Final report for the study entitled "Commensal bacteria as a novel biomarker to predict type 1 diabetes progression"

Since the last report, we have made a significant progress even in the mist of the COVID-19 pandemic, which has affected our academic activities. Since the last progress report, we carried out more studies using the oral samples collected from the patients with T1D and healthy individuals. Below are our research findings.

Altered composition of gut microbiota is associated with decreased SCFA production and increased IgA-binding in T1D patients

To explore the cross-talk of gut microbiota, SCFAs and IgA immune responses in T1D, we first studied the stool microbiota composition from newly-diagnosed pediatric donors (the average duration of diabetes was 5 months and the range was 3 days to 12 months), and age- and gender-matched healthy control subjects (Table 1 and Figure 1A). Of the 19 patients, 18 were positive for one or more of the three autoantibodies to glutamic acid decarboxylase (GAD), insulinoma associated protein 2 (IA2), and zinc transporter 8 (ZnT8). None of the healthy controls was positive for any of the islet autoantibodies. We found increased α-diversity (Figure 1B and 1C), with a significant increase in the relative abundances of *Ruminococcaceae* and *Coprococcus* (Firmicutes) at the species level in individuals with T1D, compared to control subjects (Figure 1D-E). In contrast, there was a significant reduction in the relative abundances of *Roseburia* and *Megamonas* (Firmicutes) at the species level (Figure 1F-G). To determine whether the stool microbial composition changes influenced the microbial production of SCFAs, we measured stool microbe-derived SCFAs by gas chromatographic mass spectrometer (GC-MS). We found significant reductions in acetate, butyrate and propionate concentrations in individuals with T1D compared to age- and gender-matched healthy controls (Figure 1H-I and Figure S1A), while the levels of hexanoic acid, isobutyrate, valerate and isovalerate were unchanged (Figure S1B-E). Interestingly, acetate was positively correlated with β cell function, as indicated by the level of fasting C peptide (FCP) (Figure 1J).

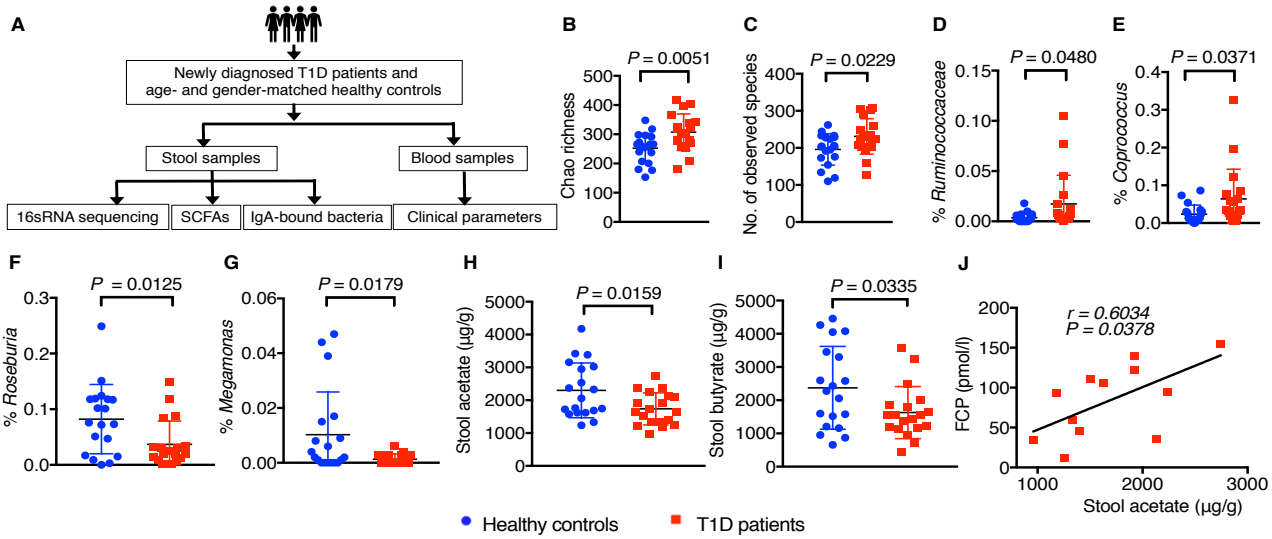

Figure 1. Stool SCFA production and bacteria-targeting IgA response in individuals with T1D compared to control subjects. (**A**) Experimental design of the study in patients with T1D and healthy control subjects. (**B-G**) Stool microbiota composition was investigated by 16S rRNA sequencing (*n* = 19/group). Changes in alpha diversity were assessed by Chao richness (B) and number of observed species (C). Altered relative microbial abundances of *Ruminococcaceae* (D), *Coprococcus* (E), *Roseburia* (F) and *Megamonas* (G) between donors with T1D and controls are shown. (**H-I**) Stool acetate (H), and butyrate (I) concentrations from individuals with T1D and control subjects (*n* = 19/group). (**J**) Correlation between stool acetate level and serum fasting C-peptide concentration (*n* = 15). Data are presented as mean ± SEM and were analyzed by a two-tailed Student's *t*-test (B-I). Data in (J) was analyzed using a two-tailed *Pearson* correlation coefficient test and linear regression.

IgA binding to bacteria are linked to T1D

We investigated the proportion of IgA-bound bacteria in our study subjects. We detected a higher proportion of IgA-bound bacteria in individuals with T1D compared to healthy control subjects (Figure 2A-B). Furthermore, the level of these IgA-bound bacteria was negatively correlated with the concentration of stool SCFAs, including acetate, butyrate and propionate (Figure 2C-E) and thus we observed a higher proportion of IgA-bound gut bacteria and lower concentrations of three SCFAs. In addition, stool acetate concentration was negatively associated with the abundance of *Eubacterium* and *Hathewayi* (Firmicutes), and stool butyrate concentration was negatively correlated to *Enterococcaceae* (Firmicutes) level (Figure 2F-H). Taken together, our data support the hypothesis that newly-diagnosed individuals with T1D have altered gut microbiota, resulting in reduced concentrations of SCFAs. Interestingly, the altered gut microbiota promoted an increased IgA response to the bacterial targets, hence there were more IgA-bound gut bacteria in patients with T1D. These results suggest that SCFA may regulate bacteria-reactive IgA immune responses.

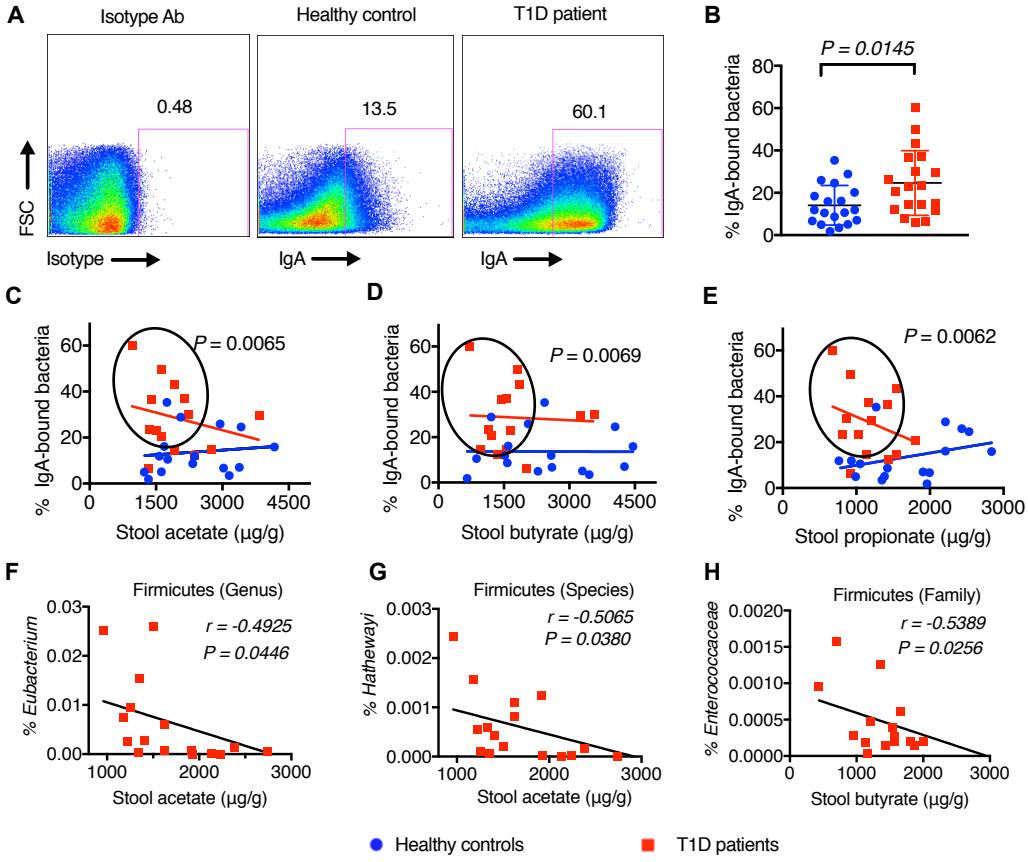

Figure 2. IgA-bound bacteria and the correlations with different SCFAs in individuals with T1D and control subjects. (A) Representative flow cytometric profiles of IgA-bound bacteria. (B) Summary of IgA-bound bacteria percentage from donors with T1D and healthy controls (n = 19/group). (C-E) Correlations between stool acetate (C), butyrate (D) or propionate (E) concentration and the level of IgA-bound bacteria (The overall elevation or intercepts between the two groups was compared. The black circles show that there were more patients in the upper left areas, i.e., with a higher percentage of IgA-bound bacteria but lower stool SCFAs, n = 14-16). (F-H) Correlations between stool acetate concentration and the relative abundances of *Eubacterium* (F) and *Hathewayi* (Firmicutes) (G), and between stool butyrate concentration and *Enterococcaceae* (Firmicutes) abundance (H) (n = 17/group). Data are presented as mean ± SEM and were assessed for statistical significance using a two-tailed Student's t-test (B). Data in (F-H) were analyzed using a two-tailed *Pearson* correlation coefficient test and/or linear regression.

Taken together, we demonstrate that gut bacteria from patients with T1D alter the host intestinal and systemic IgA immune responses, which are mediated by gut microbiota-derived SCFAs. The contribution of the host intestinal and systemic IgA immune responses, associated with gut microbiota, to directly causing T1D development in humans would be confirmed in a future longitudinal study in pre-diabetic individuals. However, our cross-sectional study provides novel insights regarding the function of gut microbiota and their metabolites in the immuno-pathogenesis of T1D. Moreover, IgA-bound gut bacteria might be used as an additional biomarker for T1D. Our findings are now in preprint for publication.

We are very grateful for the support by Diabetes Research Connection and also very grateful for the generous donors.