論文の内容の要旨
Dissertation Abstract

論文題目
Dissertation Title:

Characterization of Salivary Microbiota in Inflammatory Bowel Diseases and Its Relation to Oral Immunological Markers

（炎症性腸疾患の唾液細菌叢の特徴解明とその口腔免疫マーカーとの関係）

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Inflammatory bowel diseases (IBD) are chronic, relapsing inflammatory disorders of the gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are the major forms of IBD. It is known that gut microbiota plays a critical role in IBD pathogenesis. Furthermore, fecal and mucosa-associated microbiota qualitatively and quantitatively changed in IBD patients compared to healthy controls, termed dysbiosis. However, these studies focused on the lower part of the gastrointestinal tract, yet limited information exists about oral microbiota of IBD patients. Recent studies also showed increased frequency of oral and dental problems among IBD patients, suggesting microbial dysbiosis in the oral cavity. Testing this hypothesis requires comparison of salivary microbiota of IBD patients with that of healthy controls. In this study, I characterized the salivary microbiota of IBD patients by high-throughput pyrosequencing analysis of the bacterial 16S rDNA gene. Moreover, I evaluated inflammatory states of the saliva in IBD patients and healthy controls by measuring the level of several immunological markers such as lysozyme, immunoglobulin A (IgA) and cytokines to elucidate correlation between the microbiota and the immunological markers in saliva.

I surveyed the salivary microbial communities of 40 IBD patients, including 21 CD patients and 19 UC patients, in comparison with 24 healthy controls (HCs). After collection of saliva samples and extraction of whole genomic DNA, V1-V2 region of bacterial 16S rDNA gene was amplified using 27modF and 338R primers and sequenced using the 454 GS FLX platform. An analysis pipeline of pyrosequencing data was constructed. At first raw data

were assigned to each sample depending on its specific barcode sequence. Reads having both forward and reverse primer sequences represented ~60% of total number of reads/sample and were selected for the next step. Reads having average quality < 25 and possibly chimeric sequences were filtered out. After filtering and denoising, 3,000 reads per sample were randomly chosen, rearranged according to its average quality value, and then grouped into operational taxonomic units (OTUs) at 96% pairwise-identity cutoff. Each OTU representative sequence was assigned to the best BLAST hit phylotype.

Using UniFrac distance metric, I compared the overall bacterial composition in saliva of IBD patients and HCs. Principal coordinate analysis showed clustering of most of CD samples apart from HC samples, indicating the difference in bacterial communities between the two groups. However, UC group did not show specific clustering apart from HCs. The difference in the overall microbiota structure between CD and HC groups was significantly larger than the variability within CD or HC groups, while the difference between UC and HC groups was not significant (Fig. 1).

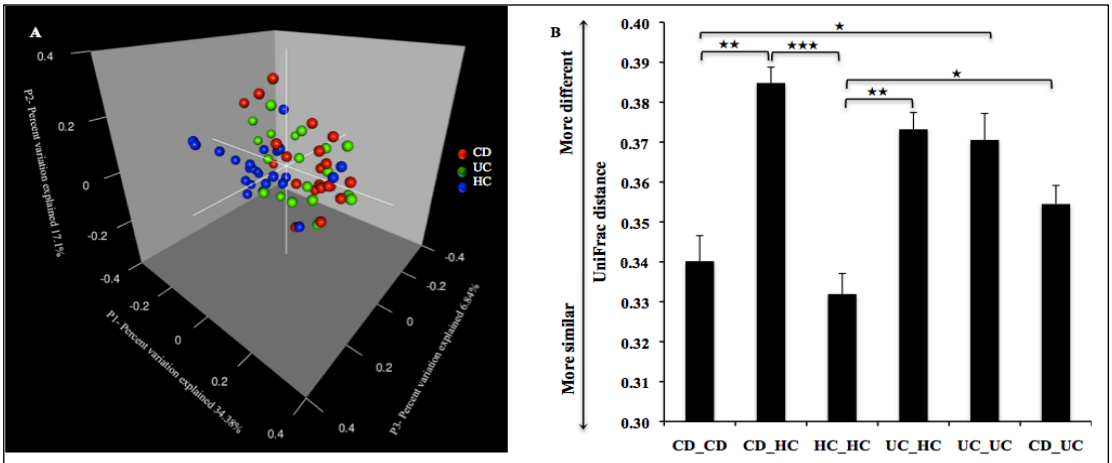

Fig. 1. (A) Principal coordinate analysis (PCoA) plot generated using weighted version of the UniFrac metric. (B) Weighted Unifrac distance between different groups (HC&CD, HC&UC, HC&HC, CD&CD, UC&UC).

Analogous to studies of the intestinal microbiota, analyzing the fecal or biopsy samples, in IBD, I demonstrated dysbiosis of the salivary microbiota of IBD patients, particularly in CD group. While the microbiota composition was not significantly changed in UC group. Species assignment revealed that the relative abundance of the phylum Bacteroidetes was significantly increased in CD group compared to HCs, while Proteobacteria was significantly decreased. At genus level, *Prevotella* (Bact) and *Veillonella* (Firm) were significantly increased, while 3 other genera, *Nisseria* (Prot), *Haemophilus* (Prot) and *Gemella* (Firm) were significantly decreased in CD group compared to HCs (Fig. 2). These data indicated that a dominant *Prevotella* largely contributed to the increase of the phylum Bacteroidetes, and dominant *Nisseria* and *Haemophilus* largely contributed to the decrease of the phylum Proteobacteria.

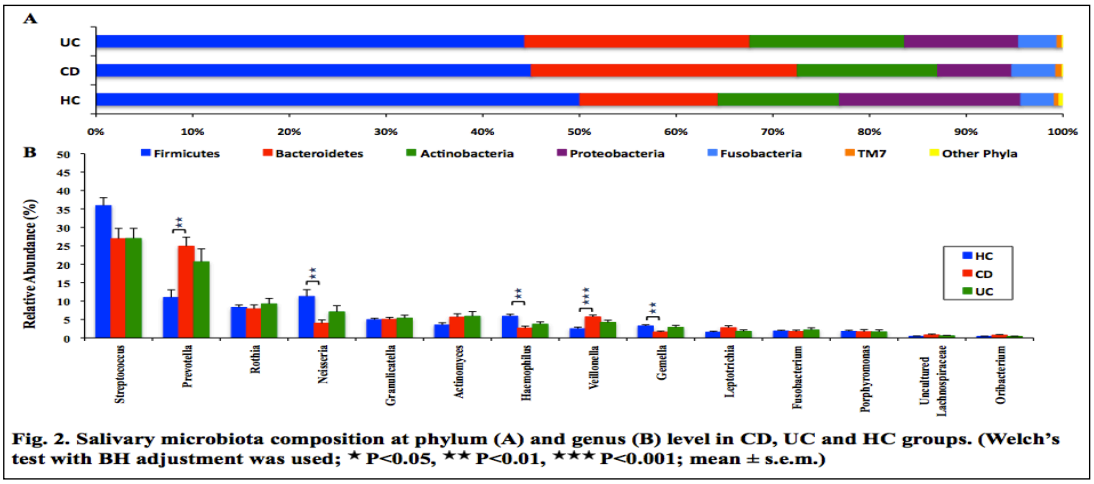

Fig. 2. Salivary microbiota composition at phylum (A) and genus (B) level in CD, UC and HC groups. (Welch's test with BH adjustment was used; ⋆ P<0.05, ⋆⋆ P<0.01, ⋆⋆⋆ P<0.001; mean ± s.e.m.)

I also evaluated inflammatory states in saliva of the 3 groups by measuring immunoglobulin A (IgA), several cytokines and enzymes including lysozyme. The level of lysozyme was significantly decreased only in CD group compared to HCs, and the lowered level of lysozyme was also found to be more striking in active CD than in CD in remission. On the other had, the level of other antimicrobial proteins, HBD2 and LL37, were similar among the 3 groups. The level of IgA showed no significant difference among the 3 groups. Among cytokines examined, only IL-1ra was shown to significantly increase both in CD and UC groups compared to HCs. On the other hand, UC group showed the higher level of IL-1b, IL-6, IL-8, MCP-1 and MIP-1 than HCs. These data indicate that the inflammatory state of the gut is reflected in saliva of IBD patients and saliva is a promising specimen for monitoring and accessing severity of the disease.

To assess correlation between the relative abundance of different bacterial genera with measured immunological markers in saliva, I used Pearson's correlation coefficient. The analysis revealed correlation between several bacterial genera and immunological markers. For example, the relative abundance of *Prevotella* in CD group was positively correlated with IL-1b, IL-1ra, and IL-6 (r=0.57, 0.71 and 0.5, respectively), while it was negatively correlated with IP-10 and lysozyme (r= -0.6 and -0.59, respectively).

In conclusion, analysis of bacterial 16S rDNA gene using high-throughput pyrosequencing revealed dysbiosis of salivary microbiota of IBD patients. Moreover, the analysis showed significant correlation of several bacterial phylotypes and immunological markers, suggesting that the dysbiosis of salivary microbiota may be linked to aberrant immunological states in oral cavity of IBD patients. The results also provide proof of salivary microbiota as an informative source for discovering systemic diseases.