COLONIC MUCOSAL BACTERIAL DIVERSITY OF DE NOVO EXTENSIVE PAEDIATRIC ULCERATIVE COLITIS BY NEXT-GENERATION SEQUENCING

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Introduction Dysbiosis may contribute to inflammatory bowel disease (IBD) pathogenesis along with a reduced bacterial diversity. Limited bacterial diversity studies have been performed at the onset of disease in adults but rarely in children. High-throughput, parallel sequencing technology (next-generation sequencing) provides the means of assessing microbial diversity in samples from diverse ecosystems such as the colonic mucosa.

Methods Paediatric patients undergoing colonoscopy were recruited to two groups: those with a new diagnosis of IBD at their first presentation and controls with a macroscopically normal colon and no evidence of IBD on biopsy. All subjects were free from systemic antibiotics, steroids and immunosuppression for 3 months. 5 extensive UC patients (E3) by Montreal criteria and 5 controls with macroscopically/microscopically normal colons were selected for assessment. The median age was 11.5 years in the UC group and 10.7 years in the controls. All patients were male. Colonic mucosal biopsies were taken from the rectum/sigmoid. DNA extraction was performed by a modified Qiagen QiAMP mini-kit method. The presence of bacteria was confirmed by universal eubacterial primers before next-generation PCR utilising V3 Forward/V6 Reverse primers. Bacterial diversity was assessed by 454 Titanium sequencing. Sequencing data was filtered, chimera and error checked and denoised before rarefaction to 13,000 reads per sample. Statistical comparisons were made by Mann–Whitney U tests using Sigma Plot 11.

Results All biopsies were positive for bacterial DNA with universal eubacterial primers. The most commonly identified bacterial phyla (comprising 95.4% of sequence reads) were Bacteroidetes (45.3%), Firmicutes (40.5%) and Proteobacteria (9.7%). Bacteroidetes were significantly more common in the control colon than in UC (7641 median reads versus 4062, p=0.032) whereas Firmicutes were significantly more common in the UC colon than in controls (5471 median reads versus...
3892, p=0.016). The difference between *Proteobacteria* was not significant (p=0.421). Bacterial diversity assessed by the Shannon index was similar in both groups (Medians of 6.1 in UC and 6.5 in controls, p=0.841).

**Conclusion** Colonic mucosal bacteria differ between paediatric patients with extensive UC at diagnosis and controls. UC microbiota was typified by a reduction in *Bacteroidetes* and an increase in *Clostridia*. Surprisingly, a reduction in bacterial diversity is not present in extensive UC at diagnosis. This is contrary to findings from previous studies in established disease and warrants further investigation.

**Competing interests** None.

**Keywords** Inflammatory Bowel Disease, Microbiota, paediatric, ulcerative colitis.