Supplementary material:

Subject information

The recruited healthy volunteers (n=24) were chosen with the following inclusion criteria: male and female of 18-65 years of age both with a normal Body Mass Index (18-28 kg/m^2). The exclusion criteria included, pregnancy, history of serious acute or chronic illness especially gastrointestinal, use of medication which is known to affect gastrointestinal transit, such as opiates and constipating drugs, substance abuse and the use of antibiotics 3 months prior the experiment. Subjects were age and gender matched and divided into the two study groups.

Predicting the functional potential of the intestinal microbiota

We employed the PICRUSt pipeline[21] to estimate the gene contents of the HITChip target species in order to study possible functional differences of the microbiota between different sample groups. The PICRUSt pipeline estimates gene contents using phylogenetic marker gene sequences by mapping the marker sequences to a phylogeny where the gene contents of target species are available. This approach allows estimating the gene contents of unknown genomes based on their phylogenetic position.

Here we have used the 16S rRNA genes targeted by the HITChip as an input to PICRUSt, where the 16S sequences are first mapped to the reference phylogeny, and the hits are then divided by 16S copy number of each corresponding reference genome to normalize for copy number variations. These normalized counts are multiplied by the gene content (i.e. KEGG orthologs) of the reference genome(s) to estimate the gene content of the unknown query genome.

To transform the HITChip data to PICRUSt format, a QIIME[23] formatted fasta file was constructed from the HITChip data using in-house scripts by multiplying the HITChip target sequences by normalized intensities (where the lowest intensity value was 1) for each sample and giving each sequence a unique identifier consisting of sample name and sequence ID (e.g. "> sample1_sequence1"). The intensity values of each HITChip target

sequence were thereafter interpreted as counts. OTUs were picked from the fasta file at 0.97 threshold using the pick_closed_reference_otus.py QIIME script[23] with Greengenes version 13_5[22]reference OTUs. The resulting biom-formatted OTU table was input to PICRUSt[21] version 1.0.0 running in Galaxy (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=PICRUSt_normalize).

KEGG ortholog (KO)[24] mappings of PICRUSt were used in further analysis, where the KO's having count number less than 13000 summed over all samples were removed to exclude background from analysis. P-values for remaining KO's in sample groupings were computed with Wilcoxon's rank sum test and Benjamini-Hochberg false discovery rate correction.

References:

1 Connor A, Tolan D, Hughes S, Carr N, Tomson C. Consensus guidelines for the safe prescription and administration of oral bowel-cleansing agents. Gut 2012;61:1525-32.

2 Strocchi A, Bond JH, Ellis C, Levitt MD. Colonic Concentrations of Hydrogen and Methane Following Colonoscopic Preparation with an Oral Lavage Solution. Gastrointest Endosc 1990;36:580-2.

3 Jalanka-Tuovinen J, Salonen A, Nikkilä J, Immonen O, Kekkonen R, Lahti L, et al. Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. PLoS ONE 2011;6:e23035.

4 Zoetendal EG, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Applied and Environmental Microbiology 1998;64:3854-9.

5 Rajilic-Stojanovic M, Heilig HG, Tims S, Zoetendal EG, de Vos WM. Long-term monitoring of the human intestinal microbiota composition. Environ Microbiol 2012;15:1146-1159.

6 Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 2010;156:3216-23.

7 Kamada N, Chen GY, Inohara N, Nunez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 2013;14:685-90.

8 Lahti L, Salojarvi J, Salonen A, Scheffer M, de Vos WM. Tipping elements in the human intestinal ecosystem. Nature communications 2014;5:4344.

9 Mai V, Greenwald B, Morris JG, Jr., Raufman JP, Stine OC. Effect of bowel preparation and colonoscopy on post-procedure intestinal microbiota composition. Gut 2006;55:1822-3.

10 Harrell L, Wang YW, Antonopoulos D, Young V, Lichtenstein L, Huang Y, et al. Standard Colonic Lavage Alters the Natural State of Mucosal-Associated Microbiota in the Human Colon. PLoS ONE 2012;7:e32545.

11 O'Brien CL, Allison GE, Grimpen F, Pavli P. Impact of colonoscopy bowel preparation on intestinal microbiota. PLoS ONE 2013;8:e62815.
12 Gorkiewicz G, Thallinger GG, Trajanoski S, Lackner S, Stocker G, Hinterleitner T, et al. Alterations in the colonic microbiota in response to osmotic diarrhea. PLoS ONE 2013;8:e55817.
13 Johansson ME, Gustafsson JK, Holmen-Larsson J, Jabbar KS, Xia L, Xu H, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. Gut 2014;63:281-91.
14 Tooth D, Garsed K, Singh G, Marciani L, Lam C, Fordham I, et al. Characterisation of faecal protease activity in irritable bowel syndrome with diarrhoea: origin and effect of gut transit. Gut 2014;63:753-60.
15 Garsed KC, Marciani L, Fields A, Fordham I, Pritchard SE, Placidi E, et al. Mode of Action of a Macrogol Formulation on Distribution of Intestinal Fluid: A MRI Study. Gastroenterology 2012;142:S814-S.
16 Salonen A, Nikkilä J, Jalanka-Tuovinen J, Immonen O, Rajilic-Stojanovic M, Kekkonen RA, et al. Comparative analysis of fecal DNA extraction methods with phylogenetic microarray: effective recovery of bacterial and archaeal DNA using mechanical cell lysis. J Microbiol Methods 2010;81:127-34.
17 Rajilic-Stojanovic M, Heilig HG, Molenaar D, Kajander K, Surakka A, Smidt H, et al. Development and application of the human intestinal tract chip, a phylogenetic microarray: analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. Environ Microbiol 2009;11:1736-51.
18 Claesson MJ, O'Sullivan O, Wang Q, Nikkilä J, Marchesi JR, Smidt H, et al. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PLoS ONE 2009;4:e6669.
19 Lahti L, Torrente A, Elo LL, Brazma A, Rung J. A fully scalable online pre-processing algorithm for short oligonucleotide microarray atlases. Nucleic acids research 2013;41.
20 Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 2002;148:257-66.
21 Ufnar JA, Wang SY, Christiansen JM, Yampara-Iquise H, Carson CA, Ellender RD. Detection of the nifH gene of Methanobrevibacter smithii: a potential tool to identify sewage pollution in recreational waters. J Appl Microbiol 2006;101:44-52.
22 Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome research 1996;6:986-94.
23 Roka R, Rosztoczy A, Leveque M, Izbeki F, Nagy F, Molnar T, et al. A pilot study of fecal serine-protease activity: a pathophysiologic factor in diarrhea-predominant irritable bowel syndrome. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association 2007;5:550-5.
24 Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013;31:814-21.

25 McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 2012;6:610-8.
26 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods 2010;7:335-6.
27 Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic acids research 2000;28:27-30.
28 Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
29 Pop M, Walker AW, Paulson J, Lindsay B, Antonio M, Hossain MA, et al. Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. Genome Biology 2014;15:R76.
30 Cohen LB. Split dosing of bowel preparations for colonoscopy: an analysis of its efficacy, safety, and tolerability. Gastrointest Endosc 2010;72:406-12.
31 Nomura T, Ohkusa T, Okayasu I, Yoshida T, Sakamoto M, Hayashi H, et al. Mucosa-associated bacteria in ulcerative colitis before and after antibiotic combination therapy. Alimentary Pharmacology & Therapeutics 2005;21:1017-27.
32 Raman M, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, et al. Fecal Microbiome and Volatile Organic Compound Metabolome in Obese Humans With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol H 2013;11:868-75.
33 Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature 2013;500:541-6.
34 Rajilic-Stojanovic M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Tims S, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology 2011;141:1792-801.
35 Gecse K, Roka R, Ferrier L, Leveque M, Eutamene H, Cartier C, et al. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. Gut 2008;57:591-9.
36 Bohe M, Borgstrom A, Genell S, Ohlsson K. Determination of Immunoreactive Trypsin, Pancreatic Elastase and Chymotrypsin in Extracts of Human Feces and Ileostomy Drainage. Digestion 1983;27:8-15.
37 Genell S, Gustafsson BE. Impaired Enteric Degradation of Pancreatic Endopeptidases in Antibiotic-Treated Rats. Scandinavian journal of gastroenterology 1977;12:801-9.
38 Carroll IM, Ringel-Kulka T, Ferrier L, Wu MC, Siddle JP, Bueno L, et al. Fecal Protease Activity Is Associated with Compositional Alterations in the Intestinal Microbiota. PLoS ONE 2013;8:e78017.