Supplementary Methods

Study design

Faecal samples were collected by the patients who were provided with two kits including an empty tube and sampling spatula. One sample was collected during the screening period, and another sample was collected during the last week of the intervention, between days 22 and 28. Samples were kept in a freezer at -20°C until being brought to the lab with an ice pack in a mini cool bag to minimise risk for DNA degradation. Once delivered to the lab the samples were stored at -20°C.

Symptom assessment

Bristol Stool Form Scale

Every day during the 10-day screening period and over the course of the 28 day intervention period, patients completed a stool diary based on the Bristol Stool Form (BSF)[1] scale. This was used to characterize patients into subgroups based on stool frequency (number of stools per day) and mean stool consistency on a seven point scale; IBS with constipation (IBS-C), IBS with diarrhoea (IBS-D), IBS with mixed bowel habits (IBS-M), or unsubtyped IBS (IBS-U)[1]. The two latter subgroups were combined into IBS nonCnonD.

IBS symptom severity assessment

All patients completed the IBS Severity Scoring System (IBS-SSS[2]) questionnaire during the 10 day screening period before intervention, during (day 14) and after the intervention on day 29. The severity of abdominal distention, severity of abdominal pain, frequency of abdominal pain, dissatisfaction with bowel habits, and interference of IBS symptoms with daily life were evaluated though five questions, each using a visual analogue scale. A score ranging from 0 (no symptoms) to 500 (maximum severity) can be achieved subsequently placing patients into subgroups of mild (75-175), moderate (176-300) or severe (>300) IBS symptoms.

Bacterial analysis

GA-map™ Dysbiosis Test[3] (Genetic Analysis AS, Oslo, Norway) was used for bacterial analysis. The test is based on regular molecular biology techniques, comprising human faecal sample homogenization and mechanical bacterial cell disruption; automated total bacterial gDNA extraction using magnetic beads; 16S rRNA PCR DNA amplification covering V3–V9; probe labelling by single nucleotide extension; hybridization to complementary probes coupled to magnetic beads; and signal detection using BioCode 1000A 128-Plex Analyzer (Applied BioCode, Santa Fe Springs, CA, USA). Quality control steps are described in detail in Casén *et al*. (2015)[3] and include a pre-labelled probe to monitor hybridization and signal retrieval, a universal control to check the presence of target material in the single-

nucleotide extension (SNE) reaction, a synthetic template which monitors the success of the SNE reaction, a negative control of nuclease-free water and a blank control comprising two blank beads which detect background sample signal [3]. The GA-map™ Dysbiosis Test[3] consists of 54 DNA probes targeting ≥300 bacteria on different taxonomic levels. Probes were selected based on the ability to distinguish between healthy controls, IBS and inflammatory bowel disease (IBD) patients[3]. The model algorithmically assesses faecal bacterial abundance, denoted as Probe Signal Intensity (PSI), a profile, and potential clinically relevant deviation in the microbiome from normobiosis[3]. The dysbiosis model output is a bacterial profile and a score of dysbiosis. A Dysbiosis Index above 2 (maximum 5) indicates a microbiota that differs from the reference Nordic (Norwegian and Swedish) group. A bacterial profile is considered to be dysbiotic when a Dysbiosis Index score of 2 or higher in the 5 point score chart is achieved. The level of dysbiosis of a bacterial profile is defined through calculating confidence regions for principal component analysis (PCA) Hotelling's T-square and Q statistics values. The principal component analysis (PCA) is centred around a normobiotic bacterial profile model based on a healthy Nordic cohort[3].

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3 Casén C, Vebø HC, Sekelja M, Hegge FT, Karlsson MK, Ciemniejewska E*, et al.* Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment Pharmacol Ther 2015;42:71-83.