Dear Sir,

Primary sclerosing cholangitis (PSC) is a liver disease often associated with IBD.1 Dysbiosis of the gut microbiota is implicated in PSC aetiology in adults,2–4 but less is known about paediatric-onset PSC.

We analysed the faecal microbiota of 27 Japanese patients with paediatric-onset PSC as well as 16 age-matched patients with UC and 23 healthy controls (HCs) (see online supplementary table S1) with pyrosequencing data of 16S rRNA gene V1-V2 region (accession #DRA004773). We assessed the influence of medications on the gut microbiota and found that salazosulfapyridine (SASP) treatment affected the microbiota structure with significant changes in the abundance of six major genera between the treated and untreated patients with PSC, perhaps due to its bactericidal property (see online supplementary figure S1). We thus report the analysis of 13 patients with PSC and 15 patients with UC and 23 HCs, all SASP untreated (see online supplementary table S2). Clustering analysis of the 16S reads revealed that the microbiota in the PSC and HC groups had significantly high species richness compared with the UC group, and the species richness of PSC samples was lower than that of HCs. The PSC group also exhibited an intermediate trend in the Shannon’s index between the UC and HC groups (figure 1A). The unweighted UniFrac metric revealed a significant difference in the overall microbiota structure among the three groups, in which the PSC samples tended to aggregate between the HC and UC samples (figure 1B, C). Collectively, the data suggested that the patients with paediatric-onset PSC had gut microbial dysbiosis, the degree of which was less than that of the patients with UC. Dysbiosis in PSC appears to be common regardless of age and the host’s genetic background.2,3 The comparison of microbial abundance identified nine genera showing significant changes among the three groups (figure 2A). The abundance of Parabacteroides and Enterococcus was significantly decreased and increased in the PSC and UC groups compared with HCs, respectively. Over-representation of the Enterococcus genus in adult PSC was also reported.3 The other seven genera showed significant changes in abundance only between the UC and HC groups. However, the abundance of Faecalibacterium, Ruminococcus and Roseburia was significantly higher in the PSC than UC group. Since these three genera include many species producing an anti-inflammatory butyrate, there might be a different degree of inflammatory states

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**Figure 1** Comparison of the overall faecal microbiota structure among the primary sclerosing cholangitis (PSC), UC and healthy control (HC) groups. (A) Microbial richness and α-diversity in the faecal microbiota of 13 PSC and 15 UC patients without salazosulfapyridine (SASP) treatment and 23 HCs. Richness was evaluated by the observed and the Chao 1-estimated Operational Taxonomic Unit (OTU) numbers generated from clustering of 3000 16S reads per sample, and diversity is based on Shannon’s index. Kruskal-Wallis test followed by Steel-Dwass test was used for multiple comparisons (*p<0.05, **p<0.01, ***p<0.001). (B) Principal coordinate analysis (PCoA) based on the unweighted UniFrac analysis of bacterial community structures of the PSC (red), UC (green), and HC (blue) groups. (C) Evaluation of dissimilarity between two groups by permutational multivariate analysis of variance (PERMANOVA). R² indicates the coefficient of determination. Significant p values are in bold.
between PSC and UC. At the species level, we identified 16 species showing significant changes in abundance among the three groups (figure 2B). The significant reduction of *Anaerostipes hadrus*, *Parabacteroides distasonis* and *Blautia obeum* was observed in both the PSC and UC groups, whereas the other seven species were significantly reduced only in the UC group. Regarding the increased species in the PSC group, the abundance of *Streptococcus parasanguinis*, *Veillonella sp. 3_1_44*, *Enterococcus faecium*, and *Enterococcus sp. NBRC 107345* was significantly increased in the PSC compared with HCs. Of them, only *S. parasanguinis* showed a significant enrichment in the PSC compared with UC group. It was also reported that the *Streptococcus* genus tended to be more abundant in PSC than UC. The significant enrichment of *Veillonella sp. 3_1_44* in the present PSC group is similar to that of the previous report on adult PSC. A concurrent proliferation of the *Veillonella* and *Streptococcus* species might involve immunomodulation mediated by symbiotic interactions between these two species in PSC. Overall, our data suggest that several distinct species belonging to the genus *Enterococcus*, *Streptococcus* and *Veillonella* are associated with the pathogenicity of paediatric-onset PSC. Further studies may confirm the PSC-associated bacteria observed here, and the influence of differences in medications as described here, disease duration, nationalities and methodologies on the gut microbiota should be considered.

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