**SUPPLEMENTAL INFORMATION**

**The effect of allogenic versus autologous faecal microbiota transfer on symptoms, visceral perception and faecal and mucosal microbiota in irritable bowel syndrome – a randomised controlled study**

Savanne Holster1, Carl M. Lindqvist1, Dirk Repsilber1, Anne Salonen2, Willem M. de Vos2,3, Julia König1\* & Robert-Jan Brummer1\*

1 Nutrition-Gut-Brain Interactions Research Centre, Faculty of Health and Medicine, School of Medical Sciences, Örebro University, Sweden

2 Immunobiology Research Program, Department of Bacteriology and Immunology, University of Helsinki, Finland

3 Laboratory of Microbiology, Wageningen University and Research Centre, The Netherlands

\* Julia König and Robert-Jan Brummer should be considered joint senior author.

**SUPPLEMENTAL METHODS**

**Data analysis - Barostat data**

The function for the fitting of the observed scores was: score = K / (1 + e^(r\*(x-d))) - K / (1 + e^(-r\*d)), with measured pressure x, and parameters K (limit of the logistic function), d (shift with respect to pressure), and r (steepness of the logistic function). The second term of the fitted function represents the fitted score at zero pressure, forcing a zero intercept, as we are fitting baseline-corrected values. The fit was obtained using the Levenberg-Marquardt algorithm, as implemented in R-package nlsLM 1,2. Optimisation of parameters for the fit involves choosing starting values for the nonlinear fitting procedure. For each individual curve, these are chosen from a broad set of possible starting values to make the fit as independent from this choice as possible. No manual interaction with regards to these choices was applied. Analysis scripts are available upon request.

1. Team RC. R: A language and environment for statistical computing. 2016; <https://www.R-project.org/>.

2. Timur V. Elzhov KMM, Andrej-Nikolai Spiess and Ben Bolker. minpack.lm: R interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, plus support for bounds. 2013; [http://CRAN.R-project.org/package=minpack.lm](http://CRAN.R-project.org/package%3Dminpack.lm).

**SUPPLEMENTAL TABLES**

Supplemental Table 1: Similarity index (Pearson correlations) between the microbiota of the recipients (R) and their corresponding donor (D) in the allogenic group.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Faecal (D) versus faecal (R) microbiota | Faecal (D) versus mucosal (R) microbiota | Mucosal (D) versus mucosal (R) microbiota |
| Baseline,median (IQR) | 0.78 (0.53-0.78) | 0.76 (0.52-0.78) | 0.62 (0.50-0.75) |
| Two weeks after FMT,median (IQR) | 0.81 (0.56-0.95) | 0.90 (0.63-0.95) | 0.57 (0.51-0.78) |
| Eight weeks after FMT,median (IQR) | 0.85 (0.76-0.96) | 0.78 (0.51-0.95) | 0.51 (0.42-061) |
| p-value | N.S. | N.S. | N.S. |

*IQR – interquartile range, N.S. – not significant*

Supplemental Table 2: Similarity index (Pearson correlations) between the microbiota of the recipients (R) and their own microbiota (D) in the autologous group.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Faecal (D) versus faecal (R) microbiota | Faecal (D) versus mucosal (R) microbiota | Mucosal (D) versus mucosal (R) microbiota |
| Baseline,median (IQR) | 1.0a,b(1.0-1.0) | 0.59 (0.27-0.96) | 1.0a (1.0-1.0) |
| Two weeks after FMT,median (IQR) | 0.60a(0.29-0.84) | 0.61 (0.27-0.86) | 0.43b(0.31-0.82) |
| Eight weeks after FMT,median (IQR) | 0.83b (0.67-0.95) | 0.36 (0.05-0.75) | 0.63b (0.31-0.71) |
| p-value | <0.01 | N.S. | <0.05 |

*IQR – interquartile range, N.S. – not significant.*

*a indicates significance between baseline and 2 weeks, b indicates significance between baseline and 8 weeks.*

Supplemental table 3: Butyrate-producing bacteria quantified with HITChip.

|  |
| --- |
| *Allistipes* et rel. |
| *Anaerostipes caccae* et rel. |
| *Anaerotruncus colihominis* et rel. |
| *Bryantella formatexigens* et rel. |
| *Butyrivibrio crossotus* et rel. |
| *Clostridium nexile* et rel. |
| *Coprobacillus catenaformis* et rel. |
| *Coprococcus eutactus* et rel. |
| *Eubacterium cylindroides* et rel. |
| *Eubacterium hallii* et rel. |
| *Eubacterium rectale* et rel. |
| *Eubacterium ventriosum* et rel. |
| *Faecalibacterium prausnitzii* et rel. |
| *Lachnospira pectinoschiza* et rel. |
| *Megasphaera elsdenii* et rel. |
| *Roseburia intestinalis* et rel. |
| *Subdoligranulum variable* at rel. |

*Rel - Relatives*

**SUPPLEMENTAL FIGURE LEGENDS**

Supplemental Figure 1: CONSORT Flow Diagram.

Supplemental Figure 2: PCA plots of the faecal and mucosal microbiota composition. Responders are depicted as open symbols. received from donor +, the other symbols received from donor x. Faecal microbiota from patients and donors (A,D), mucosal microbiota from patients and faecal microbiota from donors (B,E), and mucosal microbiota from patients and donors (C,F) are shown in both the allogenic (A-C) as well as the autologous FMT group (D-F).

Supplemental Figure 3: Baseline-corrected Shannon diversity index in faecal (A) and mucosal (B) samples two and eight weeks after FMT. No significant differences were found.

**Supplemental Figure 4**: Similarity index (Pearson correlations) between the microbiota of the recipients and their corresponding donor in the treatment group (A,C,E) and the recipients and their own microbiota in the autologous group (B,D,F). A,B. Faecal microbiota of recipients correlated to faecal microbiota of donors/own microbiota. In the allogenic group, no significant differences were found. In the autologous group, the similarity index was significantly reduced 2 and 8 weeks after the autologous FMT compared to baseline. \*\*p<0.01. C,D. Mucosal microbiota correlated to faecal microbiota of donors/own microbiota. No significant differences were found between the time points. E,F. Mucosal microbiota correlated to mucosal microbiota of donors/own microbiota. In the allogenic group, no significant differences were found. In the autologous group, the similarity index was significantly reduced 2 and 8 weeks after the autologous FMT compared to baseline. \*p<0.05. Bl - baseline.

**Supplemental Figure 5**: Relative abundance (%) of genus-like groups that include known butyrate-producing bacteria in faecal material of donors and IBS patients at different time points after FMT measured with HITChip. Mean with 95% confident intervals are shown. (A) Butyrate-producing bacteria in faecal samples from donors and IBS patients at baseline. (B) Butyrate-producing bacteria in faecal samples from donors and IBS patients in the allogenic group. (C) Butyrate-producing bacteria in faecal samples from donors and IBS patients in the autologous group. A tendency towards significance was found between the autologous group at baseline and donors (p=0.09). HITChip: Human Intestinal Tract Chip.

**SUPPLEMENTAL FIGURES**

**Supplemental Figure 1**

**Supplemental Figure 2**

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**Supplemental Figure** **3**

**Supplemental Figure 4**

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**Supplemental Figure 5**

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