Evidence from microbiome research increasingly supports the potential clinical value of therapeutic manipulation of the microbiome, using live bacteria to treat disease, known as Live Biotherapeutic Products (LBPs). Irritable Bowel Syndrome (IBS) is a chronic, debilitating, functional gastrointestinal disorder with an estimated global prevalence of 10-20% \(^1\). The gut microbiota has increasingly been implicated in the development of IBS \(^2\), with a number of studies reporting alterations in the diversity, stability and metabolic activity of the microbiota in IBS patients compared with healthy individuals \(^3\). Using 16S rRNA amplicon sequencing we profiled the microbiota of healthy and IBS subjects in i) an observational study and ii) a Phase Ib randomized control clinical trial with the LBP Blautix, a strain of *Blautia hydrogenotropha* to investigate if a) the microbiota structure of healthy and IBS subjects differ and b) a LBP could affect the structure of the microbiota in IBS subjects.

**Introduction**

Microbiota analysis was performed on faecal samples from our i) observational study (Healthy: n=64 and IBS: n=78) and Phase Ib Blautix clinical trial (Healthy-Blautix: n=16; Healthy-placebo: n=8; IBS-Blautix: n=16 and IBS-placebo; n=8) using 16S amplicon sequencing of the V3-V4 variable region and the MiSeq (2x250 bp) chemistry platform. Subjects in the Phase Ib study were randomized into the placebo or Blautix treatment group where they received twice daily doses of >10\(^{10}\) CFUs of Blautix over a 16-day treatment period followed by 2-4 weeks washout. The microbiota was assessed over the study timepoints from baseline (D1), end of treatment (D16) to washout/end of study (EOS). Microbiome data analysis was performed using an in-house bioinformatics pipeline in our MicroDx\(\textsuperscript{®}\) platform which included the USEARCH global alignment algorithm and UPARSE algorithm. Microbiome beta diversity was analysed using Principal Component Analysis (PCoA) with Spearman distance. Between Class Analysis (BCA) was performed to investigate the alteration of the microbiota. Investigation of the microbiome structure was based on co-occurrence networks, generated using the ReBoot method.

**Methods**

**Observational Study**

The microbiota of IBS subjects was distinct from that of healthy subjects (Fig. 1A) but the microbiota profile of the clinical IBS subtypes did not differ (Fig. 1B) in the observational study.

The structure of the microbiome was less interconnected in the IBS cohort when compared to the healthy cohort (Fig. 2A-B).

**Blautix Phase Ib RCT**

The microbiota of IBS subjects differed significantly from healthy subjects (not shown) and moved towards that of healthy subjects after Blautix treatment (Fig. 3A) but not after receiving placebo (Fig. 3B).

An increase in network connectivity and microbiota structure was observed in the Blautix-treated IBS subjects which was retained at washout (Fig. 4A-C). Blautix treatment had less of an impact on the healthy subjects with a slight increase in connectivity being observed which reverted back towards baseline at washout (Fig. 4D-F).

**Results**

- Global microbiota profiles differ between IBS and healthy subjects
- A less dense ecological network is observed in the IBS microbiome
- Blautix treatment altered the microbiome and improved network connectivity in IBS in a Phase Ib study
- Structural changes in the microbiota need to be considered when developing therapeutic candidates
- Any effect of therapeutics on the microbiota structure is likely to impact on the functional properties of the microbiome

**Conclusions**

- **Global microbiota profiles differ between IBS and healthy subjects**
- A less dense ecological network is observed in the IBS microbiome
- Blautix treatment altered the microbiome and improved network connectivity in IBS in a Phase Ib study
- Structural changes in the microbiota need to be considered when developing therapeutic candidates
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**References**