

Synbiotic Alters Fecal Microbiomes, but not Liver Fat or Fibrosis, in a Randomized Trial of Patients With Non-alcoholic Fatty Liver Disease

Eleonora Scorletti, Paul R. Afolabi, Elizabeth A. Miles, Debbie E. Smith, Amal Almeahmadi, Albandri Alshathry, Caroline E. Childs, Stefania Del Fabbro, Josh Beavis, Helen E. Moyses, Geraldine F. Clough, Jaswinder K. Sethi, Janisha Patel, Mark Wright, David J. Breen, Charles Peebles, Angela Darekar, Richard Aspinall, Andrew J. Fowell, Joanna K. Dowman, Valerio Nobili, Giovanni Targher, Nathalie M. Delzenne, Laure B. Bindels, Philip C. Calder, Christopher D. Byrne



PII: S0016-5085(20)30124-4
DOI: <https://doi.org/10.1053/j.gastro.2020.01.031>
Reference: YGAST 63172

To appear in: *Gastroenterology*
Accepted Date: 11 January 2020

Please cite this article as: Scorletti E, Afolabi PR, Miles EA, Smith DE, Almeahmadi A, Alshathry A, Childs CE, Del Fabbro S, Beavis J, Moyses HE, Clough GF, Sethi JK, Patel J, Wright M, Breen DJ, Peebles C, Darekar A, Aspinall R, Fowell AJ, Dowman JK, Nobili V, Targher G, Delzenne NM, Bindels LB, Calder PC, Byrne CD, Synbiotic Alters Fecal Microbiomes, but not Liver Fat or Fibrosis, in a Randomized Trial of Patients With Non-alcoholic Fatty Liver Disease, *Gastroenterology* (2020), doi: <https://doi.org/10.1053/j.gastro.2020.01.031>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Synbiotic Alters Fecal Microbiomes, but not Liver Fat or Fibrosis, in a Randomized Trial of Patients With Non-alcoholic Fatty Liver Disease

Authors:

Eleonora Scorletti^{1,2,7}, Paul R. Afolabi^{1,2}, Elizabeth A. Miles¹, Debbie E. Smith^{1,2}, Amal Almeahmadi¹, Albandri Alshathry¹, Caroline E. Childs¹, Stefania Del Fabbro¹, Josh Beavis¹, Helen E. Moyses², Geraldine F. Clough¹, Jaswinder K. Sethi^{1,9}, Janisha Patel³, Mark Wright³, David J. Breen⁴, Charles Peebles⁴, Angela Darekar⁵, Richard Aspinall⁸, Andrew J. Fowell⁸, Joanna K. Dowman⁸, Valerio Nobili^{10,11}, Giovanni Targher¹², Nathalie M. Delzenne⁶, Laure B. Bindels⁶, Philip C. Calder^{1,2,9}, Christopher D. Byrne^{1,2}

¹ Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK;

² National Institute for Health Research Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton National Health Service (NHS) Foundation Trust, Southampton, UK

³ Hepatology, Department of Medicine, University Hospital Southampton NHS Foundation Trust, Southampton, UK

⁴ Department of Radiology, University Hospital Southampton NHS Foundation Trust, Southampton, UK

⁵ Department of Medical Physics, University Hospital Southampton NHS Foundation Trust, Southampton, UK

⁶ Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université Catholique de Louvain, Brussels, Belgium

⁷ Department of Gastroenterology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA

⁸ Department of Hepatology, Portsmouth Hospitals NHS Trust, Queen Alexandra Hospital, Portsmouth, UK

⁹ Institute for Life Sciences, University of Southampton, Southampton, UK.

¹⁰ Hepatology, Gastroenterology and Nutrition Unit, IRCCS "Bambino Gesù" Children's Hospital, Rome, Italy

¹¹ Department of Pediatric, University "La Sapienza", Rome, Italy

¹² Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy

Corresponding author at: Division of Gastroenterology, 939 Biomedical Research Building, 421 Curie Boulevard University of Pennsylvania Philadelphia 19104. Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK.

E-mail address: e.scorletti@soton.ac.uk; eleonora.scorletti@pennmedicine.upenn.edu (E. Scorletti).

Author Contributions: ES-CDB-PCC-NMD designed, conducted the study, analysed data and drafted the manuscript; PRA-DES- AmalA-AA-AD-DJB-CP helped with data acquisition and data analysis; EAM-JP-MW-RA-AJF-JKD organised the recruitment and randomisation; HEM-GT-VN-LBB analysed and interpreted the data; GFC-JS critically revised the manuscript adding important intellectual content.

Disclosures/Conflicts of interest: The synbiotic and placebo were provided at no cost by Chr. Hansen Holding A/S, Boege Alle 10-12, 2970 Hoersholm, Denmark. Chr. Hansen had no input into any aspect of study design or conduct of the trial. Furthermore, Chr. Hansen will have no input into data analysis or subsequent reporting of the trial results. PCC has received consulting fees from Chr. Hansen, but not in relation to this trial. None of the other authors has any disclosures.

Abstract

Background & Aims: Dysbiosis of the intestinal microbiota has been associated with nonalcoholic fatty liver disease (NAFLD). We investigated whether administration of a synbiotic combination of probiotic and prebiotic agents affected liver fat content, biomarkers of liver fibrosis, and the composition of the fecal microbiome in patients with NAFLD.

Methods: We performed a double-blind phase 2 trial of 104 patients with NAFLD in the United Kingdom. Participants (mean age, 50.8±12.6 y; 65% men; 37% with diabetes) were randomly assigned to groups given the synbiotic agents (fructo-oligosaccharides, 4 g twice per day, plus *Bifidobacterium animalis* subsp. lactis BB-12; n=55) or placebo (n=49) for 10–14 months. Liver fat content was measured at the start and end of the study by magnetic resonance spectroscopy and liver fibrosis was determined from a validated biomarker scoring system and vibration-controlled transient elastography. Fecal samples were collected at the start and end of the study and fecal microbiomes were analyzed by 16S rDNA sequencing.

Results: Mean baseline and end of study magnetic resonance spectroscopy liver fat percentage values were 32.3%±24.8% and 28.5%±20.1% in the synbiotic group and 31.3%±22% and 25.2%±17.2% in the placebo group. In the unadjusted intention to treat analysis, we found no significant difference in liver fat reduction between groups ($\beta=2.8$; 95% CI, -2.2 to 7.8; $P=.30$). In a fully adjusted regression model (adjusted for baseline measurement of the outcome plus age, sex, weight difference, and baseline weight), only weight loss was associated with a significant decrease in liver fat ($\beta=2$; 95% CI, 1.5–2.6; $P=.03$). Fecal samples from patients who received the synbiotic had higher proportions of *Bifidobacterium* and *Faecalibacterium*, and reductions in *Oscillibacter* and *Alistipes*, compared with baseline; these changes were not observed in the placebo group. Changes in composition of fecal microbiota were not associated with liver fat or markers of fibrosis.

Conclusions: In a randomized trial of patients with NAFLD, 1 y administration of a synbiotic combination (probiotic and prebiotic) altered fecal microbiomes but did not reduce liver fat content or markers of liver fibrosis. clinicaltrials.gov no: NCT01680640.

KEY WORDS: INSYTE study, nutrition, cardiovascular disease, type 2 diabetes

1 INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a pathologic condition defined by the deposition of triglyceride (TG) in the liver greater than 5% of the total liver weight¹⁻³. The term NAFLD encompasses a spectrum of pathologic conditions where the first stage is characterized by simple steatosis with liver fat accumulation in the hepatocytes^{3, 4}. The second stage is non-alcoholic steatohepatitis (NASH) characterized by hepatocyte injury due to inflammation, ballooning and possible collagen deposition. NASH is a progressive form of fatty liver that can worsen over time and may lead to cirrhosis and liver failure².

NAFLD has become one of the most common causes of chronic liver disease and liver related mortality worldwide, and is now becoming a major reason for liver transplantation⁵. Approximately 10-20% of people with NAFLD progress to NASH⁶. NAFLD is also an independent risk factor for extra-hepatic diseases, such as type 2 diabetes and cardiovascular disease^{7, 8}. There is recent growing interest in the role of gut microbiota in NAFLD pathogenesis, and there are several metaorganismal pathways linking altered gut microbiota (termed dysbiosis) and NAFLD⁹⁻¹². Recent literature shows that the faecal microbial composition of patients with NAFLD differs from that of healthy individuals. Some studies showed a preponderance of Gram-negative bacteria, such as *Proteobacteria*, *Enterobacteriaceae* and *Escherichia*^{13, 14}. Other studies have described a gut metagenomic signature in NAFLD patients with fibrosis of an increased abundance in *Ruminococcus* associated with the worsening of liver fibrosis and/or a more complex signature, involving 37 bacterial species in a model that distinguished mild/moderate NAFLD from advanced fibrosis^{15, 16}. However, there is currently a lack of consistency in these findings due to the marked variance in the population studied, with differing ages, diets and geographical locations¹⁷. Nonetheless, despite these inconsistencies, there is the possibility that manipulation of the gut

microbiota to a more favourable profile, could provide a beneficial effect on liver disease in patients with NAFLD.

Treatments that can change the gut microbiota include, but are not limited to, probiotics, prebiotics and synbiotics. Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host¹⁸⁻²¹. A synbiotic is a mixture of one or more probiotics and one or more prebiotics that beneficially affect the host by promoting the survival and colonisation of the live microbes in the gastrointestinal tract²². Small randomized controlled trials (RCT), mostly of short duration (up to 6 months) and using ultrasonography, or surrogate markers of NAFLD, have suggested that administration of prebiotics and/or probiotics may reduce hepatic steatosis, hepatic inflammation, and insulin resistance in patients with NAFLD^{9-12, 23}. However, these studies did not test the effect of the pro-, pre- or synbiotic treatments on gut microbiota to show that modification of gut microbiota benefits the liver in patients with NAFLD. In addition, no studies have reported the effect of a synbiotic treatment on both microbiota and liver fat accumulation in NAFLD.

In a proof of concept, phase-2 RCT, we tested the hypothesis that ~12 months of synbiotic treatment was effective in: 1) ameliorating liver fat content; 2) improving two histologically validated liver fibrosis scores and 3) changing the gut microbiota in patients with NAFLD. In addition, we tested the effect of the synbiotic on liver stiffness measurement assessed by vibration-controlled transient elastography (VCTE).

2 METHODS

2.1 Study design

The INSYTE study (Investigation of synbiotic treatment in NAFLD; www.clinicaltrials.gov registration number NCT01680640) was approved by the Southampton and South West Hampshire Local Research Ethics Committee (REC: 12/SC/0614). The protocol, study design and methods for the trial have been reported previously ²⁴. 104 participants with proven NAFLD were randomised to the intervention or placebo (**Figure 1**) for a minimum of 10 months and a maximum of 14 months ²⁴. This period of intervention was chosen to allow for the potential of a maximum of two prescribed courses of antibiotics. There was a further requirement that any antibiotic therapy had ceased a minimum of one month prior to end of study tests, including collection of stool sample.

Fifty-five participants were randomised to receive synbiotic treatment consisting of fructo-oligosaccharides with a degree of polymerization <10 at 4 g/twice a day (two sachets a day, to stir into a cold drink) plus *Bifidobacterium animalis* subsp. *lactis* BB-12 at a minimum of 10 billion CFU/day (1 capsule a day) and forty-nine participants were randomised to receive placebo consisting of 4 g/twice a day of maltodextrin (1 capsule a day plus two sachets a day, to stir into a cold drink). Participants were stratified by age (<50 and ≥50 years) and sex. Block randomisation in groups of four was used to ensure a balance between the treatment groups. Synbiotic and placebo were provided free of charge by Christian Hansen A/S, Boege Alle 10-12, 2970 Hoersholm, Denmark. The prebiotic component of the synbiotic was Actilight®950P. The conduct of the trial, data analyses and writing of the manuscript were all undertaken by the authors and were completely independent from Chr. Hansen.

2.2 Liver fat measurement

Measurement of liver fat content was undertaken at baseline and at the end of the study using proton magnetic resonance spectroscopy (MRS). Three 20×20×20 mm³ spectroscopic voxels were positioned within segments 3 (inferior sub-segment of the lateral segment), 5 (inferior sub-segment of the anterior segment) and 8 (superior sub-segment of the anterior segment) of the liver, avoiding major blood vessels, intra-hepatic bile ducts and the lateral margin of the liver. For the second visit scan, these voxel positions were copied from the first scan, to ensure measurements at the end of the study were taken in the same regions of the liver, as at baseline. Values for the lipid and water peak integrals were produced for each voxel and recorded for each participant ²⁴. For each participant to test the effect of the intervention on liver fat percentage we calculated the mean of the three spectroscopic voxels for liver fat percentage at baseline and end of study.

2.3 Faecal microbiota analysis

Faecal genomic DNA was extracted using the QIAamp DNA Stool Mini Kit, including a bead-beating step. Total bacteria, *Bifidobacterium* spp. and *Bifidobacterium animalis* levels were quantified using qPCR as previously described ²⁵. The samples were PCR-enriched for the V5-V6 region of the 16S rRNA gene and then underwent a library tailing PCR as described in Pötgens et al. ²⁶. The amplicons were purified, quantified and sequenced using an Illumina MiSeq to produce 2x300 bp sequencing products. Initial quality-filtering of the reads was conducted with the Illumina Software, yielding an average of 169,858 pass-filter reads per sample. Quality scores were visualized and reads were trimmed to 220 bp (R1) and 200 bp (R2). The reads were merged with the merge-Illumina-pairs application ²⁷. For all samples but two, a subset of 26,000 reads was randomly selected using Mothur 1.32.1 ²⁸. The UPARSE pipeline implemented in USEARCH v10.0.240 ²⁹ was used to further process the

sequences. Putative chimaeras were removed using the *cluster_otus* command. Non-chimeric sequences were subjected to taxonomic classification using the RDP MultiClassifier 1.1 from the Ribosomal Database Project ³⁰. The phylotypes were computed as percent proportions based on the total number of sequences in each sample. Raw sequences can be found in the SRA database (project ID: PRJNA559052).

2.4 Statistical analysis

The sample size for the INSYTE was based on testing the effect of the synbiotic treatment on changes in MRS liver fat content as a one of three key primary outcomes for the trial [See INSYTE Protocol paper ²⁴]. Statistical analyses were performed using SPSS for Windows, Stata v14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.) and R ³¹. For each primary outcome (MRS liver fat percentage, ELF score and NAFLD fibrosis score), a change variable was calculated as the difference between end-of-study and baseline measurements. For continuous variables, multiple regression analysis was used to assess the effect of synbiotic treatment on each of the change variables of interest. Multivariable (adjusted) linear regression model testing the effects of synbiotic treatment (primary analyses) and changes in *Bifidobacterium* levels between baseline and end of study were undertaken. Models were adjusted for baseline measurement of the outcome variable only or adjusted for baseline measurement of the outcome *plus* age, sex, weight difference and baseline weight.

The ITT analysis included all patients randomized who had baseline and end-of-study measurements, regardless of whether they were later found to be ineligible, a protocol violator, given the wrong treatment allocation, or never treated. Multiple imputation (MI) was used to handle any missing data. As a secondary analysis, the above was repeated with

change levels of *Bifidobacterium* genus at the end of the study. A *P*-value of <0.05 was considered statistically significant²⁴.

In addition, for quantifying the changes in gut microbiota following intervention, we compared taxonomic and operational taxonomic unit (OTU) profiles between treatment and placebo arms. Statistical tests for changes in abundance over time and differences in changes of abundance over time were performed for each taxonomic unit, OTU and alpha-diversity indexes, by using the Wilcoxon paired rank-sum test and the Mann-Whitney U test, with a Benjamini-Hochberg correction for multiple testing (with a false discovery rate [FDR] cut-off of 0.1). Beta-diversity indexes were first visualized through a Principal Coordinates Analysis (PCoA) using Emperor³² to explore the dissimilarity between microbial communities. Beta-diversity values for each pair of samples were compared between treatment and placebo arms using Mann-Whitney U tests. Partial Spearman rank-based correlations were computed using the *pcor.test* function (<http://www.yilab.gatech.edu/pcor.html>).

3 RESULTS

Eighty-nine participants out of 104 (86%) completed the RCT (58 men and 31 women) (see **Table S1** for the reasons for participant withdrawal). **Table 1** shows the baseline clinical characteristics for the whole cohort and a comparison between the two treatment groups. At baseline, there was no significant difference in the main clinical and biochemical parameters, liver profile and *Bifidobacterium* abundance between the synbiotic and placebo groups. Abundance of *Bifidobacterium* (a component of the synbiotic) was reported as a marker of good compliance during the study. **Table 2** shows the changes between baseline and end-of-trial measurements, stratified by randomization group, for the main anthropometric and biochemical variables. Baseline intestinal permeability measurement (lactulose/mannitol ratio) in the INSYTE cohort was: median = 0.11, interquartile range (IQR)=0.25; mean $\pm$ SD 0.27 ± 0.4 (Table 2). Since the INSYTE study was not designed with a healthy control group, we compared the INSYTE baseline lactulose/mannitol ratio with that for healthy subjects described in the literature. Notably, the mean and median values of lactulose/mannitol ratio were ~10-fold greater than those reported for healthy subjects in the literature³³⁻³⁶, supporting the presence of altered intestinal permeability. That said, there were no significant changes in intestinal permeability between baseline and end-of-study measurements for the lactulose mannitol ratio in either the synbiotic or placebo groups. Interestingly, there was an overall reduction in MRS-measured liver fat content in 65% of the total 89 patients who completed the trial. With regard to LPS, there was no difference between baseline and end of study in either placebo ($p=0.80$) or treatment group ($p=0.40$) suggesting that synbiotic treatment did not affect circulating levels of LPS. At the end of the study, there was no significant association between changes in MRS-measured liver fat and changes in LPS levels ($p=0.80$). We tested the effect of synbiotic treatment on faecal SCFAs using a regression model with SCFAs difference, acetic acid difference, propionic acid difference or

butyric acid difference as separate outcomes, and adjusting for sex, age, treatment group and the baseline SCFA measure as exposures in the model. These data showed that there was no association between synbiotic treatment and change in SCFA concentrations (total SCFAs $p=0.21$; acetic acid $p=0.23$; propionic acid $p=0.24$; butyric acid $p=0.40$). With regard to MRS liver fat percentage, we undertook regression modelling using MRS difference as the outcome, adjusting the model for sex, age, weight difference, weight at baseline, liver fat content at baseline and SCFAs. There was no association between change in SCFAs and change in MRS liver fat percentage: SCFAs $p=0.63$; acetic acid $p=0.62$; propionic acid $p=0.78$; butyric acid $p=0.73$.

The results from the FFQs showed that there was an overall decrease in total calories, carbohydrates, protein and fat from visit 1 to visit 2 (after 6 months in the study). However, there was no evidence of a significant treatment effect as there was no difference between the two randomisation groups (**Table S2 and S3**). With regard to physical activity, there was also no significant difference at the end of the study in either placebo or treatment group (**Table 2**).

3.1 Intention to Treat (ITT) Analysis

Table 3 shows baseline and end-of-study data for each of the primary outcomes and the results of multivariable linear regression models for the ITT analyses. In the synbiotic group, there was only a 4% MRS liver fat reduction ($p=0.08$) and a significant increase in *Bifidobacterium* abundance ($p<0.001$). Therefore, there was no significant effect of the synbiotic treatment on MRS liver fat content. In the placebo group, although there was no change in *Bifidobacterium* abundance at the end of the study ($p=0.5$), there was a significant 6% reduction of MRS liver fat ($p=0.01$). In the unadjusted ITT analyses, there was no

difference between the two groups in changes in MRS liver fat ($\beta=2.8$; 95%CI: -2.2, 7.8; $p=0.3$). In the fully adjusted regression model (adjusted for baseline measurement of the outcome plus age, sex, weight difference and baseline weight), there was a borderline significant difference in MRS-measured liver fat ($\beta=4.1$; 95%CI: -0.02, 8.3; $p=0.05$) between placebo and synbiotic groups (**Table 3**) (N.B. However, this analysis showed a borderline significantly greater reduction in liver fat in the placebo group than in the synbiotic group). In this multivariable regression model, weight loss was the only factor that was associated with a significant reduction in MRS liver fat at the end of the study with a 2% reduction of liver fat associated with every kg of weight loss ($\beta=2.0$; 95%CI: 1.5, 2.6; $p=0.03$) (**Table 3**). We then performed regression modelling with MRS liver fat difference as the outcome and weight difference as an explanatory variable. These data showed that weight loss was strongly associated with a significant reduction liver fat (β coefficient=1.9; 95%CI 1.4, 2.5; $p<0.001$).

With regard to the two algorithmically derived fibrosis scores (i.e. the ELF and NAFLD fibrosis scores), we undertook the same regression models we used for the MRS liver fat adjusting for baseline measurement of the outcome *plus* age, sex, weight difference, and baseline weight. We did not find any significant changes in either liver fibrosis scores with synbiotic treatment (**Table 3**). We also tested the effect of the synbiotic intervention of liver stiffness measurement (kPa) (assessed by VCTE). In a regression model that included change in liver stiffness measurement between baseline and end of study as the outcome, and that adjusted for baseline measurement of the outcome *plus* age, sex, weight difference, and baseline weight in the model, synbiotic was not associated with change in liver stiffness measurement (B coefficient = -0.78 (95%CI -1.93, 0.37), $p=0.18$). Therefore, taken together, these data show that it is unlikely that the synbiotic influenced liver fat or liver fibrosis as components of NASH pathology. Weight loss was associated with significant improvements

in ELF ($p=0.039$) and NAFLD fibrosis score ($p=0.027$) (**Table 3**), and also liver stiffness measurement ($p=0.025$). Interestingly, within the synbiotic group, a negative association was found between the changes in *Bifidobacterium* spp. abundance and the changes in MRS fat liver content (Spearman's $\rho = -0.36$, $p= 0.017$). However, this association was no longer significant after controlling for body weight change, age and sex (Spearman's $\rho = -0.10$, $p=0.51$). We also tested the association between changes in *Bifidobacterium* spp. abundance at the end of the study and each of the primary outcomes (adjustments as per the ITT analysis). Notably, we did not find any significant associations between changes in *Bifidobacterium* spp. abundance at the end of the study and MRS liver fat or the two algorithmically derived fibrosis scores. Moreover, there was no association between weight loss and synbiotic treatment or *Bifidobacterium* spp. abundance at the end of the study. In a multivariable regression model using *Bifidobacterium* spp. abundance difference (end of study minus baseline) as the outcome and adjusting the model for treatment allocation, age, sex and weight difference at the end of the study, interestingly, weight difference, age and sex were not significantly associated with *Bifidobacterium* spp. abundance.

3.2 Faecal microbiota analysis

At baseline, faecal microbiome was not significantly different between the two groups of participants (**Table S4, Table S5, Figure S1**) and was characterised by a positive association between levels of *Sphingomonas* and *Sutterella* and liver fibrosis scores, whereas there was a negative association between *Olsenella* and MRS-measured liver fat and between *Clostridia* and *Firmicutes* and liver fibrosis scores (**Table 4**). Total bacteria levels were not significantly affected by the intervention (**Figure S2**). The synbiotic treatment was associated with a reduced within-sample microbial diversity (α -diversity) at the richness level, but not at the evenness or evenness and richness levels (**Table S4**), likely signalling the disappearance of taxa of low abundance. Dissimilarities between microbial communities were visualised

through a Principal Coordinates Analysis (PCoA) of the β -diversity index Morisita-Horn (**Figure 2A**). The observed distribution of β -diversity was mainly due to inter-personal variability (explaining 86% of the total variation, ANOSIM, 1000 permutations, $p=0.001$). To evaluate whether the synbiotic treatment had a greater effect upon the microbiota structure over time compared to the placebo treatment, we compared the distance between each pair of samples (baseline and end of study, computed through a β -diversity index) between the two treatment arms (**Figure 2B**). The synbiotic group displayed higher β -diversity values than the placebo group, indicating that the synbiotic treatment had a greater effect on gut microbiota structure than placebo. Similar results were obtained when looking at other β -diversity indexes, such as Binary-Jaccard and Bray-Curtis (**Figure 2B**). In accordance with these observations, compared to the placebo, the synbiotic treatment fostered the abundance of *Bifidobacterium* and *Faecalibacterium* at the expense of *Oscillibacter* and *Alistipes* (**Figure 3, Table S6**), with parent taxa showing the same trends (**Table S6**). No other significant changes were found either at the phylum level (**Figure S3**) or at any other taxonomic level (**Table S7, Figure S4**). In particular, bacteria classically linked to inflammation, obesity and NAFLD were not affected (**Figure S5**). Surprisingly, only *Oscillibacter* was negatively associated with MRS-measured liver fat after adjusting the model as per the ITT analysis ($\beta=-2.2$; 95%CI: -4.3, -0.1; $p=0.04$). The 2.2-fold increase in *Bifidobacterium* spp. was also confirmed by qPCR (**Figure S2**).

4 DISCUSSION

Our study is the first RCT to test the efficacy of a synbiotic treatment for ~12 months in NAFLD on a quantitative measurement of liver fat content, two algorithmically derived fibrosis scores in NAFLD (the ELF and NAFLD fibrosis scores), liver stiffness measurement, and changes in gut microbiota. At the end of the trial, we did not find any significant difference between synbiotic and placebo groups for MRS liver fat content, the two algorithmically derived fibrosis scores and the liver stiffness measurement. We found an overall reduction in MRS liver fat in 65% of participants independently associated only with weight loss. Although our RCT was not specifically designed to test the effect of weight loss on our primary outcomes, all participants were given healthy lifestyle advice as part of their usual care. There was only a mean weight loss of 1 kg in and yet this very small amount of weight loss was independently associated with a decrease in MRS-measured liver fat. Interestingly, from the unstandardized β coefficients, 1 kg of weight loss was associated with a 2% decrease in percentage of liver fat and weight loss was also associated with an improvement in the ELF and NAFLD fibrosis scores and liver stiffness measurement. In our proof of concept RCT, the fact that most of the patients had an improvement in MRS-measured liver fat, regardless of treatment allocation, is consistent with the so-called “clinical trial effect”³⁷, whereby participants benefit from participating in clinical trials.

We were successful in showing a change in gut microbiota in the synbiotic group, and we significantly changed the diversity of the gut microbiota after synbiotic treatment. This increased diversity was reflected by an increased abundance of *Bifidobacterium* and *Faecalibacterium* and a reduction of *Oscillibacter* and *Alistipes*. *Bifidobacterium* spp. that are known to have some beneficial effects on lipid profile and gut barrier³⁸⁻⁴⁰. However, we did not find any association between *Bifidobacterium* spp. levels at the end of the study and change in MRS-measured liver fat (after adjusting for weight loss), or on intestinal

permeability, after adjusting for age, sex and baseline measurements for intestinal permeability (the lactulose/mannitol ratio). Levels of the anti-inflammatory bacterium *Faecalibacterium prausnitzii* have been reported to be decreased in NAFLD, independently of obesity and insulin resistance, and to be increased upon consumption of inulin-type fructan prebiotics ⁴¹⁻⁴⁴. *Faecalibacterium prausnitzii* has also been shown to be negatively associated with markers of lymphocytes and Kupffer cells in the portal tract ⁴⁵. In contrast, *Alistipes* spp. has been shown to be positively associated with hepatic inflammatory and oxidative stress markers in mice ^{46, 47}. Therefore, we may speculate that the increase in *Faecalibacterium prausnitzii* and the decrease in *Alistipes* associated with the synbiotic treatment might confer beneficial effects in terms of inflammation, an effect not investigated in this study. No other significant changes were found either at the phylum level (Figure S3) or at any other taxonomic level (Table S7, Figure S4). In particular, bacteria classically linked to inflammation, obesity and NAFLD were not affected (Figure S5).

A recent study by Raman et al. characterized the faecal microbiota of obese patients with NAFLD and compared the faecal microbiota with that of healthy controls ⁴⁸. In this study these authors showed that *Oscillibacter* was significantly lower in NAFLD patients than in healthy controls ⁴⁸.

Because the INSYTE study was not designed with a healthy control cohort, we investigated the presence of gut microbiota dysbiosis in the INSYTE population by comparing the baseline microbiota composition at the phylum level with two previously published cohorts: Twins-UK and Food4Gut ^{49, 50}. As shown in Table S8, the INSYTE population presents a nearly 2-fold higher level of *Proteobacteria* compared to the two other cohorts (the first one with a similar geographical origin, the second one with identical analyses). Although we acknowledge that these two control cohorts were not assessed contemporaneously to the INSYTE study cohort, this comparison suggests that the baseline

gut microbiota of the INSYTE cohort was different from the gut microbiota found in a general UK population as *Proteobacteria* is one of the bacterial taxa presented in some studies as a signature of NAFLD¹⁴.

The synbiotic composition selected for the INSYTE study was chosen to maximize the effects of the probiotic. The probiotic (*Bifidobacterium animalis* subsp. *lactis* BB-12) was chosen based on specific beneficial effects on the host and for its ability to colonize the human intestine⁵¹ and based on its previously reported specific beneficial effects on impaired glucose tolerance, plasma lipid profile and gut barrier function^{38, 40}. The prebiotic (fructo-oligosaccharide with a degree of polymerization <10) was chosen to specifically stimulate growth and activity of Bifidobacteria.

The prebiotic was selected to have a higher affinity compared to inulin for the probiotic and was chosen to improve its survival and growth in the host^{22, 24, 52, 53}. Moreover, using a validated *in vitro* faecal bacterial culture system designed to mimic the conditions within the human colon, we have additionally investigated the effects of *Bifidobacterium lactis* BB-12 and fructo-oligosaccharide (contained within the INSYTE synbiotic) on human gut microbiota *ex vivo*⁵⁴. These data showed that with either the synbiotic or the prebiotic alone, there was a significant increase in total bacteria and in total bifidobacteria number, compared to either the probiotic alone or the negative control. Furthermore, the synbiotic supplement was well tolerated. During the trial, only one patient experienced some minor gastro-intestinal distress symptoms (bloating and flatulence). We advised that participant to divide the dose of supplement during the day and the participant was able to complete the trial without further gastro-intestinal distress.

It is important to note that previous studies in NAFLD that have tested the effects of synbiotics have also included lifestyle interventions (or medication) with the synbiotic

treatment^{11, 12, 23, 55-58}. In contrast, our RCT was primarily designed to test the effect of synbiotic alone, and not lifestyle intervention which is known to be effective in decreasing liver fat in NAFLD. Importantly, in previous studies, investigators have not adjusted their analyses for key potential confounding factors, such as changes in physical activity or weight loss. Moreover, in most of these studies, the power to detect differences with the study sample sizes has not been mentioned. With inadequate sample sizes there is high probability of type 1 statistical errors, with a high potential for different types of bias, or confounding, influencing the results^{11, 12, 23, 55-59}. Previous studies that have tested the effects of synbiotic treatment in NAFLD⁹⁻¹² have also used a combination of multiple strains of probiotics as a component of the synbiotic treatment. Therefore, it might be possible that, because the intestine harbours trillions of bacteria, adding one single type of bacterium in a synbiotic may not be as effective as adding three or six different types of bacteria^{10-12, 55, 60, 61} with the potential to influence many more bacterial species. There are some studies that have tested the effect of a single bacterium [*Bifidobacterium longum*⁹ and *L. reuteri*⁶²] in patients with NAFLD; however, these studies also included lifestyle modifications in addition to the synbiotic treatment. An additional parameter to be considered is the fact that patient responsiveness to microbiota-targeting intervention may be highly individualized^{63, 64}. Our RCT was not designed to stratify patients based either on baseline gut microbiome or microbiome changes overtime, and it is therefore not possible to determine whether a subset of patients did respond more favorably.

There are strengths and limitations of our study that should be considered. The strengths of our study were: the rigorous design of the placebo-controlled trial; the long duration of the trial (~12 months); the detailed phenotyping of participants; the adjustment for potential confounders; and the fact that we have also evaluated the effect of the intervention on changes in gut microbiota using an *ex vivo* system designed to mimic the

human colon. Our RCT also had some limitations. Firstly, we choose one strain of bacteria for inclusion in the synbiotic. The prebiotic was selected based on its effect on lipid metabolism^{22, 52, 65, 66}. The probiotic *Bifidobacterium animalis subspecies lactis* (BB12) was selected based on its ability to colonize the intestine⁵¹ and its beneficial properties on the gut barrier^{39, 40}. With dysbiosis, there is an increased production of endotoxins from Gram-negative bacteria that can damage the intestinal barrier with the potential for toxic metabolites to enter the portal circulation ultimately damaging the liver⁶⁷. We reasoned that by improving the gut barrier using this *Bifidobacterium* as a probiotic, we could reduce the toxic effect of the gut microbiota and therefore improve liver fat. Secondly, the INSYTE study was designed as a phase 2 clinical trial to test the effect of the intervention on liver fat content using MRS and not liver biopsy. NAFLD is known to be a “patchy” disease and liver biopsy is invasive, expensive, and subject to sampling variability. Consequently, we did not include the requirement for liver biopsy in the design of this proof of concept RCT. MRS is currently considered the non-invasive, gold-standard technique for accurately measuring changes in liver fat percentage and has excellent reproducibility and sensitivity. Additionally, MRS is a very sensitive technique for measuring liver fat and liver fat signals of only 0.2% above the background noise level can be measured⁶⁸. Furthermore, because synbiotics are safe, inexpensive nutritional supplements that are widely available, we also reasoned that if synbiotics have a place in the treatment of NAFLD, that place would be early in the course of the disease process, targeted at decreasing liver fat content. Therefore, our intention was to test the effect of the synbiotic intervention on liver fat content, rather than to assess the histological resolution of NASH, improvements in NAFLD activity score, or improvements in liver fibrosis, as required in phase 3 RCTs testing novel medications that would be potentially licensed as pharmaceutical agents for the treatment of NASH. Finally, we

deliberately did not include a lifestyle intervention because *a priori*, our intention was to test the effect of the synbiotic intervention alone.

In conclusion, the results of our double-blinded, randomised, placebo-controlled phase 2 trial show that synbiotic treatment (consisting of fructo-oligosaccharides with a degree of polymerization <10 at 4 g/twice a day plus *Bifidobacterium animalis* subsp. *lactis* BB-12 at a minimum of 10 billion CFU/day) for a period of ~12 months was effective in changing the gut microbiota in patients with NAFLD. However, this treatment was ineffective in decreasing MRS-measured liver fat content, or in improving two validated biomarker scores for liver fibrosis, or liver stiffness measurement, compared with placebo. Interestingly despite there only being a mean weight loss of 1 kg in both the synbiotic and placebo groups, weight loss of 1 kg was significantly associated with an improvement of 2% in MRS-measured liver fat content, and with improvement in the two algorithmically-derived fibrosis scores^{69, 70} and liver stiffness measurement. Thus, our RCT suggests that changing the gut microbiota with this synbiotic may occur without clinically significant effects on the liver in NAFLD. The changes in specific microbes known to be involved with inflammation that we observed in the study, support a need to evaluate the effect of synbiotic treatment on inflammatory/immune-related parameters in NAFLD.

Post Script

Sadly, our friend and respected colleague, Professor Valerio Nobili died suddenly and unexpectedly after completion of this manuscript. We honour Valerio's contribution as an author of this work and as a highly respected clinician scientist in NAFLD research.

Journal Pre-proof

Acknowledgements

The authors would like to thank the INSYTE Trial Investigators who helped with the recruitment. We would also like to thank the INSYTE Trial participants and also Chr. Hansen Holding A/S, Boege Alle 10-12, 2970 Hoersholm, Denmark, who provided the synbiotic and placebo at no cost. This work was supported by the National Institute of Health Research through the NIHR Southampton Biomedical Research Centre and by the Parnell Diabetes Trust. The authors thank Sanchia Triggs, Gemma Rood, Andria Staniford, Norma Diaper and Jennifer Hedges who were the research nurses at Southampton General Hospital; Kirsty Fancey and all the nurses in the Hepatology Unit at Portsmouth Hospitals; Lucinda England for research governance administration; Cheng Yueqing who helped with the data management and data cleaning, Colin Newell, and Karen Long who helped with data entry; Bouazza Es Saadi at the Université catholique de Louvain, Louvain Drug Research Institute (Belgium) for helping with the gut microbiota analysis.

Figure 1. Consort diagram

Figure 2. Synbiotics increase microbial beta-diversity. (A) Principal coordinate analysis of the Morisita-Horn beta-diversity index computed based on the OTU table, for each group of patients, colored by time (baseline in red, end of study in blue) or patient ID. (B) Pairwise distance between baseline and end of study samples for each patient, computed using beta-diversity metrics. P-value of the Mann-Whitney test comparing placebo and synbiotics is presented below each graph.

Figure 3. Synbiotics boost *Bifidobacterium* and *Faecalibacterium* at the expense of *Oscilibacter* and *Alistipes*. Genera presenting a significant difference in change of abundance over time between arms (q-value < 0.1). The bold line represents the mean changes.

5 REFERENCES

1. Chalasani N, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012;142:1592-609.
2. Calzadilla Bertot L, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2016;17.
3. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346:1221-31.
4. Byrne CD. Fatty liver: role of inflammation and fatty acid nutrition. *Prostaglandins Leukot Essent Fatty Acids* 2010;82:265-71.
5. Sherif ZA, et al. Global Epidemiology of Nonalcoholic Fatty Liver Disease and Perspectives on US Minority Populations. *Dig Dis Sci* 2016;61:1214-25.
6. Younossi Z, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11-20.
7. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015;62:S47-64.
8. Adams LA, et al. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* 2017;66:1138-1153.
9. Malaguarnera M, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Dig Dis Sci* 2012;57:545-53.
10. Hadi A, et al. Efficacy of synbiotic supplementation in patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis of clinical trials: Synbiotic supplementation and NAFLD. *Crit Rev Food Sci Nutr* 2018:1-12.
11. Mofidi F, et al. Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. *Br J Nutr* 2017;117:662-668.
12. Eslamparast T, et al. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Am J Clin Nutr* 2014;99:535-42.
13. Zhu L, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013;57:601-9.
14. Betrapally NS, et al. Changes in the Intestinal Microbiome and Alcoholic and Nonalcoholic Liver Diseases: Causes or Effects? *Gastroenterology* 2016;150:1745-1755.e3.
15. Boursier J, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764-75.
16. Loomba R, et al. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2017;25:1054-1062.e5.
17. Delzenne NM, et al. Contribution of the gut microbiota to the regulation of host metabolism and energy balance: a focus on the gut-liver axis. *Proc Nutr Soc* 2019:1-10.
18. Roberfroid M, et al. Prebiotic effects: metabolic and health benefits. *Br J Nutr* 2010;104 Suppl 2:S1-63.
19. Gibson GR, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017;14:491-502.

20. FAO/WHO. Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food. WHO 2002;London, Ontario, Can.
21. Bindels LB, et al. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol* 2015;12:303-10.
22. Kolida S, Gibson GR. Synbiotics in health and disease. *Annu Rev Food Sci Technol* 2011;2:373-93.
23. Bakhshimoghaddam F, et al. Daily Consumption of Synbiotic Yogurt Decreases Liver Steatosis in Patients with Nonalcoholic Fatty Liver Disease: A Randomized Controlled Clinical Trial. *J Nutr* 2018;148:1276-1284.
24. Scorletti E, et al. Design and rationale of the INSYTE study: A randomised, placebo controlled study to test the efficacy of a synbiotic on liver fat, disease biomarkers and intestinal microbiota in non-alcoholic fatty liver disease. *Contemp Clin Trials* 2018;71:113-123.
25. Salazar N, et al. Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clin Nutr* 2015;34:501-7.
26. Potgens SA, et al. Klebsiella oxytoca expands in cancer cachexia and acts as a gut pathobiont contributing to intestinal dysfunction. *Sci Rep* 2018;8:12321.
27. Eren AM, et al. A filtering method to generate high quality short reads using illumina paired-end technology. *PLoS One* 2013;8:e66643.
28. Schloss PD, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009;75:7537-41.
29. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013;10:996-8.
30. Cole JR, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res* 2014;42:D633-42.
31. Team RDC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria 2012;. ISBN 3-900051-07-0, URL <http://www.R-project.org/>. In.
32. Vazquez-Baeza Y, et al. EMPERor: a tool for visualizing high-throughput microbial community data. *Gigascience* 2013;2:16.
33. Benjamin J, et al. Intestinal permeability and its association with the patient and disease characteristics in Crohn's disease. *World J Gastroenterol* 2008;14:1399-405.
34. Penalva JC, et al. A study of intestinal permeability in relation to the inflammatory response and plasma endocab IgM levels in patients with acute pancreatitis. *J Clin Gastroenterol* 2004;38:512-7.
35. Linsalata M, et al. Comparison of an enzymatic assay with liquid chromatography-pulsed amperometric detection for the determination of lactulose and mannitol in urine of healthy subjects and patients with active celiac disease. *Clin Chem Lab Med* 2014;52:e61-4.
36. Dasty M, et al. Lactulose/mannitol test and specificity, sensitivity, and area under curve of intestinal permeability parameters in patients with liver cirrhosis and Crohn's disease. *Dig Dis Sci* 2008;53:2789-92.
37. McCarney R, et al. The Hawthorne Effect: a randomised, controlled trial. *BMC Med Res Methodol* 2007;7:30.
38. Stenman LK, et al. Potential probiotic Bifidobacterium animalis ssp. lactis 420 prevents weight gain and glucose intolerance in diet-induced obese mice. *Benef Microbes* 2014;5:437-45.

39. Guardamagna O, et al. Bifidobacteria supplementation: effects on plasma lipid profiles in dyslipidemic children. *Nutrition* 2014;30:831-6.
40. Aoki R, et al. A proliferative probiotic Bifidobacterium strain in the gut ameliorates progression of metabolic disorders via microbiota modulation and acetate elevation. *Sci Rep* 2017;7:43522.
41. Dewulf EM, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112-21.
42. Ramirez-Farias C, et al. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. *Br J Nutr* 2009;101:541-50.
43. Sokol H, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A* 2008;105:16731-6.
44. Da Silva HE, et al. Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. *Sci Rep* 2018;8:1466.
45. Schwenger KJP, et al. Markers of activated inflammatory cells are associated with disease severity and intestinal microbiota in adults with nonalcoholic fatty liver disease. *Int J Mol Med* 2018;42:2229-2237.
46. Neyrinck AM, et al. Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an effect related to the modulation of the gut microbiota. *Mol Nutr Food Res* 2017;61.
47. Moschen AR, et al. Lipocalin 2 Protects from Inflammation and Tumorigenesis Associated with Gut Microbiota Alterations. *Cell Host Microbe* 2016;19:455-69.
48. Raman M, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013;11:868-75.e1-3.
49. Goodrich JK, et al. Human genetics shape the gut microbiome. *Cell* 2014;159:789-99.
50. Hiel S, al. e. Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. *Am J Clin Nutr* 2019;109:1683-1695.
51. Izquierdo E, et al. Resistance to simulated gastrointestinal conditions and adhesion to mucus as probiotic criteria for Bifidobacterium longum strains. *Curr Microbiol* 2008;56:613-8.
52. Betrapally NS, et al. Gut microbiome and liver disease. *Transl Res* 2017;179:49-59.
53. Rossi M, al. e. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. *Appl Environ Microbiol* 2005;71:6150-8.
54. Henrique-Bana FC et al. In vitro effects of Bifidobacterium lactis-based synbiotics on human faecal bacteria. *Food Research International* 2018.
55. Asgharian A, et al. The effect of synbiotic supplementation on body composition and lipid profile in patients with NAFLD: A randomized, double blind, placebo-controlled clinical trial study. *Iranian Red Crescent Medical* 2017;Journal 19.
56. Eslamparast T, et al. Effects of synbiotic supplementation on insulin resistance in subjects with the metabolic syndrome: a randomised, double-blind, placebo-controlled pilot study. *Br J Nutr* 2014;112:438-45.
57. Manzhali E, et al. Treatment efficacy of a probiotic preparation for non-alcoholic steatohepatitis: A pilot trial. *J Dig Dis* 2017;18:698-703.
58. Wong VW, et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. *Ann Hepatol* 2013;12:256-62.

59. Ahn SB, et al. Randomized, Double-blind, Placebo-controlled Study of a Multispecies Probiotic Mixture in Nonalcoholic Fatty Liver Disease. *Sci Rep* 2019;9:5688.
60. Javadi L et al. The effect of probiotic and/or prebiotic on liver function tests in patients with nonalcoholic fatty liver disease: a double blind randomized clinical trial. *. Iran Red Crescent Med J.* 2017.
61. Ekhlasi G, et al. Effects of symbiotic and vitamin E supplementation on blood pressure, nitric oxide and inflammatory factors in non-alcoholic fatty liver disease. *Excli j* 2017;16:278-290.
62. Ferolla SM, et al. Beneficial Effect of Synbiotic Supplementation on Hepatic Steatosis and Anthropometric Parameters, But Not on Gut Permeability in a Population with Nonalcoholic Steatohepatitis. *Nutrients* 2016;8.
63. Salonen A, et al. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *Isme j* 2014;8:2218-30.
64. Walker AW, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *Isme j* 2011;5:220-30.
65. Rivero-Gutierrez B, et al. A synbiotic composed of *Lactobacillus fermentum* CECT5716 and FOS prevents the development of fatty acid liver and glycemic alterations in rats fed a high fructose diet associated with changes in the microbiota. *Mol Nutr Food Res* 2017;61.
66. Trevisi P, et al. Effect of fructo-oligosaccharides and different doses of *Bifidobacterium animalis* in a weaning diet on bacterial translocation and Toll-like receptor gene expression in pigs. *Nutrition* 2008;24:1023-9.
67. Schuster S, et al. Triggering and resolution of inflammation in NASH. *Nat Rev Gastroenterol Hepatol* 2018;15:349-364.
68. Machann J, et al. Hepatic lipid accumulation in healthy subjects: a comparative study using spectral fat-selective MRI and volume-localized ¹H-MR spectroscopy. *Magn Reson Med* 2006;55:913-7.
69. Angulo P, et al The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846-54.
70. Guha IN, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008;47:455-60.

Table 1. Baseline characteristics of study participants

	Total (n=89)	Placebo (n=44)	Synbiotic (n=45)
Age	50.8 (12.6)	51.6 (13.1)	50.2 (12.4)
Sex (M/F)%	65/35	61/39	69/31
Physical activity (METs)	1.2 (0.3)	1.26 (0.3)	1.16 (0.3)
Diabetes/No Diabetes (%)	37/63	36/64	38/62
HbA1c (mmol/mol)	40 (22.5)	39 (24.5)	41 (21.5)
Height (cm)	172.3 (0.2)	170.8 (10.3)	173.8 (10)
Weight (kg)	98.0 (16.5)	96.8 (17.1)	99.2 (16.1)
BMI (kg/m ²)	33.1 (5.2)	33.2 (4.9)	32.9 (5.5)
DEXA body fat %*	34.0 (11.2)	34.5 (11.0)	32.9 (12.1)
Systolic blood pressure (mmHg)	133.6 (16.1)	134.3 (18.9)	132.9 (12.9)
Diastolic blood pressure (mmHg)	74.1 (9.1)	72.5 (9.8)	75.7(8.2)
Fasting glucose (mmol/L)*	6.1 (2.3)	6.1 (2.2)	6.2 (2.5)
Fasting insulin (µU/mL)*	14.2 (9.0)	14.9 (9.5)	13.5 (7.9)
Total cholesterol (mmol/L)*	4.8 (1.4)	4.8 (1.2)	4.9 (1.2)
HDL-cholesterol (mmol/L)*	1.2 (0.34)	1.2 (0.4)	1.2 (0.2)
LDL-cholesterol (mmol/L)	2.7 (0.9)	2.2 (1.0)	2.8 (0.8)
Total cholesterol/HDL-cholesterol ratio	4.1 (0.8)	4.0 (0.8)	4.1 (0.8)
Triglycerides (mmol/L)*	1.8 (0.9)	1.7 (0.8)	1.8 (1.1)
ALT (U/L)*	58.0 (42.0)	61.7 (30.9)	59.0 (39.3)
AST (U/L)*	35.0 (23.5)	40.5 (29.3)	34.0 (18.0)
GGT (U/L)*	55.0 (53.5)	56.0 (56.0)	51.0 (48.0)
HA (ug/L)*	35.0 (40.0)	38 (28)	31 (47)
PIIINP (ug/L)*	8.3 (3.7)	8.2 (3.2)	8.3 (3.4)
TIMP-1 (ug/L)	158.6 (28.6)	160.2 (28.9)	155.5 (35.5)
MRS-measured liver fat (%)	25 (26.6)	22.9 (12.9, 44.7)	26.9 (9.7, 38.4)
†ELF score	6.8 (0.3)	6.9 (0.3)	6.9 (0.4)
NAFLD fibrosis score	-1.15 (1.3)	-1.2 (1.3)	-1.3 (1.3)
Fibroscan (kPa)	7.5 (3.2)	8.4 (3.5)	6.7 (2.7)
Fibroscan CAP score (dB/m)*	306 (60)	312 (84)	312 (65)
Lactulose (%)*	0.62 (1.4)	0.60 (1.2)	0.66 (1.7)
Mannitol (%)*	6.2 (3.3)	6.0 (4.1)	6.47 (2.7)
Lactulose-mannitol ratio	0.12 (0.3)	0.11 (0.2)	0.11 (0.3)
LPS (EU/mL)*	0.13 (0.1)	0.13 (0.1)	0.13 (0.1)
<i>Bifidobacterium</i> spp. (%)*	0.78 (1.85)	0.63 (1.89)	0.88 (1.75)
Acetic Acid (umol/g)*	146.2 (187.7)	155.68 (193.3)	145.99 (194.6)
Propionic Acid (umol/g)*	8.82 (6.1)	8.38 (5.1)	9.47 (6.4)
Butyric Acid (umol/g)*	6.53 (5.2)	6.22 (5.7)	6.97 (6.1)

Variables that are normally distributed are expressed as mean (SD). *Variables that are non-normally distributed are expressed as median (IQR). Acetic acid, propionic acid and butyric acid concentrations expression per gram of faeces.

Table 2. Anthropometric and biochemical characteristics at baseline and end of study according to

randomization group

Parameters	Placebo		Synbiotic	
	Baseline	End of Study	Baseline	End of Study
Weight (kg)	96.8 (17.1)	96.8 (17.9)	99.2 (16.1)	98.4 (17.4)
Physical activity (METs)	1.26 (0.3)	1.19 (0.2)	1.16 (0.3)	1.2 (0.3)
BMI (kg/m ²)	33.2 (4.9)	33.1 (5.1)	32.9 (5.5)	32.6 (5.6)
DEXA body fat %*	34.5 (11.0)	33.9 (10.7)	32.9 (12.1)	31.9 (10.9)
Systolic blood pressure (mmHg)	134.3 (18.9)	132.6 (15.2)	132.9 (12.9)	131.4 (12.2)
Diastolic blood pressure (mmHg)	72.5 (9.8)	73 (9.2)	75.7 (8.2)	75.4 (7.7)
Fasting glucose (mmol/L)*	6.1 (2.2)	5.9 (2.8)	6.2 (2.5)	5.9 (2.6)
Fasting insulin (μU/mL)	14.9 (9.5)	13.6 (7.6)	13.5 (7.9)	13.1 (7.5)
HbA1c (mmol/mol)*	39 (24.5)	41.5 (17.2)	41.0 (21.5)	41.0 (19.5)
Total cholesterol (mmol/L)	4.8 (1.2)	4.7 (1.1)	4.9 (1.2)	4.96 (1.2)
HDL-cholesterol (mmol/L)	1.2 (0.4)	1.2 (0.3)	1.2 (0.2)	1.21 (0.2)
LDL-cholesterol (mmol/L)	2.2 (1.0)	2.2 (0.9)	2.8 (0.8)	2.6 (0.8)
Total cholesterol/HDL-cholesterol ratio	4.0 (0.8)	3.9 (0.89)	4.1 (0.8)	4.2 (1.1)
Triglycerides (mmol/L)*	1.7 (0.8)	1.7 (0.6)	1.8 (1.1)	1.6 (1.2)
ALT (U/L)	61.7 (30.9)	55.38 (30.72)	59.0 (39.3)	57.0 (35.0)
AST (U/L)	40.5 (29.3)	40.8 (24)	34.0 (18.0)	34.0 (16.8)
GGT (U/L)*	56.0 (56.0)	42 (61)	51.0 (48.0)	40 (36)
HA (ug/L)*	38 (28)	51 (42)**	31 (47)	39 (58)
PIIINP (ug/L)	8.2 (3.2)	9.53 (4.6)**	8.3 (3.4)	8.9 (3.3)
TIMP-1 (ug/L)	160.2 (28.9)	201.2 (91.1)	155.5 (35.5)	202.9 (77.5)
MRS-measured liver fat (%)	22.9 (12.9, 44.7)	21.4 (10.7, 35.9)**	26.9 (9.7, 38.4)	23.7 (13.0, 42.0)
ELF score	6.9 (0.3)	7.0 (0.4)	6.9 (0.4)	7.0 (0.3)
NAFLD fibrosis score	-1.2 (1.3)	-1.3 (1.3)	-1.3 (1.3)	-1.6 (1.4)**
Fibroscan (kPa)	8.4 (3.5)	8.0 (3.7)	6.7 (2.7)	7.1 (3.8)
Fibroscan CAP score (dB/m)*	312 (78)	332 (110)	312 (65)	327 (51)
Lactulose-mannitol ratio	0.11 (0.2)	0.10 (0.19)	0.11 (0.3)	0.11 (0.26)
LPS (EU/mL)*	0.13 (0.1)	0.15 (0.1)	0.13 (0.1)	0.11 (0.1)
Bifidobacterium spp. (%)*	0.63 (1.89)	0.45 (1.28)	0.88 (1.75)	2.03(3.70)
Acetic Acid (umol/g)*	155.68 (193.3)	152.38 (184.4)	145.99 (194.6)	127 (158.7)
Propionic Acid (umol/g)*	8.38 (5.1)	8.34 (7.7)	9.47 (6.4)	8.3 (5.4)
Butyric Acid (umol/g)*	6.22 (5.7)	5.7 (5.9)	6.97 (6.1)	5.69 (3.3)

butyric acid concentrations expression per gram of faeces.

Variables that are normally distributed are expressed as mean (SD). Variables that are non-normally distributed (*) are expressed as median (IQR). ** p<0.05. Acetic acid, propionic acid and

Table 3. Baseline and end-of-study primary outcome results and regression models testing the effects of the synbiotic intervention and changes in *Bifidobacterium* spp. on the primary outcomes, adjusted for baseline measurement of primary outcome only* and fully adjusted**

	Placebo		Synbiotic		Difference in change from baseline to end of study* (95% CI) Synbiotic treatment Primary analysis	Adjusted difference in change from baseline to end of study** (95% CI) Synbiotic treatment Primary analysis	Adjusted difference in change from baseline to end of study** (95% CI) <i>Bifidobacterium</i> spp	Difference in change in weight from baseline to end of study*
Primary outcomes	Baseline	End of study	Baseline	End of study				
MRS-measured liver fat (%)	22.9 (12.9, 44.7)	21.4 (10.7, 35.9)	26.9 (9.7, 38.4)	23.7 (13.0, 42.0)	2.8 (-2.2, 7.8) p=0.30	4.1 (-0.02, 8.3) p=0.05	0.1 (-1.3, 1) p=0.80	1.9 (1.4, 2.5) p<0.001
ELF score	12.5 (0.7)	12.8 (0.8)	12.5 (0.9)	12.9 (0.8)	-0.0008 (-0.2, 0.2) p=1.0	0.02 (-0.2, 0.2) p=0.90	0.020 (-0.08, 0.04) p=0.50	0.015 (0.001, 0.03) p=0.039
NAFLD fibrosis score	-1.2 (1.3)	-1.3 (1.3)	-1.3 (1.3)	-1.6 (1.4)	-0.03 (-0.3, 0.2) p=0.80	-0.009 (-0.3, 0.3) p=0.90	0.005 (-0.02, 0.03) p=0.60	0.046 (0.008, 0.085) p=0.027

Results of multivariable (adjusted) linear regression models testing the effects of synbiotic treatment (primary analyses) and changes in *Bifidobacterium* levels between baseline and end of study.

For each pre-specified primary outcome (MRS liver fat content, ELF score and NAFLD fibrosis score; the difference in outcome represents the change in the outcome between baseline and end of study (* adjusted for baseline measurement of the outcome variable only, or ** adjusted for baseline measurement of the outcome *plus* age, sex, weight difference and baseline weight).

Table 4. Associations of baseline MRS-measured liver fat content, NAFLD fibrosis score and ELF score with gut microbiota at baseline

	MRS-measured Liver fat	NAFLD Fibrosis Score	ELF score	MRS-measured Liver fat	NAFLD Fibrosis Score	ELF score
pSRBC adjusted for age, sex, baseline weight	Correlation coefficients (rho)			p-value		
<i>Olsenella</i> genus	-0.32	-0.17	-0.10	0.001	0.108	0.354
Unclassified <i>Clostridia</i>	0.07	-0.37	-0.14	0.515	0.000	0.187
Unclassified <i>Clostridiales</i>	0.04	-0.36	-0.15	0.727	0.000	0.155
Unclassified <i>Firmicutes</i>	-0.03	-0.35	-0.11	0.803	0.000	0.284
<i>Sphingomonadales</i> order	-0.06	0.32	0.14	0.533	0.001	0.202
<i>Sphingomonadaceae</i> family	-0.06	0.32	0.14	0.533	0.001	0.202
<i>Sphingomonas</i> genus	-0.06	0.32	0.14	0.533	0.001	0.202
<i>Sutterella</i> genus	-0.02	0.10	0.31	0.833	0.320	0.002

pSRBC: partial Spearman rank-based correlation (adjusted for age, sex and body weight).

Table R2. Predictive metagenomics using Picrust. 42 patients in the placebo group and 44 patients in the synbiotics group.

Predicted function	Mean baseline placebo	Mean end of study placebo	Mean baseline synbiotics	Mean end of study synbiotics	SD baseline placebo	SD end of study placebo	SD baseline synbiotics	SD end of study synbiotics	p-value Wilcoxon paired test placebo	q-value Wilcoxon paired test placebo	p-value Wilcoxon paired test synbiotics	q-value Wilcoxon paired test synbiotics	p-value Mann-Whitney U test	q-value Mann-Whitney U test
D-Arginine and D-ornithine metabolism	265.35	247.275	226.75	268.4090909	113.5252259	158.4401509	95.24561273	91.05743605	0.091618561	1	0.008493193	1	0.003073386	0.875915116
Stilbenoid, diarylheptanoid and gingerol biosynthesis	315.45	356.475	337.0909091	272.0454545	201.4382835	271.3138014	252.3117137	184.8082544	0.343316391	1	0.020843012	1	0.017409232	1
N-Glycan biosynthesis	4089.9	4425.55	4524.909091	4211.136364	673.9313592	835.6731955	1192.938249	852.7657218	0.017996261	1	0.290894474	1	0.026645237	1
Glycosphingolipid biosynthesis - ganglio series	11725.9	12377.925	12917.86364	12168.43182	2648.473414	2713.935778	3542.441587	3194.183867	0.012015921	1	0.27263814	1	0.029345058	1
Glycosaminoglycan degradation	17333.625	18376.675	19184.68182	18314.25	4868.289615	4849.443777	5510.224048	4712.820328	0.048164485	1	0.327444308	1	0.059131923	1
Phosphotransferase system (PTS)	55580.15	55783.6	61816.34091	78986.70455	25139.32885	26845.79131	52093.74738	75151.51739	0.685015903	1	0.025976988	1	0.061616132	1
Phosphonate and phosphinate metabolism	11593.6	11356.225	12620.95455	13839.06818	2695.113858	2570.091972	3809.017629	5393.891025	0.375005878	1	0.111746752	1	0.076848615	1
Ion channels	2914.175	2869.9	3208.590909	3790.454545	1022.747579	1030.212321	2095.412634	2211.062004	0.549739024	1	0.067789929	1	0.087973434	1
G protein-coupled receptors	1.175	0.325	0.522727273	1.545454545	3.72026674	0.944281032	1.731898224	6.926372068	0.112851646	1	0.665167912	1	0.110246446	1
Flavone and flavonol biosynthesis	1796.35	1575.525	1715.5	1761.181818	931.0459393	799.3028369	864.241501	904.3488284	0.023110385	1	0.759441067	1	0.115989335	1
Fatty acid elongation in mitochondria	132.35	215.7	188.1590909	199.7727273	223.9309131	352.9061908	347.5197798	331.4798295	0.085204152	1	0.779917425	1	0.119595134	1
Ribosome biogenesis in eukaryotes	8709.45	8604.075	8915.386364	9480.340909	955.8157464	1071.427095	1177.245054	1880.55123	0.535971383	1	0.144623883	1	0.120605472	1
Apoptosis	473.325	532.2	441.9772727	410.6136364	357.8528123	456.7068408	262.9355292	326.4625208	0.497268916	1	0.161297816	1	0.126744429	1
Lipoic acid metabolism	5890.275	6327.35	6921.613636	7110.681818	2087.25473	1636.499823	3290.226233	3223.433802	0.072506332	1	0.598826471	1	0.129482361	1
Caffeine metabolism	132.025	215.75	188.0681818	200.0909091	222.7991492	352.6897198	347.5689925	331.2905574	0.069784189	1	0.890293307	1	0.133554146	1
Steroid biosynthesis	398.4	649.55	566.4545455	601.8863636	668.2836723	1057.056653	1042.629222	993.3265047	0.081413935	1	0.965081866	1	0.140576094	1
Cell cycle	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
Hepatitis C	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
Measles	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
mTOR signaling pathway	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
Lysosome	26215.9	27144.55	28820.68182	27967.54545	6949.390476	6505.006387	7100.053471	6476.906545	0.131558151	1	0.427798625	1	0.156480982	1
Systemic lupus erythematosus	132.2	215.825	188.1136364	199.9772727	222.6958602	352.7615514	347.7748012	331.613216	0.091143182	1	0.916564672	1	0.171585971	1
Vibrio cholerae pathogenic cycle	12883.425	12333.975	13656.27273	14347.65909	2414.361984	2199.304266	5908.147612	5698.759294	0.17435351	1	0.623183719	1	0.184996257	1
Benzoate degradation	33402.65	33515.4	34350.95455	40256.27273	8386.477334	8694.823396	9849.473833	24297.66802	0.857582336	1	0.114442471	1	0.190524847	1
Styrene degradation	2911.525	3096.275	2908.977273	3959.022727	1167.164589	1614.810221	1154.054158	4121.353466	0.93115225	1	0.06747004	1	0.193588091	1
Staphylococcus aureus infection	276.825	282.075	340.7727273	434.3181818	260.4241006	247.1907171	382.5460522	1104.597765	0.581548464	1	0.391013371	1	0.198700279	1
Chagas disease (American trypanosomiasis)	341.5	440.675	523.5909091	773.3409091	448.3272614	472.6734867	939.8197828	1032.964912	0.378634579	1	0.012302676	1	0.198702552	1
Flavonoid biosynthesis	909.85	937.325	1061.181818	1010.613636	579.4428602	590.4054838	957.4740818	1014.995342	0.636765143	1	0.078940093	1	0.205041544	1
Proximal tubule bicarbonate reclamation	4115.8	4564.875	4476.863636	5004.704545	1543.641868	1586.845026	1665.702207	3258.455245	0.064573524	1	0.925615712	1	0.23889665	1
Transporters	1145095.425	1118833.45	1209885.886	1318781.341	245476.8552	233538.6022	346038.7809	525069.4566	0.608505822	1	0.253094487	1	0.247541898	1
Various types of N-glycan biosynthesis	38.7	38.775	18.56818182	14.72727273	79.19052002	68.65278208	39.58999035	34.13813501	0.234768753	1	0.62285655	1	0.24817544	1
Cyanoamino acid metabolism	64057.9	60655.35	67152.13636	69173.77273	15127.75429	12455.40037	15842.44391	18907.45015	0.094498974	1	0.750653466	1	0.251229375	1
ABC transporters	575218.175	563192.625	606455.7273	662503.7273	119496.7638	115947.359	190002.4531	283699.9383	0.535971383	1	0.421055814	1	0.25871762	1
Toluene degradation	24612.95	25149.9	25985.27273	28180.11364	7521.147798	9263.18218	11216.33071	11420.5661	0.845470442	1	0.117188016	1	0.261008152	1
Synthesis and degradation of ketone bodies	3970.625	3859.425	3926.318182	4517.522727	1238.646484	1177.981213	1220.667541	2575.953447	0.847155624	1	0.205428083	1	0.262518546	1
Geraniol degradation	6610.075	7177.075	8214.772727	9193.318182	4072.888129	3715.322103	9879.39754	10672.17123	0.086652737	1	0.823234617	1	0.262912669	1
Linoleic acid metabolism	11371.325	11169.7	12196.77273	12515.27273	2757.890985	2501.594386	2340.676039	2868.302694	0.857582336	1	0.248265122	1	0.266357439	1
Protein processing in endoplasmic reticulum	12601.525	12734.75	13217.31818	13076.84091	1609.862217	1516.079116	1867.254766	2485.946761	0.805726984	1	0.356548966	1	0.266748008	1
Chlorocyclohexane and chlorobenzene degradation	1864.225	2059.325	2128.886364	2593.113636	778.5571628	941.4875406	1167.796558	1828.911014	0.307391545	1	0.030327584	1	0.270234366	1
Inositol phosphate metabolism	17425.625	18182.1	17950.06818	21826.202436	4477.321004	4344.202436	3220.47534	13911.89344	0.375380561	1	0.017059172	1	0.278484107	1
Mineral absorption	1137.075	1146.5	976.0227273	847.5681818	778.324347	686.0515044	558.3755296	441.0999675	0.840212451	1	0.07113398	1	0.274541107	1
RIG-I-like receptor signaling pathway	171.8	181.175	345.2727273	286.6136364	137.0242726	139.3861796	886.8046471	500.819896	0.951765431	1	0.181453222	1	0.276490934	1
1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	321.925	325.05	321.9545455	348.1363636	205.7924505	255.1205798	248.0736907	252.0691665	0.774995235	1	0.30441897	1	0.278484426	1
Phagosome	6.075	7.05	1.636363636	0.045454545	27.07670728	32.76329247	6.531109486	0.210707055	0.580712162	1	0.278517024	1	0.289448042	1
mRNA surveillance pathway	12.15	14.1	3.272727273	0.068181818	54.15341455	65.52658494	13.06221897	0.333949395	0.580712162	1	0.280712665	1	0.289456512	1
Ascorbate and aldarate metabolism	18403.475	19149.9	20843.84091	25648.25	6976.762092	7010.80623	14596.87402	24001.74036	0.665550807	1	0.150821772	1	0.290191463	1
Circadian rhythm - plant	135.625	218.575	192.6590909	205.8863636	222.5502176	351.7117765	347.0122271	329.1478932	0.230222285	1	0.896845278	1	0.298640489	1
D-Alanine metabolism	17659.425	17347.15	18358.61364	19071.43182	2405.819972	2284.375048	3528.857448	4418.733126	0.40493774	1	0.559201957	1	0.311106253	1
Pentose and glucuronate interconversions	102926.825	101050.275	111502.2045	119850.2955	25159.51313	21173.99942	31927.78049	47409.58672	0.627285164	1	0.327444308	1	0.311106253	1
Glutathione metabolism	31114.65	31229.55	34342.13636	37740.84091	6911.698951	6366.609861	18168.10339	20479.89986	0.941724097	1	0.150821772	1	0.315811704	1
Adipocytokine signaling pathway	14045.675	14417.6	14717.11364	14928.15909	1952.317169	1738.795189	1990.760498	2365.47233	0.01924554	1	0.690081471	1	0.320151978	1
Amoebiasis	1858.375	1944.75	2085.545455	1963.090909	686.2430865	758.8966941	817.7263275	655.8215447	0.642837453	1	0.437705051	1	0.324523721	1
Proteasome	8138.55	7963.225	8518.863636	8773.613636	1211.711651	1181.754009	1314.442982	1770.829621	0.261703594	1	0.639651458	1	0.328948971	1

Biosynthesis of ansamycins	21582.45	20949.9	22195.61364	24122.88636	4777.366461	4476.931675	5168.685174	9566.995461	0.484520777	1	0.448392587	1	0.337901143	1
Adherens junction	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Clavulanic acid biosynthesis	0	0	0	0.090909091	0	0	0	0.603022689 NA	NA	1	1	1	0.352564748	1
Cytochrome P450	0	0	0	0.045454545	0	0	0	0.301511345 NA	NA	1	1	1	0.352564748	1
Dilated cardiomyopathy (DCM)	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Focal adhesion	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Isoflavonoid biosynthesis	0	0	0	0.090909091	0	0	0	0.603022689 NA	NA	1	1	1	0.352564748	1
Leukocyte transendothelial migration	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Phototransduction - fly	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Regulation of actin cytoskeleton	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Tight junction	0	0	0	0.022727273	0	0	0	0.150755672 NA	NA	1	1	1	0.352564748	1
Vasopressin-regulated water reabsorption	0	0	0	0.045454545	0	0	0	0.301511345 NA	NA	1	1	1	0.352564748	1
Galactose metabolism	142630.1	139314.475	152419.4773	159322.4545	31638.97696	26942.3534	33108.92078	43885.16577	0.617864972	1	0.51336551	1	0.355813166	1
Phenylpropanoid biosynthesis	39677.075	36853.625	42224.95455	42842.22727	12729.71235	10179.83666	13262.18629	14204.31922	0.089208928	1	0.958542366	1	0.355813166	1
Transcription factors	291144.575	283762.35	310239.3864	338426.3409	67624.67163	62830.26558	107538.0651	158934.2659	0.420212251	1	0.606898554	1	0.369970246	1
African trypanosomiasis	411.675	516.275	605.8863636	826.8181818	442.9810662	500.4347334	926.4896796	1031.39847	0.484579933	1	0.059456808	1	0.3700449756	1
Pores ion channels	67264.175	68930.925	74829.04545	78730.13636	13777.79539	13142.87191	36930.81184	40299.08062	0.264874036	1	0.894310661	1	0.3700452179	1
Steroid hormone biosynthesis	4892.15	5200.15	5473.227273	5488.977273	1825.802185	2063.996466	2506.6107	2480.609523	0.443733318	1	0.839774901	1	0.379597873	1
Vibrio cholerae infection	6.85	8.075	11.65909091	0.477272727	27.02093206	32.65231566	53.34004263	0.927327652	0.248072348	1	0.7716228	1	0.379756316	1
Retinol metabolism	4085.175	4283.625	4547.818182	5420.818182	1380.2473	1489.369298	3053.09222	4151.711237	0.628446886	1	0.115076215	1	0.38252722	1
Lipopolysaccharide biosynthesis	45705.975	48063.725	49272.63636	50556.09091	9515.587464	9914.262355	21941.28501	21388.72843	0.170088119	1	0.995391589	1	0.38937636	1
Porphyrin and chlorophyll metabolism	156677.6	151184.95	163079.75	169578.1591	33687.18977	28199.39884	36299.26626	50725.9964	0.361103988	1	0.707209643	1	0.38937636	1
Renin-angiotensin system	2.375	3.025	3.181818182	3.636363636	4.650268809	5.811074749	6.085716129	8.531994257	0.793772688	1	0.636605762	1	0.400561908	1
Prostate cancer	8701.225	8599.1	9130.545455	8983.136364	1271.060177	1163.591804	1345.744291	1847.218595	0.553699557	1	0.299440771	1	0.404325165	1
beta-Alanine metabolism	33094.575	33301.225	36215.70455	37274.61364	6692.646584	5839.096411	14908.03781	17925.58026	0.826382224	1	0.407755058	1	0.40938259	1
Hypertrophic cardiomyopathy (HCM)	0.2	0.35	0.204545455	0.386363636	0.516397779	0.622237485	0.509424913	1.4177596	0.250456648	1	0.804814717	1	0.420528677	1
Photosynthesis proteins	71893.625	69489.575	74692.20455	76522.04545	12790.94599	12811.29686	16249.87601	17319.21016	0.397423832	1	0.785982868	1	0.445813324	1
Carbohydrate metabolism	32505.85	31865.925	35815.81818	38171.90909	8676.293763	7601.971999	13580.23551	16052.44907	0.65589929	1	0.491188694	1	0.451162787	1
Glyoxylate and dicarboxylate metabolism	94440.925	93372.1	100983.0455	108045.7727	19033.67714	16500.71495	26508.24348	40232.42375	0.724554494	1	0.400762053	1	0.451162787	1
Lipopolysaccharide biosynthesis proteins	65148.375	67618.175	70678.90909	74806.65909	13819.39174	13678.11901	30038.37754	35729.64188	0.196854553	1	0.976690955	1	0.451162787	1
Nitrotoluene degradation	17438.925	17016.55	17641.02273	18284.15909	3314.836449	3365.170632	4811.647068	5842.162127	0.646303622	1	0.543709192	1	0.456547993	1
Photosynthesis	71240.275	68805.575	73949.54545	75656	12825.34132	12832.80016	16074.41796	17051.42794	0.368200169	1	0.794886249	1	0.456547993	1
Butanoate metabolism	114792.3	114314.35	120161.0455	129067.4091	18039.08954	17992.3076	31112.55162	44654.14297	0.816038972	1	0.462422613	1	0.467424862	1
Cysteine and methionine metabolism	170378.15	167225.6	179380.25	187864.6818	25409.96345	22785.22997	38944.72671	52355.19866	0.40493774	1	0.733174042	1	0.467424862	1
RNA polymerase	27341.825	27108.375	28310.75	28839.22727	3007.367747	2914.043148	4554.883891	5279.984984	0.553699557	1	0.687221542	1	0.468166834	1
Other ion-coupled transporters	231652.275	228000.6	248596.7273	265986.8636	39989.3075	36803.4657	80080.37118	99800.03132	0.459813673	1	0.72448427	1	0.468171338	1
Bacterial toxins	19378.7	19425.025	20983.84091	21368.20455	3809.503522	3468.288357	3623.645087	4250.110885	0.994697614	1	0.304912347	1	0.472916121	1
D-Glutamine and D-glutamate metabolism	26579.875	26300.775	27685	28669.5	3522.961998	3223.347014	4265.663791	6184.70709	0.646303622	1	0.647951695	1	0.484003227	1
Dioxin degradation	8810.05	9083.075	9504.545455	11411.5	3377.012524	3495.049806	4159.566147	8368.303516	0.685015903	1	0.238796771	1	0.484003227	1
Fructose and mannose metabolism	165917.925	165405.4	177250.5	188934.2045	31556.6343	29101.96473	41653.06052	63695.14345	0.9410677	1	0.421055814	1	0.493246486	1
Glycerolipid metabolism	68159	67651.325	71703.84091	76595.40909	13952.06723	13442.92185	16150.29024	26714.0752	0.952305993	1	0.483910104	1	0.49522829	1
Glycosphingolipid biosynthesis - globo series	24712.65	24966.8	26482.45455	26212.56818	4505.31178	3934.132043	4553.837387	5119.069035	0.553699557	1	0.639651458	1	0.49522829	1
Xylene degradation	8515.775	8727.575	8943.022727	10391.13636	2971.161402	3053.717154	3252.478979	6229.474779	0.665550807	1	0.273050394	1	0.49522829	1
Sporulation	137676.4	129739.15	140812.2955	140791.0682	18174.87016	38135.51342	42261.52225	54548.4235	0.28865785	1	0.866965215	1	0.50089197	1
Prion diseases	968.6	976.075	1047.454545	1171.568182	395.6379334	402.4920058	872.7960271	913.9123513	0.840212451	1	0.465761149	1	0.501759793	1
Huntington's disease	4800.85	5124.35	5280.295455	5552.772727	1554.834339	1419.483394	2809.776798	2932.47155	0.178696416	1	0.631395182	1	0.507481876	1
Secondary bile acid biosynthesis	7701.95	7500.3	8448.477273	8634.045455	2446.042591	2103.315627	2195.358746	2696.462713	0.816038982	1	0.567026264	1	0.507481876	1
Starch and sucrose metabolism	208639.675	200722.175	222650.6591	230954.5	49257.92565	41861.5703	58619.99305	71233.27056	0.28865785	1	0.839774901	1	0.512320394	1
Primary bile acid biosynthesis	7734.525	7531.45	8488.477273	8679.977273	2457.438545	2109.025391	2188.60639	2714.949256	0.805726984	1	0.590802441	1	0.518084628	1
Primary immunodeficiency	8741.2	8595.2	9308.931818	9617.522727	1391.170015	1454.164933	2217.223084	2336.07217	0.793237981	1	0.59541997	1	0.519019379	1
Bisphenol degradation	13922.275	13819.425	14868.90909	15094.11364	3198.196679	2962.268958	2633.286282	3132.78308	0.893074824	1	0.559201957	1	0.523881855	1
Tuberculosis	27527.025	27103.825	28652.11364	29303.90909	3981.788618	4137.121859	4401.652751	5745.580112	0.412534034	1	0.759441067	1	0.523881855	1
Electron transfer carriers	3433.625	3615.625	4314.818182	5245.136364	2896.863365	2624.389254	6052.612952	7209.621567	0.329805829	1	0.995391589	1	0.524842798	1
Sphingolipid metabolism	48464.75	47193.025	52283.86364	53116.20455	12562.02366	10386.88264	10891.54617	11952.25377	0.571703779	1	0.958171297	1	0.539542954	1
Cytoskeleton proteins	70910.475	68867.05	72737.18182	74118.77273	14036.88586	13170.09544	14165.14973	19016.65195	0.397423832	1	0.930958095	1	0.547395156	1
Thiamine metabolism	92252.875	90710.525	96280.31818	99669.06818	14656.0104	13422.0636	17263.17937	23034.58983	0.636765143	1	0.647951695	1	0.547395156	1
Pathogenic Escherichia coli infection	0	0.125	1.136363636	0.022727273	0	0.790569415	7.537783614	0.150755672	1	1	1	1	0.549231636	1
Bacterial chemotaxis	91821.6	85859.6	92743.20455	92663.93182	29648.56399	26552.74258	37705.38236	43826.19516	0.237050311	1	0.894310661	1	0.553353132	1
Isoquinoline alkaloid biosynthesis	9807.275	9717.775	10264.29545	10735.90909	1211.414431	1186.647302	2728.331312	3484.312623	0.435623973	1	0.71751715	1	0.55444443	1

Penicillin and cephalosporin biosynthesis	3817.825	4175.525	4052.159091	4933.272727	1336.360593	1382.438963	1109.050127	3709.481142	0.183117418	1	0.866965215	1	0.5544443	1
Nucleotide excision repair	70964.075	70124.05	73684.40909	75060.52273	9106.827054	9052.748734	11301.36598	14259.62777	0.382644861	1	0.912611615	1	0.559342368	1
Signal transduction mechanisms	82237.575	80667.05	88144.93182	92190.20455	16681.68587	15533.31991	26293.50779	31217.1453	0.562667682	1	0.750653466	1	0.559342368	1
Nitrogen metabolism	132454.525	131435.8	139176.9318	146674.6591	21402.2044	18382.14143	29565.86385	46676.79697	0.704687674	1	0.567026264	1	0.56536255	1
Streptomycin biosynthesis	62887.85	62536.275	66067.20455	67688.06818	10744.20339	9328.772852	10501.00393	15347.04057	0.805726984	1	0.647951695	1	0.56536255	1
Atrazine degradation	3743.95	4197.95	4392.75	4835.068182	1407.161353	2168.432885	2923.892445	3927.708779	0.439591436	1	0.921779757	1	0.575638595	1
Terpenoid backbone biosynthesis	99686.6	98567.05	103115.0909	105480.5682	12462.94187	11654.76899	14953.76133	19221.87605	0.459813673	1	0.768259206	1	0.577494475	1
Amyotrophic lateral sclerosis (ALS)	2366.55	2522.075	2651.545455	2962.545455	1023.038735	1002.831667	1924.525667	2561.042079	0.319892672	1	0.884016207	1	0.578695391	1
Nucleotide metabolism	8873.475	9029.45	9691.75	11330.97727	3497.697316	3374.987042	8086.951325	10285.57263	1	1	0.363173909	1	0.583605562	1
Carotenoid biosynthesis	511.425	566.775	728.1818182	724.0681818	485.691254	508.3849484	927.0061657	1033.437308	0.910045961	1	0.603532038	1	0.587913319	1
Amino sugar and nucleotide sugar metabolism	263903.775	260125	280377.5227	290886.2727	43365.35059	37676.64036	58485.71712	72721.34884	0.646303622	1	0.857882999	1	0.589746284	1
Chloroalkane and chloroalkene degradation	28685.075	29325.55	30660.06818	32673.81818	6486.369283	6408.808709	7416.039397	11184.51657	0.571703779	1	0.199286615	1	0.589746284	1
Bladder cancer	124.325	139.275	228.5227273	330.2045455	416.512898	368.4977116	900.5087577	942.16442	0.404920269	1	0.989272691	1	0.596973947	1
Alanine, aspartate and glutamate metabolism	200315.4	198122.5	211132.8864	217232.0682	31087.35913	27049.3594	34402.0662	44836.36164	0.826382224	1	0.520869619	1	0.602115252	1
Alzheimer's disease	8928.175	8944.625	9230.727273	9717.681818	1073.824053	1167.296914	1741.880117	2770.135013	0.857582336	1	0.567026264	1	0.608342793	1
Others	166085.525	164632.1	177017.9091	187776.9318	32023.73636	29374.04638	45959.53119	64901.98601	0.764824907	1	0.574901594	1	0.608342793	1
Ubiquitin system	1195.325	1376.9	1505.159091	1990.818182	726.7036354	690.5043419	1098.210954	1906.887251	0.03128888	1	0.02466878	1	0.612863238	1
Peptidases	339035.425	333670.05	355561.0455	368387.8409	45657.06525	41877.66375	66561.22068	88426.5951	0.484520777	1	0.777106828	1	0.614598556	1
Glycosphingolipid biosynthesis - lacto and neolacto series	38.6	37.1	35.54545455	31.95454545	43.40081531	49.99784611	48.39010784	43.16973237	0.683714769	1	0.299798966	1	0.615863689	1
Selenocompound metabolism	65957.525	65127.9	69796.79545	73243.86364	10148.99434	9268.168621	15246.2264	21116.73921	0.617864972	1	0.664680578	1	0.620882176	1
Tryptophan metabolism	21480.125	22525.8	23930.36364	27192.15909	7529.089253	7544.542477	15520.19102	21670.77316	0.361103988	1	0.777106828	1	0.620882176	1
PPAR signaling pathway	21014.675	21275.45	22002.79545	22711.09091	2961.201267	2585.163844	2739.358095	3798.781756	0.50137474	1	0.273050394	1	0.627193277	1
Biosynthesis and biodegradation of secondary metabolites	11770.754	12076.625	13345.99049	14911.45455	4199.12777	3984.407717	9742.123072	12020.51038	0.704687674	1	0.72448427	1	0.62864251	1
Glycolysis / Gluconeogenesis	200310.9	198897.725	212274.2273	223620.4091	34407.09901	32207.69548	50157.5095	68889.41612	0.805726984	1	0.582827231	1	0.62864251	1
Propanoate metabolism	84227.8	84628.725	88736.18182	95062.52273	14713.69591	14229.62857	22971.65386	34691.82025	0.910045961	1	0.375595505	1	0.633531479	1
Purine metabolism	387706.175	383510.15	406837.4545	420021.5227	46654.17783	42818.64056	75182.49109	94918.77273	0.544800451	1	0.759441067	1	0.633531479	1
Pentose phosphate pathway	158244.425	156311.7	168082.1818	176283.9318	29721.75845	27249.17455	40245.38907	54041.05128	0.826382224	1	0.690081471	1	0.639896397	1
Glycine, serine and threonine metabolism	146096.6	144697.75	155442.0227	162390.75	21316.65726	19030.13843	35337.4801	45938.22647	0.608505822	1	0.794886249	1	0.64628764	1
Lysine biosynthesis	148685.825	146682.825	156562.4773	161058.0227	25413.67784	23001.15439	29363.7957	37042.49721	0.685015903	1	0.707209643	1	0.64628764	1
Arachidonic acid metabolism	3700.4	3765.725	3498.613636	3982.977273	2345.024354	2232.633698	2326.236068	3618.228704	0.914367263	1	0.640631778	1	0.651059016	1
Calcium signaling pathway	0.75	0.65	1.090909091	0.5	1.514883426	1.687814548	2.540995793	1.130959667	0.486398021	1	0.239069339	1	0.654360334	1
Peptidoglycan biosynthesis	147166.775	144844.45	153814.5	157558.25	20716.48578	19050.21275	26634.92663	34079.4605	0.382644861	1	0.958542366	1	0.659147505	1
Sulfur relay system	45496.75	44847.6	48811.31818	52271.84091	9851.126357	9277.331059	17457.60804	21943.645	0.617864972	1	0.803815884	1	0.659147505	1
Two-component system	282466.025	277022.875	300888.4318	319452.5455	61306.66169	54307.57601	110277.798	138976.835	0.65589929	1	0.812770678	1	0.659147505	1
Limonene and pinene degradation	13556.1	14136.525	15095.79545	16164.88636	3510.50864	3430.109933	8584.91616	9390.175903	0.326894475	1	0.861036905	1	0.665615315	1
Phenylalanine, tyrosine and tryptophan biosynthesis	156028.9	153709.1	165381.8864	170501.9545	27195.70122	23533.88407	31945.8746	42227.62597	0.724544494	1	0.759441067	1	0.665615315	1
Plant-pathogen interaction	30248.575	29223.075	31384.34091	32013.63636	5931.176019	5269.761326	6790.771203	8900.412256	0.313807068	1	0.986175368	1	0.665615315	1
Replication, recombination and repair proteins	138781.1	137703.05	144339.75	149036.5909	20873.19078	21122.134	29030.58892	37940.01995	0.675256809	1	0.876064671	1	0.665615315	1
Endocytosis	0.15	0.075	3.409090909	0.022727273	0.662164284	0.474341649	22.61335084	0.150755672	0.772829993	1	1	1	0.670186016	1
Fc gamma R-mediated phagocytosis	0.15	0.075	3.409090909	0.022727273	0.662164284	0.474341649	22.61335084	0.150755672	0.772829993	1	1	1	0.670186016	1
GnRH signaling pathway	0.15	0.075	3.409090909	0.022727273	0.662164284	0.474341649	22.61335084	0.150755672	0.772829993	1	1	1	0.670186016	1
Non-homologous end-joining	1188.925	1350.55	1654.75	1706.477273	1057.328743	1291.021797	1973.020411	2181.759419	0.816038982	1	0.656295029	1	0.672107828	1
Pyrimidine metabolism	324611.425	320097.65	336672	344290.1364	37818.05044	34922.9556	55089.81756	69470.77008	0.459813673	1	0.995391589	1	0.672107828	1
Pyruvate metabolism	183701.2	183746.8	195438.3864	206440.7045	33873.93906	30948.97681	42196.20274	66598.76049	0.89951513	1	0.528428679	1	0.678624624	1
Ether lipid metabolism	193.6	184	192.3863636	195.0227273	145.7544123	84.37735752	107.8351921	92.67936441	0.716640805	1	0.540068468	1	0.683607592	1
Bacterial motility proteins	184504.1	177491.15	190078.8636	188423.0455	58605.68262	55402.86904	91116.3237	96527.07641	0.599208943	1	0.986175368	1	0.685165279	1
Parkinson's disease	118.125	76.6	82.68181818	119	302.1600177	134.3126471	185.7335892	353.0472661	0.913357745	1	0.441814265	1	0.686809043	1
Valine, leucine and isoleucine biosynthesis	134737.875	134289.175	142880.0682	148510.5909	24738.4698	23221.57705	29235.87672	38717.47233	0.984093759	1	0.606898554	1	0.691729363	1
Valine, leucine and isoleucine degradation	33862.85	34607.375	36256.75	39903.75	7022.122648	6759.612186	13230.54406	20613.2165	0.704687674	1	0.333238581	1	0.69352512	1
Function unknown	220496.3	219856.875	239913.5	257916.7273	44762.36371	40302.56807	96454.30139	117944.9476	0.636765143	1	0.606898554	1	0.69831644	1
Lysine degradation	20582.9	21002.225	23470.68182	24981.77273	6151.542041	5624.64286	16403.59576	16470.80687	0.636765143	1	0.839774901	1	0.69831644	1
RNA degradation	85553.35	84622.2	89682.97727	91736.52273	10398.19559	9870.966924	14491.21412	17404.75686	0.589975534	1	0.930958095	1	0.69831644	1
Citrate cycle (TCA cycle)	118470.9	119741.975	123630.5909	130075.1818	11743.71869	13193.90051	23420.60615	36366.07642	0.910045961	1	0.51336551	1	0.7001489	1
Fluorobenzoate degradation	479.825	754.075	665.3409091	1194.181818	931.102459	1092.645587	1121.651812	2709.306882	0.125433671	1	0.309955508	1	0.7001489	1
C5-Branched dibasic acid metabolism	57548.85	57279.25	61008.95455	63896.43182	10715.74081	9754.830868	14479.97867	18996.87186	0.984093759	1	0.647951695	1	0.703467971	1
Fatty acid metabolism	36916.875	37675.2	40476.54545	44207.38636	8783.595228	8141.276121	18430.14658	22392.53296	0.646303622	1	0.321714505	1	0.704926072	1
Glycan biosynthesis and metabolism	6791.6	7010.4	7440.477273	8179.545455	2603.680618	2334.568722	6853.567092	6540.581307	0.294816961	1	0.700148783	1	0.706795566	1
Protein kinases	54983.55	54306.45	60170.88636	64055.18182	12902.56717	11427.79782	23527.84099	29071.15565	0.714597445	1	0.803815884	1	0.706795566	1
Zeatin biosynthesis	9905.825	9773.225	10267.75	10362.86364	1208.827868	1119.223139	1305.0315	1482.184742	0.455667159	1	0.940145439	1	0.710127339	1
alpha-Linolenic acid metabolism	824.475	889	1164.022727	1444.409091	1230.522194	1099.019003	2752.111997	2807.196858	0.435623973	1	0.829065385	1	0.710127339	1

Caprolactam degradation	2794.35	3195.75	3954.863636	5050.181818	3435.070809	3196.134362	8006.530243	8643.642789	0.397423832	1	0.211899533	1	0.711557812	1
Glutamatergic synapse	20175.575	20065.925	21264.81818	21656.81818	3466.277216	3058.488938	3260.579027	4500.531469	0.96289612	1	0.623183719	1	0.713464662	1
Drug metabolism - other enzymes	57945.55	57326.625	59681.43182	61193.70455	7200.354101	6590.120561	9234.532016	13099.96199	0.627285164	1	0.750653466	1	0.718211212	1
Metabolism of xenobiotics by cytochrome P450	3760.15	4129.55	4737.840909	5895.227273	2461.250115	2243.908129	5475.657395	6729.667627	0.270692344	1	0.16848305	1	0.718211212	1
Restriction enzyme	36488.775	36668	36954.40909	37977.13636	6045.951383	6004.625969	5318.609113	8755.100564	0.704687674	1	0.921779757	1	0.718211212	1
Vitamin B6 metabolism	38092.9	37963.8	39879.20455	41732.09091	5032.96194	4880.614091	8183.885143	10950.53672	0.836755011	1	0.715829138	1	0.720155728	1
Nicotinate and nicotinamide metabolism	77500.05	76386.625	81785.25	84779.81818	11054.83914	10114.4322	19300.30405	23063.96702	0.608505822	1	0.866965215	1	0.724885816	1
Tyrosine metabolism	57691.875	58048.225	61794.84091	66233	10560.48898	9887.735686	18120.52807	25690.06118	0.984093759	1	0.673107444	1	0.724885816	1
Carbon fixation in photosynthetic organisms	117774.375	117002.625	123482.2045	128736.2045	17032.53467	15857.54644	23371.48173	34314.69314	0.764824907	1	0.623183719	1	0.731581165	1
Cell cycle - Caulobacter	90207.45	89254.875	93563.61364	94716.95455	11155.02353	10359.90711	14107.3959	16652.91833	0.562667682	1	0.949340595	1	0.731581165	1
Phosphatidylinositol signaling system	16580.7	16729.825	17702.15909	18482.02273	2635.546722	2383.884676	3151.440025	4395.795256	0.857582336	1	0.441467386	1	0.731581165	1
beta-Lactam resistance	4075.975	4236.35	4627.25	5126.795455	1344.379821	1244.626513	1329.996103	2648.75853	0.65589929	1	0.921779757	1	0.733601898	1
Folate biosynthesis	66057.025	66994.7	70565.36364	72698.02273	7063.824694	6782.689767	13958.41861	16700.90554	0.571703779	1	0.967749554	1	0.738296795	1
Metabolism of cofactors and vitamins	19544	19867.825	21500.25	23309.86364	3720.818128	4051.150023	10009.4307	11916.15304	0.984093759	1	0.567026264	1	0.738296795	1
Renal cell carcinoma	943.9	1050.3	1101.136364	1321.522727	493.5328841	488.2722129	960.7115239	1005.888673	0.196754649	1	0.106502698	1	0.743738189	1
Polycyclic aromatic hydrocarbon degradation	19827	19671.125	20641.90909	21281.52273	3523.565953	3353.109611	3540.06273	4691.723497	0.847155624	1	0.750653466	1	0.758560664	1
Arginine and proline metabolism	224413.6	223444.175	239404.7955	248760.2995	40780.69385	36146.74638	48369.31793	69118.87989	0.984093759	1	0.639651458	1	0.765352689	1
DNA repair and recombination proteins	502759.225	498016.35	525502.0455	538996.7727	60276.27071	56648.04935	92274.79356	114638.0745	0.599208943	1	0.866965215	1	0.765352689	1
Energy metabolism	167918.325	168128.225	176014.4091	180399.9545	22055.99196	21025.16095	37567.63112	48838.30033	0.97349265	1	0.759441067	1	0.765352689	1
Fatty acid biosynthesis	82992.65	82775.525	87599.27273	91076.02273	13024.92541	12502.45754	17576.18732	24154.37048	0.816038982	1	0.777106828	1	0.765352689	1
Peroxisome	31685.625	32072.6	33707.29545	34807.86364	4235.304813	3626.211414	5881.980087	7358.097285	0.24244683	1	0.375595505	1	0.765352689	1
Ubiquinone and other terpenoid-quinone biosynthesis	30910.075	32119.425	34083.47727	37280.52273	8055.756885	8269.93031	18144.05176	22306.1081	0.40493774	1	0.543709192	1	0.765352689	1
Cell division	14846.5	14802.05	16054.79545	16216.29545	1952.946531	1986.895645	3771.25773	3826.678909	0.919700599	1	0.715829138	1	0.770991151	1
Drug metabolism - cytochrome P450	3915.025	4365.7	4947.227273	6108.886364	2492.960587	2312.63065	5493.411406	6802.203816	0.333141171	1	0.216226733	1	0.777848558	1
Ribosome Biogenesis	253897.95	249921.45	264857.9773	272811.0227	33284.62747	31907.47667	53537.93778	66689.16302	0.459813673	1	0.995391589	1	0.778989933	1
Other glycan degradation	63144.575	61980.375	69018.72727	68938.31818	15597.57174	12841.45908	15166.42466	15507.64206	0.742951676	1	0.579346956	1	0.784725508	1
Meiosis - yeast	850.5	1045.325	946.4772727	1012.454545	500.5194225	701.2136507	794.1778691	665.7811099	0.091824062	1	0.285592331	1	0.788169783	1
Cardiac muscle contraction	89.85	59.05	67.88636364	91.20454545	225.7847341	101.0450395	152.629848	265.2227304	0.969872339	1	0.621783779	1	0.791359047	1
Membrane and intracellular structural molecules	102094.35	102826.225	111742.0455	116772.0227	16161.81826	15629.05812	44805.40587	48310.40725	0.752098107	1	0.940145439	1	0.791618204	1
Bacterial secretion system	107592.275	109089.6	114269.9318	124602.6591	19375.83876	19019.84911	33324.07504	56286.25374	0.984093759	1	0.590802441	1	0.792694818	1
Butirosin and neomycin biosynthesis	14832.025	14581.275	15760.97727	15926.25	3472.92058	3055.409763	3420.580486	4523.628591	0.685015903	1	0.949340595	1	0.792694818	1
Mismatch repair	147373.3	145291.025	153072.0227	155834.6364	18946.42243	17928.27922	25701.41012	31856.98725	0.451733939	1	0.976960955	1	0.792694818	1
Photosynthesis - antenna proteins	13.25	10.825	19	9.227272727	25.58119301	28.10957269	42.82088167	18.68199436	0.385091151	1	0.223166927	1	0.799441795	1
Aminoacyl-tRNA biosynthesis	212230.3	210666.575	219044.4091	224134.2273	27486.68789	28768.44913	34186.42852	45876.45843	0.675256809	1	0.921779757	1	0.80646335	1
Riboflavin metabolism	42590.2	41967.6	44483.43182	46179.86364	4868.61946	4504.460404	11024.85547	13649.01652	0.313807068	1	0.741897447	1	0.80646335	1
Amino acid metabolism	33585.975	34354.275	35443.29545	38837.31818	7347.669376	7177.063741	9616.702528	19616.68202	0.627285164	1	0.469526576	1	0.813370219	1
Methane metabolism	230055.15	227391.825	241938.5455	247816.6818	40418.02813	36179.31373	48970.84481	64010.05536	0.93115225	1	0.894310661	1	0.813370219	1
Amino acid related enzymes	268732.425	266292.5	280249.4091	287883.4318	35026.98497	33246.03237	47576.69266	62667.15173	0.646303622	1	0.903454856	1	0.820291476	1
Cellular antigens	7774.45	7941.025	8319.25	8628.818182	1505.62511	1949.160509	2430.555924	3118.999029	0.686769122	1	0.498524484	1	0.820291476	1
Epithelial cell signaling in Helicobacter pylori infection	16696.8	16522.425	17721.54545	17860.79545	2648.517275	2423.234868	3913.733312	4853.75675	0.774995235	1	0.623183719	1	0.820291476	1
Flagellar assembly	76898.4	73354.65	78905.93182	75722.34091	26161.31041	25079.37424	41721.25624	43589.51609	0.608505822	1	0.839774901	1	0.820291476	1
Homologous recombination	168266.1	166741.25	174746.3182	178620.6818	19647.27553	19031.34502	28335.35464	35485.99229	0.599208943	1	0.986175368	1	0.820291476	1
Protein export	107002.1	105703.05	111852.9091	113790.6364	12838.94466	11514.01966	17871.07673	21650.43697	0.49291002	1	0.967749554	1	0.820291476	1
Tropane, piperidine and pyridine alkaloid biosynthesis	21436.825	21379.4	22494.97727	23450.25	3075.098013	2724.000892	4359.300933	6352.677118	0.87850707	1	0.761564023	1	0.820291476	1
Novobiocin biosynthesis	24466.375	24192.05	25543.43182	26439.40909	3504.846203	3107.346976	4769.000199	6047.579771	0.731781314	1	0.785982868	1	0.826316242	1
Chromosome	288724.775	283907.325	301372.5455	309732.0455	38323.78269	35268.5928	56172.2786	72772.1159	0.467971859	1	0.866965215	1	0.827226605	1
NOD-like receptor signaling pathway	8232.025	8081.45	8667.5	8721.568182	1229.51069	1136.127001	1331.66473	1920.783917	0.535971383	1	0.894310661	1	0.827226605	1
Pantothenate and CoA biosynthesis	115043.725	113979.1	120543.0682	124286.8864	18756.54582	17462.16039	23553.60948	30993.4619	0.847155624	1	0.967749554	1	0.827226605	1
DNA replication	117169.175	116086.25	121777.1364	123909.4091	12860.42847	12406.58173	19145.00501	23277.14834	0.608505822	1	0.995391589	1	0.834175088	1
Polyketide sugar unit biosynthesis	39961.3	39621.35	41792.47727	41808.29545	6557.129806	5680.421186	6360.111969	8017.624132	0.65589929	1	1	1	0.834175088	1
Cell motility and secretion	31908.05	32833.9	34315.15909	35660.90909	4474.767852	5452.562135	10185.53391	10958.94607	0.764824907	1	0.759441067	1	0.836795491	1
Shigellosis	0.7	0.55	2.454545455	0.431818182	3.27539889	1.678674691	15.21543669	1.561122543	1	1	0.786030953	1	0.838192162	1
Betalain biosynthesis	0.875	1.125	0.409090909	1.068181818	3.25172538	2.936835031	2.265188342	4.479398461	0.483303242	1	0.14343262	1	0.840516585	1
Indole alkaloid biosynthesis	0.875	1.125	0.409090909	1.068181818	3.25172538	2.936835031	2.265188342	4.479398461	0.483303242	1	0.14343262	1	0.840516585	1
Biosynthesis of unsaturated fatty acids	21067.925	21708.125	23230.88636	25433.86364	5388.600467	5823.089022	11482.44653	14523.10111	1	1	0.631395182	1	0.841136405	1
Translation proteins	162728.05	160957.2	169380.4773	174655.2727	20992.73808	19948.86993	32763.5107	42281.77417	0.580806766	1	0.949340595	1	0.841136405	1
Protein digestion and absorption	4281.775	4286.325	4602.636364	4545.136364	1121.624245	1376.435836	1175.133067	1224.467888	0.795447917	1	0.575360752	1	0.847303016	1
Biotin metabolism	28318.075	27997.6	30564.34091	30908.34091	5023.049944	4344.356167	7023.399776	7764.423985	0.836755011	1	0.785982868	1	0.84811003	1
Biosynthesis of type II polyketide products	0.225	0.45	0.090909091	0.295454545	0.73336247	1.431334325	0.290803363	1.111871363	0.528232777	1	0.203091788	1	0.848509167	1
Biosynthesis of vancomycin group antibiotics	12768.575	12704.725	13289.34091	13330.06818	1958.082328	1781.945753	2023.910548	2467.140284	0.685015903	1	0.81544628	1	0.855095437	1

Prenyltransferases	56927.225	57094.525	59242.56818	60130.36364	5596.567916	5519.140327	8223.557436	9633.735191	0.868033403	1	0.976960955	1	0.855095437	1
Ribosome	421786.525	417882.2	434660	441573.0455	45601.12518	45583.67618	61799.67383	78077.58961	0.527213339	1	0.894310661	1	0.855095437	1
Type I diabetes mellitus	10090	10118.225	10550.52273	10555.86364	1428.27574	1272.218343	1418.654841	1677.062598	0.704687674	1	0.876064671	1	0.855095437	1
Transcription machinery	188452.475	186519.25	196306.0455	197438.5227	28203.5931	26844.02869	27593.7338	36292.50102	0.65589929	1	0.949340595	1	0.876117004	1
Sulfur metabolism	52859.925	52689.75	57171.81818	59761.56818	9948.578629	9075.868293	14013.46063	18077.28471	0.96289612	1	0.664680578	1	0.890180573	1
Type II diabetes mellitus	8611.9	8537.575	9203.590909	9411.522727	1424.939833	1233.095789	2375.050005	2865.289169	0.984093759	1	0.995391589	1	0.900216597	1
Ethylbenzene degradation	7589.775	7580.025	7962.363636	8052.181818	1252.917344	1168.494923	2960.746795	2711.489613	0.962477305	1	0.876064671	1	0.903762636	1
DNA replication proteins	225357.375	224291.525	234142.4091	237597	25058.66813	23726.13551	36782.44729	44630.53915	0.459813673	1	0.848819179	1	0.904278373	1
Lipid biosynthesis proteins	107183.1	107299.125	112808.1591	116951.9091	15695.46452	14480.22938	21183.8485	30262.16874	1	1	0.912611615	1	0.904278373	1
Histidine metabolism	119706.575	116968.4	127294.2273	129149.75	22325.11264	19410.32732	21992.74155	29006.43163	0.774995235	1	0.995391589	1	0.911338763	1
Inorganic ion transport and metabolism	34059.4	34767	39291.43182	42611.95455	10100.96435	9339.162495	24310.43244	27331.1939	0.882456461	1	0.812770678	1	0.911338763	1
Other transporters	49863.025	49311.2	53087.13636	55295.65909	8607.787102	7399.634177	13965.57893	17843.55011	0.675256809	1	0.876064671	1	0.911338763	1
Pertussis	4656.975	4961.55	6778.75	8363.704545	5950.650132	5463.354717	14441.73379	14424.90257	0.467971859	1	0.51336551	1	0.911338763	1
Aminobenzoate degradation	21214.325	21922.875	22067.97727	24258.79545	3809.263671	4178.444909	3808.00644	10815.3561	0.571703779	1	0.715829138	1	0.914411997	1
Bacterial invasion of epithelial cells	124.225	115.275	81.77272727	101.1136364	295.0329393	203.3590973	158.8560653	193.7684084	0.635065983	1	0.716619367	1	0.917948326	1
MAPK signaling pathway - yeast	8054.2	8278.2	8674.113636	8791.545455	1893.50882	1607.835497	1904.802819	2103.769789	0.382284815	1	0.606898554	1	0.918406085	1
Tetracycline biosynthesis	22933.825	23077.3	24717.5	25568.5	5040.12074	5009.011015	6215.368546	7804.087753	0.816038982	1	0.498524484	1	0.918406085	1
Lipid metabolism	24796.15	24501.25	26890.59091	28094.54545	4780.932366	4650.193041	11128.31162	12853.65009	0.571703779	1	0.733174042	1	0.925479789	1
Oxidative phosphorylation	206496.825	205896.225	216951.3636	221300.8864	22658.29231	22541.26263	38864.04576	44079.50447	0.826382224	1	0.894310661	1	0.925479789	1
Phenylalanine metabolism	34395.85	35168.6	36672.72727	40490.75	6839.974181	6446.779056	10171.87419	19465.34447	0.599208943	1	0.388270715	1	0.925479789	1
General function prediction only	652733.425	646978.975	689703.7273	711428.2273	96268.95778	86752.10117	147259.5771	191205.6051	0.744604358	1	0.976960955	1	0.932559327	1
One carbon pool by folate	113768.45	112675.875	117475.5	119233.5455	12400.80579	11751.96192	16786.17201	20983.03631	0.484520777	1	0.930958095	1	0.932559327	1
Protein folding and associated processing	110859.75	112221.85	119507.0227	124736.2955	15098.22253	14536.2257	35045.42908	40258.67569	0.889001579	1	0.848819179	1	0.939644145	1
Chaperones and folding catalysts	188000.925	186739.5	198752.2727	204070.5682	22218.92687	20640.92516	40734.06232	49797.64553	0.608505822	1	0.857882999	1	0.953827416	1
Insulin signaling pathway	16212.475	16161.6	17372.38636	17956.75	3783.035242	3563.372832	4304.81263	6206.890028	0.941724097	1	0.912611615	1	0.953827416	1
Naphthalene degradation	22851.4	23508.85	24328.02273	25622.20455	4353.341839	4304.673915	6191.352734	8666.079384	0.397423832	1	0.407755058	1	0.953827416	1
Carbon fixation pathways in prokaryotes	185915.65	186928	194295.9091	200526.0682	19714.64677	19203.61374	32664.70785	44513.6927	0.795447917	1	0.967749554	1	0.960924758	1
Chronic myeloid leukemia	0.1	0.05	0.090909091	0.181818182	0.496138938	0.220721428	0.290803363	0.921897116	0.586213681	1	1	1	0.961883767	1
Notch signaling pathway	0.1	0.05	0.090909091	0.181818182	0.496138938	0.220721428	0.290803363	0.921897116	0.586213681	1	1	1	0.961883767	1
Wnt signaling pathway	0.1	0.05	0.090909091	0.181818182	0.496138938	0.220721428	0.290803363	0.921897116	0.586213681	1	1	1	0.961883767	1
Base excision repair	77464.65	77248.375	81688.09091	83431.38636	10422.53013	9933.420006	15535.30549	19064.84099	0.910045961	1	0.986175368	1	0.968025166	1
Secretion system	214159.525	215882.6	228369.2727	245627.5	48113.36622	45666.53853	94484.24756	120560.6961	0.994697614	1	0.986175368	1	0.968025166	1
Basal transcription factors	41.425	44.85	35.81818182	33.06818182	109.919758	135.6690071	53.89493341	63.07411577	0.884266723	1	0.94313501	1	0.971375748	1
Pathways in cancer	8911.875	8829.75	9414.840909	9639.5	1055.10311	1082.296152	1813.496083	2240.056981	0.617864972	1	0.976960955	1	0.971421845	1
p53 signaling pathway	27.725	17.025	14.22727273	27.70454545	75.7001465	33.51806822	37.82632505	88.00279582	0.786136951	1	0.823046278	1	0.974922327	1
Germination	8397.525	8205.875	8498.636364	8193.840909	2360.980609	2391.749399	2379.579542	3029.998664	0.617864972	1	0.430846051	1	0.974992861	1
Taurine and hypotaurine metabolism	19926.55	20141.85	21110.75	21844.75	2684.668217	2533.876209	3978.273759	5590.417753	0.694826674	1	0.698626764	1	0.975128082	1
Viral myocarditis	27.725	17.025	14.22727273	27.68181818	75.7001465	33.51806822	37.82632505	88.00363965	0.786136951	1	0.823046278	1	0.978503803	1
Carbohydrate digestion and absorption	3001.3	2852.85	3396	3687.090909	1299.092291	1292.511579	2655.839201	3002.692852	0.518804353	1	0.794886249	1	0.978564378	1
Colorectal cancer	27.725	17.025	14.22727273	27.65909091	75.7001465	33.51806822	37.82632505	88.00474173	0.786136951	1	0.823046278	1	0.982085931	1
Small cell lung cancer	27.725	17.025	14.22727273	27.65909091	75.7001465	33.51806822	37.82632505	88.00474173	0.786136951	1	0.823046278	1	0.982085931	1
Toxoplasmosis	27.725	17.025	14.22727273	27.65909091	75.7001465	33.51806822	37.82632505	88.00474173	0.786136951	1	0.823046278	1	0.982085931	1
Antigen processing and presentation	7940.15	7762.375	8299.386364	8290.136364	1164.486561	1088.75249	1287.654894	1803.059991	0.339910107	1	0.528428679	1	0.98223295	1
Glycosyltransferases	60624.625	60661.275	65733.56818	68317.95455	8781.384651	8189.004808	19178.97215	22218.18832	0.694826672	1	0.986175368	1	0.98223295	1
Progesterone-mediated oocyte maturation	7940.15	7762.375	8299.386364	8290.136364	1164.486561	1088.75249	1287.654894	1803.059991	0.339910107	1	0.528428679	1	0.98223295	1
RNA transport	25597.8	25123.3	26650.29545	27233.27273	4647.622917	4359.725757	5434.287149	7084.408562	0.774995235	1	0.866965215	1	0.989339212	1
Transcription related proteins	893.7	1035.775	1102.909091	1566.909091	985.0564789	917.1830477	1817.461708	2683.229838	0.282584112	1	0.29356813	1	0.989339212	1
Influenza A	33.8	24.075	15.86363636	27.70454545	79.18339634	46.12045961	39.83663787	87.99671758	0.793354632	1	0.727506649	1	0.992832553	1
Bile secretion	0.5	0.375	2.227272727	1.636363636	2.063106943	1.371645091	10.65279445	8.683292991	1	1	0.952682032	1	1	1
Biosynthesis of siderophore group nonribosomal peptides	3261.925	3475.525	3966.772727	6188.159091	3846.567203	3911.686894	6304.988047	11919.34363	0.941724097	1	0.861036905	1	1	1
Glycerophospholipid metabolism	104042.75	102896.6	110081.8182	113539.0455	10809.10669	16515.99822	24715.06583	32457.08271	0.685015903	1	0.885180209	1	1	1
Translation factors	96376	95669.175	100102.1591	101603.5909	10441.80737	10051.60751	15030.5834	18771.7787	0.589975534	1	0.912611615	1	1	1
Aldosterone-regulated sodium reabsorption	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Biosynthesis of 12-, 14- and 16-membered macrolides	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Biosynthesis of type II polyketide backbone	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
CAM ligands	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Cell cycle - yeast	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Cholinergic synapse	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Complement and coagulation cascades	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Cytokine receptors	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA

Cytokine-cytokine receptor interaction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Cytosolic DNA-sensing pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
ECM-receptor interaction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Endocrine and other factor-regulated calcium reabsorption	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
ErbB signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Fat digestion and absorption	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Fc epsilon RI signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
GTP-binding proteins	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Gastric acid secretion	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glioma	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glycan binding proteins	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glycosaminoglycan biosynthesis - chondroitin sulfate	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Hedgehog signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Hematopoietic cell lineage	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Leishmaniasis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Long-term depression	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Long-term potentiation	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
MAPK signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Melanogenesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Neuroactive ligand-receptor interaction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Neurotrophin signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Olfactory transduction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Oocyte meiosis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Other types of O-glycan biosynthesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Pancreatic cancer	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Pancreatic secretion	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Phototransduction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Rheumatoid arthritis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Salivary secretion	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Sesquiterpenoid biosynthesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Spliceosome	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
TGF-beta signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
VEGF signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Vascular smooth muscle contraction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA

Table SZ. Predictive metagenomics using Picrust. 42 patients in the placebo group and 44 patients in the synbiotics group.

Predicted function	Mean baseline placebo	Mean end of study placebo	Mean baseline synbiotics	Mean end of study synbiotics	SD baseline placebo	SD end of study placebo	SD baseline synbiotics	SD end of study synbiotics	p-value Wilcoxon paired test placebo	q-value Wilcoxon paired test placebo	p-value Wilcoxon paired test synbiotics	q-value Wilcoxon paired test synbiotics	p-value Mann-Whitney U test	q-value Mann-Whitney U test
D-Arginine and D-orithine metabolism	265.35	247.275	226.75	268.4090909	113.5252259	158.4401509	95.24561273	91.05743605	0.091618561	1	0.008493193	1	0.003073386	0.875915116
Stilbenoid, diarylheptanoid and gingerol biosynthesis	315.45	356.475	337.0909091	272.0454545	201.4382835	271.3138014	252.3117137	184.8082544	0.343316391	1	0.020843012	1	0.017409232	1
N-Glycan biosynthesis	4089.9	4425.55	4524.909091	4211.136364	673.9313592	835.6731955	1192.938249	852.7657218	0.017996261	1	0.290894474	1	0.026645237	1
Glycosphingolipid biosynthesis - ganglio series	11725.9	12377.925	12917.86364	12168.43182	2648.473414	2713.935778	3542.441587	3194.183867	0.012015921	1	0.27263814	1	0.029345058	1
Glycosaminoglycan degradation	17333.625	18376.675	19184.68182	18314.25	4868.289615	4849.443777	5510.224048	4712.820328	0.048164485	1	0.327444308	1	0.059131923	1
Phosphotransferase system (PTS)	55580.15	55783.6	61816.34091	78986.70455	25139.32885	26845.79131	52093.74738	75151.51739	0.685015903	1	0.025976988	1	0.061616132	1
Phosphonate and phosphinate metabolism	11593.6	11356.225	12620.95455	13839.06818	2695.113858	2570.091972	3809.017629	5393.891025	0.375005878	1	0.111746752	1	0.076848615	1
Ion channels	2914.175	2869.9	3208.590909	3790.454545	1022.747579	1030.212321	2095.412634	2211.062004	0.549739024	1	0.067789929	1	0.087973434	1
G protein-coupled receptors	1.175	0.325	0.522727273	1.545454545	3.72026674	0.944281032	1.731898224	6.926372068	0.112851646	1	0.665167912	1	0.110246446	1
Flavone and flavonol biosynthesis	1796.35	1575.525	1715.5	1761.181818	931.0459393	799.3028369	864.241501	904.3488284	0.023110385	1	0.759441067	1	0.115989335	1
Fatty acid elongation in mitochondria	132.35	215.7	188.1590909	199.7727273	223.9309131	352.9061908	347.5197798	331.4798295	0.085204152	1	0.779917425	1	0.119595134	1
Ribosome biogenesis in eukaryotes	8709.45	8604.075	8915.386364	9480.340909	955.8157464	1071.427095	1177.245054	1880.55123	0.535971383	1	0.144623883	1	0.120605472	1
Apoptosis	473.325	532.2	441.9772727	410.6136364	357.8528123	456.7068408	262.9355292	326.4625208	0.497268916	1	0.161297816	1	0.126744429	1
Lipoic acid metabolism	5890.275	6327.35	6921.613636	7110.681818	2087.25473	1636.499823	3290.226233	3223.433802	0.072506332	1	0.598826471	1	0.129482361	1
Caffeine metabolism	132.025	215.75	188.0681818	200.0909091	222.7991492	352.6897198	347.5689925	331.2905574	0.890293307	1	0.135541446	1	0.135541446	1
Steroid biosynthesis	398.4	649.55	566.4545455	601.8863636	668.2836723	1057.056653	1042.629222	993.3265047	0.081413935	1	0.965081866	1	0.140576094	1
Cell cycle	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
Hepatitis C	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
Measles	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
mTOR signaling pathway	6.075	7.05	1.636363636	0.022727273	27.07670728	32.76329247	6.531109486	0.150755672	0.580712162	1	0.201242621	1	0.151794019	1
Lysosome	26215.9	27144.55	28820.68182	27967.54545	6949.390476	6505.006387	7100.053471	6476.906545	0.131558151	1	0.427798625	1	0.156480982	1
Systemic lupus erythematosus	132.2	215.825	188.1136364	199.9772727	222.6958602	352.7615514	347.7748012	331.613216	0.091143182	1	0.916564672	1	0.171585971	1
Vibrio cholerae pathogenic cycle	12883.425	12333.975	13656.27273	14347.65909	2414.361984	2199.304266	5908.147612	5698.759294	0.17435351	1	0.623183719	1	0.184996257	1
Benzoate degradation	33402.65	33515.4	34350.95455	40256.27273	8386.477334	8694.823396	9849.473833	24297.66802	0.857582336	1	0.114442471	1	0.190524847	1
Styrene degradation	2911.525	3096.275	2908.977273	3959.022727	1167.164589	1614.810221	1154.054158	4121.353466	0.93115225	1	0.06747004	1	0.193588091	1
Staphylococcus aureus infection	276.825	282.075	340.7727273	434.3181818	260.4241006	247.1907171	382.5460522	1104.597765	0.581548464	1	0.391013371	1	0.198700279	1
Chagas disease (American trypanosomiasis)	341.5	440.675	523.5909091	773.3409091	448.3272614	472.6734867	939.8197828	1032.964912	0.378634579	1	0.012302676	1	0.198702552	1
Flavonoid biosynthesis	909.85	937.325	1061.181818	1010.613636	579.4428602	590.4054838	957.4740818	1014.995342	0.636765143	1	0.078940093	1	0.205041544	1
Proximal tubule bicarbonate reclamation	4115.8	4564.875	4476.863636	5004.704545	1543.641868	1586.845026	1665.702207	3258.455245	0.064573524	1	0.925615712	1	0.23889665	1
Transporters	1145095.425	1118833.45	1209885.886	1318781.341	245476.8552	233538.6022	346038.7809	525069.4566	0.608505822	1	0.253094487	1	0.247541898	1
Various types of N-glycan biosynthesis	38.7	38.775	18.56818182	14.72727273	79.19052002	68.65278208	39.58999035	34.13813501	0.234768753	1	0.62285655	1	0.24817544	1
Cyanoamino acid metabolism	64057.9	60655.35	67152.13636	69173.72723	15127.75429	12455.40037	15842.44391	18907.45015	0.094498974	1	0.750653466	1	0.251229375	1
ABC transporters	575218.175	563192.625	606455.7273	662503.7273	119496.7638	115947.359	190002.4531	283699.9383	0.535971383	1	0.421055814	1	0.25871762	1
Toluene degradation	24612.95	25149.9	25985.27273	28180.11364	7521.147798	9263.18218	11216.33071	11420.5661	0.845470442	1	0.117188016	1	0.261008152	1
Synthesis and degradation of ketone bodies	3970.625	3859.425	3926.318182	4517.522727	1238.646484	1177.981213	1220.667541	2575.953447	0.847155624	1	0.205428083	1	0.262518546	1
Geraniol degradation	6610.075	7177.075	8214.772727	9193.318182	4072.888129	3715.322103	9879.39754	10672.17123	0.086652737	1	0.823234617	1	0.262912669	1
Linoleic acid metabolism	11371.325	11169.7	12196.77273	12515.27273	2757.890985	2501.594386	2340.676039	2868.302694	0.857582336	1	0.248265122	1	0.266357439	1
Protein processing in endoplasmic reticulum	12601.525	12734.75	13217.31818	13076.84091	1609.862217	1516.079116	1867.254766	2485.946761	0.805726984	1	0.356548966	1	0.266748008	1
Chlorocyclohexane and chlorobenzene degradation	1864.225	2059.325	2128.886364	2593.113636	778.5571628	941.4875406	1167.796558	1828.911014	0.307391545	1	0.030327584	1	0.270234366	1
Inositol phosphate metabolism	17425.625	18182.1	17950.06818	21826.202436	4477.321004	4477.321004	3220.47534	13911.89344	0.375380561	1	0.017059172	1	0.278484107	1
Mineral absorption	1137.075	1146.5	976.0227273	847.5681818	778.324347	686.0515044	558.3755296	441.0999675	0.840212451	1	0.071133398	1	0.274541107	1
RIG-I-like receptor signaling pathway	171.8	181.175	345.2727273	286.6136364	137.0242726	139.3861796	886.8046471	500.819896	0.951765431	1	0.181453222	1	0.276490934	1
1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	321.925	325.05	321.9254545	348.1363636	205.7924505	255.1205798	248.0736907	252.0691665	0.774995235	1	0.30441897	1	0.278484426	1
Phagosome	6.075	7.05	1.636363636	0.045454545	27.07670728	32.76329247	6.531109486	0.210707055	0.580712162	1	0.278517024	1	0.289448042	1
mRNA surveillance pathway	12.15	14.1	3.272727273	0.068181818	54.15341455	65.52658494	13.06221897	0.333949395	0.580712162	1	0.280712665	1	0.289456512	1
Ascorbate and aldarate metabolism	18403.475	19149.9	20843.84091	25648.25	6976.762092	7010.80623	14596.87402	24001.74036	0.665550807	1	0.150821772	1	0.290191463	1
Circadian rhythm - plant	135.625	218.575	192.6590909	205.8863636	222.5502176	351.7117765	347.0122271	329.1478932	0.230222285	1	0.896845278	1	0.298640489	1
D-Alanine metabolism	17659.425	17347.15	18358.61364	19071.43182	2405.819972	2284.375048	3528.857448	4418.733126	0.40493774	1	0.559201957	1	0.311106253	1
Pentose and glucuronate interconversions	102926.825	101050.275	111502.2045	119850.2955	25159.51313	21173.99942	31927.84049	47409.58672	0.627285164	1	0.327444308	1	0.311106253	1
Glutathione metabolism	31114.65	31229.55	34342.13636	37740.84091	6911.698951	6366.609861	18168.10339	20479.89986	0.941724097	1	0.150821772	1	0.315811704	1
Adipocytokine signaling pathway	14045.675	14417.6	14717.11364	14928.15909	1952.317169	1738.795189	1990.760498	2365.47233	0.01924554	1	0.690081471	1	0.320151978	1
Amoebiasis	1858.375	1944.75	2085.545455	1963.090909	686.2430865	758.8966941	817.7263275	655.8215447	0.642837453	1	0.437705051	1	0.324523721	1
Proteasome	8138.55	7963.225	8518.863636	8773.613636	1211.711651	1181.754009	1314.442982	1770.829621	0.261703594	1	0.639651458	1	0.328948971	1

Biosynthesis of ansamycins	21582.45	20949.9	22195.61364	24122.88636	4777.366461	4476.931675	5168.685174	9566.995461	0.484520777	1	0.448392587	1	0.337901143	1
Adherens junction	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Clavulanic acid biosynthesis	0	0	0	0.090909091	0	0	0	0.603022689	NA	NA	1	1	0.352564748	1
Cytochrome P450	0	0	0	0.045454545	0	0	0	0.301511345	NA	NA	1	1	0.352564748	1
Dilated cardiomyopathy (DCM)	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Focal adhesion	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Isoflavonoid biosynthesis	0	0	0	0.090909091	0	0	0	0.603022689	NA	NA	1	1	0.352564748	1
Leukocyte transendothelial migration	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Phototransduction - fly	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Regulation of actin cytoskeleton	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Tight junction	0	0	0	0.022727273	0	0	0	0.150755672	NA	NA	1	1	0.352564748	1
Vasopressin-regulated water reabsorption	0	0	0	0.045454545	0	0	0	0.301511345	NA	NA	1	1	0.352564748	1
Galactose metabolism	142630.1	139314.475	152419.4773	159322.4545	31638.97696	26942.3534	33108.92078	43885.16577	0.617864972	1	0.51336551	1	0.355813166	1
Phenylpropanoid biosynthesis	39677.075	36853.625	42224.95455	42842.22727	12729.71235	10179.83666	13262.18629	14204.31922	0.089208928	1	0.958542366	1	0.355813166	1
Transcription factors	291144.575	283762.35	310239.3864	338426.3409	67624.67163	62830.26558	107538.0651	158934.2659	0.420212251	1	0.606898554	1	0.369970246	1
African trypanosomiasis	411.675	516.275	605.8863636	826.8181818	442.9810662	500.4347334	926.4896796	1031.39847	0.484579933	1	0.059456808	1	0.3700449756	1
Pores ion channels	67264.175	68930.925	74829.04545	78730.13636	13777.79539	13142.87191	36930.81184	40299.08062	0.264874036	1	0.894310661	1	0.370452179	1
Steroid hormone biosynthesis	4892.15	5200.15	5473.227273	5488.977273	1825.802185	2063.996466	2506.6107	2480.609523	0.443733318	1	0.839774901	1	0.379597873	1
Vibrio cholerae infection	6.85	8.075	11.65909091	0.477272727	27.02093206	32.65231566	53.34004263	0.927327652	0.248072348	1	0.7716228	1	0.379756316	1
Retinol metabolism	4085.175	4283.625	4547.818182	5420.818182	1380.2473	1489.369298	3053.09222	4151.711237	0.628446886	1	0.115076215	1	0.38252722	1
Lipopolysaccharide biosynthesis	45705.975	48063.725	49272.63636	50556.09091	9515.587464	9914.262355	21941.28501	21388.72843	0.170088119	1	0.995391589	1	0.38937636	1
Porphyrin and chlorophyll metabolism	156677.6	151184.95	163079.75	169578.1591	33687.18977	28199.39884	36299.26626	50725.9964	0.361103988	1	0.707209643	1	0.38937636	1
Renin-angiotensin system	2.375	3.025	3.181818182	3.636363636	4.650268809	5.811074749	6.085716129	8.531994257	0.793772688	1	0.636605762	1	0.400561908	1
Prostate cancer	8701.225	8599.1	9130.545455	8983.136364	1271.060177	1163.591804	1345.744291	1847.218595	0.553699557	1	0.299440771	1	0.404325165	1
beta-Alanine metabolism	33094.575	33301.225	36215.70455	37274.61364	6692.646584	5839.096411	14908.03781	17925.58026	0.826382224	1	0.407755058	1	0.40938259	1
Hypertrophic cardiomyopathy (HCM)	0.2	0.35	0.204545455	0.386363636	0.516397779	0.622237485	0.509424913	1.4177596	0.250456648	1	0.804814717	1	0.420528677	1
Photosynthesis proteins	71893.625	69489.575	74692.20455	76522.04545	12790.94599	12811.29686	16249.87601	17319.21016	0.397423832	1	0.785982868	1	0.445813324	1
Carbohydrate metabolism	32505.85	31865.925	35815.81818	38171.90909	8676.293763	7601.971999	13580.23551	16052.44907	0.65589929	1	0.491188694	1	0.451162787	1
Glyoxylate and dicarboxylate metabolism	94440.925	93372.1	100983.0455	108045.7727	19033.67714	16500.71495	26508.24348	40232.42375	0.724554494	1	0.400762053	1	0.451162787	1
Lipopolysaccharide biosynthesis proteins	65148.375	67618.175	70678.90909	74806.65909	13819.39174	13678.11901	30038.37754	35729.64188	0.196854553	1	0.976690955	1	0.451162787	1
Nitrotoluene degradation	17438.925	17016.55	17641.02273	18284.15909	3314.836449	3365.170632	4811.647068	5842.162127	0.646303622	1	0.543709192	1	0.456547993	1
Photosynthesis	71240.275	68805.575	73949.54545	75656	12825.34132	12832.80016	16074.41796	17051.42794	0.368200169	1	0.794886249	1	0.456547993	1
Butanoate metabolism	114792.3	114314.35	120161.0455	129067.4091	18039.08954	17992.3076	31112.55162	44654.14297	0.816038982	1	0.462422613	1	0.467424862	1
Cysteine and methionine metabolism	170378.15	167225.6	179380.25	187864.6818	25409.96345	22785.22997	38944.72671	52355.19866	0.40493774	1	0.733174042	1	0.467424862	1
RNA polymerase	27341.825	27108.375	28310.75	28839.22727	3007.367747	2914.043148	4554.883891	5279.984984	0.553699557	1	0.687221542	1	0.468166834	1
Other ion-coupled transporters	231652.275	228000.6	248596.7273	265986.8636	39989.3075	36803.4657	80080.37118	99800.03132	0.459813673	1	0.72448427	1	0.468171338	1
Bacterial toxins	19378.7	19425.025	20983.84091	21368.20455	3809.503522	3468.288357	3623.645087	4250.110885	0.994697614	1	0.304912347	1	0.472916121	1
D-Glutamine and D-glutamate metabolism	26579.875	26300.775	27685	28669.5	3522.961998	3223.347014	4265.663791	6184.70709	0.646303622	1	0.647951695	1	0.484003227	1
Dioxin degradation	8810.05	9083.075	9504.545455	11411.5	3377.012524	3495.049806	4159.566147	8368.303516	0.685015903	1	0.238796771	1	0.484003227	1
Fructose and mannose metabolism	165917.925	165405.4	177250.5	188934.2045	31556.6343	29101.96473	41653.06052	63695.14345	0.9410677	1	0.421055814	1	0.493246486	1
Glycerolipid metabolism	68159	67651.325	71703.84091	76595.40909	13952.06723	13442.92185	16150.29024	26714.0752	0.952305993	1	0.483910104	1	0.49522829	1
Glycosphingolipid biosynthesis - globo series	24712.65	24966.8	26482.45455	26212.56818	4505.31178	3934.132043	4553.837387	5119.069035	0.553699557	1	0.639651458	1	0.49522829	1
Xylene degradation	8515.775	8727.575	8943.022727	10391.13636	2971.161402	3053.717154	3252.478979	6229.474779	0.665550807	1	0.273050394	1	0.49522829	1
Sporulation	137676.4	129739.15	140812.2955	140791.0682	18174.87016	38135.51342	42261.52225	54548.4235	0.28865785	1	0.866965215	1	0.50089197	1
Prion diseases	968.6	976.075	1047.454545	1171.568182	395.6379334	402.4920058	872.7960271	913.9123513	0.840212451	1	0.465761149	1	0.501759793	1
Huntington's disease	4800.85	5124.35	5280.295455	5552.772727	1554.834339	1419.483394	2809.776798	2932.47155	0.178696416	1	0.631395182	1	0.507481876	1
Secondary bile acid biosynthesis	7701.95	7500.3	8448.477273	8634.045455	2446.042591	2103.315627	2195.358746	2696.462713	0.816038982	1	0.567026264	1	0.507481876	1
Starch and sucrose metabolism	208639.675	200722.175	222650.6591	230954.5	49257.92565	41861.5703	58619.99305	71233.27056	0.28865785	1	0.839774901	1	0.512320394	1
Primary bile acid biosynthesis	7734.525	7531.45	8488.477273	8679.977273	2457.438545	2109.025391	2188.60639	2714.949256	0.805726984	1	0.590802441	1	0.518084628	1
Primary immunodeficiency	8741.2	8595.2	9308.931818	9617.522727	1391.170015	1454.164933	2217.223084	2336.07217	0.793237981	1	0.59541997	1	0.519019379	1
Bisphenol degradation	13922.275	13819.425	14868.90909	15094.11364	3198.196679	2962.268958	2633.286282	3132.78308	0.893074824	1	0.559201957	1	0.523881855	1
Tuberculosis	27527.025	27103.825	28652.11364	29303.90909	3981.788618	4137.121859	4401.652751	5745.580112	0.412534034	1	0.759441067	1	0.523881855	1
Electron transfer carriers	3433.625	3615.625	4314.818182	5245.136364	2896.863365	2624.389254	6052.612952	7209.621567	0.329805829	1	0.995391589	1	0.524842798	1
Sphingolipid metabolism	48464.75	47193.025	52283.86364	53116.20455	12562.02366	10386.88264	10891.54617	11952.25377	0.571703779	1	0.958171297	1	0.539542954	1
Cytoskeleton proteins	70910.475	68867.05	72737.18182	74118.77273	14036.88586	13170.09544	14165.14973	19016.65195	0.397423832	1	0.930958095	1	0.547395156	1
Thiamine metabolism	92252.875	90710.525	96280.31818	99669.06818	14656.0104	13422.0636	17263.17937	23034.58983	0.636765143	1	0.647951695	1	0.547395156	1
Pathogenic Escherichia coli infection	0	0.125	1.136363636	0.022727273	0	0.790569415	7.537783614	0.150755672	1	1	1	1	0.549231636	1
Bacterial chemotaxis	91821.6	85859.6	92743.20455	92663.93182	29648.56399	26552.74258	37705.38236	43826.19516	0.237050311	1	0.894310661	1	0.553353132	1
Isoquinoline alkaloid biosynthesis	9807.275	9717.775	10264.29545	10735.90909	1211.414431	1186.647302	2728.331312	3484.312623	0.435623973	1	0.71751715	1	0.55444443	1

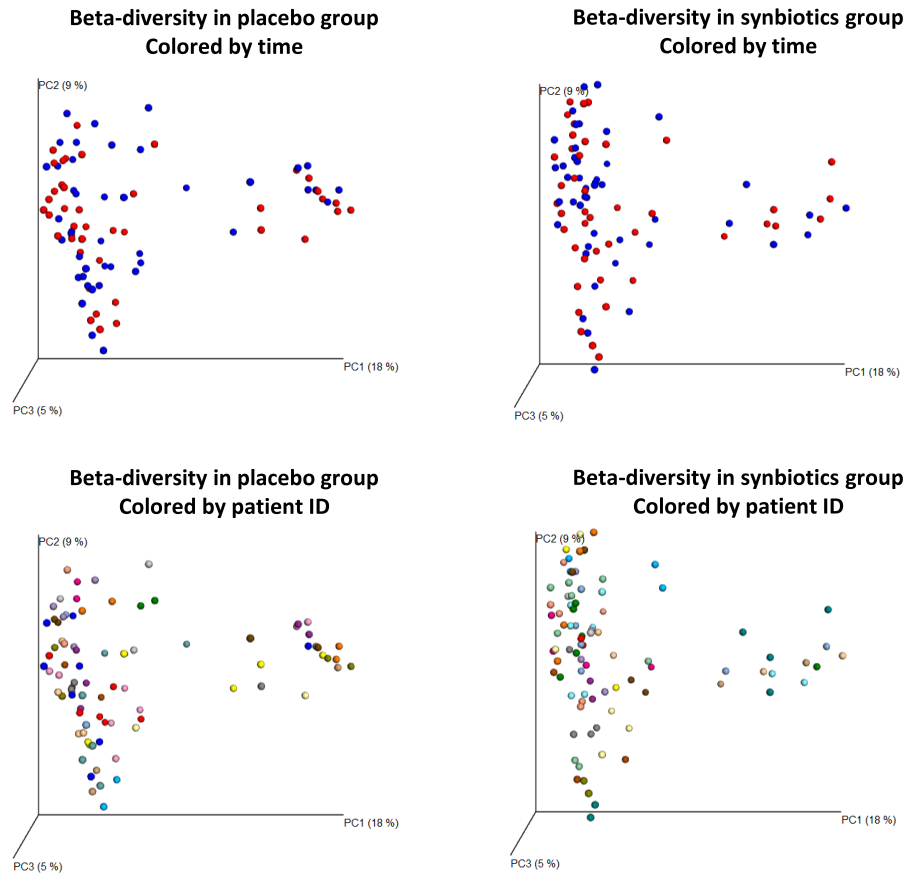
Penicillin and cephalosporin biosynthesis	3817.825	4175.525	4052.159091	4933.272727	1336.360593	1382.438963	1109.050127	3709.481142	0.183117418	1	0.866965215	1	0.5544443	1
Nucleotide excision repair	70964.075	70124.05	73684.40909	75060.52273	9106.827054	9052.748734	11301.36598	14259.62777	0.382644861	1	0.912611615	1	0.559342368	1
Signal transduction mechanisms	82237.575	80667.05	88144.93182	92190.20455	16681.68587	15533.31991	26293.50779	31217.1453	0.562667682	1	0.750653466	1	0.559342368	1
Nitrogen metabolism	132454.525	131435.8	139176.9318	146674.6591	21402.2044	18382.14143	29565.86385	46676.79697	0.704687674	1	0.567026264	1	0.56536255	1
Streptomycin biosynthesis	62887.85	62536.275	66067.20455	67688.06818	10744.20339	9328.772852	10501.00393	15347.04057	0.805726984	1	0.647951695	1	0.56536255	1
Atrazine degradation	3743.95	4197.95	4392.75	4835.068182	1407.161353	2168.432885	2923.892445	3927.708779	0.439591436	1	0.921779757	1	0.575638595	1
Terpenoid backbone biosynthesis	99686.6	98567.05	103115.0909	105480.5682	12462.94187	11654.76899	14953.76133	19221.87605	0.459813673	1	0.768259206	1	0.577494475	1
Amyotrophic lateral sclerosis (ALS)	2366.55	2522.075	2651.545455	2962.545455	1023.038735	1002.831667	1924.525667	2561.042079	0.319892672	1	0.884016207	1	0.578695391	1
Nucleotide metabolism	8873.475	9029.45	9691.75	11330.97727	3497.697316	3374.987042	8086.951325	10285.57263	1	1	0.363173909	1	0.583605562	1
Carotenoid biosynthesis	511.425	566.775	728.1818182	724.0681818	485.691254	508.3849484	927.0061657	1033.437308	0.910045961	1	0.603532038	1	0.587913319	1
Amino sugar and nucleotide sugar metabolism	263903.775	260125	280377.5227	290886.2727	43365.35059	37676.64036	58485.71712	72721.34884	0.646303622	1	0.857882999	1	0.589746284	1
Chloroalkane and chloroalkene degradation	28685.075	29325.55	30660.06818	32673.81818	6486.369283	6408.808709	7416.039397	11184.51657	0.571703779	1	0.199286615	1	0.589746284	1
Bladder cancer	124.325	139.275	228.5227273	330.2045455	416.512898	368.4977116	900.5087577	942.16442	0.404920269	1	0.989272691	1	0.596973947	1
Alanine, aspartate and glutamate metabolism	200315.4	198122.5	211132.8864	217232.0682	31087.35913	27049.3594	34402.0662	44836.36164	0.826382224	1	0.520869619	1	0.602115252	1
Alzheimer's disease	8928.175	8944.625	9230.727273	9717.681818	1073.824053	1167.296914	1741.880117	2770.135013	0.857582336	1	0.567026264	1	0.608342793	1
Others	166085.525	164632.1	177017.9091	187776.9318	32023.73636	29374.04638	45959.53119	64901.98601	0.764824907	1	0.574901594	1	0.608342793	1
Ubiquitin system	1195.325	1376.9	1505.159091	1990.818182	726.7036354	690.5043419	1098.210954	1906.887251	0.03128888	1	0.02466878	1	0.612863238	1
Peptidases	339035.425	333670.05	355561.0455	368387.8409	45657.06525	41877.66375	66561.22068	88426.5951	0.484520777	1	0.777106828	1	0.614598556	1
Glycosphingolipid biosynthesis - lacto and neolacto series	38.6	37.1	35.54545455	31.95454545	43.40081531	49.99784611	48.39010784	43.16973237	0.683714769	1	0.299798966	1	0.615863689	1
Selenocompound metabolism	65957.525	65127.9	69796.79545	73243.86364	10148.99434	9268.168621	15246.2264	21116.73921	0.617864972	1	0.664680578	1	0.620882176	1
Tryptophan metabolism	21480.125	22525.8	23930.36364	27192.15909	7529.089253	7544.542477	15520.19102	21670.77316	0.361103988	1	0.777106828	1	0.620882176	1
PPAR signaling pathway	21014.675	21275.45	22002.79545	22711.09091	2961.201267	2585.163844	2739.358095	3798.781756	0.50137474	1	0.273050394	1	0.627193277	1
Biosynthesis and biodegradation of secondary metabolites	11770.754	12076.625	13345.99049	14911.45455	4199.12777	3984.407717	9742.123072	12020.51038	0.704682764	1	0.72448427	1	0.62864251	1
Glycolysis / Gluconeogenesis	200310.9	198897.725	212274.2273	223620.4091	34407.09901	32207.69548	50157.5095	68889.41612	0.805726984	1	0.582827231	1	0.62864251	1
Propanoate metabolism	84227.8	84628.725	88736.18182	95062.52273	14713.69591	14229.62857	22971.65386	34691.82025	0.910045961	1	0.375595505	1	0.633531479	1
Purine metabolism	387706.175	383510.15	406837.4545	420021.5227	46654.17783	42818.64056	75182.49109	94918.77273	0.544800451	1	0.759441067	1	0.633531479	1
Pentose phosphate pathway	158244.425	156311.7	168082.1818	176283.9318	29721.75845	27249.17455	40245.38907	54041.05128	0.826382224	1	0.690081471	1	0.639896397	1
Glycine, serine and threonine metabolism	146096.6	144697.75	155442.0227	162390.75	21316.65726	19030.13843	35337.4801	45938.22647	0.608505822	1	0.794886249	1	0.64628764	1
Lysine biosynthesis	148685.825	146682.825	156562.4773	161058.0227	25413.67784	23001.15439	29363.7957	37042.49721	0.685015903	1	0.707209643	1	0.64628764	1
Arachidonic acid metabolism	3700.4	3765.725	3498.613636	3982.977273	2345.024354	2232.633698	2326.236068	3618.228704	0.914367263	1	0.640631778	1	0.651059016	1
Calcium signaling pathway	0.75	0.65	1.090909091	0.5	1.514883426	1.687814548	2.540995793	1.130959667	0.486398021	1	0.239069339	1	0.654360334	1
Peptidoglycan biosynthesis	147166.775	144844.45	153814.5	157558.25	20716.48578	19050.21275	26634.92663	34079.4605	0.382644861	1	0.958542366	1	0.659147505	1
Sulfur relay system	45496.75	44847.6	48811.31818	52271.84091	9851.126357	9277.331059	17457.60804	21943.645	0.617864972	1	0.803815884	1	0.659147505	1
Two-component system	282466.025	277022.875	300888.4318	319452.5455	61306.66169	54307.57601	110277.798	138976.835	0.65589929	1	0.812770678	1	0.659147505	1
Limonene and pinene degradation	13556.1	14136.525	15095.79545	16164.88636	3510.50864	3430.109933	8584.91616	9390.175903	0.326894475	1	0.861036905	1	0.665615315	1
Phenylalanine, tyrosine and tryptophan biosynthesis	156028.9	153709.1	165381.8864	170501.9545	27195.70122	23533.88407	31945.8746	42227.62597	0.724554494	1	0.759441067	1	0.665615315	1
Plant-pathogen interaction	30248.575	29223.075	31384.34091	32013.63636	5931.176019	5269.761326	6790.771203	8900.412256	0.313807068	1	0.986175368	1	0.665615315	1
Replication, recombination and repair proteins	138781.1	137703.05	144339.75	149036.5909	20873.19078	21122.134	29030.58892	37940.01995	0.675256809	1	0.876064671	1	0.665615315	1
Endocytosis	0.15	0.075	3.409090909	0.022727273	0.662164284	0.474341649	22.61335084	0.150755672	0.772829993	1	1	1	0.670186016	1
Fc gamma R-mediated phagocytosis	0.15	0.075	3.409090909	0.022727273	0.662164284	0.474341649	22.61335084	0.150755672	0.772829993	1	1	1	0.670186016	1
GnRH signaling pathway	0.15	0.075	3.409090909	0.022727273	0.662164284	0.474341649	22.61335084	0.150755672	0.772829993	1	1	1	0.670186016	1
Non-homologous end-joining	1188.925	1350.55	1654.75	1706.477273	1057.328743	1291.021797	1973.020411	2181.759419	0.816038982	1	0.656295029	1	0.672107828	1
Pyrimidine metabolism	324611.425	320097.65	336672	344290.1364	37818.05044	34922.9556	55089.81756	69470.77008	0.459813673	1	0.995391589	1	0.672107828	1
Pyruvate metabolism	183701.2	183746.8	195438.3864	206440.7045	33873.93906	30948.97681	42196.20274	66598.76049	0.89951513	1	0.528428679	1	0.678624624	1
Ether lipid metabolism	193.6	184	192.3863636	195.0227273	145.7544123	84.37735752	107.8351921	92.67936441	0.716640805	1	0.540068468	1	0.683607592	1
Bacterial motility proteins	184504.1	177491.15	190078.8636	188423.0455	58605.68262	55402.86904	91116.3237	96527.07641	0.599208943	1	0.986175368	1	0.685165279	1
Parkinson's disease	118.125	76.6	82.68181818	119	302.1600177	134.3126471	185.7335892	353.0472661	0.913357745	1	0.441814265	1	0.686809043	1
Valine, leucine and isoleucine biosynthesis	134737.875	134289.175	142880.0682	148510.5909	24738.4698	23221.57705	29235.87672	38717.47233	0.984093759	1	0.606898554	1	0.691729363	1
Valine, leucine and isoleucine degradation	33862.85	34607.375	36256.75	39903.75	7022.122648	6759.612186	13230.54406	20613.2165	0.704687674	1	0.333238581	1	0.69352512	1
Function unknown	220496.3	219856.875	239913.5	257916.7273	44762.36371	40302.56807	96454.30139	117944.9476	0.636765143	1	0.606898554	1	0.69831644	1
Lysine degradation	20582.9	21002.225	23470.68182	24981.77273	6151.542041	5624.64286	16403.59576	16470.80687	0.636765143	1	0.839774901	1	0.69831644	1
RNA degradation	85553.35	84622.2	89682.97727	91736.52273	10398.19559	9870.966924	14491.21412	17404.75686	0.589975534	1	0.930958095	1	0.69831644	1
Citrate cycle (TCA cycle)	118470.9	119741.975	123630.5909	130075.1818	11743.71869	13193.90051	23420.60615	36366.07642	0.910045961	1	0.51336551	1	0.7001489	1
Fluorobenzoate degradation	479.825	754.075	665.3409091	1194.181818	931.102459	1092.645587	1121.651812	2709.306882	0.125433671	1	0.309955508	1	0.7001489	1
C5-Branched dibasic acid metabolism	57548.85	57279.25	61008.95455	63896.43182	10715.74081	9754.830868	14479.97867	18996.87186	0.984093759	1	0.647951695	1	0.703467971	1
Fatty acid metabolism	36916.875	37675.2	40476.54545	44207.38636	8783.595228	8141.276121	18430.14658	22392.53296	0.646303622	1	0.321714505	1	0.704926072	1
Glycan biosynthesis and metabolism	6791.6	7010.4	7440.477273	8179.545455	2603.680618	2334.568722	6853.567092	6540.581307	0.294816961	1	0.700148783	1	0.706795566	1
Protein kinases	54983.55	54306.45	60170.88636	64055.18182	12902.56717	11427.79782	23527.84099	29071.15565	0.714597445	1	0.803815884	1	0.706795566	1
Zeatin biosynthesis	9905.825	9773.225	10267.75	10362.86364	1208.827868	1119.223139	1305.0315	1482.184742	0.455667159	1	0.940145439	1	0.710127339	1
alpha-Linolenic acid metabolism	824.475	889	1164.022727	1444.409091	1230.522194	1099.019003	2752.111997	2807.196858	0.435623973	1	0.829065385	1	0.710127339	1

Caprolactam degradation	2794.35	3195.75	3954.863636	5050.181818	3435.070809	3196.134362	8006.530243	8643.642789	0.397423832	1	0.211899533	1	0.711557812	1
Glutamatergic synapse	20175.575	20065.925	21264.81818	21656.81818	3466.277216	3058.488938	3260.579027	4500.531469	0.96289612	1	0.623183719	1	0.713464662	1
Drug metabolism - other enzymes	57945.55	57326.625	59681.43182	61193.70455	7200.354101	6590.120561	9234.532016	13099.96199	0.627285164	1	0.750653466	1	0.718211212	1
Metabolism of xenobiotics by cytochrome P450	3760.15	4129.55	4737.840909	5895.227273	2461.250115	2243.908129	5475.657395	6729.667627	0.270692344	1	0.16848305	1	0.718211212	1
Restriction enzyme	36488.775	36668	36954.40909	37977.13636	6045.951383	6004.625969	5318.609113	8755.100564	0.704687674	1	0.921779757	1	0.718211212	1
Vitamin B6 metabolism	38092.9	37963.8	39879.20455	41732.09091	5032.96194	4880.614091	8183.885143	10950.53672	0.836755011	1	0.715829138	1	0.720155728	1
Nicotinate and nicotinamide metabolism	77500.05	76386.625	81785.25	84779.81818	11054.83914	10114.4322	19300.30405	23063.96702	0.608505822	1	0.866965215	1	0.724885816	1
Tyrosine metabolism	57691.875	58048.225	61794.84091	66233	10560.48898	9887.735686	18120.52807	25690.06118	0.984093759	1	0.673107444	1	0.724885816	1
Carbon fixation in photosynthetic organisms	117774.375	117002.625	123482.2045	128736.2045	17032.53467	15857.54644	23371.48173	34314.69314	0.764824907	1	0.623183719	1	0.731581165	1
Cell cycle - Caulobacter	90207.45	89254.875	93563.61364	94716.95455	11155.02353	10359.90711	14107.3959	16652.91833	0.562667682	1	0.949340595	1	0.731581165	1
Phosphatidylinositol signaling system	16580.7	16729.825	17702.15909	18482.02273	2635.546722	2383.884676	3151.440025	4395.795256	0.857582336	1	0.441467386	1	0.731581165	1
beta-Lactam resistance	4075.975	4236.35	4627.25	5126.795455	1344.379821	1244.626513	1329.996103	2648.75853	0.65589929	1	0.921779757	1	0.733601898	1
Folate biosynthesis	66057.025	66994.7	70565.36364	72698.02273	7063.824694	6782.689767	13958.41861	16700.90554	0.571703779	1	0.967749554	1	0.738296795	1
Metabolism of cofactors and vitamins	19544	19867.825	21500.25	23309.86364	3720.818128	4051.150023	10009.4307	11916.15304	0.984093759	1	0.567026264	1	0.738296795	1
Renal cell carcinoma	943.9	1050.3	1101.136364	1321.522727	493.5328841	488.2722129	960.7115239	1005.888673	0.196754649	1	0.106502698	1	0.743738189	1
Polycyclic aromatic hydrocarbon degradation	19827	19671.125	20641.90909	21281.52273	3523.565953	3353.109611	3540.06273	4691.723497	0.847155624	1	0.750653466	1	0.758560664	1
Arginine and proline metabolism	224413.6	223444.175	239404.7955	248760.2995	40780.69385	36146.74638	48369.31793	69118.87989	0.984093759	1	0.639651458	1	0.765352689	1
DNA repair and recombination proteins	502759.225	498016.35	525502.0455	538996.7727	60276.27071	56648.04935	92274.79356	114638.0745	0.599208943	1	0.866965215	1	0.765352689	1
Energy metabolism	167918.325	168128.225	176014.4091	180399.9545	22055.99196	21025.16095	37567.63112	48838.30033	0.97349265	1	0.759441067	1	0.765352689	1
Fatty acid biosynthesis	82992.65	82775.525	87599.27273	91076.02273	13024.92541	12502.45754	17576.18732	24154.37048	0.816038982	1	0.777106828	1	0.765352689	1
Peroxisome	31685.625	32072.6	33707.29545	34807.86364	4235.304813	3626.211414	5881.980087	7358.097285	0.24244683	1	0.375595505	1	0.765352689	1
Ubiquinone and other terpenoid-quinone biosynthesis	30910.075	32119.425	34083.47727	37280.52273	8055.756885	8269.93031	18144.05176	22306.1081	0.40493774	1	0.543709192	1	0.765352689	1
Cell division	14846.5	14802.05	16054.79545	16216.29545	1952.946531	1986.895645	3771.25773	3826.678909	0.919700599	1	0.715829138	1	0.770991151	1
Drug metabolism - cytochrome P450	3915.025	4365.7	4947.227273	6108.886364	2492.960587	2312.63065	5493.411406	6802.203816	0.333141171	1	0.216226733	1	0.777848558	1
Ribosome Biogenesis	253897.95	249921.45	264857.9773	272811.0227	33284.62747	31907.47667	53537.93778	66689.16302	0.459813673	1	0.995391589	1	0.778989933	1
Other glycan degradation	63144.575	61980.375	69018.72727	68938.31818	15597.57174	12841.45908	15166.42466	15507.64206	0.742951676	1	0.579346956	1	0.784725508	1
Meiosis - yeast	850.5	1045.325	946.4772727	1012.454545	500.5194225	701.2136507	794.1778691	665.7811099	0.091824062	1	0.285592331	1	0.788169783	1
Cardiac muscle contraction	89.85	59.05	67.88636364	91.20454545	225.7847341	101.0450395	152.629848	265.2227304	0.969872339	1	0.621783779	1	0.791359047	1
Membrane and intracellular structural molecules	102094.35	102826.225	111742.0455	116772.0227	16161.81826	15629.05812	44805.40587	48310.40725	0.752098107	1	0.940145439	1	0.791618204	1
Bacterial secretion system	107592.275	109089.6	114269.9318	124602.6591	19375.83876	19019.84911	33324.07504	56286.25374	0.984093759	1	0.590802441	1	0.792694818	1
Butirosin and neomycin biosynthesis	14832.025	14581.275	15760.97727	15926.25	3472.92058	3055.409763	3420.580486	4523.628591	0.685015903	1	0.949340595	1	0.792694818	1
Mismatch repair	147373.3	145291.025	153072.0227	155834.6364	18946.42243	17928.27922	25701.41012	31856.98725	0.451733939	1	0.976960955	1	0.792694818	1
Photosynthesis - antenna proteins	13.25	10.825	19	9.227272727	25.58119301	28.10957269	42.82088167	18.68199436	0.385091151	1	0.223166927	1	0.799441795	1
Aminoacyl-tRNA biosynthesis	212230.3	210666.575	219044.4091	224134.2273	27486.68789	28768.44913	34186.42852	45876.45843	0.675256809	1	0.921779757	1	0.80646335	1
Riboflavin metabolism	42590.2	41967.6	44483.43182	46179.86364	4868.61946	4504.460404	11024.85547	13649.01652	0.313807068	1	0.741897447	1	0.80646335	1
Amino acid metabolism	33585.975	34354.275	35443.29545	38837.31818	7347.669376	7177.063741	9616.702528	19616.68202	0.627285164	1	0.469526576	1	0.813370219	1
Methane metabolism	230055.15	227391.825	241938.5455	247816.6818	40418.02813	36179.31373	48970.84481	64010.05536	0.93115225	1	0.894310661	1	0.813370219	1
Amino acid related enzymes	268732.425	266292.5	280249.4091	287883.4318	35026.98497	33246.03237	47576.69266	62667.15173	0.646303622	1	0.903454856	1	0.820291476	1
Cellular antigens	7774.45	7941.025	8319.25	8628.818182	1505.62511	1949.160509	2430.555924	3118.999029	0.686769122	1	0.498524484	1	0.820291476	1
Epithelial cell signaling in Helicobacter pylori infection	16696.8	16522.425	17721.54545	17860.79545	2648.517275	2423.234868	3913.733312	4853.75675	0.774995235	1	0.623183719	1	0.820291476	1
Flagellar assembly	76898.4	73354.65	78905.93182	75722.34091	26161.31041	25079.37424	41721.25624	43589.51609	0.608505822	1	0.839774901	1	0.820291476	1
Homologous recombination	168266.1	166741.25	174746.3182	178620.6818	19647.27553	19031.34502	28335.35464	35485.99229	0.599208943	1	0.986175368	1	0.820291476	1
Protein export	107002.1	105703.05	111852.9091	113790.6364	12838.94466	11514.01966	17871.07673	21650.43697	0.49291002	1	0.967749554	1	0.820291476	1
Tropane, piperidine and pyridine alkaloid biosynthesis	21436.825	21379.4	22494.97727	23450.25	3075.098013	2724.000892	4359.300933	6352.677118	0.87850707	1	0.761564023	1	0.820291476	1
Novobiocin biosynthesis	24466.375	24192.05	25543.43182	26439.40909	3504.846203	3107.346976	4769.000199	6047.579771	0.731781314	1	0.785982868	1	0.826316242	1
Chromosome	288724.775	283907.325	301372.5455	309732.0455	38323.78269	35268.5928	56172.2786	72772.1159	0.467971859	1	0.866965215	1	0.827226605	1
NOD-like receptor signaling pathway	8232.025	8081.45	8667.5	8721.568182	1229.51069	1136.127001	1331.66473	1920.783917	0.535971383	1	0.894310661	1	0.827226605	1
Pantothenate and CoA biosynthesis	115043.725	113979.1	120543.0682	124286.8864	18756.54582	17462.16039	23553.60948	30993.4619	0.847155624	1	0.967749554	1	0.827226605	1
DNA replication	117169.175	116086.25	121777.1364	123909.4091	12860.42847	12406.58173	19145.00501	23277.14834	0.608505822	1	0.995391589	1	0.834175088	1
Polyketide sugar unit biosynthesis	39961.3	39621.35	41792.47727	41808.29545	6557.129806	5680.421186	6360.111969	8017.624132	0.65589929	1	1	1	0.834175088	1
Cell motility and secretion	31908.05	32833.9	34315.15909	35660.90909	4474.767852	5452.562135	10185.53391	10958.94607	0.764824907	1	0.759441067	1	0.836795491	1
Shigellosis	0.7	0.55	2.454545455	0.431818182	3.27539889	1.678674691	15.21543669	1.561122543	1	1	0.786030953	1	0.838192162	1
Betalain biosynthesis	0.875	1.125	0.409090909	1.068181818	3.25172538	2.936835031	2.265188342	4.479398461	0.483303242	1	0.14343262	1	0.840516585	1
Indole alkaloid biosynthesis	0.875	1.125	0.409090909	1.068181818	3.25172538	2.936835031	2.265188342	4.479398461	0.483303242	1	0.14343262	1	0.840516585	1
Biosynthesis of unsaturated fatty acids	21067.925	21708.125	23230.88636	25433.86364	5388.600467	5823.089022	11482.44653	14523.10111	1	1	0.631395182	1	0.841136405	1
Translation proteins	162728.05	160957.2	169380.4773	174655.2727	20992.73808	19948.86993	32763.5107	42281.77417	0.580806766	1	0.949340595	1	0.841136405	1
Protein digestion and absorption	4281.775	4286.325	4602.636364	4545.136364	1121.624245	1376.435836	1175.133067	1224.467888	0.795447917	1	0.575360752	1	0.847303016	1
Biotin metabolism	28318.075	27997.6	30564.34091	30908.34091	5023.049944	4344.356167	7023.399776	7764.423985	0.836755011	1	0.785982868	1	0.84811003	1
Biosynthesis of type II polyketide products	0.225	0.45	0.090909091	0.295454545	0.73336247	1.431334325	0.290803363	1.111871363	0.528232777	1	0.203091788	1	0.848509167	1
Biosynthesis of vancomycin group antibiotics	12768.575	12704.725	13289.34091	13330.06818	1958.082328	1781.945753	2023.910548	2467.140284	0.685015903	1	0.81544628	1	0.855095437	1

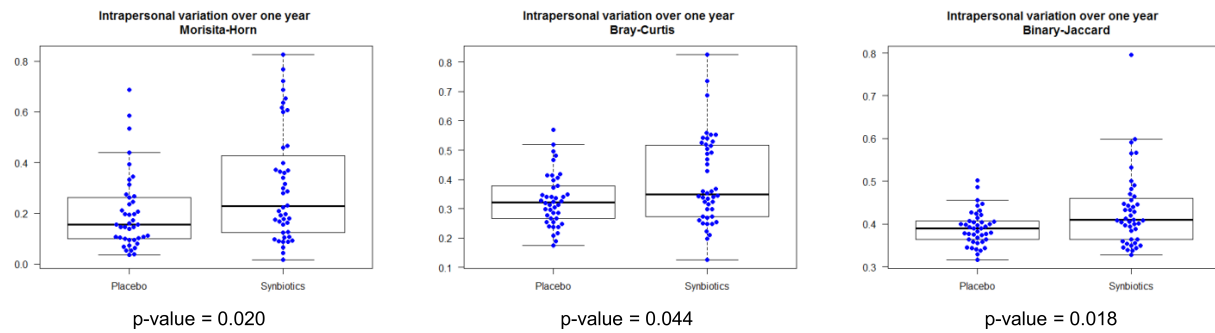
Prenyltransferases	56927.225	57094.525	59242.56818	60130.36364	5596.567916	5519.140327	8223.557436	9633.735191	0.868033403	1	0.976960955	1	0.855095437	1
Ribosome	421786.525	417882.2	434660	441573.0455	45601.12518	45583.67618	61799.67383	78077.58961	0.527213339	1	0.894310661	1	0.855095437	1
Type I diabetes mellitus	10090	10118.225	10550.52273	10555.86364	1428.27574	1272.218343	1418.654841	1677.062598	0.704687674	1	0.876064671	1	0.855095437	1
Transcription machinery	188452.475	186519.25	196306.0455	197438.5227	28203.5931	26844.02869	27593.7338	36292.50102	0.65589929	1	0.949340595	1	0.876117004	1
Sulfur metabolism	52859.925	52689.75	57171.81818	59761.56818	9948.578629	9075.868293	14013.46063	18077.28471	0.96289612	1	0.664680578	1	0.890180573	1
Type II diabetes mellitus	8611.9	8537.575	9203.590909	9411.522727	1424.939833	1233.095789	2375.050005	2865.289169	0.984093759	1	0.995391589	1	0.900216597	1
Ethylbenzene degradation	7589.775	7580.025	7962.363636	8052.181818	1252.917344	1168.494923	2960.746795	2711.489613	0.962477305	1	0.876064671	1	0.903762636	1
DNA replication proteins	225357.375	222491.525	234142.4091	237597	25058.66813	23726.13551	36782.44729	44630.53915	0.459813673	1	0.848819179	1	0.904278373	1
Lipid biosynthesis proteins	107183.1	107299.125	112808.1591	116951.9091	15695.46452	14480.22938	21183.8485	30262.16874	1	1	0.912611615	1	0.904278373	1
Histidine metabolism	119706.575	116968.4	127294.2273	129149.75	22325.11264	19410.32732	21992.74155	29006.43163	0.774995235	1	0.995391589	1	0.911338763	1
Inorganic ion transport and metabolism	34059.4	34767	39291.43182	42611.95455	10100.96435	9339.162495	24310.43244	27331.1939	0.882456461	1	0.812770678	1	0.911338763	1
Other transporters	49863.025	49311.2	53087.13636	55295.65909	8607.787102	7399.634177	13965.57893	17843.55011	0.675256809	1	0.876064671	1	0.911338763	1
Pertussis	4656.975	4961.55	6778.75	8363.704545	5950.650132	5463.354717	14441.73379	14424.90257	0.467971859	1	0.51336551	1	0.911338763	1
Aminobenzoate degradation	21214.325	21922.875	22067.97727	24258.79545	3809.263671	4178.444909	3808.006044	10815.3561	0.571703779	1	0.715829138	1	0.914411997	1
Bacterial invasion of epithelial cells	124.225	115.275	81.77272727	101.1136364	295.0329393	203.3590973	158.8560653	193.7684084	0.635065983	1	0.716619367	1	0.917948326	1
MAPK signaling pathway - yeast	8054.2	8278.2	8674.113636	8791.545455	1893.50882	1607.835497	1904.802819	2103.769789	0.382284815	1	0.606898554	1	0.918406085	1
Tetracycline biosynthesis	22933.825	23077.3	24717.5	25568.5	5040.12074	5009.011015	6215.368546	7804.087753	0.816038982	1	0.498524484	1	0.918406085	1
Lipid metabolism	24796.15	24501.25	26890.59091	28094.54545	4780.932366	4650.193041	11128.31162	12853.65009	0.571703779	1	0.733174042	1	0.925479789	1
Oxidative phosphorylation	206496.825	205896.225	216951.3636	221300.8864	22658.29231	22541.26263	38864.04576	44079.50447	0.826382224	1	0.894310661	1	0.925479789	1
Phenylalanine metabolism	34395.85	35168.6	36672.72727	40490.75	6839.974181	6446.779056	10171.87419	19465.34447	0.599208943	1	0.388270715	1	0.925479789	1
General function prediction only	652733.425	646978.975	689703.7273	711428.2273	96268.95778	86752.10117	147259.5771	191205.6051	0.744604358	1	0.976960955	1	0.932559327	1
One carbon pool by folate	113768.45	112675.875	117475.5	119233.5455	12400.80579	11751.96192	16786.17201	20983.03631	0.484520777	1	0.930958095	1	0.932559327	1
Protein folding and associated processing	110859.75	112221.85	119507.0227	124736.2955	15098.22253	14536.2257	35045.42908	40258.67569	0.889001579	1	0.848819179	1	0.939644145	1
Chaperones and folding catalysts	188000.925	186739.5	198752.2727	204070.5682	22218.92687	20640.92516	40734.06232	49797.64553	0.608505822	1	0.857882999	1	0.953827416	1
Insulin signaling pathway	16212.475	16161.6	17372.38636	17956.75	3783.035242	3563.372832	4304.81263	6206.890028	0.941724097	1	0.912611615	1	0.953827416	1
Naphthalene degradation	22851.4	23508.85	24328.02273	25622.20455	4353.341839	4304.673915	6191.352734	8666.079384	0.397423832	1	0.407755058	1	0.953827416	1
Carbon fixation pathways in prokaryotes	185915.65	186928	194295.9091	200526.0682	19714.64677	19203.61374	32664.70785	44513.6927	0.795447917	1	0.967749554	1	0.960924758	1
Chronic myeloid leukemia	0.1	0.05	0.090909091	0.181818182	0.496138938	0.220721428	0.290803363	0.921897116	0.586213681	1	1	1	0.961883767	1
Notch signaling pathway	0.1	0.05	0.090909091	0.181818182	0.496138938	0.220721428	0.290803363	0.921897116	0.586213681	1	1	1	0.961883767	1
Wnt signaling pathway	0.1	0.05	0.090909091	0.181818182	0.496138938	0.220721428	0.290803363	0.921897116	0.586213681	1	1	1	0.961883767	1
Base excision repair	77464.65	77248.375	81688.09091	83431.38636	10422.53013	9933.420006	15535.30549	19064.84099	0.910045961	1	0.986175368	1	0.968025166	1
Secretion system	214159.525	215882.6	228369.2727	245627.5	48113.36622	45666.53853	94484.24756	120560.6961	0.994697614	1	0.986175368	1	0.968025166	1
Basal transcription factors	41.425	44.85	35.81818182	33.06818182	109.919758	135.6690071	53.89493341	63.07411577	0.884266723	1	0.94313501	1	0.971375748	1
Pathways in cancer	8911.875	8829.75	9414.840909	9639.5	1055.10311	1082.296152	1813.496083	2240.056981	0.617864972	1	0.976960955	1	0.971421845	1
p53 signaling pathway	27.725	17.025	14.22727273	27.70454545	75.7001465	33.51806822	37.82632505	88.00279582	0.786136951	1	0.823046278	1	0.974922327	1
Germination	8397.525	8205.875	8498.636364	8193.840909	2360.980609	2391.749399	2379.579542	3029.998664	0.617864972	1	0.430846051	1	0.974992861	1
Taurine and hypotaurine metabolism	19926.55	20141.85	21110.75	21844.75	2684.668217	2533.876209	3978.273759	5590.417753	0.694826674	1	0.698626764	1	0.975128082	1
Viral myocarditis	27.725	17.025	14.22727273	27.68181818	75.7001465	33.51806822	37.82632505	88.00363965	0.786136951	1	0.823046278	1	0.978503803	1
Carbohydrate digestion and absorption	3001.3	2852.85	3396	3687.090909	1299.092291	1292.511579	2655.839201	3002.692852	0.518804353	1	0.794886249	1	0.978564378	1
Colorectal cancer	27.725	17.025	14.22727273	27.65909091	75.7001465	33.51806822	37.82632505	88.00474173	0.786136951	1	0.823046278	1	0.982085931	1
Small cell lung cancer	27.725	17.025	14.22727273	27.65909091	75.7001465	33.51806822	37.82632505	88.00474173	0.786136951	1	0.823046278	1	0.982085931	1
Toxoplasmosis	27.725	17.025	14.22727273	27.65909091	75.7001465	33.51806822	37.82632505	88.00474173	0.786136951	1	0.823046278	1	0.982085931	1
Antigen processing and presentation	7940.15	7762.375	8299.386364	8290.136364	1164.486561	1088.75249	1287.654894	1803.059991	0.339910107	1	0.528428679	1	0.98223295	1
Glycosyltransferases	60624.625	60661.275	65733.56818	68317.95455	8781.384651	8189.004808	19178.97215	22218.18832	0.694826672	1	0.986175368	1	0.98223295	1
Progesterone-mediated oocyte maturation	7940.15	7762.375	8299.386364	8290.136364	1164.486561	1088.75249	1287.654894	1803.059991	0.339910107	1	0.528428679	1	0.98223295	1
RNA transport	25597.8	25123.3	26650.29545	27233.27273	4647.622917	4359.725757	5434.287149	7084.408562	0.774995235	1	0.866965215	1	0.989339212	1
Transcription related proteins	893.7	1035.775	1102.909091	1566.909091	985.0564789	917.1830477	1817.461708	2683.229838	0.282584112	1	0.29356813	1	0.989339212	1
Influenza A	33.8	24.075	15.86363636	27.70454545	79.18339634	46.12045961	39.83663787	87.99671758	0.793354632	1	0.727506649	1	0.992832553	1
Bile secretion	0.5	0.375	2.227272727	1.636363636	2.063106943	1.371645091	10.65279445	8.683292991	1	1	0.952682032	1	1	1
Biosynthesis of siderophore group nonribosomal peptides	3261.925	3475.525	3966.772727	6188.159091	3846.567203	3911.686894	6304.988047	11919.34363	0.941724097	1	0.861036905	1	1	1
Glycerophospholipid metabolism	104042.75	102896.6	110081.8182	113539.0455	10809.10669	16515.99822	24715.06583	32457.08271	0.685015903	1	0.885180209	1	1	1
Translation factors	96376	95669.175	100102.1591	101603.5909	10441.80737	10051.60751	15030.5834	18771.7787	0.589975534	1	0.912611615	1	1	1
Aldosterone-regulated sodium reabsorption	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Biosynthesis of 12-, 14- and 16-membered macrolides	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Biosynthesis of type II polyketide backbone	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
CAM ligands	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Cell cycle - yeast	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Cholinergic synapse	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Complement and coagulation cascades	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA
Cytokine receptors	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA	NA

Cytokine-cytokine receptor interaction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Cytosolic DNA-sensing pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
ECM-receptor interaction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Endocrine and other factor-regulated calcium reabsorption	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
ErbB signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Fat digestion and absorption	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Fc epsilon RI signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
GTP-binding proteins	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Gastric acid secretion	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glioma	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glycan binding proteins	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glycosaminoglycan biosynthesis - chondroitin sulfate	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Hedgehog signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Hematopoietic cell lineage	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Leishmaniasis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Long-term depression	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Long-term potentiation	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
MAPK signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Melanogenesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Neuroactive ligand-receptor interaction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Neurotrophin signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Olfactory transduction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Oocyte meiosis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Other types of O-glycan biosynthesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Pancreatic cancer	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Pancreatic secretion	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Phototransduction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Rheumatoid arthritis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Salivary secretion	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Sesquiterpenoid biosynthesis	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Spliceosome	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
TGF-beta signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
VEGF signaling pathway	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA
Vascular smooth muscle contraction	0	0	0	0	0	0	0	0 NA	NA	NA	NA	NA	NA

A



B



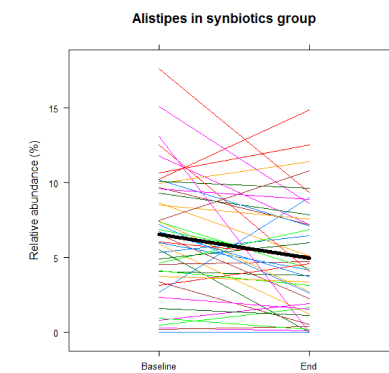
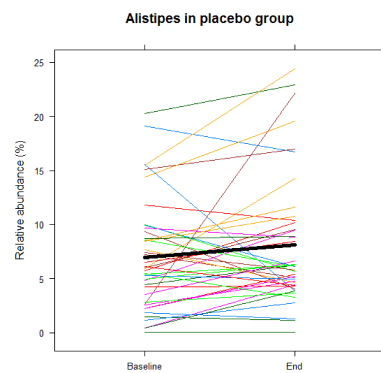
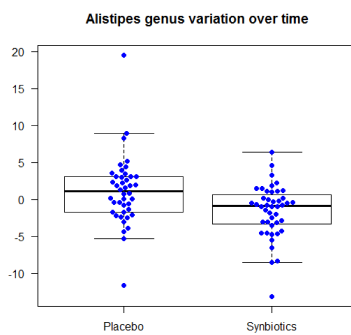
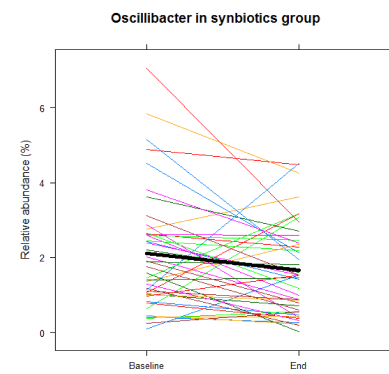
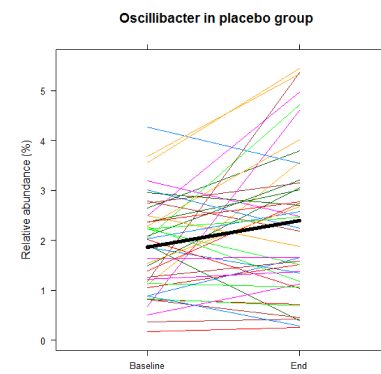
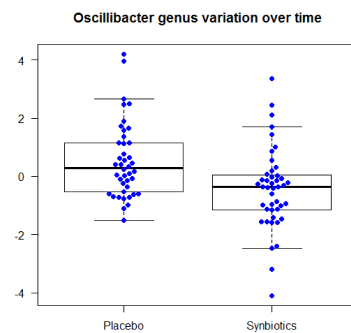
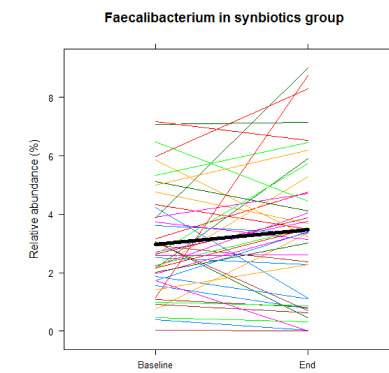
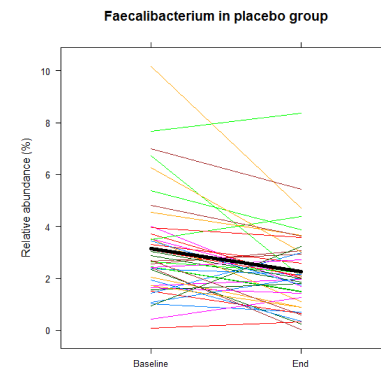
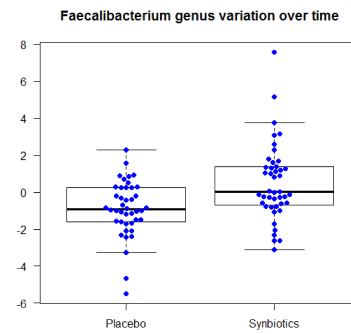
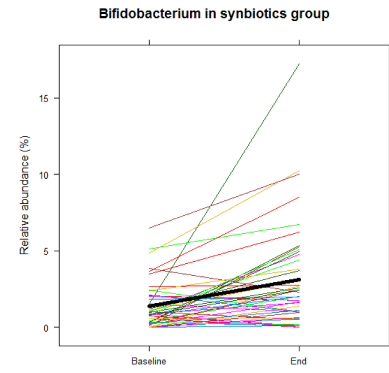
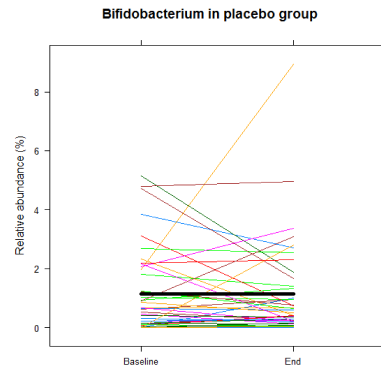
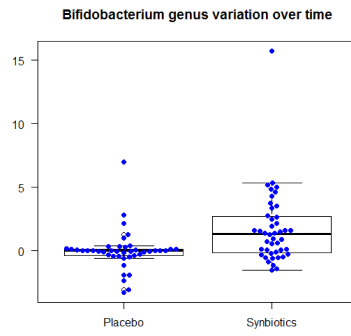
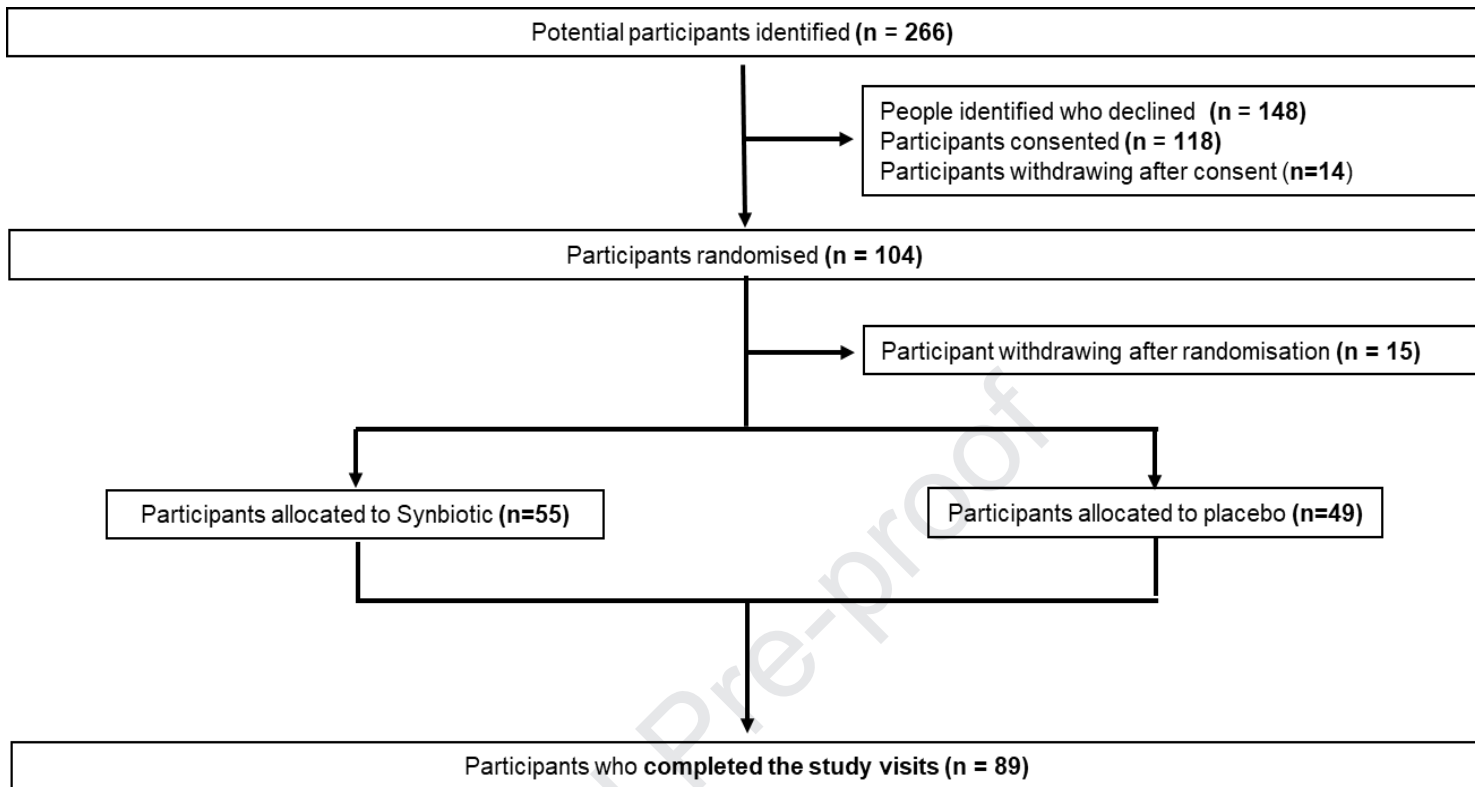


Figure 1. Consort diagram

For reasons for participant withdrawal see Table S1.

Figure 2. Effect of synbiotic supplementation on microbial beta-diversity.**Include text below in the legend.**

(A) Principal coordinate analysis of the Morisita-Horn beta-diversity index computed based on the OTU table, for each group of patients, coloured by time (baseline in red, end of study in blue) or patient ID. (B) Changes of beta-diversity indexes over the period of intervention in each arm. 42 patients in the placebo group and 44 patients in the synbiotic group.

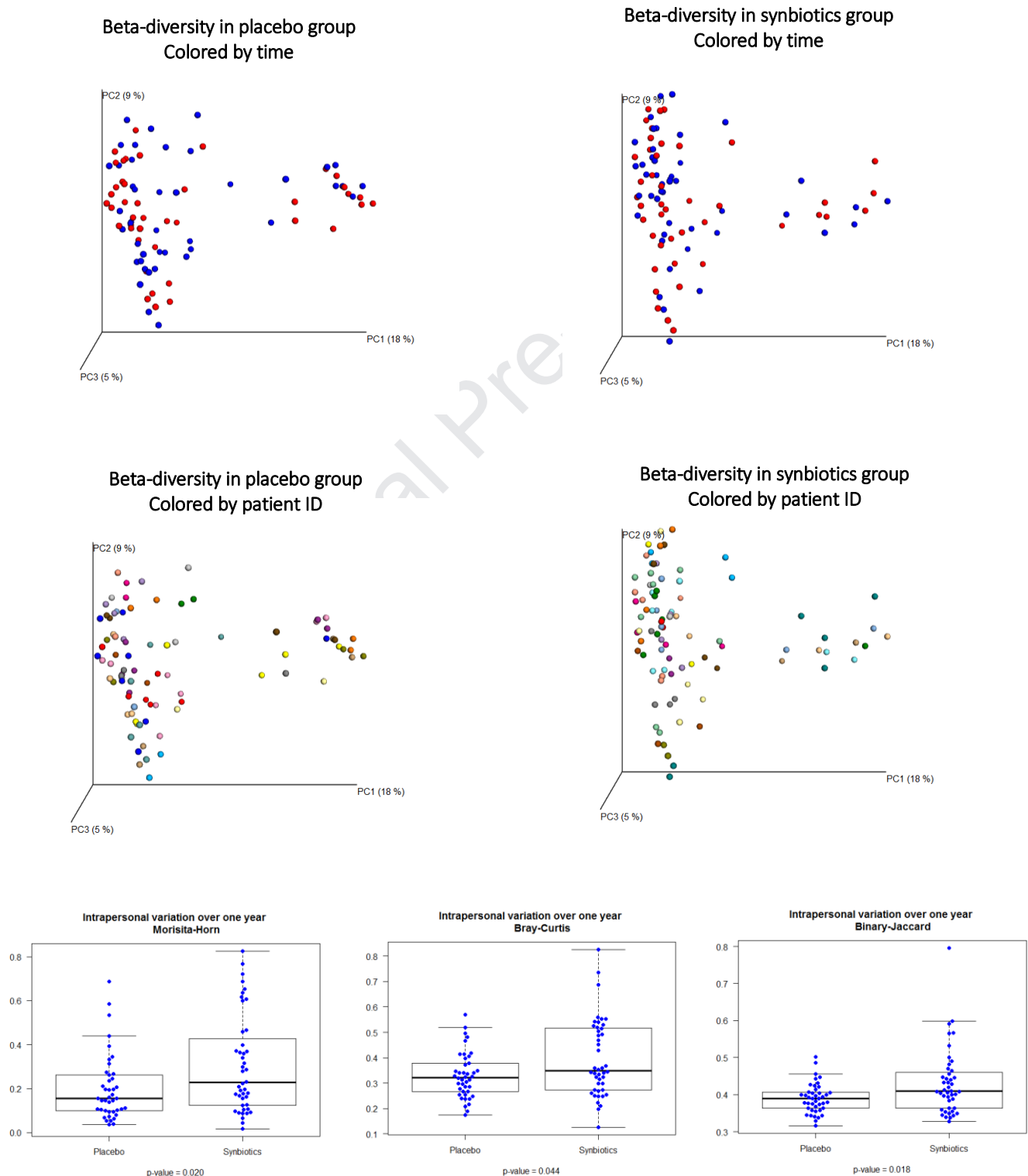
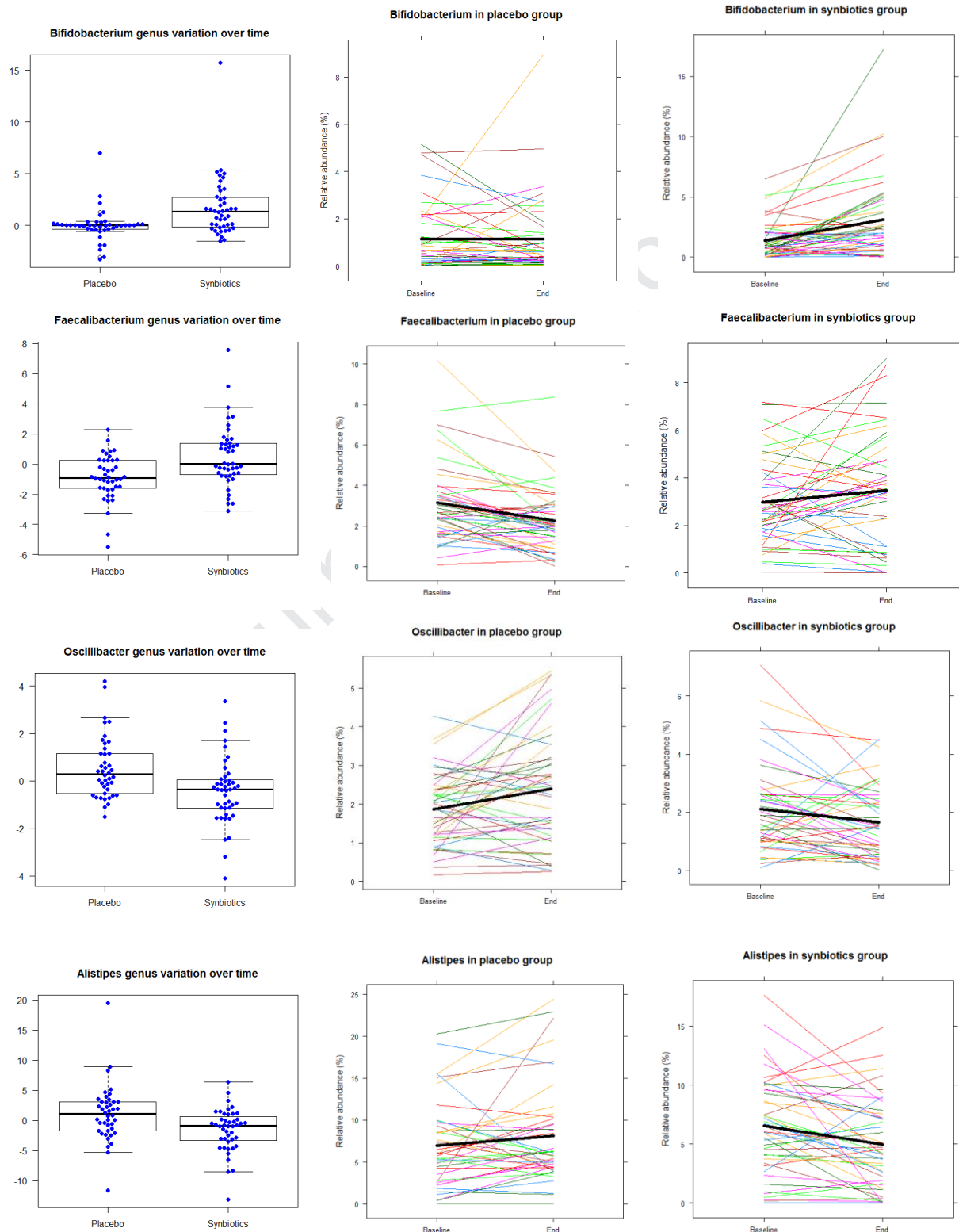


Figure 3. Effects of synbiotic supplementation on *Bifidobacterium*, *Faecalibacterium*, *Oscilibacter* and *Alistipes*.

Genera presenting a significant difference in change of abundance over time between arms (q-value < 0.1). The bold line represents the mean changes. Descriptive statistics and test results are presented in Table S2. 42 patients in the placebo group and 44 patients in the synbiotic group.



What you need to know:

BACKGROUND AND CONTEXT: Dysbiosis of the intestinal microbiota has been associated with nonalcoholic fatty liver disease (NAFLD). Synbiotic combinations (probiotic and prebiotic) that reduce dysbiosis might be used to treat NAFLD, but there have been no randomized placebo-controlled trials of their effects in these patients.

NEW FINDINGS: In a randomized trial of patients with NAFLD, 10–14 months administration of a synbiotic combination (fructo-oligosaccharides and *Bifidobacterium animalis* subsp. lactis BB-12) altered fecal microbiomes, increasing proportions of *Bifidobacterium* and *Faecalibacterium*, but did not reduce liver fat content or markers of liver fibrosis

LIMITATIONS: This study tested only 1 probiotic and prebiotic combination. Studies are needed to determine which intestinal microbes or metabolites affect liver fat and fibrosis.

IMPACT: A synbiotic reduced fecal dysbiosis in patients with NAFLD, but not liver fat or fibrosis, after 1 y. Studies are needed to identify intestinal microbes that promote NAFLD and to determine the effects of their modification.

Lay Summary: Patients with NAFLD were given a combination of probiotic and prebiotic agents (synbiotic) or a placebo for 1 year. The synbiotic altered the composition of microbes in fecal samples, but did not affect liver fat or markers of liver scarring.