [35,39].

2.1 Cohort Development

To deliver health insights from stool samples, we design and validate wellness scores in the context of two cohorts representative of the general adult population. The "development" and "validation" cohorts began with around 10,000 and 80,000 samples, respectively. We further exclude outliers based on age, birth sex, and body mass index (BMI). Additional criteria mitigate artifacts linked to sequencing depth or data sparseness within each cohort. Samples in the final cohorts contain at least 1,700 KOs, with a minimum total read count of 70,000. The final score development cohort consists of 9,350 stool samples from adults (59.4% female; Table 1). The final independent validation cohort consists of 71,220 stool samples from adults (66.5% female; Table 1).

2.2 Metatranscriptomic Analysis

Stool samples are collected and analyzed from individuals who undergo a minimum 8-hour fasting period. With established metatranscriptomic techniques [40], the entire set of transcripts (RNA molecules) from each stool sample is measured, providing both the primary sequence and read count for each transcript. Each sequencing read is aligned to microbial genomes as follows: Viome utilizes a custom reference catalog that encompasses 32,599 genomes from NCBI RefSeq release 205 "complete genome" category, as well as 4,644 representative human gut genomes of UHGG [41], ribosomal RNA (rRNA) gene sequences, and the human genome GRCh38 [42]. Altogether, the custom catalog spans archaea, bacteria, fungi, protozoa, phages, viruses, and the human host, with a total of 98,527,909 annotated microbial genes. To annotate microbial gene functions, the KEGG Orthology [31,32] is adopted, and KEGG orthologs (KOs) are assigned via eggNOG-mapper [43]. Taxonomy classification (at all taxonomic levels) is accomplished through mapping paired-end reads to the custom catalog using Centrifuge [44]. Any reads mapped to the host genome or rRNA sequences are excluded from further analysis but tracked for monitoring.

To estimate expression level (or activity) in the sample, an Expectation-Maximization (EM) algorithm [45] is applied to mapped microbial reads. Respective taxonomic ranks (strains, species, genera, etc.) can be aggregated from the genomes. For the current study, species activity is used in the downstream analyses. The reads mapped to microbial genomes are specifically extracted and mapped to gene or open reading frame (ORF) regions for molecular function or KO annotation and quantification. The identified KOs from stool samples are used in downstream analyses, such as score development, validation, and ultimately scoring new samples.

2.3 Score Development

Our stool-based scores are intended to deliver early insights using only microbial functions (i.e., gene expression) from the gut microbiome. We follow a similar approach for score development as we previously reported for saliva-based scores with the oral microbiome [34].

The final scores are composed entirely of microbial KOs, quantified in stool, which contributed to the signal intended by the score design. In developing the stool-based scores, we explore domain concepts including carbon and energy metabolism, aerobic/anaerobic metabolism, activities related to vitamins and protein cofactors, metabolism of amino acids and nucleic acids, environmental stressors, known pathogenic components, etc. The microbial KOs included in the selection process are identified because of their association with one or more of these physiological processes. In the final step of our development process, we leverage the covariance of the feature set within a normalized expression dataset, and we aggregate the computationally determined weights, based on the first component (PC1) of Principal Component Analysis (PCA). When the final scores are computed on a large cohort, they exhibit a gaussian-like curve which represents the stratification of health insights and distribution of scores across the population (shown in Supplemental Figure S1).

Our five-step process for score development was previously explained in detail [34], including: 1) Domain exploration; 2) Metadata curation; 3) Signal definition; 4) Feature selection; and 5) Pathway activity quantification. The complete details of each step are available in our earlier report but for context are briefly reviewed.

Table 1. Sociodemographic, anthropometric, medication use, and IBS-related characteristics of the cohorts.

	Score Development Cohort (*n* = 9,350 stool samples)		**Independent Validation Cohort (*n* = 71,220 stool samples)**	
Female	59.4%		66.5%	
Age (years)	46.2 ± 13.0		44.5 ± 13.6	
BMI (kg/m^2)	26.1 ± 5.4		25.8 ± 5.3	
Ethnicity	74.2% White From the remaining 25.8%: 28.9% Hispanic or Latino 28.1% Multi-ethnic 19.2% Asian 11.1% Black or African American 5.6% Arab or Middle Eastern 7.0% Other		74.1% White From the remaining 25.9%: 28.7% Hispanic or Latino 27.4% Multi-ethnic 18.5% Asian 12.2% Black or African American 5.9% Arab or Middle Eastern 7.4% Other	
IBS Prevalence	(*self-reported*)	(*Rome IV*)	(*self-reported*)	(*Rome IV*)
No IBS	93.6%	81.2%	91.1%	74.5%
Any IBS	6.4%	10.0%	8.9%	12.1%
IBS-C	1.4%	2.2%	2.4%	2.7%
IBS-D	1.1%	3.3%	1.4%	4.1%
IBS-M	0.9%	3.9%	1.5%	4.6%
IBS-U	–	0.6%	–	0.6%
PPI use[1]	4.2%		4.3%	
Antibiotic use[2]	15.4%		13.1%	
Medication use	0.5% Antidiarrheal meds 2.4% Laxatives/stool softeners		0.3% Antidiarrheal meds 3.1% Laxatives/stool softeners	
Sample BSFS[3]				
< 3	19.4%		19.6%	
3 < 6	52.6%		51.8%	
≥ 6	28.0%		28.7%	
No answer	0.02%		0.1%	
Abdominal Pain				
Never	42.4%		37.3%	
< 1 day/wk	35.8%		36.0%	
1-6 days/wk	18.4%		22.7%	
7 days/wk	3.3%		3.9%	
No answer	0.2%		0.3%	
Bloating				
Never	16.0%		12.7%	
Rarely	25.8%		22.1%	
Sometimes	30.6%		30.5%	
Often	20.0%		24.3%	
Always	7.5%		10.1%	
No answer	0.2%		0.3%	

[1] PPI = Self-reported proton-pump inhibitor use.
[2] Self-reported antibiotics, "currently taking them", or "have taken them in the past month".
[3] Bristol Stool Form Score (average of multiple selection, rounded down). < 3: indicative of decreased gut transit time (1 = Separate hard pellets; 2 = Clumpy log shaped); 3 - 6: indicative of average gut transit time (3 = Log shape with cracks on the surface; 4 = Smooth snake shape; 5 = Soft chunks); ≥ 6: indicative of increased gut transit time (6 = Paste consistency with ragged edges; 7 = Liquid consistency with no solid pieces)

Through domain exploration, we define both the conceptual landscape for the score and the entire set of microbial KOs relevant to the score. The metadata curation process includes identifying and creating case/control labels which enable downstream steps as well as the validation presented in this manuscript. Signal definition is the early draft of a score, meaning a small set of KOs demonstrating covariance which stratifies health insights across the development cohort. The feature selection process is extremely iterative and serves to strengthen the score's ability to stratify health insights in a manner consistent with domain knowledge. The final step, pathway activity quantification, is the aggregate quantification of the entire set of KO features in a score by combining the expression levels of the selected KOs. As we previously reported [34], "we derive a pathway score as a weighted function Score = $C1F1 + C2F2 + \ldots + CnFn$, where Fi is the expression level of the feature and Ci is its weight."

The overall implementation of scores is a normative approach [46]; the development cohort serves as a reference population which provides weightings of each feature that can be used to compute scores for all new samples. After using the development cohort to design the scores with a numeric value between 0 and 100, the numerical values are further signed with one of the categories, "Good", "Average", or "Not Optimal", based on the score distribution in the development cohort. We adopt MAD (median absolute deviation), a measure of dispersion and robust to outliers. The categorical thresholds are calculated using $median \pm 0.75 * MAD$ of the respective score distribution in the development cohort. "Good" refers to the higher end, "Not Optimal" the lower end, and "Average" the middle range.

2.4 Score Validation

The validation cohort, consisting of 71,220 stool samples, is used to confirm the reproducibility of signals identified during score development. This cohort is a previously unseen sample set; there is no overlap between the independent validation cohort and score development cohort. For the validation process, we take a general wellness approach and evaluate the ability for each of the eight scores to differentiate gastrointestinal diseases known to be associated with the gut microbiome.

The focus of the validation process in this paper employs odds ratios (OR) to test scores with IBS and each subtype (IBS-C, IBS-D, IBS-M). Cases for each self-reported IBS phenotype were defined by responses to the question: "Please list all of the illnesses you are currently suffering from or diagnosed with." The list included IBS, IBS-C, IBS-D, and IBS-M, (with "IBS" as the composite of all IBS subtypes) and all such metadata labels defined in the development cohort are also applied to the validation cohort. Cases for Rome IV-determined IBS classifications were defined using the independent and clinically validated Rome IV Diagnostic Questionnaire. Controls are carefully curated using domain knowledge to identify and exclude phenotypes likely to have a "Not Optimal" score; therefore, control definitions are score-specific (see Supplementary Table S1). Cases are matched to controls (1:5) on age, birth sex, BMI, and month sequenced. Age matching allows for ±5 years with respect to case. For BMI, matching is performed within defined cates: BMI < 15 is classified as an outlier, 15 ≤ BMI < 18.5 (classified as "underweight"), 18.5 ≤ BMI < 24.9 ("healthy"), 24.9 ≤ BMI < 29.9 ("overweight"), and 29.9 ≤ BMI ("obese"). For month sequenced, matching was performed within a window of ±3 months. All odds ratios are further adjusted for BMI, age, PPI use or antibiotic use, gastrointestinal surgeries, and use of medication specific to IBS subtypes.

Across the cohorts, every score was assigned one of three values: "Good", "Average", or "Not Optimal"; for odds ratios, we focus on the odds for "Not Optimal" scores as compared to the odds for "Good" scores. Using score category (i.e., "Not Optimal" or "Good") as the independent variable and IBS subtype as the dependent variable, a logistic regression model is applied while adjusting for age, BMI, and disease-specific medication as confounders. Odds ratio is defined as the odds of having a "Not Optimal" score with an IBS subtype over having a "Good" score with the same IBS subtype. Statistical comparison of scores between matched case/control samples is carried out using the two-tailed Mann-Whitney U test, and the Benjamini-Hochberg correction is applied to control the False Discovery Rate.

Venn diagrams included in supplementary materials were created through differential expression of species for cases of IBS-C, IBS-D, or IBS-M, excluding cases with multiple IBS subtypes. Controls were defined as those without any self-reported comorbidities. Individuals taking PPIs or antibiotics were also excluded from both cases and controls. Methodology for matching is the same for odds ratios, where cases are matched to controls (1:5) on age, birth sex, BMI, and month sequenced. For visualization purposes, differentially expressed species [$p \leq 0.05$ and log(fold-change) ≥ 0.6] were aggregated to the genus level.

3. Results

3.1 Cohort Characteristics

We employ two large cohorts: 1) a score development cohort is used to facilitate a heuristic approach for feature (KO) selection during our score design process; and 2) an independent validation cohort is used to assess the performance of each score. The composition of both cohorts are comparable; the development cohort (9,350 stool samples from adults; 59.4% female) has an average age of 46.2 ± 13.0 years and BMI of 26.1 ± 5.4 kg/m^2, while the validation cohort (71,220 stool samples from adults; 66.5% female) has an average age of 44.5 ± 13.6 years and BMI of 25.8 ± 5.3 kg/m^2.

Table 1 shows characteristics for each cohort at the time of sample collection, including sociodemographics (age, birth sex, ethnicity), anthropometrics (BMI), and medication use (PPI, antibiotics, anti-diarrhea meds, and laxatives/stool softeners). Additional characteristics related to IBS include both self-reported and Rome IV-defined IBS phenotypes, the Bristol Stool classification of the bowel movement that was sampled for the cohort, abdominal pain frequency over the 3 months prior to sampling, and frequency of bloating.

Since our validation of stool-based scores focuses on individuals with IBS, we present the IBS-related characteristics across the two cohorts in Tables 2-5. In each table, IBS is defined as the complete set of individuals who self-reported IBS or any IBS subtype (IBS-C, IBS-D, IBS-M). The trends reported are similar across both the score development cohort and the independent validation cohort.

Table 2 shows the Bristol Stool classification of the sampled bowel movement, stratified by IBS subtype; a higher proportion of individuals with IBS-C self-report slower gut transit times while a higher proportion of those with IBS-D self-report faster gut transit times. Individuals with IBS-M report Bristol Stool classifications similar to the entire set of individuals with IBS, which also closely follows the general cohort population.

Table 3 shows responses of recent abdominal pain, broken down for those with IBS and those without IBS; across both cohorts, individuals with IBS reported more frequent abdominal pain than those without IBS. When stratified by IBS subtype, the numbers were generally similar across all subtypes (not shown).

Table 2. Gut transit time as measured by Bristol Stool Scores for the sampled (analyzed) bowel movement from IBS (any subtype) and IBS subtypes across cohorts (totaling 6,972 individuals). Gut transit times are as expected across subtypes, and the two cohorts have similar characteristics.

	Slower transit time (BFSF* < 3)	Average transit time (BFSF 3 < 6)	Faster transit time (BFSF ≥ 6)
Score Development Cohort			
IBS (*n* = 599)	17.7%	49.4%	32.9%
IBS-C (*n* = 132)	**23.9%**	50.8%	25.8%
IBS-D (*n* = 101)	10.9%	43.6%	**45.5%**
IBS-M (*n* = 85)	16.5%	47.1%	36.5%
Independent Validation Cohort			
IBS (*n* = 6,373)	20.6%	44.4%	35.0%
IBS-C (*n* = 1,732)	**29.6%**	43.4%	27.1%
IBS-D (*n* = 1,006)	12.9%	39.4%	**47.7%**
IBS-M (*n* = 1,091)	21.4%	42.3%	36.4%

Bold numbers indicate the highest values across columns with abnormal gut transit time.
* Bristol Stool Form Score (see Table 1 for categorical definitions)

Table 3. Abdominal pain reported by individuals with IBS (*n* = 6,972) and Non-IBS (*n* = 73,598). Breakdown of abdominal pain is as expected for IBS/Non-IBS, and the two cohorts are similar.

Score Development Cohort	IBS (any subtype; *n* = 599)	Non-IBS (*n* = 8,751)
Abdominal Pain		
Never	13.0%	**44.4%**
< 1 day/wk	28.0%	36.3%
1-6 days/wk	**44.7%**	16.6%
7 days/wk	13.9%	2.5%
No answer	0.3%	0.2%
Independent Validation Cohort	**IBS (any subtype; *n* = 6,373)**	**Non-IBS (*n* = 64,847)**
Abdominal Pain		
Never	11.0%	**39.8%**
< 1 day/wk	27.7%	36.8%
1-6 days/wk	**47.8%**	20.2%
7 days/wk	13.3%	2.9%
No answer	0.2%	0.3%

Bold numbers indicate the highest values across columns regarding abdominal pain frequency.

Table 4. Gut transit time as measured by Bristol Stool Scores for recent atypical bowel movements from IBS (any subtype) and IBS subtypes across cohorts (totaling 6,972 individuals). Breakdown of gut transit time pain is as expected across subtypes, and the two cohorts are similar.

	Slower transit time (BFSF** < 3)	Average transit time (BFSF 3 < 6)	Faster transit time (BFSF ≥ 6)	Mixed transit time (BFSF < 3 & ≥ 6)	No answer*
Score Development Cohort					
IBS (*n* = 599)	21.7%	4.8%	28.7%	31.4%	13.4%
IBS-C (*n* = 132)	**43.9%**	3.8%	6.1%	28.8%	17.4%
IBS-D (*n* = 101)	2.0%	0.0%	**67.3%**	18.8%	11.9%
IBS-M (*n* = 85)	11.8%	2.4%	29.4%	**49.4%**	7.1%
Independent Validation Cohort					
IBS (*n* = 6,373)	22.3%	5.0%	30.0%	31.5%	11.2%
IBS-C (*n* = 1,732)	**50.3%**	4.1%	6.6%	26.0%	13.0%
IBS-D (*n* = 1,006)	2.7%	1.8%	**65.2%**	15.8%	14.5%
IBS-M (*n* = 1,091)	9.4%	3.3%	24.4%	**53.7%**	9.2%

Bold numbers indicate the highest values across columns with abnormal gut transit time.
* Responses were not recorded for individuals who never experienced abdominal pain.
** Bristol Stool Form Score (see Table 1 for categorical definitions)

Table 5. Bloating frequency reported by individuals with IBS (*n* = 6,972) and Non-IBS (*n* = 73,598). Bloating is as expected for IBS/Non-IBS, and the two cohorts have similar characteristics.

Score Development Cohort	IBS (any subtype; *n* = 599)	Non-IBS (*n* = 8,751)
Bloating frequency		
Less than average	15.4%	**43.5%**
Average	30.4%	30.6%
More than average	**53.9%**	25.7%
No answer	0.3%	0.2%
Independent Validation Cohort	**IBS (any subtype; *n* = 6,373)**	**Non-IBS (*n* = 64,847)**
Bloating frequency		
Less than average	13.5%	**36.9%**
Average	25.7%	31.0%
More than average	**60.5%**	31.8%
No answer	0.2%	0.3%

Bold numbers indicate the highest values across columns regarding bloating frequency.

Those individuals who reported any abdominal pain (Table 3) were also asked for the Bristol Stool classification of their recent atypical bowel movements, and these are stratified across IBS subtypes in Table 4. As the numbers show, the trends highlighted in bold in Table 2 are also present in Table 4. A higher proportion of individuals with IBS-C indicated decreased gut transit time ("usually constipation; BSFS 1-2"), while a higher proportion of individuals with IBS-D indicated increased gut transit time ("usually diarrhea; BSFS 6-7"). A higher proportion of individuals with IBS-M indicated mixed gut transit time ("both constipation and diarrhea").

Table 5 shows responses of typical bloating, broken down for those with IBS and those without IBS; across both cohorts, individuals with IBS reported more frequent bloating than those without IBS. When stratified by IBS subtype, the numbers were generally similar across all subtypes (not shown).

3.2 Metatranscriptomic Analysis

We present high-level metrics of development cohort (n = 9,350) and independent validation cohort (n = 71,220) to demonstrate sample data quality. The total number of reads in paired-end fastq files in millions for both cohorts combined is 10.6 ± 4.28. The average KO richness or distinct KOs associated with each sample for development and validation cohorts combined is 3,641 ± 699. Species richness, defined as distinct species identified in each sample, across development and validation cohorts combined, is 1,451 ± 518. Effective sequencing depth, defined as the total reads in millions mapped to a recognized element like KO or species for both cohorts combined, is 3.32 ± 2.20.

3.3 Score Development

Our Score Development Framework is a 5-step process [34], and herein, we use *UricAcidProduction-Pathways* as an example while we talk through each step. A high level summary for each of the eight gut pathway scores appears in Table 6, including key functions and a brief description of microbial activities. Altogether, the eight scores comprise a total of 278 KOs (259 distinct KOs), where 242 KOs (or 93%) appear only once across all scores and 17 KOs (or 6.5%) each appear in 2-3 scores. Any two scores share a maximum of three KOs. The number of KOs in each score ranges from 19 to 63, with the final number determined heuristically rather than stringent limits. While the process is similar for each score, all scores are developed independently from each other, with key functions, components, metadata and phenotype labels driving feature selection.

The first step in the framework is domain-driven curation for potential inclusion of features (KOs) in score development. As an example, the *UricAcid-ProductionPathways* score was designed by curating "key components" and "key functions" centered around microbial nucleotide, nucleoside, and purine degradation generating uric acid (Table 7). KOs were considered for inclusion if they impacted these "in scope" parameters.

The second and third steps involve metadata curation and signal definition in our development cohort. These steps provide information useful for step four, feature selection, which involves the iterative refinement of the KOs that define a score.

The final step involves pathway activity quantification. Depicted in Table 8 is the score definition for *UricAcidProductionPathways*, along with the corresponding weights (loadings) derived from the first principal component (PC1) of a principal component analysis (PCA) (see Materials and Methods). The normalized expression data for the development cohort (n = 9,350) appears in Figure 2A (including colorized PC1), and the loadings from Table 8 are visualized in Figure 2B. The KOs with negative loadings represent microbial uric acid production, which is undesirable and therefore higher expression of these features would result in a lower score. The loadings in Table 8 also include KOs with positive loadings, which represent uric acid degradation or alternative metabolism, and a higher expression of these features would result in a higher score.

3.4 Score Validation

We use the independent validation cohort to examine how the eight gut pathway scores behave with IBS and its subtypes; our hypothesis, based on the domain knowledge used to design the score, was that non-optimal scores would associate with IBS and its subtypes. We choose IBS and IBS subtypes (IBS-C, IBS-D, IBS-M) to stratify the scores, because these IBS subtypes have altered gut microbiomes and, to some extent, share underlying symptoms such as altered gut transit, altered stool consistency, abdominal pain, and bloating [47,48].

For simplicity, we only report on "Not Optimal" scores, using "Good" scores as a reference point. The translational interpretation for each "Not Optimal" score is presented in Table 9. For example, in the case of *UricAcidProductionPathways*, a "Not Optimal" score indicates the microbial gene expression contains more functions with negative loadings and fewer functions with positive loadings from Table 8; this may be interpreted as increased uric acid production (Table 9).

Table 6. High level overview of Stool Pathways Scores

Score Name	Key Functions	Microbial Activities	KOs
ButyrateProductionPathways	Butanoate metabolism Lysine degradation Fatty acid degradation BCAA[1] degradation Propanoate metabolism	Accumulation of beneficial butyrate from pyruvate and by degradation of fatty acids, lysine, and BCAAs.	39
GABAProductionPathways	Butanoate metabolism Pentose/glucuronate interconversions Vitamin B6 metabolism Amino/nucleotide sugar metabolism	Accumulation of GABA[2] from various substrates including butanoate. Along with synthesis of cofactors needed for enzymes that produce GABA, including vitamin B6.	63
LacticAcidProductionPathways	Lactate transport Pyruvate metabolism Other glycan degradation	Metabolism and transport of butyrate, pyruvate, and lactate, including carbon flow specific to the accumulation of lactic acid.	30
LPSBiosynthesisPathways	Lipopolysaccharide biosynthesis KDO2-lipid A biosynthesis Nucleotide sugar biosynthesis	Accumulation of beneficial and deleterious LPS modifications, nucleotide sugars, and related processing of glutamine/glutamate/ammonia.	24
MethanogenesisPathways	Methane metabolism Formaldehyde assimilation Fructose/mannose metabolism Pentose phosphate pathway	Several methods of methane production, ammonia release from amino acids, and pentose phosphate activity.	47
OxalateMetabolismPathways	Oxalate degradation Glyoxylate/dicarboxylate metabolism Ascorbate/aldarate metabolism	Accumulation of oxalate and glyoxylate precursors, including the metabolism of related vitamins and carbon compounds.	28
SulfideProductionPathways	Sulfur metabolism Taurine/hypotaurine metabolism Glycine/serine/threonine metabolism	Tricarboxylic acid-related energy processes as well as the generation of sulfide from organic sulfur-containing amino acids and inorganic sources like sulfite and sulfate.	28
UricAcidProductionPathways	Nucleotide metabolism Purine metabolism Hypoxanthine oxidation C4-Dicarboxylate sensing	Beneficial production of trehalose and processing of purines like adenine, guanine, inosine, xanthine, and urate.	19

[1] BCAA = Branched-chain amino acid.
[2] GABA = Gamma-aminobutyric acid.

Table 7. Scope of the *UricAcidProductionPathways* score, which serves as one example from the suite of available wellness scores.

Key components	Nucleotides	AMP, GMP, 3′-GMP, IMP
	Nucleosides	Adenosine, guanosine, inosine, xanthosine
	Purines	Adenine, guanine, hypoxanthine, xanthine, uric acid
	Enzymes	Oxidoreductases, deaminases, nucleotidases
	Cofactors	NADH, molybdenum, FAD
Key functions	Purine metabolism	Hypoxanthine oxidation
	Nucleotide metabolism	Adenosine degradation, guanosine degradation
	Energy extraction	Anaerobic fermentation
	Two-component systems	C4-Dicarboxylate sensing and transport
	Other	Molybdenum biosynthesis, quorum sensing

Table 8. Score design for *UricAcidProductionPathways*. Loadings originate from the first principal component of PCA analysis using the development cohort.

Loadings	KEGG Ortholog (KO) and Description
0.42707	K07651 (resE); two-component system, OmpR family, sensor histidine kinase
0.30347	K04076 (lonB); ATP-dependent Lon protease
0.27966	K18011 (kamE); beta-lysine 5,6-aminomutase beta subunit
0.27711	K18532 (AK6, FAP7); adenylate kinase
0.24838	K01236 (treZ, glgZ); maltooligosyltrehalose trehalohydrolase
0.06793	K01430 (ureA); urease subunit gamma
0.05184	K00948 (PRPS, prsA); ribose-phosphate pyrophosphokinase
0.04766	K00839 (pucG); (S)-ureidoglycine-glyoxylate transaminase
-0.02885	K21053 (ade); adenine deaminase
-0.06887	K20967 (MOCS1); GTP 3',8-cyclase / cyclic pyranopterin monophosphate synthase
-0.08136	K00087 (ygeS, xdhA); xanthine dehydrogenase molybdenum-binding subunit
-0.10569	K13479 (ygeT, xdhB); xanthine dehydrogenase FAD-binding subunit
-0.18014	K15973 (mhqR); MarR family transcriptional regulator
-0.22229	K13483 (yagT); xanthine dehydrogenase YagT iron-sulfur-binding subunit
-0.22525	K00364 (guaC), GMPR; GMP reductase
-0.26568	K11711 (dctS); two-component system, LuxR family, sensor histidine kinase
-0.26922	K03787 (surE); 5'/3'-nucleotidase
-0.30322	K07084 (yuiF); putative amino acid transporter
-0.32907	K10558 (lsrA, ego); AI-2 transport system ATP-binding protein

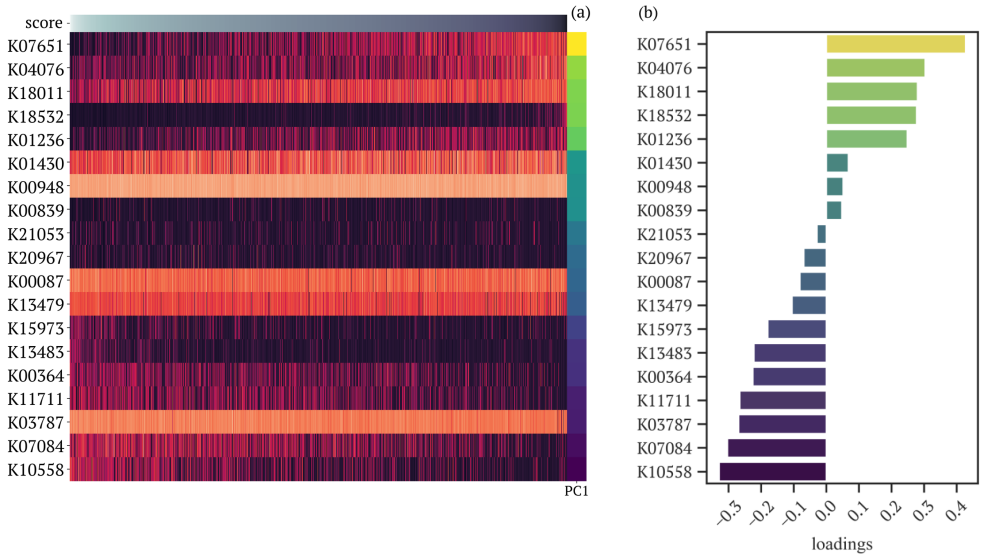


Figure 2. Raw data and loadings from Principal Component Analysis for *UricAcidProduction-Pathways*. **(a)** Normalized expression data from the development cohort (n = 9,350) is subsetted by the 19 features for *UricAcidProductionPathways*. All samples are sorted along the x-axis using score values (colorized at top) and all features are sorted along the y-axis using the loadings from Principal Component 1 (PC1; colorized loadings at right). **(b)** Loadings for each of the 19 features of PC1 are visualized for *UricAcidProductionPathways*. Colorization of PC1 in **(a)** is aligned with the loadings in **(b)**.

Table 9. Translational interpretation and potential actions for a "Not Optimal" score.

Score Name	"Not Optimal" Interpretation	Potential Actionability	Supporting Evidence
ButyrateProductionPathways	Functions indicate *decreased* butyrate production.	Influence gut microbiota to *increase* butyrate levels.	[49–52]
GABAProductionPathways	Functions indicate *decreased* GABA production.	Influence gut microbiota to *increase* GABA levels.	[53,54]
LacticAcidProductionPathways	Functions indicate *increased* lactic acid production.	Influence gut microbiota to *decrease* lactate levels.	[55–57]
LPSBiosynthesisPathways	Functions indicate *increased* deleterious LPS production.	Influence gut microbiota to *decrease* deleterious LPS levels.	[58–60]
MethanogenesisPathways	Functions indicate *increased* methane production from SCFAs.	Influence gut microbiota to *decrease* methane levels.	[61,62]
OxalateMetabolismPathways	Functions indicate *decreased* oxalate metabolism.	Influence gut microbiota to *increase* oxalate metabolism.	[63–65]
SulfideProductionPathways	Functions indicate *increased* sulfide production.	Influence gut microbiota to *decrease* sulfide levels.	[47,62,66]
UricAcidProductionPathways	Functions indicate *increased* uric acid production.	Influence gut microbiota to *decrease* uric acid levels.	[67,68]

Focusing on "Not Optimal" scores, we present odds ratios for IBS and its subtypes as defined using self-reported IBS phenotypes (Figure 3) or as defined by the independent and clinically validated Rome IV Diagnostic Questionnaire (Figure 4), where the vertical lines at 1 represents the reference group, "Good" scores. Numerical values for these are shown in Supplementary Tables S2 and S3, respectively. The odds ratios indicate the likelihood that someone with a "Not Optimal" score also has the IBS subtype (without informing about causality). As seen in Figures 3 and 4, odds ratios demonstrate that the gut pathway scores can variably differentiate IBS subtypes. The scores also behave similarly whether IBS and its subtypes are defined using self-reported IBS phenotypes or using the Rome IV Diagnostic Questionnaire.

Although this manuscript focuses on gut pathway scores, we also make our findings more accessible to the readership by including context for the microbiota (Figures S2, S3, S4, and S5). For IBS cases, all significantly increased species are depicted in Figure S2 (self-reported IBS phenotypes) or Figure S4 (Rome IV-determined IBS phenotypes) while all significantly decreased species are depicted in Figure S3 (self-reported IBS phenotypes) or Figure S5 (Rome IV-determined IBS phenotypes). For simplicity, all information in the Venn Diagrams has been collapsed to the genus level.

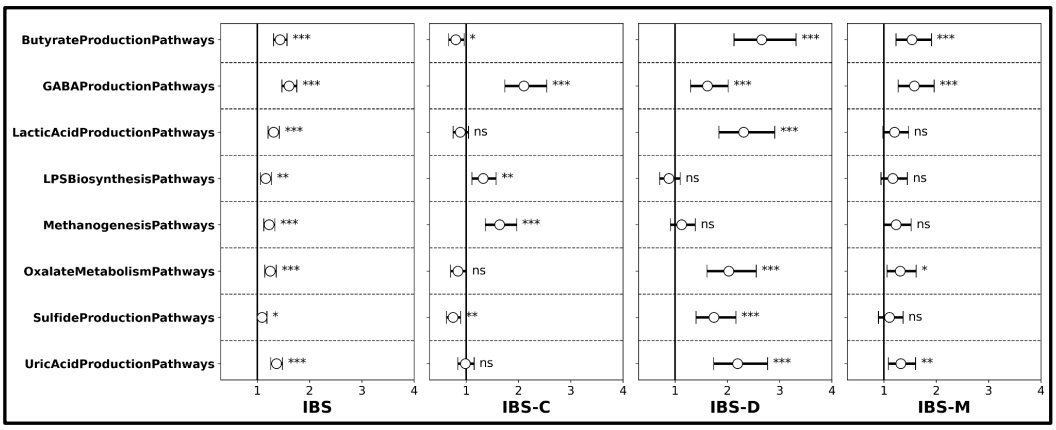


Figure 3. Odds ratios for each "Not Optimal" score stratified with cases defined by self-reported IBS phenotypes. "Good" scores serve as the reference group and are indicated by the vertical line at 1. The maximum number of cases available for 1:5 matching include: 6,373 for IBS; 1,732 for IBS-C; 1,006 for IBS-D; and 1,091 for IBS-M. Definitions for "controls" are score-specific and specified in Table S1, including the minimum size of the control pool available for 1:5 matching. $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***); not significant (ns). Numerical values for all odds ratios in Table S2.

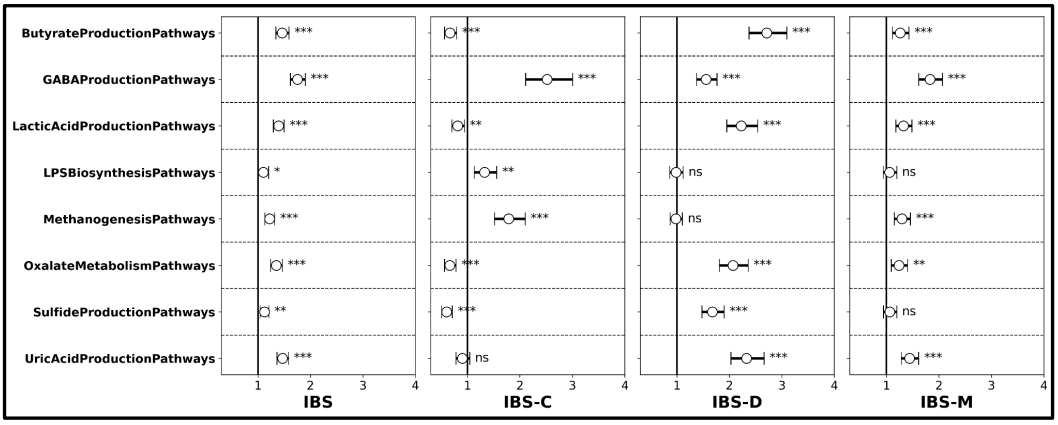


Figure 4. Odds ratios for each "Not Optimal" score stratified with cases defined by Rome IV Diagnostic Questionnaire. "Good" scores serve as the reference group and are indicated by the vertical line at 1. The maximum number of cases available for 1:5 matching include: 8,629 for IBS; 1,942 for IBS-C; 2,952 for IBS-D; and 3,309 for IBS-M. Definitions for "controls" are score-specific and specified in Table S1, including the minimum size of the control pool available for 1:5 matching. $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***); not significant (ns). Numerical values for all odds ratios are shown in Table S3.

4. Discussion

Microbial functions within the gut are important for understanding, preventing, and treating many health issues, including DGBIs like IBS. The wellness scores we validate in this manuscript provide early health insights based on microbial functions (KOs) detected through metatranscriptomic analysis of the gut microbiome without focusing on the composition of the microbiota. Rather than serving as diagnostics, the eight gut scores (Table 6) provide generalized insights across both systemic and chronic health issues with particular focus on gastrointestinal conditions. For validation of the scores, we select IBS phenotypes in order to leverage both the underlying symptoms shared across IBS subtypes as well as their divergent characteristics like altered gut transit [41,42]. This independent validation demonstrates that gut pathway scores can consistently stratify molecular insights based on signals identified during score development.

4.1. Considerations for score development and cohort definitions

The nature of the independent cohort is important to the validation of gut wellness scores. First and foremost, in order to show consistent functionality of the gut pathway scores, it is important to perform validations with an independent cohort that resembles the development cohort. At the highest level, Table 1 establishes general similarity across the two cohorts while Tables 2-5 show they are comparable in terms of anthropometrics, sociodemographics, medication use (PPIs, antibiotics, anti-diarrheals, and laxatives/stool softeners), and IBS phenotypes (i.e., Rome IV responses, stool quality, abdominal pain, bloating, and gut transit time).

Beyond their similarity, the size of each cohort is also important to the process, because large cohorts facilitate both an array of metadata labels and a large sample count for each definition of "case." For the current study, most of the cohort metadata labels used to define "case" (IBS phenotypes) and "controls" (Supplementary Table S1) are identified through self-reported questionnaires, which could raise concerns since self-reported health information is sometimes questionable [69,70]. However, the odds ratios based on self-reported IBS phenotypes (Figure 3) are consistent with Figure 4, where cases are defined by the independent and clinically validated Rome IV Diagnostic Questionnaire. Furthermore, Tables 2-5 corroborate the labels for IBS and its subtypes. In Tables 2 and 4, variability in self-reported gut transit times align with expectations for self-reported IBS subtypes; those with slower transit times most often self-report IBS-C, while those with faster transit times most often self-reported IBS-D, and those with mixed atypical bowel movements most often self-reported IBS-M. Similar trends are shown in Tables 3 and 5 for the frequency of abdominal pain and bloating (respectively) between those who self-reported IBS and those who did not ("Non-IBS"). As expected, there was no noticeable trend for abdominal pain and bloating when tabulated across IBS subtypes (not shown). Altogether, the corroborating evidence supports our definitions of "case" and "control" as well as our use of

the two cohorts in development and validation of the gut pathway scores.

4.2. Unpacking the UricAcidProductionPathways gut score

To maximize transparency and understandability of the gut pathway scores, we disclose the complete score design for *UricAcidProductionPathways*. The overall design process remains similar for each score, and we fully detail this process in our earlier report for saliva pathway scores [34].

During score development, we deeply explore the domain knowledge to identify biological phenomena central to each score before finalizing an appropriate set of features to define the score. In the case of *UricAcidProductionPathways*, score KOs are related to the biochemical production of uric acid by the gut microbiome; domain-wise, they are associated with the production or degradation of purines (Tables 7-8), and a "Not Optimal" *UricAcidProductionPathways* score indicates increased levels of uric acid (Table 9). The set of KOs in the final score definition (Table 8) are used to subset the normalized expression data of the entire cohort, and the loadings for each KO represent the feature's contribution to total RNA variability across the subsetted expression data. Since the selection of each feature is tied to its ability to stratify prioritized phenotypes, like IBS, the subsetted expression data is inherently designed to stratify those phenotypes.

A brief interpretation of the odds ratios in Figures 3 and 4 offers additional context for these gut pathway scores. First, the odds of having a "Not Optimal" *UricAcidProductionPathways* score is significantly higher for those who reported IBS, IBS-D, or IBS-M, but not for those with IBS-C. The clinical implication is that those with IBS, IBS-D, or IBS-M are more likely to have activity related to increased uric acid levels, as measured with the *UricAcidProductionPathways* gut score. Understanding the scientific implications of our findings requires further research, because the relationship between uric acid levels and IBS has not been fully elucidated. However, several studies support a link between purine levels and IBS subtypes [71–74], and there are well-documented associations between high uric acid levels (hyperuricemia) and inflammatory diseases (i.e., gout [75,76], hypertension [77], metabolic syndrome [78], and chronic kidney disease [79]) which are, in turn, associated with IBS or its subtypes [68,80–82]. Furthermore, purinergic receptors are implicated in secretomotor reflexes of the gastrointestinal tract [83–85] and are actively pursued as IBS drug targets [86,87].

Finally, it may be helpful to contextualize the attention we give to *UricAcidProductionPathways* and its relationship to IBS. Briefly, we disclose the score with the smallest set of KOs in order to maximize conceptual development and discussion within the paper while minimizing confusion. To be clear, the current study is not intended to produce any diagnostic nor to reveal a novel role for uric acid in relation to IBS. Furthermore, we could have reframed *UricAcidProductionPathways* in context of many other diseases, such as gout, but IBS phenotypes are more common, they traverse a variety of symptoms, and a link between IBS and purines like uric acid has been established.

4.3. Interpreting the gut pathway scores with IBS subtypes

The primary goal for the current study is to validate the ability for gut pathway scores (based on KEGG functions) to assess altered microbiomes in an independent cohort. To this end, we report thirty-two odds ratios for the eight gut pathway scores, where individuals with self-reported IBS phenotypes have generally higher likelihood to present "Not Optimal" scores (Figure 3). For twenty two of the odds ratios, IBS phenotypes are significantly more likely to present "Not Optimal" scores. Eight other odds ratios are indistinguishable from "Good", and for the remaining two odds ratios, IBS-C is less likely to present "Not Optimal" scores. In addition to Figure 3, we also include odds ratios with cases defined by the Rome IV Diagnostic Questionnaire (Figure 4). Between the two definitions for IBS phenotypes, the scores behave quite similarly, with the primary differences being the level of significance for four of the values in distinguishing "Good" from "Not Optimal" scores.

A single high-level interpretation of the odds ratios (Figures 3 and 4) is challenging; conceptually, each score is designed to cover distinct biological phenomena. Furthermore, each odds ratio is computed with its own case/control definitions (Tables S1-S3), but highlighting specific findings may prove useful for identifying future areas of research.

Overall, Figures 3 and 4 indicate those with IBS-D present with more "Not Optimal" scores than those with IBS-C, IBS-M, and even the IBS composite. One explanation for this is that faster gut transit times are associated with an altered microbiome [88] and altered fecal metabolites [89], and it is possible that the gut is impacted to a greater degree with IBS-D. For three of the scores which fit this pattern: "Not Optimal" corresponds to increased production of lactic acid with *LacticAcidProductionPathways*, decreased degradation of oxalates with *OxalateMetabolismPathways*, and increased production of sulfide with *SulfideProductionPathways* (Table 9). These odds ratios agree with the literature, which indicates that IBS-D and/or diarrhea is associated with increased levels of lactic acid [90,91], oxalates [63,92], and sulfides [62,66,93].

Of all the odds ratios, the largest values are for *ButyrateProductionPathways* with IBS-D, for which a "Not Optimal" score corresponds to decreased

production of butyrate (Table 9). Thus, individuals with IBS-D may have reduced activity related to butyrate production, as measured with the *Butyrate-ProductionPathways* gut score. While there are conflicting reports regarding fecal butyrate levels in IBS-D, IBS-D is associated with fewer butyrate-producing microbes [48,50], and butyrate supplementation is known to improve symptoms [94,95]. Of course, the gastrointestinal role of butyrate and other short-chain fatty acids (SCFAs) is quite complex and their specific impact on IBS-D remains to be elucidated.

One striking trend is the increased likelihood for each of IBS, IBS-C, IBS-D, and IBS-M to present "Not Optimal" *GABAProductionPathways* scores (Figures 3 and 4), corresponding to decreased production of gamma-aminobutyric acid (GABA) as seen in Table 9. GABA is a major inhibitory neurotransmitter and can be produced by gut microbes [96]. One role GABA plays in the gastrointestinal tract is to dampen pain, and consistent with our cohort metadata (Table 3), increased abdominal pain is a cardinal feature of IBS [97]. It is possible that reduced GABA impairs gastrointestinal pain processing for IBS and its subtypes [98]. Beyond the gut, reduced GABA function is associated with depression and anxiety [99], and IBS patients are at higher risk of developing depression or anxiety compared to the general population [100]. Many studies support that GABA dysregulation plays a role in IBS, and this is consistent with our findings.

Our findings also indicate that those with IBS-C are more likely to present "Not Optimal" *LPSBiosynthesisPathways* scores (Figures 3 and 4), corresponding to increased deleterious microbial LPS production (Table 9). LPS is the major surface membrane component of almost all Gram-negative bacteria [101], and its role in promoting inflammation is well-documented [102]. However, there is tremendous heterogeneity in LPS structural modifications [103], and associations between microbes and IBS symptoms have been inconsistent or even contradictory [104]. In contrast to expectations, some components and versions of LPS are even antagonistic to inflammatory responses [105,106]. Most studies which link IBS to LPS focus on levels of LPS in blood, and much of the information regarding levels of LPS in stool is conflicting. Although LPS can cause diarrhea through endotoxemia [107], the only consistent reports we could find linking IBS to fecal LPS levels indicated those with constipation were more likely to have elevated fecal LPS [60,108–110].

The odds ratios for IBS-C highlight one more notable finding: for two scores, individuals with IBS-C are less likely to present with "Not Optimal" scores for *ButyrateProductionPathways* and *SulfideProduction-Pathways*). These values are inverted from IBS-D, and this is best explained in the context of IBS-C individuals being more likely to present with "Not Optimal" *MethanogenesisPathways* scores, which corresponds to increased methane produced from SCFAs (Table 9). Methane production in the gut is well-documented and correlated with IBS-C [62,111]. Recently, it has been shown to affect gut transit time and intestinal peristaltic activity, eventually leading to constipation [111]. It is also naturally correlated with bloating and flatulence [112]. In light of increased methane among those with IBS-C, it is not surprising for IBS-C individuals to have increased butyrate production (i.e., reduced likelihood of "Not Optimal" scores for *ButyrateProduction-Pathways*), because butyrate is a SCFA that directly or indirectly (i.e., via acetate) promotes both methanogenesis [113] and constipation [114]). We can also expect a trend for IBS-C individuals to generally present with decreased levels of sulfide (i.e., decreased likelihood of "Not Optimal" scores for *Sulfide-ProductionPathways*), because sulfate reduction often restricts methanogenesis via several pathways [115], with some exceptions [116,117]. Furthermore, IBS studies often report sulfide as a driver of IBS-D and methane as a driver of IBS-C [62,118].

4.4. Application of gut scores to dietary modifications

The implementation of dietary modifications based on these scores is beyond the scope of this manuscript, but we wanted to include some examples of such recommendations in order to benefit those interested in the application of biomarkers to wellness. Within our wellness platform, *UricAcidProduction-Pathways* is implemented such that those with a "Not Optimal" score are encouraged to avoid foods high in purines, such as scallops, clams, and lobster, effectively reducing substrates for the microbial production of uric acid [119,120]. Additionally, an increase in the consumption of polyphenols known to modulate both host and microbial uric acid metabolism are encouraged through both diet and supplementation. These can include citrus flavonoids like naringin, chlorogenic acid, fisetin, quercetin, and ferulic acid [121–126]. For "Not Optimal" *GABAProductionPathways* scores our wellness program encourages substrates and cofactors, such as L-glutamate and vitamin B6, known to be involved in GABA production [127–131]. Probiotics observed to produce GABA are also supplemented [132–134]. Excessive sulfide and methane gas, as indicated by "Not Optimal" *SulfideProductionPathways* and *MethanogenesisPathways* scores, are addressed through the restriction of sulfur containing substrates known to increase the microbial production of sulfide gas; the restriction of substrates

like carnitine, choline, and fructooligosaccharides known to contribute to the production of microbial methane gas; as well as the introduction of polyphenols, prebiotics, and probiotics shown to reduce both the abundance and activity of gas producing microbes [135–145]. The impact these and other personalized interventions have on gastrointestinal function and mental health are currently being investigated (ClinicalTrials.gov ID's NCT06190184; NCT05465629.)

4.5. Limitations

Several limitations exist for this study. The analysis includes case/control comparisons as part of its validation but may not address all potential confounding factors. The current analyses cannot establish causality due to the composition of scores, which likely consist of KO features comprising both causal and consequential elements. Future studies will be conducted to delineate causal features through prospective interventional trials. Metabolite levels could not be assessed in the current study, but we are planning to accomplish these measurements in future studies. While our cohorts are extensive and encompass various demographics, they may not entirely mirror every demographic group in the USA or other nations. Additionally, the metadata labels rely on self-reported data, which may be prone to misreporting; however, given the very large size of our cohorts, any such discrepancies are likely to be insignificant. Furthermore, incorporation of the independent and clinically validated Rome IV Diagnostic Questionnaire should mitigate concerns related to the odds ratios for self-reported IBS phenotypes.

5. Conclusions

To conclude, we summarize here the novel methods, findings, and some implications for current applications and future research.

Using stool metatranscriptomic data from a large adult population (n = 9,350), we have developed a suite of gut microbiome functional pathway scores that represent the biochemical activity of well-known processes in the gastrointestinal tract. The process for developing these pathway scores includes 5 iterative steps: domain exploration, metadata curation, signal definition, feature selection and pathway activity quantification. We employ a normative approach to determining the optimal and non-optimal segments of the score distributions within the development cohort. To verify that these pathways are consistent with their intended purpose, we confirm that the pathways are differentially active in a case/control analysis of IBS and subtypes within a large independent adult population (n = 71,220). Furthermore, we compute the odds that a non-optimal pathway activity of each of the scores is associated with IBS or its subtypes.

The main finding in this paper is that there is a significantly higher odds of IBS and subtypes for several of the scores, regardless of whether the IBS phenotype was determined via Rome IV or via self-reporting. In particular, individuals with not optimal *GABAProductionPathways* have higher odds of IBS and its subtypes (IBS-C, IBS-D, and IBS-M). In addition, individuals with not optimal *LacticAcidProduction-Pathways*, *OxalateMetabolismPathways*, or *Sulfide-ProductionPathways* have higher odds of IBS-D. Finally, individuals with not optimal *Methanogenesis-Pathways* have higher odds of IBS-C, while at the same time, individuals with not optimal *ButyrateProduction-Pathways* or *SulfideProductionPathways* have a lower odds of IBS-C.

Our findings demonstrate the use of metatranscriptomics for wellness applications like early health insights and nutritional recommendations, which we have previously implemented within a large-scale health application [146].

Supplementary Materials Figure S1: Landscape of score distribution; Table S1: Control definitions for each score; Table S2: Odds Ratio and Confidence Intervals shown in Figure 3; Table S3: Odds Ratio and Confidence Intervals shown in Figure 4; Figure S2: Venn Diagram for genera of significantly increased species across self-reported IBS phenotypes; Figure S3: Venn Diagram for genera of significantly decreased species across self-reported IBS phenotypes; Figure S4: Venn Diagram for genera of significantly increased species across Rome IV-defined IBS phenotypes; Figure S5: Venn Diagram for genera of significantly decreased species across Rome IV-defined IBS phenotypes.

Author Contributions: Conceptualization, E.P., G.B.; Data curation, E.P., A.G., M.M.; Formal analysis, E.P., A.G., M.M., O.O.; Funding acquisition, M.V., G.B.; Investigation, E.P., A.G., M.M., C.J., L.H., M.V., G.B.; Methodology, E.P., A.G., M.M.; Project administration, G.B.; Resources, G.B.; Software, E.P., A.G., M.M., O.O., L.H.; Supervision, G.B.; Validation, C.J.; Visualization, E.P., A.G., M.M.; Writing – original draft, E.P., A.G., M.M., G.B.; Writing – review & editing, E.P., A.G., M.M., C.J., L.H., G.A., M.V., R.W., G.B.

Data Availability Statement: The raw data used in this study cannot be shared publicly due to privacy and legal reasons. However, if data is specifically requested, we may be able to share a summary and/or portions of the data. Researchers requiring more data for non-commercial purposes can request via: https://www.viomelifesciences.com/data-access.

Viome may provide access to summary statistics through a Data Transfer Agreement that protects the privacy of participants' data.

Acknowledgements: We would like to thank Angel Janevski for leading data and analytics, which is responsible for computing scores and recommendations on a regular basis.

Conflicts of Interest: All authors are stockholders and either employees or paid advisors of Viome Inc, a commercial for-profit company.

Funding: This research received no funding outside of Viome.

References

1. Schmulson MJ, Drossman DA. What Is New in Rome IV. J Neurogastroenterol Motil. 2017;23(2):151–63.
2. Sperber AD, Bangdiwala SI, Drossman DA, Ghoshal UC, Simren M, Tack J, et al. Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study. Gastroenterology. 2021;160(1):99-114.e3.
3. Drossman DA. Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features, and Rome IV. Gastroenterology. 2016;150(6):1262-1279.e2.
4. Fairlie T, Shah A, Talley NJ, Chey WD, Koloski N, Lee YY, et al. Overlap of disorders of gut–brain interaction: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2023;8(7):646–59.
5. Black CJ, Drossman DA, Talley NJ, Ruddy J, Ford AC. Functional gastrointestinal disorders: advances in understanding and management. Lancet. 2020;396(10263):1664–74.
6. Singh R, Zogg H, Ghoshal UC, Ro S. Current Treatment Options and Therapeutic Insights for Gastrointestinal Dysmotility and Functional Gastrointestinal Disorders. Front Pharmacol. 2022;13:808195.
7. Chandran S, Prakrithi S, Mathur S, Kishor M, Rao TS. A review of functional gastrointestinal disorders: A primer for mental health professionals. Arch Ment Heal. 2018;19(2):70.
8. Scalese G, Severi C. The pathophysiology of gut-brain connection. In: Stasi C, editor. The complex interplay between gut-brain, gut-liver, and liver-brain axes. Academic Press; 2021.
9. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. Clin Epidemiology. 2014;6:71–80.
10. Chey WD, Kurlander J, Eswaran S. Irritable Bowel Syndrome: A Clinical Review. JAMA. 2015;313(9):949–58.
11. Ma W, Drew DA, Staller K. The Gut Microbiome and Colonic Motility Disorders: A Practical Framework for the Gastroenterologist. Curr Gastroenterol Rep. 2022;24(10):115–26.
12. Hasler WL, Saad RJ, Rao SS, Wilding GE, Parkman HP, Koch KL, et al. Heightened colon motor activity measured by a wireless capsule in patients with constipation: relation to colon transit and IBS. Am J Physiol-Gastrointest Liver Physiol. 2009;297(6):G1107–14.
13. Simrén M. Quality of life in irritable bowel syndrome: measurement techniques and relevance of current knowledge. Expert Rev Pharmacoeconomics Outcomes Res. 2003;3(1):75–88.
14. Camilleri M, BouSaba J. Potential Value of Biomarker-Based Approaches for Evaluation and Management of Costly Functional Gastrointestinal Diseases. Clin Gastroenterol Hepatol. 2023;21(10):2462–72.
15. Liu HN, Wu H, Chen YZ, Chen YJ, Shen XZ, Liu TT. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: A systematic review and meta-analysis. Dig Liver Dis. 2017;49(4):331–7.
16. Sasso JM, Ammar RM, Tenchov R, Lemmel S, Kelber O, Grieswelle M, et al. Gut Microbiome–Brain Alliance: A Landscape View into Mental and Gastrointestinal Health and Disorders. ACS Chem Neurosci. 2023;14(10):1717–63.
17. Hou Y, Dong L, Lu X, Shi H, Xu B, Zhong W, et al. Distinctions Between Fecal and Intestinal Mucosal Microbiota in Subgroups of Irritable Bowel Syndrome. Dig Dis Sci. 2022;67(12):5580–92.
18. Garcia-Mazcorro JF, Amieva-Balmori M, Triana-Romero A, Wilson B, Smith L, Reyes-Huerta J, et al. Fecal Microbial Composition and Predicted Functional Profile in Irritable Bowel Syndrome Differ between Subtypes and Geographical Locations. Microorganisms. 2023;11(10):2493.
19. Ng QX, Yau CE, Yaow CYL, Chong RIH, Chong NZY, Teoh SE, et al. What Has Longitudinal 'Omics' Studies Taught Us about Irritable Bowel Syndrome? A Systematic Review. Metabolites. 2023;13(4):484.
20. Huang X, Ding L. Relationship between intestinal microecology and serum microRNA in different types of irritable bowel syndrome. Chinese Journal of Microecology. 35(4):454–7.
21. Nakov R, Snegarova V, Dimitrova-Yurukova D, Velikova T. Biomarkers in Irritable Bowel Syndrome: Biological Rationale and Diagnostic Value. Dig Dis. 2022;40(1):23–32.
22. Legg M. The role of informatics in the shift from reactive to proactive healthcare. EPMA J. 2014;5(Suppl 1):A50.
23. Domenico MD, Ballini A, Boccellino M, Scacco S, Lovero R, Charitos IA, et al. The Intestinal Microbiota May Be a Potential Theranostic Tool for Personalized Medicine. J Pers Med. 2022;12(4):523.
24. Hippe B, Schiller M. Advances in Precision Nutrition, Personalization and Healthy Aging. 2022;279–99.
25. Pritchard DE, Moeckel F, Villa MS, Housman LT, McCarty CA, McLeod HL. Strategies for integrating personalized medicine into healthcare practice. Pers Med. 2017;14(2):141–52.
26. Buono JL, Mathur K, Averitt AJ, Andrae DA. Economic Burden of Irritable Bowel Syndrome with Diarrhea: Retrospective Analysis of a U.S. Commercially Insured Population. J Manag Care Spéc Pharm. 2017;23(4):453–60.
27. Bidwell S, Thompson L, McKerchar C, Miles H. Delayed Diagnosis of Gut Disease & Delay-Reduction Interventions [Internet]. University of Otago; 2021 [cited 2024 Feb 5]. Available from: https://www.otago.ac.nz/__data/assets/pdf_file/0013/321430/delayed-diagnosis-of-gut-symptoms-a-literature-review-0233231.pdf
28. Heintz-Buschart A, Wilmes P. Human Gut Microbiome: Function Matters. Trends Microbiol. 2018;26(7):563–74.
29. Rosen CE, Palm NW. Functional Classification of the Gut Microbiota: The Key to Cracking the Microbiota Composition Code. BioEssays. 2017;39(12).
30. Visconti A, Roy CIL, Rosa F, Rossi N, Martin TC, Mohney RP, et al. Interplay between the human gut microbiome and host metabolism. Nat Commun. 2019;10(1):4505.
31. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 2019;28(11):1947–51.

32. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000 Jan 1;28(1):27–30.
33. Hatch A, Horne J, Toma R, Twibell BL, Somerville KM, Pelle B, et al. A Robust Metatranscriptomic Technology for Population-Scale Studies of Diet, Gut Microbiome, and Human Health. Int J Genom. 2019;2019:1718741.
34. Patridge E, Gorakshakar A, Molusky MM, Ogundijo O, Janevski A, Julian C, et al. Microbial functional pathways based on metatranscriptomic profiling enable effective saliva-based health assessments for precision wellness. Comput Struct Biotechnol J. 2024;23:834–42.
35. Palsson OS, Whitehead WE, Tilburg MAL van, Chang L, Chey W, Crowell MD, et al. Development and Validation of the Rome IV Diagnostic Questionnaire for Adults. Gastroenterology. 2016;150(6):1481–91.
36. Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther. 2016;44(7):693–703.
37. Lewis SJ, Heaton KW. Stool Form Scale as a Useful Guide to Intestinal Transit Time. Scand J Gastroenterol. 1997;32(9):920–4.
38. Saad RJ, Rao SS, Koch KL, Kuo B, Parkman HP, McCallum RW, et al. Do Stool Form and Frequency Correlate With Whole-Gut and Colonic Transit? Results From a Multicenter Study in Constipated Individuals and Healthy Controls. Am J Gastroenterol. 2010;105(2):403–11.
39. Rome Foundation. Rome IV [Internet]. 2016 [cited 2024 Apr 15]. Available from: https://theromefoundation.org/rome-iv/rome-iv-questionnaire/
40. Toma R, Duval N, Pelle B, Parks MM, Gopu V, Torres PJ, et al. A clinically validated human capillary blood transcriptome test for global systems biology studies. BioTechniques. 2020;69(4):289–301.
41. Almeida A, Nayfach S, Boland M, Strozzi F, Beracochea M, Shi ZJ, et al. A unified catalog of 204,938 reference genomes from the human gut microbiome. Nat Biotechnol. 2021;39(1):105–14.
42. Schneider VA, Graves-Lindsay T, Howe K, Bouk N, Chen HC, Kitts PA, et al. Evaluation of GRCh38 and de novo haploid genome assemblies demonstrates the enduring quality of the reference assembly. Genome Res. 2017;27(5):849–64.
43. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, Mering C von, et al. Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. Mol Biol Evol. 2017;34(8):2115–22.
44. Kim D, Song L, Breitwieser FP, Salzberg SL. Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res. 2016;26(12):1721–9.
45. Henderson NC, Varadhan R. Damped Anderson Acceleration With Restarts and Monotonicity Control for Accelerating EM and EM-like Algorithms. J Comput Graph Stat. 2019;28(4):834–46.
46. Schmidt S, Pardo Y. Encyclopedia of Quality of Life and Well-Being Research. 2014;4375–9.
47. Su Q, Tun HM, Liu Q, Yeoh YK, Mak JWY, Chan FK, et al. Gut microbiome signatures reflect different subtypes of irritable bowel syndrome. Gut Microbes. 2023;15(1):2157697.
48. Wang Y, Ma W, Mehta R, Nguyen LH, Song M, Drew DA, et al. Diet and gut microbial associations in irritable bowel syndrome according to disease subtype. Gut Microbes. 2023;15(2):2262130.
49. Zhuang X, Tian Z, Li L, Zeng Z, Chen M, Xiong L. Fecal Microbiota Alterations Associated With Diarrhea-Predominant Irritable Bowel Syndrome. Front Microbiol. 2018;9:1600.
50. Pozuelo M, Panda S, Santiago A, Mendez S, Accarino A, Santos J, et al. Reduction of butyrate- and methane-producing microorganisms in patients with Irritable Bowel Syndrome. Sci Rep-uk. 2015 Oct 1;5(1).
51. Zhou Y, Zhang F, Mao L, Feng T, Wang K, Xu M, et al. Bifico relieves irritable bowel syndrome by regulating gut microbiota dysbiosis and inflammatory cytokines. Eur J Nutr. 2023;62(1):139–55.
52. Skrzydło-Radomańska B, Prozorow-Król B, Cichoż-Lach H, Majsiak E, Bierła JB, Kanarek E, et al. The Effectiveness and Safety of Multi-Strain Probiotic Preparation in Patients with Diarrhea-Predominant Irritable Bowel Syndrome: A Randomized Controlled Study. Nutrients. 2021;13(3):756.
53. Mishima Y, Ishihara S. Molecular Mechanisms of Microbiota-Mediated Pathology in Irritable Bowel Syndrome. Int J Mol Sci. 2020;21(22):8664.
54. Pokusaeva K, Johnson C, Luk B, Uribe G, Fu Y, Oezguen N, et al. GABA-producing Bifidobacterium dentium modulates visceral sensitivity in the intestine. Neurogastroenterol Motil. 2017;29(1):e12904.
55. Carroll IM, Chang YH, Park J, Sartor RB, Ringel Y. Luminal and mucosal-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Gut Pathog. 2010;2(1):19.
56. König J, Rangel I, Brummer RJ. The Role of Lactic Acid Bacteria in the Pathophysiology and Treatment of Irritable Bowel Syndrome (IBS). Food Nutr Sci. 2013;04(11):27–39.
57. Xiao L, Liu Q, Luo M, Xiong L. Gut Microbiota-Derived Metabolites in Irritable Bowel Syndrome. Front Cell Infect Microbiol. 2021;11:729346.
58. Brown GC, Camacho M, Williams-Gray CH. The Endotoxin Hypothesis of Parkinson's Disease. Mov Disord. 2023;38(7):1143–55.
59. Zhou SY, Gillilland M, Wu X, Leelasinjaroen P, Zhang G, Zhou H, et al. FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated intestinal inflammation and barrier dysfunction. J Clin Investig. 2017;128(1):267–80.
60. Zhuang M, Shang W, Ma Q, Strappe P, Zhou Z. Abundance of Probiotics and Butyrate-Production Microbiome Manages Constipation via Short-Chain Fatty Acids Production and Hormones Secretion. Mol Nutr Food Res. 2019;63(23):e1801187.
61. Ghoshal U, Shukla R, Srivastava D, Ghoshal UC. Irritable Bowel Syndrome, Particularly the Constipation-Predominant Form, Involves an Increase in Methanobrevibacter smithii, Which Is Associated with Higher Methane Production. Gut Liver. 2016;10(6):932–8.

62. Villanueva-Millan MJ, Leite G, Wang J, Morales W, Parodi G, Pimentel ML, et al. Methanogens and Hydrogen Sulfide Producing Bacteria Guide Distinct Gut Microbe Profiles and Irritable Bowel Syndrome Subtypes. Am J Gastroenterol. 2022;117(12):2055–66.
63. Gravina AG, Dallio M, Romeo M, Somma AD, Cotticelli G, Loguercio C, et al. Adherence and Effects Derived from FODMAP Diet on Irritable Bowel Syndrome: A Real Life Evaluation of a Large Follow-Up Observation. Nutrients. 2020;12(4):928.
64. Lei WY, Chang CY, Wu JH, Lin FH, Chen CH, Chang CF, et al. An Initial Attack of Urinary Stone Disease Is Associated with an Increased Risk of Developing New-Onset Irritable Bowel Syndrome: Nationwide Population-Based Study. PLoS ONE. 2016;11(6):e0157701.
65. Kyrian O, Derkach I, Dorofeyev A, Rudenko M. Changes in the intestinal microbiota in patients with ulcerative colitis and irritable bowel syndrome combined with urolithiasis. World of Medicine and Biology. 2021;17(77):77–81.
66. Villanueva-Millan MJ, Leite G, Morales W, Sanchez M, Parodi G, Weitsman S, et al. Hydrogen Sulfide Producers Drive a Diarrhea-Like Phenotype and a Methane Producer Drives a Constipation-Like Phenotype in Animal Models. Dig Dis Sci. 2023;1–11.
67. Mars RAT, Yang Y, Ward T, Houtti M, Priya S, Lekatz HR, et al. Longitudinal Multi-omics Reveals Subset-Specific Mechanisms Underlying Irritable Bowel Syndrome. Cell. 2020;183(4):1137–40.
68. Chou Y, Huang C, Li W, Huang S, Wu W, Tsai C, et al. Clinical study of ammonium acid urate urolithiasis. Kaohsiung J Méd Sci. 2012;28(5):259–64.
69. Heyden JV der, Bacquer DD, Tafforeau J, Herck KV. Reliability and validity of a global question on self-reported chronic morbidity. J Public Heal. 2014;22(4):371–80.
70. Kountur R. Detecting Careless Responses to Self-Reported Questionnaires. Eurasian Journal of Educational Research. 2016;16(64).
71. Kasahara K, Kerby RL, Zhang Q, Pradhan M, Mehrabian M, Lusis AJ, et al. Gut bacterial metabolism contributes to host global purine homeostasis. Cell Host Microbe. 2023;31(6):1038-1053.e10.
72. Liu Y, Jarman JB, Low YS, Augustijn HE, Huang S, Chen H, et al. A widely distributed gene cluster compensates for uricase loss in hominids. Cell. 2023;186(16):3400-3413.e20.
73. Moss M. Purines, Alcohol and Boron in the Diets of People with Chronic Digestive Problems. Journal of Nutritional and Environmental Medicine. 2009 Jul;11:23–32.
74. Mars RAT, Yang Y, Ward T, Houtti M, Priya S, Lekatz HR, et al. Longitudinal Multi-omics Reveals Subset-Specific Mechanisms Underlying Irritable Bowel Syndrome. Cell. 2020;182(6):1460-1473.e17.
75. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440(7081):237–41.
76. Suttenfield FD. Arthritis after intrasynovial injection of sodium urate crystals. Lancet. 1962;280(7270):1380–1.
77. Agarwal V, Hans N, Messerli FH. Effect of Allopurinol on Blood Pressure: A Systematic Review and Meta-Analysis. J Clin Hypertens. 2013;15(6):435–42.
78. Sun HL, Pei D, Lue KH, Chen YL. Uric Acid Levels Can Predict Metabolic Syndrome and Hypertension in Adolescents: A 10-Year Longitudinal Study. PLoS ONE. 2015;10(11):e0143786.
79. Ohno I. Relationship Between Hyperuricemia and Chronic Kidney Disease. Nucleosides, Nucleotides Nucleic Acids. 2011;30(12):1039–44.
80. Guo Y, Niu K, Momma H, Kobayashi Y, Chujo M, Otomo A, et al. Irritable Bowel Syndrome Is Positively Related to Metabolic Syndrome: A Population-Based Cross-Sectional Study. PLoS ONE. 2014;9(11):e112289.
81. Zhou Q, Fillingim RB, Riley JL, Malarkey WB, Verne NG. Central and peripheral hypersensitivity in the irritable bowel syndrome. Pain. 2010;148(3):454–61.
82. Liu M, He P, Ye Z, Yang S, Zhang Y, Wu Q, et al. Functional gastrointestinal disorders, mental health, genetic susceptibility, and incident chronic kidney disease. Chin Méd J. 2023;
83. Christofi FL. Purinergic receptors and gastrointestinal secretomotor function. Purinergic Signal. 2008;4(3):213–36.
84. An Q, Yue G, Yang X, Lou J, Shan W, Ding J, et al. Pathophysiological Role of Purinergic P2X Receptors in Digestive System Diseases. Front Physiol. 2021;12:781069.
85. Keating C, Pelegrin P, Martínez CM, Grundy D. P2X7 Receptor-Dependent Intestinal Afferent Hypersensitivity in a Mouse Model of Postinfectious Irritable Bowel Syndrome. J Immunol. 2011;187(3):1467–74.
86. Burnstock G, Jacobson KA, Christofi FL. Purinergic drug targets for gastrointestinal disorders. Curr Opin Pharmacol. 2017;37:131–41.
87. Ochoa-Cortes F, Liñán-Rico A, Jacobson KA, Christofi FL. Potential for Developing Purinergic Drugs for Gastrointestinal Diseases. Inflamm Bowel Dis. 2014;20(7):1259–87.
88. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. Gut. 2016;65(1):57.
89. Shankar V, Homer D, Rigsbee L, Khamis HJ, Michail S, Raymer M, et al. The networks of human gut microbe–metabolite associations are different between health and irritable bowel syndrome. ISME J. 2015;9(8):1899–903.
90. Xue H, Zhang M, Ma J, Chen T, Wang F, Tang X. Lactose-Induced Chronic Diarrhea Results From Abnormal Luminal Microbial Fermentation and Disorder of Ion Transport in the Colon. Front Physiol. 2020;11:877.
91. Wang SP, Rubio LA, Duncan SH, Donachie GE, Holtrop G, Lo G, et al. Pivotal Roles for pH, Lactate, and Lactate-Utilizing Bacteria in the Stability of a Human Colonic Microbial Ecosystem. mSystems. 2020;5(5):e00645-20.
92. Semrad CE. Approach to the Patient with Diarrhea and Malabsorption. In: Goldman L, Schafer AI, editors. Goldman's Cecil Medicine. 2012. p. 895–913. (Section XII: Gastrointestinal Diseases).
93. Banik GD, De A, Som S, Jana S, Daschakraborty SB, Chaudhuri S, et al. Hydrogen sulphide in exhaled breath: a potential biomarker for small intestinal bacterial overgrowth in IBS. J Breath Res. 2016;10(2):026010.

94. Banasiewicz T, Krokowicz Ł, Stojcev Z, Kaczmarek BF, Kaczmarek E, Maik J, et al. Microencapsulated sodium butyrate reduces the frequency of abdominal pain in patients with irritable bowel syndrome. Color Dis. 2013;15(2):204–9.
95. Scarpellini E, Lauritano EC, Lupascu A, Petruzzellis C, Novi ML, Roccarina D, et al. Efficacy of butyrate in the treatment of diarrhoea-predominant irritable bowel syndrome. Dig Liver Dis Suppl. 2007;1(1):19–22.
96. Dicks LMT. Gut Bacteria and Neurotransmitters. Microorganisms. 2022;10(9):1838.
97. BouSaba J, Sannaa W, Camilleri M. Pain in irritable bowel syndrome: Does anything really help? Neurogastroenterol Motil. 2022;34(1):e14305.
98. Bednarska O, Icenhour A, Tapper S, Witt ST, Tisell A, Lundberg P, et al. Reduced excitatory neurotransmitter levels in anterior insulae are associated with abdominal pain in irritable bowel syndrome. Pain. 2019;160(9):2004–12.
99. Möhler H. The GABA system in anxiety and depression and its therapeutic potential. Neuropharmacology. 2012;62(1):42–53.
100. Mayer EA, Ryu HJ, Bhatt RR. The neurobiology of irritable bowel syndrome. Mol Psychiatry. 2023;28(4):1451–65.
101. Maldonado RF, Sá-Correia I, Valvano MA. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiol Rev. 2016;40(4):480–93.
102. Mazgaeen L, Gurung P. Recent Advances in Lipopolysaccharide Recognition Systems. Int J Mol Sci. 2020;21(2):379.
103. Valvano MA. Remodelling of the Gram-negative bacterial Kdo2-lipid A and its functional implications. Microbiology. 2022;168(4).
104. Duan R, Zhu S, Wang B, Duan L. Alterations of Gut Microbiota in Patients With Irritable Bowel Syndrome Based on 16S rRNA-Targeted Sequencing: A Systematic Review. Clin Transl Gastroenterol. 2019;10(2):e00012.
105. d'Hennezel E, Abubucker S, Murphy LO, Cullen TW. Total Lipopolysaccharide from the Human Gut Microbiome Silences Toll-Like Receptor Signaling. mSystems. 2017;2(6):e00046-17.
106. Saitoh S, Akashi S, Yamada T, Tanimura N, Kobayashi M, Konno K, et al. Lipid A antagonist, lipid IVa, is distinct from lipid A in interaction with Toll-like receptor 4 (TLR4)-MD-2 and ligand-induced TLR4 oligomerization. Int Immunol. 2004;16(7):961–9.
107. Benson S, Kattoor J, Wegner A, Hammes F, Reidick D, Grigoleit JS, et al. Acute experimental endotoxemia induces visceral hypersensitivity and altered pain evaluation in healthy humans. PAIN®. 2012;153(4):794–9.
108. LIU X, Meiling Y, XIE J, ZHANG L, Wenfang X, Ming Y, et al. Study on the relationship between constipation, colonic mucosal inflammation, intestinal LPS and Parkinson's disease. Chinese Journal of Clinical Pharmacology and Therapeutics. 2017;22(4):456.
109. Reichardt F, Chassaing B, Nezami BG, Li G, Tabatabavakili S, Mwangi S, et al. Western diet induces colonic nitrergic myenteric neuropathy and dysmotility in mice via saturated fatty acid- and lipopolysaccharide-induced TLR4 signalling. J Physiol. 2017;595(5):1831–46.
110. Anitha M, Reichardt F, Tabatabavakili S, Nezami BG, Chassaing B, Mwangi S, et al. Intestinal Dysbiosis Contributes to the Delayed Gastrointestinal Transit in High-Fat Diet Fed Mice. CMGH Cell Mol Gastroenterol Hepatol. 2016;2(3):328–39.
111. Waqar SHB, Rehan A. Methane and Constipation-predominant Irritable Bowel Syndrome: Entwining Pillars of Emerging Neurogastroenterology. Cureus. 2019;11(5):e4764.
112. Triantafyllou K, Chang C, Pimentel M. Methanogens, Methane and Gastrointestinal Motility. J Neurogastroenterol Motil. 2013;20(1):31–40.
113. Abell GCJ, Conlon MA, McOrist AL. Methanogenic archaea in adult human faecal samples are inversely related to butyrate concentration. Microb Ecol Heal Dis. 2009;18(3–4):154–60.
114. Wang L, Lv WQ, Yang JT, Lin X, Liu HM, Tan HJ, et al. Enteric nervous system damage caused by abnormal intestinal butyrate metabolism may lead to functional constipation. Front Microbiol. 2023;14:1117905.
115. Ferry JG. Methanogenesis: ecology, physiology, biochemistry & genetics. Springer Science & Business Media; 1993.
116. Braccia DJ, Jiang X, Pop M, Hall AB. The Capacity to Produce Hydrogen Sulfide (H2S) via Cysteine Degradation Is Ubiquitous in the Human Gut Microbiome. Front Microbiol. 2021;12:705583.
117. Sela-Adler M, Ronen Z, Herut B, Antler G, Vigderovich H, Eckert W, et al. Co-existence of Methanogenesis and Sulfate Reduction with Common Substrates in Sulfate-Rich Estuarine Sediments. Front Microbiol. 2017;8:766.
118. Chin K, Ivanina EA, Robbins DDH. The Role of the Microbiome And Probiotics in IBS. 2022 Dec 7;
119. Kaneko K, Takayanagi F, Fukuuchi T, Yamaoka N, Yasuda M, Mawatari K ichi, et al. Determination of total purine and purine base content of 80 food products to aid nutritional therapy for gout and hyperuricemia. Nucleosides, Nucleotides Nucleic Acids. 2020;39(10–12):1449–57.
120. Kaneko K, Aoyagi Y, Fukuuchi T, Inazawa K, Yamaoka N. Total Purine and Purine Base Content of Common Foodstuffs for Facilitating Nutritional Therapy for Gout and Hyperuricemia. Biol Pharm Bull. 2014;37(5):709–21.
121. Park KY, Kim HJ, Ahn HS, Kim SH, Park EJ, Yim SY, et al. Effects of coffee consumption on serum uric acid: systematic review and meta-analysis. Semin Arthritis Rheum. 2016;45(5):580–6.
122. Zhao M, Zhu D, Sun-Waterhouse D, Su G, Lin L, Wang X, et al. In Vitro and In Vivo Studies on Adlay-Derived Seed Extracts: Phenolic Profiles, Antioxidant Activities, Serum Uric Acid Suppression, and Xanthine Oxidase Inhibitory Effects. J Agric Food Chem. 2014;62(31):7771–8.
123. Wang SH, Chen CS, Huang SH, Yu SH, Lai ZY, Huang ST, et al. Hydrophilic Ester-Bearing Chlorogenic Acid Binds to a Novel Domain to Inhibit Xanthine Oxidase. Planta Med. 2009;75(11):1237–40.
124. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Poel BV, et al. Structure−Activity Relationship and Classification of Flavonoids as Inhibitors of Xanthine Oxidase and Superoxide Scavengers. J Nat Prod. 1998;61(1):71–6.

125. Lou Y, Gao Q, Fan M, Waleed AA, Wang L, Li Y, et al. Ferulic acid ameliorates hyperuricemia by regulating xanthine oxidase. Int J Biol Macromol. 2023;253(Pt 3):126542.
126. Roumeliotis S, Roumeliotis A, Dounousi E, Eleftheriadis T, Liakopoulos V. Dietary Antioxidant Supplements and Uric Acid in Chronic Kidney Disease: A Review. Nutrients. 2019;11(8):1911.
127. Skonieczna-Żydecka K, Jakubczyk K, Maciejewska-Markiewicz D, Janda K, Kaźmierczak-Siedlecka K, Kaczmarczyk M, et al. Gut Biofactory—Neurocompetent Metabolites within the Gastrointestinal Tract. A Scoping Review. Nutrients. 2020;12(11):3369.
128. Otaru N, Ye K, Mujezinovic D, Berchtold L, Constancias F, Cornejo FA, et al. GABA Production by Human Intestinal Bacteroides spp.: Prevalence, Regulation, and Role in Acid Stress Tolerance. Front Microbiol. 2021;12:656895.
129. Radford-Smith DE, Anthony DC. Prebiotic and Probiotic Modulation of the Microbiota–Gut–Brain Axis in Depression. Nutrients. 2023;15(8):1880.
130. Perna S, Alalwan TA, Alaali Z, Alnashaba T, Gasparri C, Infantino V, et al. The Role of Glutamine in the Complex Interaction between Gut Microbiota and Health: A Narrative Review. Int J Mol Sci. 2019 Oct 22;20(20):5232.
131. Tomé D. The Roles of Dietary Glutamate in the Intestine. Ann Nutr Metab. 2018;73(Suppl 5):15–20.
132. Tette FM, Kwofie SK, Wilson MD. Therapeutic Anti-Depressant Potential of Microbial GABA Produced by Lactobacillus rhamnosus Strains for GABAergic Signaling Restoration and Inhibition of Addiction-Induced HPA Axis Hyperactivity. Curr Issues Mol Biol. 2022;44(4):1434–51.
133. Song X, Zhao Z, Zhao Y, Wang Z, Wang C, Yang G, et al. Lactobacillus plantarum DP189 prevents cognitive dysfunction in D-galactose/AlCl3 induced mouse model of Alzheimer's disease via modulating gut microbiota and PI3K/Akt/GSK-3β signaling pathway. Nutr Neurosci. 2022;25(12):2588–600.
134. Oleskin, Zhilenkova OG, Shenderov BA, Amerhanova AM, Kudrin VS, Klodt. and PM. Lactic-acid bacteria supplement fermented dairy products with human behavior-modifying neuroactive compounds. J Pharm Nutr Sci. 2014;4(3):199–206.
135. Yao CK, Rotbart A, Ou JZ, Kalantar-Zadeh K, Muir JG, Gibson PR. Modulation of colonic hydrogen sulfide production by diet and mesalazine utilizing a novel gas-profiling technology. Gut Microbes. 2018;9(6):510–22.
136. OIKE H, ARAMAKI K, TOKUNACA T, IINO H, HIRAYAMA M. Suppressive Effect of Fructooligosaccharides on the Generation of Volatile Methanethiol in a Fecal Incubation Method and Human Feces. Biosci Microflora. 2000;19(1):51–9.
137. Borrel G, McCann A, Deane J, Neto MC, Lynch DB, Brugère JF, et al. Genomics and metagenomics of trimethylamine-utilizing Archaea in the human gut microbiome. ISME J. 2017;11(9):2059–74.
138. Kumpitsch C, Fischmeister FPhS, Mahnert A, Lackner S, Wilding M, Sturm C, et al. Reduced B12 uptake and increased gastrointestinal formate are associated with archaeome-mediated breath methane emission in humans. Microbiome. 2021;9(1):193.
139. Patra A, Park T, Kim M, Yu Z. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. J Anim Sci Biotechnol. 2017;8(1):13.
140. Doyle N, Mbandlwa P, Kelly WJ, Attwood G, Li Y, Ross RP, et al. Use of Lactic Acid Bacteria to Reduce Methane Production in Ruminants, a Critical Review. Front Microbiol. 2019;10:2207.
141. Barton LL, Ritz NL, Fauque GD, Lin HC. Sulfur Cycling and the Intestinal Microbiome. Dig Dis Sci. 2017;62(9):2241–57.
142. Gasaly N, Gotteland M. Interference of dietary polyphenols with potentially toxic amino acid metabolites derived from the colonic microbiota. Amino Acids. 2022;54(3):311–24.
143. Yu X, Gurry T, Nguyen LTT, Richardson HS, Alm EJ. Prebiotics and Community Composition Influence Gas Production of the Human Gut Microbiota. mBio. 2020;11(5):e00217-20.
144. Seo M, Heo J, Yoon J, Kim SY, Kang YM, Yu J, et al. Methanobrevibacter attenuation via probiotic intervention reduces flatulence in adult human: A non-randomised paired-design clinical trial of efficacy. PLoS ONE. 2017;12(9):e0184547.
145. Nogacka AM, Reyes-Gavilán CG de los, Arboleya S, Ruas-Madiedo P, Martínez-Faedo C, Suarez A, et al. In vitro Selection of Probiotics for Microbiota Modulation in Normal-Weight and Severely Obese Individuals: Focus on Gas Production and Interaction With Intestinal Epithelial Cells. Front Microbiol. 2021;12:630572.
146. Connell J, Toma R, Ho C, Shen N, Moura P, Cai Y, et al. Evidence-based precision nutrition improves clinical outcomes by analyzing human and microbial molecular data with artificial intelligence. Biorxiv. 2021;2021.04.24.441290.
147. Usuda H, Okamoto T, Wada K. Leaky Gut: Effect of Dietary Fiber and Fats on Microbiome and Intestinal Barrier. Int J Mol Sci. 2021;22(14):7613.
148. Xiong J, Chen X, Zhao Z, Liao Y, Zhou T, Xiang Q. A potential link between plasma short-chain fatty acids, TNF-α level and disease progression in non-alcoholic fatty liver disease: A retrospective study. Exp Ther Med. 2022;24(3):598.
149. Jin M, Kalainy S, Baskota N, Chiang D, Deehan EC, McDougall C, et al. Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. Liver Int. 2019;39(8):1437–47.
150. Flaig B, Garza R, Singh B, Hamamah S, Covasa M. Treatment of Dyslipidemia through Targeted Therapy of Gut Microbiota. Nutrients. 2023;15(1):228.
151. Xiao X, Hu X, Yao J, Cao W, Zou Z, Wang L, et al. The role of short-chain fatty acids in inflammatory skin diseases. Front Microbiol. 2023;13:1083432.
152. Eslick S, Williams EJ, Berthon BS, Wright T, Karihaloo C, Gately M, et al. Weight Loss and Short-Chain Fatty Acids Reduce Systemic Inflammation in Monocytes and Adipose Tissue Macrophages from Obese Subjects. Nutrients. 2022;14(4):765.

153. Kim HK, Rutten NBMM, Vaart IB van der, Niers LEM, Choi YH, Rijkers GT, et al. Probiotic supplementation influences faecal short chain fatty acids in infants at high risk for eczema. Benef Microbes. 2015;6(6):783–90.
154. Gio-Batta M, Sjöberg F, Jonsson K, Barman M, Lundell AC, Adlerberth I, et al. Fecal short chain fatty acids in children living on farms and a link between valeric acid and protection from eczema. Sci Rep. 2020;10(1):22449.
155. Gio-Batta M, Spetz K, Barman M, Bråbäck L, Norin E, Björkstén B, et al. Low Concentration of Fecal Valeric Acid at 1 Year of Age Is Linked with Eczema and Food Allergy at 13 Years of Age: Findings from a Swedish Birth Cohort. Int Arch Allergy Immunol. 2022;183(4):398–408.
156. Kanda N, Hoashi T, Saeki H. The Defect in Regulatory T Cells in Psoriasis and Therapeutic Approaches. J Clin Med. 2021;10(17):3880.
157. Reddel S, Chierico FD, Quagliariello A, Giancristoforo S, Vernocchi P, Russo A, et al. Gut microbiota profile in children affected by atopic dermatitis and evaluation of intestinal persistence of a probiotic mixture. Sci Rep. 2019;9(1):4996.
158. Nylund L, Nermes M, Isolauri E, Salminen S, Vos WM, Satokari R. Severity of atopic disease inversely correlates with intestinal microbiota diversity and butyrate-producing bacteria. Allergy. 2015;70(2):241–4.
159. Deng Y, Wang H, Zhou J, Mou Y, Wang G, Xiong X. Patients with Acne Vulgaris Have a Distinct Gut Microbiota in Comparison with Healthy Controls. Acta Derm Venereol. 2018;98(8):783–90.
160. Park HK, Lee SJ. Treatment of gouty arthritis is associated with restoring the gut microbiota and promoting the production of short-chain fatty acids. Arthritis Res Ther. 2022;24(1):51.
161. Jang YO, Kim OH, Kim SJ, Lee SH, Yun S, Lim SE, et al. High-fiber diets attenuate emphysema development via modulation of gut microbiota and metabolism. Sci Rep. 2021;11(1):7008.
162. Wu Y, Xu H, Tu X, Gao Z. The Role of Short-Chain Fatty Acids of Gut Microbiota Origin in Hypertension. Front Microbiol. 2021;12:730809.
163. Groot PF de, Belzer C, Aydin Ö, Levin E, Levels JH, Aalvink S, et al. Distinct fecal and oral microbiota composition in human type 1 diabetes, an observational study. PLoS ONE. 2017;12(12):e0188475.
164. Vatanen T, Franzosa EA, Schwager R, Tripathi S, Arthur TD, Vehik K, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. Nature. 2018 Oct 1;562(7728):589–94.
165. Huang J, Pearson JA, Peng J, Hu Y, Sha S, Xing Y, et al. Gut microbial metabolites alter IgA immunity in type 1 diabetes. JCI Insight. 2020;5(10).
166. Skonieczna-Żydecka K, Grochans E, Maciejewska D, Szkup M, Schneider-Matyka D, Jurczak A, et al. Faecal Short Chain Fatty Acids Profile is Changed in Polish Depressive Women. Nutrients. 2018;10(12):1939.
167. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol Psychiatr. 2016;21(6):786–96.
168. Lucas S, Omata Y, Hofmann J, Böttcher M, Iljazovic A, Sarter K, et al. Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss. Nat Commun. 2018 Jan 4;9(1):55.
169. Vitetta L, Coulson S, Linnane AW, Butt H. The Gastrointestinal Microbiome and Musculoskeletal Diseases: A Beneficial Role for Probiotics and Prebiotics. Pathogens. 2013;2(4):606–26.
170. Olejniczak-Staruch I, Ciążyńska M, Sobolewska-Sztychny D, Narbutt J, Skibińska M, Lesiak A. Alterations of the Skin and Gut Microbiome in Psoriasis and Psoriatic Arthritis. Int J Mol Sci. 2021;22(8):3998.
171. Sencio V, Gallerand A, Machado MG, Deruyter L, Heumel S, Soulard D, et al. Influenza Virus Infection Impairs the Gut's Barrier Properties and Favors Secondary Enteric Bacterial Infection through Reduced Production of Short-Chain Fatty Acids. Infect Immun. 2021;89(9):e00734-20.
172. Mueller NT, Differding MK, Zhang M, Maruthur NM, Juraschek SP, Miller ER, et al. Metformin Affects Gut Microbiome Composition and Function and Circulating Short-Chain Fatty Acids: A Randomized Trial. Diabetes Care. 2021;44(7):1462–71.
173. Yang T, Richards EM, Pepine CJ, Raizada MK. The gut microbiota and the brain–gut–kidney axis in hypertension and chronic kidney disease. Nat Rev Nephrol. 2018 Jul 1;14(7):442–56.
174. Aggarwal S, Ahuja V, Paul J. Attenuated GABAergic Signaling in Intestinal Epithelium Contributes to Pathogenesis of Ulcerative Colitis. Dig Dis Sci. 2017;62(10):2768–79.
175. Aggarwal S, Ahuja V, Paul J. Dysregulation of GABAergic Signalling Contributes in the Pathogenesis of Diarrhea-predominant Irritable Bowel Syndrome. J Neurogastroenterol Motil. 2018;24(3):422–30.
176. Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW, et al. Inhibitory role for GABA in autoimmune inflammation. Proc Natl Acad Sci. 2010;107(6):2580–5.
177. Kang DW, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaffer M, et al. Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe. 2018 Feb 1;49:121–31.
178. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc National Acad Sci. 2011 Sep 20;108(38):16050–5.
179. Strandwitz P, Kim KH, Terekhova D, Liu JK, Sharma A, Levering J, et al. GABA-modulating bacteria of the human gut microbiota. Nat Microbiol. 2018 Dec 10.
180. Yu L, Han X, Cen S, Duan H, Feng S, Xue Y, et al. Beneficial effect of GABA-rich fermented milk on insomnia involving regulation of gut microbiota. Microbiol Res. 2020;233:126409.
181. Patterson E, Ryan PM, Wiley N, Carafa I, Sherwin E, Moloney G, et al. Gamma-aminobutyric acid-producing lactobacilli positively affect metabolism and depressive-like behaviour in a mouse model of metabolic syndrome. Sci Rep. 2019;9(1):16323.

182. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. Cell. 2018 Jun 1;173(7):1728-1741.e13.
183. Cookson TA. Bacterial-Induced Blood Pressure Reduction: Mechanisms for the Treatment of Hypertension via the Gut. Front Cardiovasc Med. 2021;8:721393.
184. Kimura M, Hayakawa K, Sansawa H. Involvement of γ-Aminobutyric Acid (GABA) B Receptors in the Hypotensive Effect of Systemically Administered GABA in Spontaneously Hypertensive Rats. Jpn J Pharmacol. 2002;89(4):388–94.
185. Bajic SS, Djokic J, Dinic M, Veljovic K, Golic N, Mihajlovic S, et al. GABA-Producing Natural Dairy Isolate From Artisanal Zlatar Cheese Attenuates Gut Inflammation and Strengthens Gut Epithelial Barrier in vitro. Front Microbiol. 2019;10:527.
186. Abdelazez A, Abdelmotaal H, Evivie SE, Melak S, Jia FF, Khoso MH, et al. Screening Potential Probiotic Characteristics of Lactobacillus brevis Strains In Vitro and Intervention Effect on Type I Diabetes In Vivo. BioMed Res Int. 2018;2018:7356173.
187. Khrais A, Ali H, Choi S, Ahmed A, Ahlawat S. D-Lactic Acidosis in Short Bowel Syndrome. Cureus. 2022;14(5):e25471.
188. Washington C, Carmichael J. Management of Ischemic Colitis. Clin Colon Rectal Surg. 2012;25(04):228–35.
189. Hove H, Nordgaard-Andersen I, Mortensen PB. Faecal DL-Lactate Concentration in 100 Gastrointestinal Patients. Scand J Gastroenterol. 1994;29(3):255–9.
190. Bjerrum JT, Wang Y, Hao F, Coskun M, Ludwig C, Günther U, et al. Metabonomics of human fecal extracts characterize ulcerative colitis, Crohn's disease and healthy individuals. Metabolomics. 2015;11(1):122–33.
191. Hove H, Holtug K, Jeppesen PB, Mortensen BP. Butyrate absorption and lactate secretion in ulcerative colitis. Dis Colon Rectum. 1995;38(5):519–25.
192. Hove H, Mortensen PB. Influence of intestinal inflammation (IBD) and small and large bowel length on fecal short-chain fatty acids and lactate. Dig Dis Sci. 1995;40(6):1372–80.
193. Montgomery RD, Frazer AC, Hood C, Goodhart JM, Holland MR, Schneider R. Studies of intestinal fermentation in ulcerative colitis. Gut. 1968;9(5):521.
194. Vernia P, Caprilli R, Latella G, Barbetti F, Magliocca FM, Cittadini M. Fecal Lactate and Ulcerative Colitis. Gastroenterology. 1988;95(6):1564–8.
195. Vernia P, Gnaedinger A, Hauck W, Breuer RI. Organic anions and the diarrhea of inflammatory bowel disease. Dig Dis Sci. 1988;33(11):1353–8.
196. Gillis CC, Hughes ER, Spiga L, Winter MG, Zhu W, Carvalho TF de, et al. Dysbiosis-Associated Change in Host Metabolism Generates Lactate to Support Salmonella Growth. Cell Host Microbe. 2018;23(1):54-64.e6.
197. Sheedy JR, Wettenhall REH, Scanlon D, Gooley PR, Lewis DP, McGregor N, et al. Increased d-lactic Acid intestinal bacteria in patients with chronic fatigue syndrome. vivo (Athens, Greece). 2009;23(4):621–8.
198. Crawford SO, Hoogeveen RC, Brancati FL, Astor BC, Ballantyne CM, Schmidt MI, et al. Association of blood lactate with type 2 diabetes: the Atherosclerosis Risk in Communities Carotid MRI Study. Int J Epidemiology. 2010;39(6):1647–55.
199. Bouter K, Bakker G, Levin E, Hartstra A, Kootte R, Udayappan S, et al. Differential metabolic effects of oral butyrate treatment in lean versus metabolic syndrome subjects. Clin Transl Gastroenterol. 2018;9(5):155.
200. Piper DW, Kemp ML, Fenton BH, Croydon MJ, Clarke AD. Gastric juice lactic acidosis in the presence of gastric carcinoma. Gastroenterology. 1970;58(6):766–71.
201. Stevens BR, Goel R, Seungbum K, Richards EM, Holbert RC, Pepine CJ, et al. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. Gut. 2018;67(8):1555.
202. Bigatello LM, Broitman SA, Fattori L, Paoli MD, Pontello M, Bevilacqua G, et al. Endotoxemia, encephalopathy, and mortality in cirrhotic patients. Am J Gastroenterol. 1987;82(1):11–5.
203. Lin RS, Lee FY, Lee SD, Tsai YT, Lin HC, Rei-Hwa L, et al. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophaegeal varices, and hyperdynamic circulation. J Hepatol. 1995;22(2):165–72.
204. Chen G, Shi F, Yin W, Guo Y, Liu A, Shuai J, et al. Gut microbiota dysbiosis: The potential mechanisms by which alcohol disrupts gut and brain functions. Front Microbiol. 2022;13:916765.
205. Mohammad S, Thiemermann C. Role of Metabolic Endotoxemia in Systemic Inflammation and Potential Interventions. Front Immunol. 2021;11:594150.
206. Ramendra R, Isnard S, Mehraj V, Chen J, Zhang Y, Finkelman M, et al. Circulating LPS and (1→3)-β-D-Glucan: A Folie à Deux Contributing to HIV-Associated Immune Activation. Front Immunol. 2019;10:465.
207. Tulkens J, Vergauwen G, Deun JV, Geeurickx E, Dhondt B, Lippens L, et al. Increased levels of systemic LPS-positive bacterial extracellular vesicles in patients with intestinal barrier dysfunction. Gut. 2020;69(1):191.
208. Sieve I, Ricke-Hoch M, Kasten M, Battmer K, Stapel B, Falk CS, et al. A positive feedback loop between IL-1β, LPS and NEU1 may promote atherosclerosis by enhancing a pro-inflammatory state in monocytes and macrophages. Vasc Pharmacol. 2018;103:16–28.
209. Jaw JE, Tsuruta M, Oh Y, Schipilow J, Hirano Y, Ngan DA, et al. Lung exposure to lipopolysaccharide causes atherosclerotic plaque destabilisation. Eur Respir J. 2016;48(1):205–15.
210. Yoshida N, Emoto T, Yamashita T, Watanabe H, Hayashi T, Tabata T, et al. Bacteroides vulgatus and Bacteroides dorei Reduce Gut Microbial Lipopolysaccharide Production and Inhibit Atherosclerosis. Circulation. 2018 Nov 27;138(22):2486–98.
211. Dong Y, Wang P, Yang X, Chen M, Li J. Potential of gut microbiota for lipopolysaccharide biosynthesis in European women with type 2 diabetes based on metagenome. Front Cell Dev Biol. 2022;10:1027413.

212. An L, Wirth U, Koch D, Schirren M, Drefs M, Koliogiannis D, et al. The Role of Gut-Derived Lipopolysaccharides and the Intestinal Barrier in Fatty Liver Diseases. J Gastrointest Surg. 2022;26(3):671–83.
213. Violi F, Cammisotto V, Bartimoccia S, Pignatelli P, Carnevale R, Nocella C. Gut-derived low-grade endotoxaemia, atherothrombosis and cardiovascular disease. Nat Rev Cardiol. 2023;20(1):24–37.
214. Maes M, Kubera M, Leunis JC. The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. Neuro Endocrinol Lett. 2008;29(1):117–24.
215. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, et al. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe. 2010;16(4):444–53.
216. Nadal I, Donant E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalance in the composition of the duodenal microbiota of children with coeliac disease. J Méd Microbiol. 2007;56(12):1669–74.
217. Feehley T, Belda-Ferre P, Nagler CR. What's LPS Got to Do with It? A Role for Gut LPS Variants in Driving Autoimmune and Allergic Disease. Cell Host Microbe. 2016;19(5):572–4.
218. Huang Z, Kraus VB. Does lipopolysaccharide-mediated inflammation have a role in OA? Nat Rev Rheumatol. 2016;12(2):123–9.
219. Mehta NN, McGillicuddy FC, Anderson PD, Hinkle CC, Shah R, Pruscino L, et al. Experimental Endotoxemia Induces Adipose Inflammation and Insulin Resistance in Humans. Diabetes. 2010;59(1):172–81.
220. Brown GC. The endotoxin hypothesis of neurodegeneration. J Neuroinflammation. 2019;16(1):180.
221. Kim KA, Jeong JJ, Yoo SY, Kim DH. Gut microbiota lipopolysaccharide accelerates inflamm-aging in mice. BMC Microbiol. 2016;16(1):9.
222. Xia L, Xu Z, Zhou X, Bergmann F, Grabe N, Büchler MW, et al. Impaired autophagy increases susceptibility to endotoxin-induced chronic pancreatitis. Cell Death Dis. 2020;11(10):889.
223. Ren Z, Jiang J, Xie H, Li A, Lu H, Xu S, et al. Gut microbial profile analysis by MiSeq sequencing of pancreatic carcinoma patients in China. Oncotarget. 2017;8(56):95176–91.
224. Liu Q, Tian X, Maruyama D, Arjomandi M, Prakash A. Lung immune tone via gut-lung axis: gut-derived LPS and short-chain fatty acids' immunometabolic regulation of lung IL-1β, FFAR2, and FFAR3 expression. Am J Physiol-Lung Cell Mol Physiol. 2021;321(1):L65–78.
225. Yuan X, Wang R, Han B, Sun C, Chen R, Wei H, et al. Functional and metabolic alterations of gut microbiota in children with new-onset type 1 diabetes. Nat Commun. 2022;13(1):6356.
226. Maes M, Mihaylova I, Leunis JC. Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): Indication for the involvement of gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut–intestinal permeability. J Affect Disord. 2007;99(1–3):237–40.
227. Weaver GA, Krause JA, Miller TL, Wolin MJ. Incidence of methanogenic bacteria in a sigmoidoscopy population: an association of methanogenic bacteria and diverticulosis. Gut. 1986;27(6):698.
228. Lecours PB, Marsolais D, Cormier Y, Berberi M, Haché C, Bourdages R, et al. Increased Prevalence of Methanosphaera stadtmanae in Inflammatory Bowel Diseases. PLoS ONE. 2014;9(2):e87734.
229. Gottlieb K, Wacher V, Sliman J, Pimentel M. Review article: inhibition of methanogenic archaea by statins as a targeted management strategy for constipation and related disorders. Aliment Pharmacol Ther. 2016;43(2):197–212.
230. Chaudhary PP, Conway PL, Schlundt J. Methanogens in humans: potentially beneficial or harmful for health. Appl Microbiol Biotechnol. 2018;102(7):3095–104.
231. Mathur R, Chua KS, Mamelak M, Morales W, Barlow GM, Thomas R, et al. Metabolic effects of eradicating breath methane using antibiotics in prediabetic subjects with obesity. Obesity. 2016;24(3):576–82.
232. Mathur R, Amichai M, Chua KS, Mirocha J, Barlow GM, Pimentel M. Methane and Hydrogen Positivity on Breath Test Is Associated With Greater Body Mass Index and Body Fat. J Clin Endocrinol Metab. 2013;98(4):E698–702.
233. Basseri RJ, Basseri B, Pimentel M, Chong K, Youdim A, Low K, et al. Intestinal methane production in obese individuals is associated with a higher body mass index. Gastroenterol Hepatol. 2012;8(1):22–8.
234. Mathur R, Kim G, Morales W, Sung J, Rooks E, Pokkunuri V, et al. Intestinal Methanobrevibacter smithii but not total bacteria is related to diet-induced weight gain in rats. Obesity. 2013;21(4):748–54.
235. Cesario V, Rienzo TAD, Campanale M, D'angelo G, Barbaro F, Gigante G, et al. Methane intestinal production and poor metabolic control in type I diabetes complicated by autonomic neuropathy. Minerva Endocrinol. 2014;39(3):201–7.
236. Barlow GM, Yu A, Mathur R. Role of the Gut Microbiome in Obesity and Diabetes Mellitus. Nutr Clin Pr. 2015;30(6):787–97.
237. Pimentel M, Lin HC, Enayati P, Burg B van den, Lee HR, Chen JH, et al. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. Am J Physiol-Gastrointest Liver Physiol. 2006;290(6):G1089–95.
238. Zompo FD, Ojetti V, Feliciani D, Mangiola F, Petruzziello C, Tesori V, et al. Helicobacter pylori infection is associated with high methane production during lactulose breath test. Eur Rev Méd Pharmacol Sci. 2016;20(16):3452–6.
239. Miele L, Valenza V, Torre GL, Montalto M, Cammarota G, Ricci R, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology. 2009;49(6):1877–87.

240. Ghoshal UC, Baba CS, Ghoshal U, Alexander G, Misra A, Saraswat VA, et al. Low-grade small intestinal bacterial overgrowth is common in patients with non-alcoholic steatohepatitis on quantitative jejunal aspirate culture. Indian J Gastroenterol. 2017;36(5):390–9.
241. Rodriguez DA, Ryan PM, Monjaraz EMT, Mayans JAR, Quigley EM. Small Intestinal Bacterial Overgrowth in Children: A State-Of-The-Art Review. Front Pediatr. 2019;7:363.
242. Leite G, Morales W, Weitsman S, Celly S, Parodi G, Mathur R, et al. The duodenal microbiome is altered in small intestinal bacterial overgrowth. PLoS ONE. 2020;15(7):e0234906.
243. Djemai K, Drancourt M, Alou MT. Bacteria and Methanogens in the Human Microbiome: a Review of Syntrophic Interactions. Microb Ecol. 2022;83(3):536–54.
244. Yazici C, Arslan DC, Abraham R, Cushing K, Keshavarzian A, Mutlu EA. Breath Methane Levels Are Increased Among Patients with Diverticulosis. Dig Dis Sci. 2016;61(9):2648–54.
245. Jang SI, Kim JH, Youn YH, Park H, Lee SI, Conklin JL. Relationship Between Intestinal Gas and the Development of Right Colonic Diverticula. J Neurogastroenterol Motil. 2010;16(4):418–23.
246. Patil DP, Dhotre DP, Chavan SG, Sultan A, Jain DS, Lanjekar VB, et al. Molecular analysis of gut microbiota in obesity among Indian individuals. J Biosci. 2012;37(4):647–57.
247. Mbakwa CA, Penders J, Savelkoul PH, Thijs C, Dagnelie PC, Mommers M, et al. Gut colonization with methanobrevibacter smithii is associated with childhood weight development. Obesity. 2015;23(12):2508–16.
248. Haines A, Dilawari J, Metz G, Blendis L, Wiggins H. Breath-methane in patience with cancer of the large bowel. Lancet. 1977;310(8036):481–3.
249. Peled Y. Methane Production and Colon Cancer. Gastroenterology. 1985;88(5):1294.
250. Scanlan PD, Shanahan F, Marchesi JR. Human methanogen diversity and incidence in healthy and diseased colonic groups using mcrA gene analysis. BMC Microbiol. 2008;8(1):79.
251. Ghavami SB, Rostami E, Sephay AA, Shahrokh S, Balaii H, Aghdaei HA, et al. Alterations of the human gut Methanobrevibacter smithii as a biomarker for inflammatory bowel diseases. Microb Pathog. 2018;117:285–9.
252. Hwang L, Low K, Khoshini R, Melmed G, Sahakian A, Makhani M, et al. Evaluating Breath Methane as a Diagnostic Test for Constipation-Predominant IBS. Dig Dis Sci. 2010;55(2):398–403.
253. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. Am J Gastroenterol. 2003;98(2):412–9.
254. Reddymasu SC, McCallum RW. Small Intestinal Bacterial Overgrowth in Gastroparesis. J Clin Gastroenterol. 2010;44(1):e8–13.
255. Jangi S, Gandhi R, Cox LM, Li N, Glehn F von, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun. 2016;7(1):12015.
256. Qian Y, Yang X, Xu S, Wu C, Song Y, Qin N, et al. Alteration of the fecal microbiota in Chinese patients with Parkinson's disease. Brain, Behav, Immun. 2018;70:194–202.
257. Bedarf JR, Hildebrand F, Coelho LP, Sunagawa S, Bahram M, Goeser F, et al. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. Genome Med. 2017;9(1):39.
258. Lim MY, You HJ, Yoon HS, Kwon B, Lee JY, Lee S, et al. The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. Gut. 2017;66(6):1031.
259. Fiedorek SC, Pumphrey CL, Casteel HB. Breath Methane Production in Children With Constipation and Encopresis. J Pediatr Gastroenterol Nutr. 1990;10(4):473–7.
260. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring Bacterial Community of Human Gut Microbiota Reveals an Increase in Lactobacillus in Obese Patients and Methanogens in Anorexic Patients. PLoS ONE. 2009;4(9):e7125.
261. Million M, Angelakis E, Maraninchi M, Henry M, Giorgi R, Valero R, et al. Correlation between body mass index and gut concentrations of Lactobacillus reuteri, Bifidobacterium animalis, Methanobrevibacter smithii and Escherichia coli. Int J Obes. 2013;37(11):1460–6.
262. Borgo F, Riva A, Benetti A, Casiraghi MC, Bertelli S, Garbossa S, et al. Microbiota in anorexia nervosa: The triangle between bacterial species, metabolites and psychological tests. PLoS ONE. 2017;12(6):e0179739.
263. Mack I, Cuntz U, Grämer C, Niedermaier S, Pohl C, Schwiertz A, et al. Weight gain in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid profiles and gastrointestinal complaints. Sci Rep. 2016;6(1):26752.
264. Prochazkova P, Roubalova R, Dvorak J, Kreisinger J, Hill M, Tlaskalova-Hogenova H, et al. The intestinal microbiota and metabolites in patients with anorexia nervosa. Gut Microbes. 2021;13(1):1902771.
265. Piqué JM, Pallarés M, Cusó E, Vilar-Bonet J, Gassull MA. Methane production and colon cancer. Gastroenterology. 1984;87(3):601–5.
266. Waikar SS, Srivastava A, Palsson R, Shafi T, Hsu CY, Sharma K, et al. Association of Urinary Oxalate Excretion With the Risk of Chronic Kidney Disease Progression. JAMA Intern Med. 2019;179(4):542–51.
267. Liu M, Koh H, Kurtz ZD, Battaglia T, PeBenito A, Li H, et al. Oxalobacter formigenes-associated host features and microbial community structures examined using the American Gut Project. Microbiome. 2017;5(1):108.
268. Katoh R, Kawaoi A, Muramatsu A, Hemmi A, Suzuki K. Birefringent (Calcium Oxalate) Crystals in Thyroid Diseases. Am J Surg Pathol. 1993;17(7):698–705.
269. Pusztaszeri M. Birefringent crystals in thyroid fine-needle aspiration cytology. Diagn Cytopathol. 2016;44(10):814–5.
270. Reid JD, Choi CH, Oldroyd NO. Calcium Oxalate Crystals in the Thyroid: Their Identification, Prevalence, Origin, and Possible Significance. Am J Clin Pathol. 1987;87(4):443–54.

271. Cury DB, Moss AC, Schor N. Nephrolithiasis in patients with inflammatory bowel disease in the community. Int J Nephrol Renov Dis. 2013;6:139–42.
272. Liu M, Devlin JC, Hu J, Volkova A, Battaglia TW, Ho M, et al. Microbial genetic and transcriptional contributions to oxalate degradation by the gut microbiota in health and disease. eLife. 2021;10:e63642.
273. Kharlamb V, Schelker J, Francois F, Jiang J, Holmes RP, Goldfarb DS. Oral Antibiotic Treatment of Helicobacter pylori Leads to Persistently Reduced Intestinal Colonization Rates with Oxalobacter formigenes. J Endourol. 2011;25(11):1781–5.
274. Mulay SR, Eberhard JN, Pfann V, Marschner JA, Darisipudi MN, Daniel C, et al. Oxalate-induced chronic kidney disease with its uremic and cardiovascular complications in C57BL/6 mice. Am J Physiol-Ren Physiol. 2016;310(8):F785–95.
275. Liu Y, Li S, Zeng Z, Wang J, Xie L, Li T, et al. Kidney Stones and Cardiovascular Risk: A Meta-analysis of Cohort Studies. Am J Kidney Dis. 2014;64(3):402–10.
276. Mirmiran P, Bahadoran Z, Azizi F. Dietary oxalate-calcium balance and the incidence of hypertension and chronic kidney disease: a prospective study among an Asian population. Nutr Metab. 2022;19(1):74.
277. Nazzal L, Puri S, Goldfarb DS. Enteric hyperoxaluria: an important cause of end-stage kidney disease. Nephrol Dial Transplant. 2016;31(3):375–82.
278. Singh SB, Lin HC. Hydrogen Sulfide in Physiology and Diseases of the Digestive Tract. Microorg. 2015;3(4):866–89.
279. Hertel J, Harms AC, Heinken A, Baldini F, Thinnes CC, Glaab E, et al. Integrated Analyses of Microbiome and Longitudinal Metabolome Data Reveal Microbial-Host Interactions on Sulfur Metabolism in Parkinson's Disease. Cell Rep. 2019;29(7):1767-1777.e8.
280. Teigen L, Mathai PP, Lopez S, Matson M, Elkin B, Kozysa D, et al. Differential hydrogen sulfide production by a human cohort in response to animal- and plant-based diet interventions. Clin Nutr. 2022;41(6):1153–62.
281. Blachier F, Andriamihaja M, Larraufie P, Ahn E, Lan A, Kim E. Production of hydrogen sulfide by the intestinal microbiota and epithelial cells and consequences for the colonic and rectal mucosa. Am J Physiol-Gastrointest Liver Physiol. 2021;320(2):G125–35.
282. Portolese AC, McMullen BN, Baker SK, See JRC, Yochum GS, Koltun WA, et al. The Microbiome of Complicated Diverticulitis: An Imbalance of Sulfur-Metabolizing Bacteria. Dis Colon Rectum. 2023;66(5):707–15.
283. Wang Y, Nguyen LH, Mehta RS, Song M, Huttenhower C, Chan AT. Association Between the Sulfur Microbial Diet and Risk of Colorectal Cancer. JAMA Netw Open. 2021;4(11):e2134308.
284. Loubinoux J, Bronowicki J, Pereira IAC, Mougenel J, Faou AE. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiol Ecol. 2002;40(2):107–12.
285. Worcester EM. Stones from bowel disease. Endocrinol Metab Clin North Am. 2002;31(4):979–99.
286. Coe FL, Parks JH, Asplin JR. The Pathogenesis and Treatment of Kidney Stones. N Engl J Med. 1992;327(16):1141–52.
287. Rock KL, Kataoka H, Lai JJ. Uric acid as a danger signal in gout and its comorbidities. Nat Rev Rheumatol. 2013;9(1):13–23.
288. Li CC, Chien TM, Wu WJ, Huang CN, Chou YH. Uric acid stones increase the risk of chronic kidney disease. Urolithiasis. 2018;46(6):543–7.
289. King C, Lanaspa MA, Jensen T, Tolan DR, Sánchez-Lozada LG, Johnson RJ. Uric Acid as a Cause of the Metabolic Syndrome. Contrib Nephrol. 2018;192:88–102.
290. Daudon M, Traxer O, Conort P, Lacour B, Jungers P. Type 2 Diabetes Increases the Risk for Uric Acid Stones. J Am Soc Nephrol. 2006;17(7):2026–33.
291. Cupisti A, Morelli E, Meola M, Cozza V, Parrucci M, Barsotti G. Hypertension in Kidney Stone Patients. Nephron. 1996;73(4):569–72.
292. Devarajan A. Cross-talk between renal lithogenesis and atherosclerosis: an unveiled link between kidney stone formation and cardiovascular diseases. Clin Sci. 2018;132(6):615–26.
293. Yoo TW, Sung KC, Shin HS, Kim BJ, Kim BS, Kang JH, et al. Relationship Between Serum Uric Acid Concentration and Insulin Resistance and Metabolic Syndrome. Circ J. 2005;69(8):928–33.
294. Carbone A, Salhi YA, Tasca A, Palleschi G, Fuschi A, Nunzio CD, et al. Obesity and kidney stone disease: a systematic review. Minerva Urol e Nefrol. 2018;70(4):393–400.

Supplementary Materials

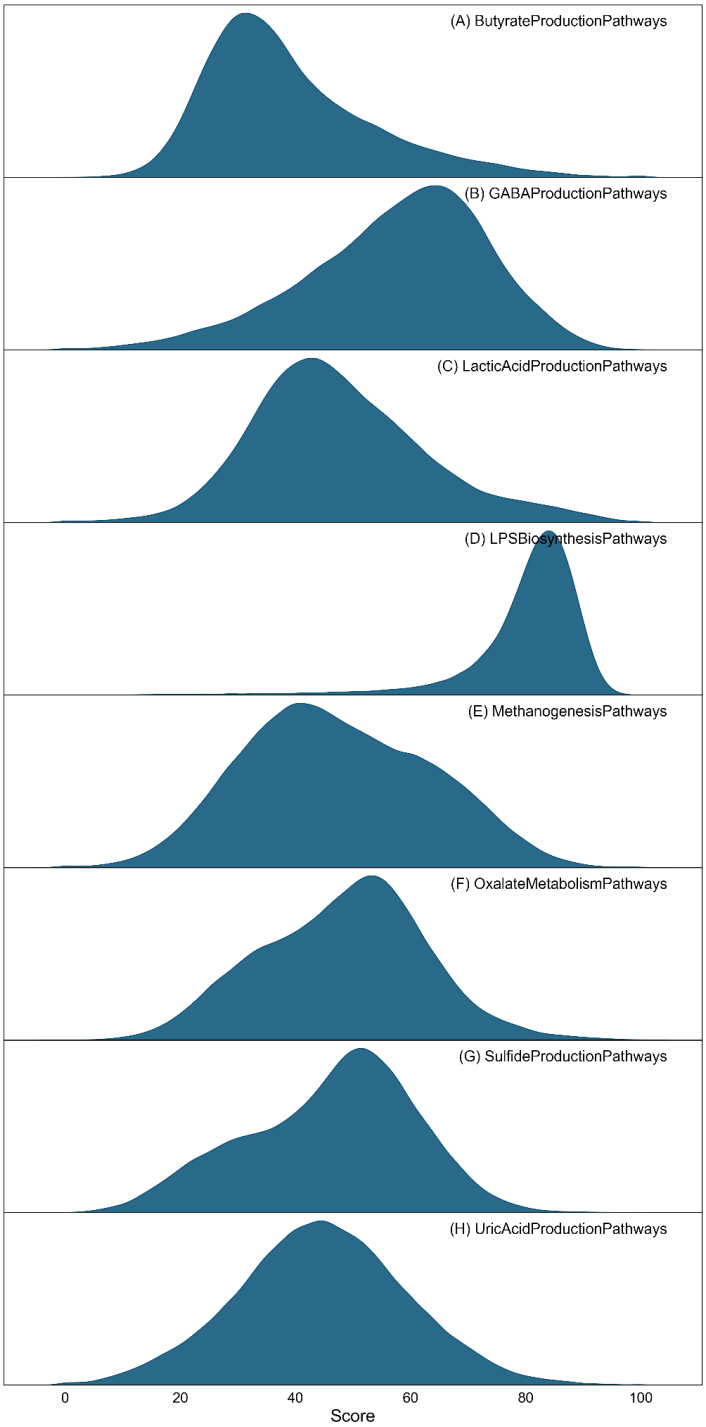


Figure S1. Landscape of score distribution. The distribution of eight functional pathway scores (A-H) in an independent cohort (n = 71,220 samples). The distribution of each pathway score across the entire cohort is expected to follow a Gaussian normal distribution, where a high score is "Good". Some scores deviate significantly from this normal distribution, which can be attributed to missing molecular signatures in the reference cohort, resulting in extremely high or low scores. Scores exceeding 100 or falling below 0 were clipped.

Table S1. Control definitions for each score. The definition of "controls" shown in Figure 3 is different for each score, where all diseases listed for each score were removed from the control pool, along with anyone who reported IBS or its subtypes. The count of comorbidities (*n*) is shown alongside the minimum size of the control pool (*x*) available for 1:5 matching, which was determined by subtracting the number of excluded cases (plus all IBS cases) from the size of the validation cohort (*n* = 71,220). For odds ratios based on Rome IV criteria, any positive IBS classification was also excluded from controls.

Score Name (*n* = comorbidity count; *x* = minimum control pool)	**Comorbidities excluded from controls**	**References**
ButyrateProductionPathways (*n* = 37 ; *x* = 39,021)	Acne, Atherosclerosis, Atopic dermatitis, *C. difficile* infection, Cardiovascular condition, Colon polyps, Colorectal cancer, Congestive heart failure, COPD, Coronary heart disease, Crohn's disease, Cystic acne, Depression, Dermatitis (Skin rashes or irritation), Dysglycemia / Irregular blood sugar, Eczema, Emphysema, GI inflammation, Gout, *H. pylori* infection, High lipids, Hypercholesterolemia, Hypertension, Inflammatory bowel disease, Insomnia, Leaky gut, Liver cirrhosis, Major depressive disorder, Osteoarthritis, Osteoporosis, Prediabetes, Psoriasis, Psoriatic arthritis, Rheumatoid arthritis, Type 1 diabetes, Type 2 diabetes, Ulcerative colitis	[147–173]
GABAProductionPathways (*n* = 17 ; *x* = 46,045)	Anxiety, Autism, Behavioral / Mood disorder, Colon polyps, Colorectal cancer, Crohn's disease, Depression, Dysmotility, Epilepsy, Inflammatory bowel disease, Irritable bowel syndrome-Diarrhea, Insomnia, Leaky gut, Metabolic syndrome, NAFLD, Type 1 diabetes, Type 2 diabetes, Ulcerative colitis	[174–186]
LacticAcidProductionPathways (*n* = 16 ; *x* = 51,028)	Chronic fatigue syndrome, Colon polyps, Colorectal cancer, Crohn's disease, Dysbiosis, Gastritis, Infections, Irritable bowel syndrome-Diarrhea, Inflammatory bowel disease, Insulin resistance, Ischemic colitis, Metabolic syndrome, Prediabetes, Short bowel syndrome, Type 2 diabetes, Ulcerative colitis	[90,187–200]
LPSBiosynthesisPathways (*n* = 39 ; *x* = 38,062)	Alcohol addiction, Allergies, ALS, Alzheimer's disease, Anxiety, Atherosclerosis, Autism, Cardiovascular condition, Celiac disease, Cognitive dysfunction / Cognitive impairment, Colon polyps, Colorectal cancer, Coronary heart disease, Crohn's disease, Depression, Dysbiosis, GI inflammation, HIV, Inflammatory bowel disease, Influenza, Insulin resistance, Kidney disease, Leaky gut, Liver cirrhosis, Metabolic syndrome, Mild cognitive impairment, NAFLD, Obesity, Osteoarthritis, Pancreatic Cancer, Pancreatitis, Parkinson's disease, Peripheral artery disease, Prediabetes, Rheumatoid arthritis, Sepsis, SIBO, Type 2 diabetes, Ulcerative colitis	[58,201–226]
MethanogenesisPathways (*n* = 32 ; *x* = 44,552)	Abdominal weight, Abnormal fasting glucose, Anorexia, Colon polyps, Colorectal cancer, Crohn's disease, Diverticulitis, Diverticulosis, Dysbiosis, Dysglycemia / Irregular blood sugar, Encopresis, Gastritis, Gastroparesis, GI inflammation, *H. pylori*, High lipids, Hypercholesterolemia, Infections, Irritable bowel syndrome-Constipation, Inflammatory bowel disease, Insulin resistance, Leaky gut, Metabolic syndrome, Multiple sclerosis, NAFLD, Obesity, Parkinson's disease, Prediabetes, SIBO, Type 1 diabetes, Type 2 diabetes, Ulcerative colitis	[112,227–265]
OxalateMetabolismPathways (*n* = 19 ; *x* = 43,222)	Abdominal weight, Cardiovascular condition, Colon polyps, Colorectal cancer, Coronary heart disease, Crohn's disease, Hashimoto's disease, Hypertension, Hyperthyroidism, Hypothyroidism, Inflammatory bowel disease, Kidney disease, Kidney stones, Obesity, Short bowel disease, Stroke, Thyroid cancer, Thyroid nodule(s), Ulcerative colitis	[266–277]
SulfideProductionPathways (*n* = 16 ; *x* = 53,736)	Colon polyps, Colorectal cancer, Crohn's disease, Diverticulitis, Diverticulosis, GI inflammation, *H. pylori* infection, Infections, Inflammatory bowel disease, Irritable bowel syndrome-Constipation, Irritable bowel syndrome-Diarrhea, Irritable bowel syndrome, Leaky gut, Parkinson's disease, SIBO, Ulcerative colitis	[278–284]
UricAcidProductionPathways (*n* = 14 ; *x* = 49,983)	Colon polyps, Colorectal cancer, Crohn's disease, Gout, Hypertension, Inflammatory bowel disease, Insulin resistance, Kidney disease, Kidney stones, Metabolic syndrome, Obesity, Prediabetes, Type 2 diabetes, Ulcerative colitis	[285–294]

Table S2. Odds Ratio and Confidence Intervals shown in Figure 3. IBS cases are the composite of IBS and all IBS subtypes while "control" definitions are score-specific (Table S1). Values represent Odds Ratios. Significance levels of the p-values are shown in superscript, with 95% Confidence Interval in parentheses. $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***); not significant (ns).

Score Name	**IBS** (max *n* = 6,373)	**IBS-C** (max *n* = 1,732)	**IBS-D** (max *n* = 1,006)	**IBS-M** (max *n* = 1,091)
ButyrateProductionPathways	1.3*** (1.2 - 1.5)	0.8* (0.7 - 0.9)	2.6*** (2.1 - 3.3)	1.5*** (1.2 - 1.9)
GABAProductionPathways	1.7*** (1.5 - 1.8)	2.1*** (1.8 - 2.6)	1.6*** (1.3 - 2.0)	1.5*** (1.2 - 1.9)
LacticAcidProductionPathways	1.4*** (1.3 - 1.5)	0.9^{ns} (0.8 - 1.1)	2.3*** (1.8 - 2.9)	1.2^{ns} (1.0 - 1.5)
LPSBiosynthesisPathways	1.2** (1.1 - 1.3)	1.3** (1.1 - 1.5)	0.9^{ns} (0.7 - 1.1)	1.1^{ns} (0.9 - 1.4)
MethanogenesisPathways	1.2*** (1.1 - 1.4)	1.6*** (1.4 - 2.0)	1.2^{ns} (1.0 - 1.5)	1.3* (1.0 - 1.5)
OxalateMetabolismPathways	1.3*** (1.2 - 1.4)	0.9^{ns} (0.7 - 1.0)	2.1*** (1.6 - 2.6)	1.2* (1.0 - 1.5)
SulfideProductionPathways	1.1^{ns} (1.0 - 1.2)	0.7*** (0.6 - 0.9)	1.8*** (1.5 - 2.3)	1.1^{ns} (0.9 - 1.3)
UricAcidProductionPathways	1.5*** (1.4 - 1.6)	1.0^{ns} (0.8 - 1.1)	2.3*** (1.8 - 2.9)	1.3* (1.1 - 1.6)

Table S3. Odds Ratio and Confidence Intervals shown in Figure 4. IBS cases are the composite of any IBS subtype classification, and cases for each IBS subtype classification are defined by the Rome IV Diagnostic Questionnaire. "Control" definitions are score-specific (Table S1). Values represent Odds Ratios. Significance levels of the p-values are shown in superscript, with 95% Confidence Interval in parentheses. $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***); not significant (ns).

Score Name	**IBS** (max *n* = 8,629)	**IBS-C** (max *n* = 1,942)	**IBS-D** (max *n* = 2,952)	**IBS-M** (max *n* = 3,309)
ButyrateProductionPathways	1.5*** (1.3 - 1.6)	0.7*** (0.6 - 0.8)	2.7*** (2.4 - 3.1)	1.3*** (1.1 - 1.4)
GABAProductionPathways	1.8*** (1.6 - 1.9)	2.5*** (2.1 - 3.0)	1.6*** (1.4 - 1.8)	1.8*** (1.6 - 2.1)
LacticAcidProductionPathways	1.4*** (1.3 - 1.5)	0.8** (0.7 - 0.9)	2.2*** (2.0 - 2.5)	1.3*** (1.2 - 1.5)
LPSBiosynthesisPathways	1.1* (1.0 - 1.2)	1.3** (1.1 - 1.6)	1.0^{ns} (0.9 - 1.1)	1.1^{ns} (0.9 - 1.2)
MethanogenesisPathways	1.2*** (1.1 - 1.3)	1.8*** (1.5 - 2.1)	1.0^{ns} (0.9 - 1.1)	1.3*** (1.2 - 1.5)
OxalateMetabolismPathways	1.3*** (1.2 - 1.5)	0.7*** (0.6 - 0.8)	2.1*** (1.8 - 2.4)	1.2** (1.1 - 1.4)
SulfideProductionPathways	1.1** (1.0 - 1.2)	0.6*** (0.5 - 0.7)	1.7*** (1.5 - 1.9)	1.1^{ns} (0.9 - 1.2)
UricAcidProductionPathways	1.5*** (1.4 - 1.6)	0.9^{ns} (0.8 - 1.0)	2.3*** (2.0 - 2.7)	1.4*** (1.3 - 1.6)

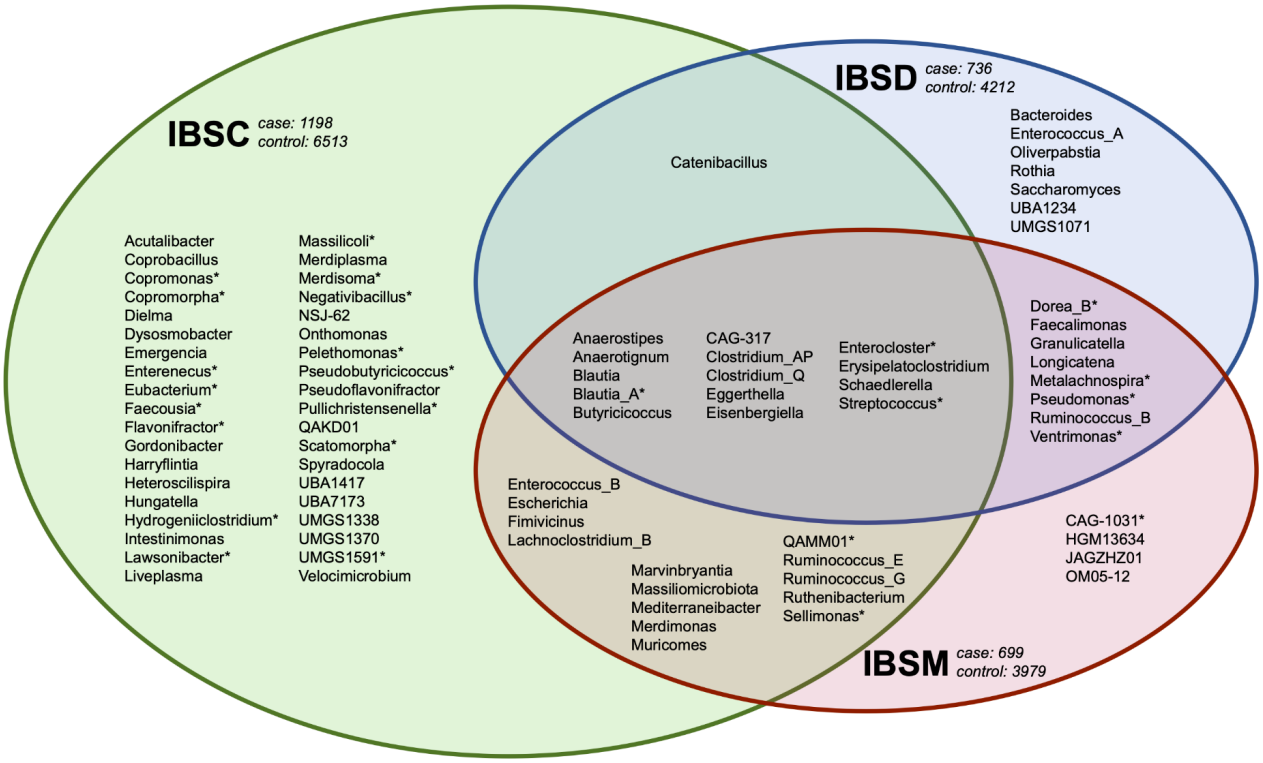


Figure S2. Genera of significantly increased species (differentially expressed) between self-reported IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for "IBS-C" (green; n = 1,198), "IBS-D" (blue; n = 736), and "IBS-M" (red; n = 699). All significantly increased species ($p \leq 0.05$ and log "Fold Change" ≥ 0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to Rome IV-determined IBS phenotypes (Figure S4) are indicated with an asterisk (*).

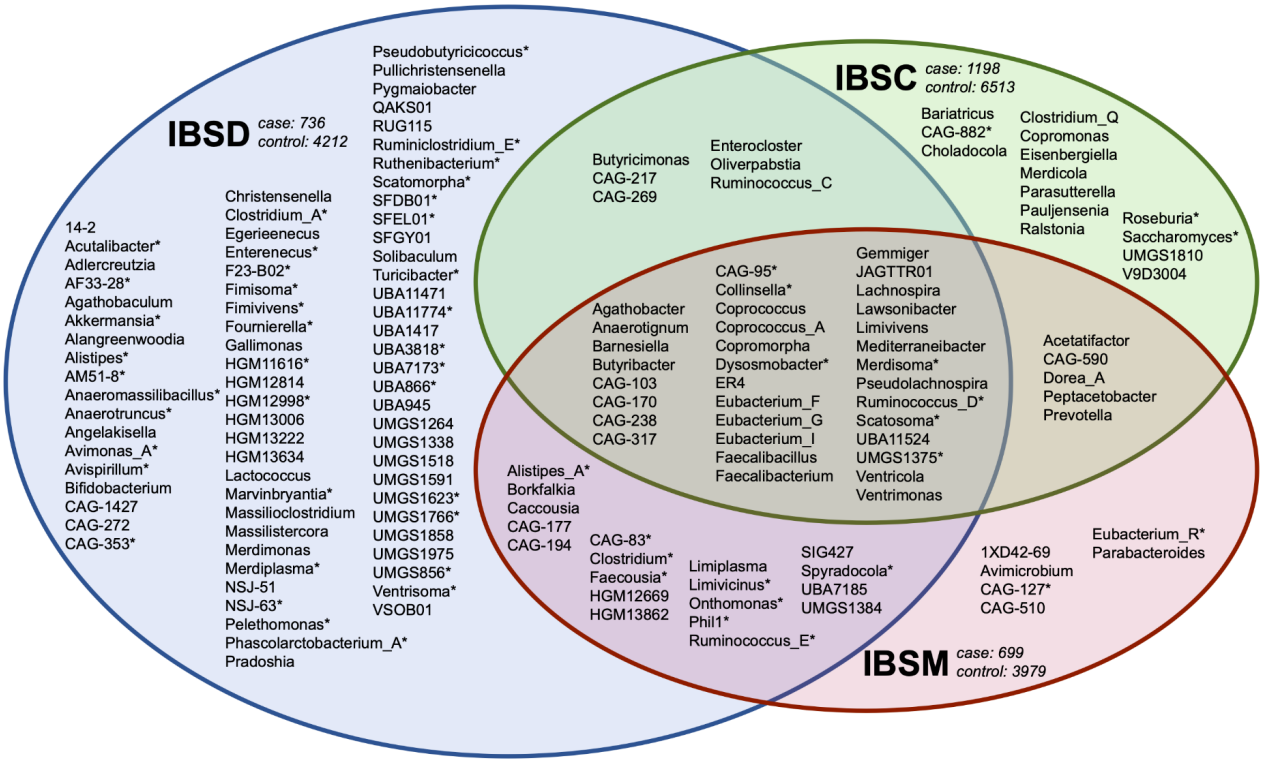


Figure S3. Genera of significantly decreased species (differentially expressed) between self-reported IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for "IBS-C" (green; n = 1,198), "IBS-D" (blue; n = 736), and "IBS-M" (red; n = 699). All significantly decreased species ($p \leq 0.05$ and log "Fold Change" ≤ -0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to Rome IV-determined IBS phenotypes (Figure S5) are indicated with an asterisk (*).

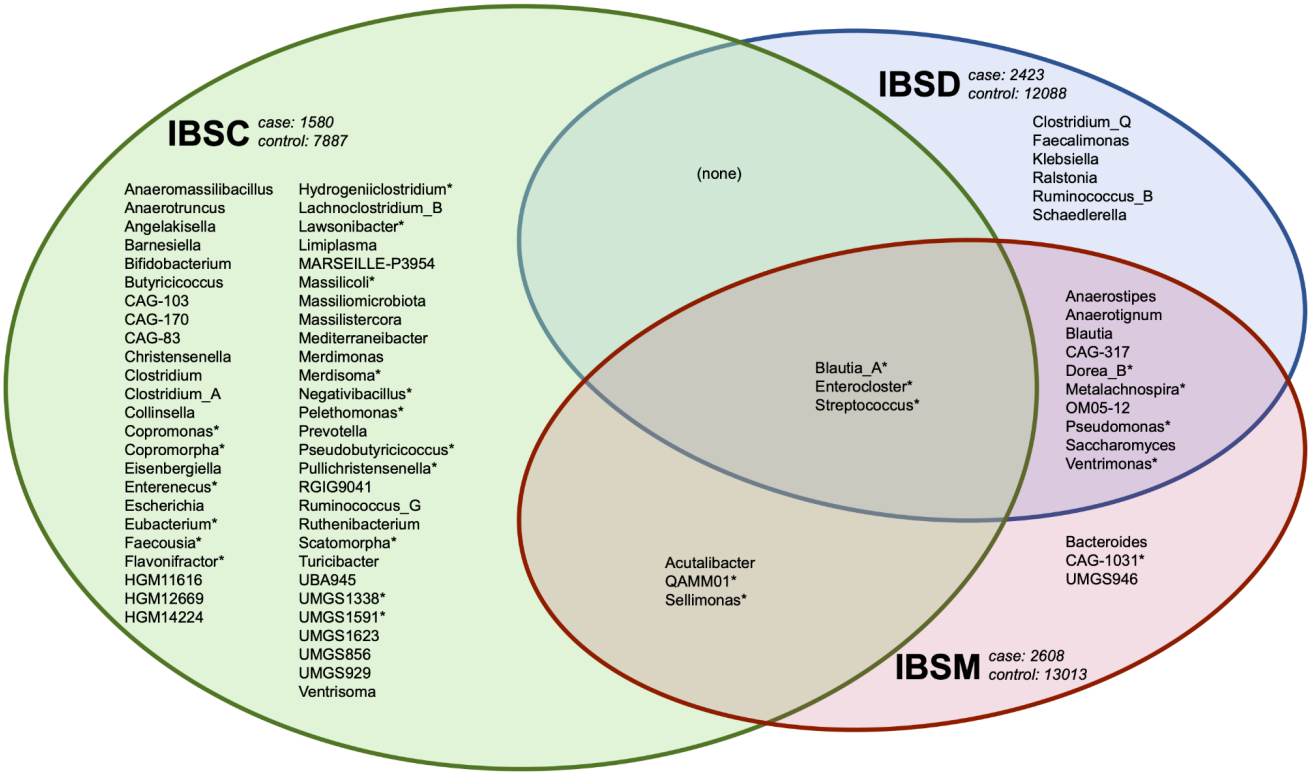


Figure S4. Genera of significantly increased species (differentially expressed) between Rome IV-defined IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for "IBS-C" (green; n = 1,198), "IBS-D" (blue; n = 736), and "IBS-M" (red; n = 699). All significantly increased species ($p \leq 0.05$ and log "Fold Change" ≥ 0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to self-reported IBS phenotypes (Figure S2) are indicated with an asterisk (*).

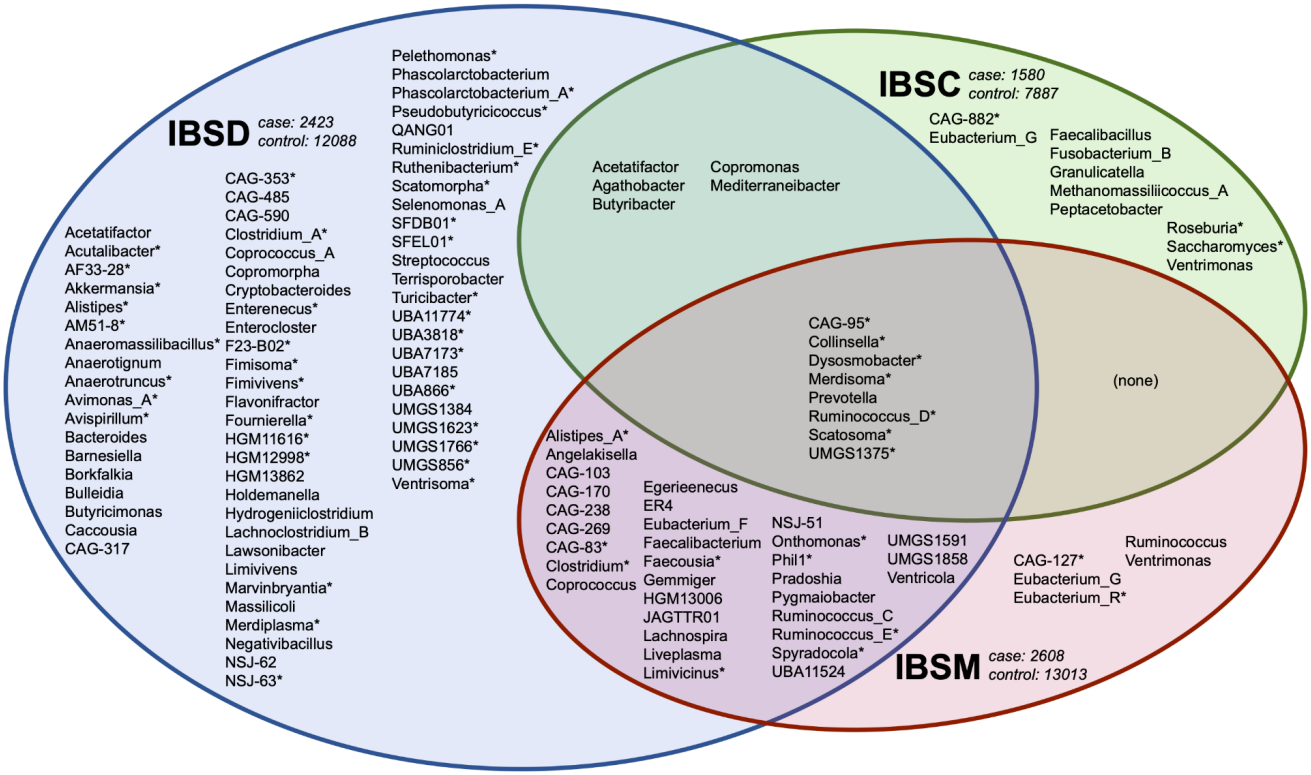


Figure S5. Genera of significantly decreased species (differentially expressed) between Rome IV-defined IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for "IBS-C" (green; n = 1,198), "IBS-D" (blue; n = 736), and "IBS-M" (red; n = 699). All significantly increased species ($p \leq 0.05$ and log "Fold Change" ≥ 0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to self-reported IBS phenotypes (Figure S3) are indicated with an asterisk (*).