The positive correlation between *Porphyromonas gingivalis* and *Prevotella* spp. Response to: 'Comment on 'Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population' by Kishikawa *et al.*' by Kitamura *et al*

We thank Kitamura *et al.* for their interest in our study and for providing their thoughts through correspondence.¹ They reported interesting characteristics of *Porphyromonas gingivalis* (*Pg*) regarding rheumatoid arthritis (RA) as follows: (1) oral infection of *Pg* increased serum levels of lipopolysaccharide

(LPS)^{2,3}; (2) oral administration of *Pg* decreased the proportion of phylum *Bacteroidetes*³; and (3) serum LPS levels were inversely associated with *Bacteroides* counts. In our metagenome-wide association study (MWAS) of the RA gut microbiome,^{4,5} we had identified high abundances of five species belonging to the genus *Prevotella* (i.e., *P. denticola*, *P. marshii*, *P. disiens*, *P. corporis*, and *P. amnii*) in the RA metagenome. Considering that *Prevotella* in the RA gut microbiome showed an inverse relationship with *Bacteroides*,^{6,7} disentanglement of gut microbiome link between *Pg* and the *Prevotella* spp. should be of interest.

In our RA MWAS, we had excluded *Pg* from the analysis because the average relative abundance of *Pg* was below the quality control threshold of 0.001%. Here, we additionally examined the case-control association test of *Pg* and found no significant association ($P = 0.78$). However, we found significant

Figure 1 Characteristics of *Porphyromonas gingivalis* (*Pg*) in the rheumatoid arthritis (RA) gut microbiome. (A) Correlation between the relative abundance of *Pg* and that of *Prevotella* spp. The x-axes indicate the relative abundance of *Pg* in a logarithmic scale. The y-axes indicate the relative abundance of each of the five *Prevotella* species with significant RA-control discrepancy (ie, *P. denticola*, *P. marshii*, *P. disiens*, *P. corporis*, and *P. amnii*) and the total abundance of the five *Prevotella* spp. in a logarithmic scale. (B) Correlations between the relative abundance of the taxa and RA activity indices and biomarkers. Only correlations with p values less than 0.05 were coloured (positive correlations in red and negative correlations in blue). Wilcoxon rank-sum tests were used to compare the high-level and low-level groups. Stage indicates Steinbrocker classification of the joint X-rays. RF, rheumatoid factor; ACPA, anti-citrullinated peptide antibody; DAS28CRP, Disease Activity Score 28-joint count C reactive protein.

positive correlations of the relative abundance between *Pg* and three of the RA-associated five *Prevotella* species (i.e., *P. denticola*, *P. corporis* and *P. amnii*; $P < 0.017$; figure 1A). When we focused on the total abundance of the five *Prevotella* species, significant positive correlation was also found ($r = 0.291$, $P = 0.0011$). This result suggests that *Pg* and the *Prevotella* spp. in the RA gut microbiome cooperate in the RA pathophysiology.

Another concern by Kitamura *et al.* was the distinct distributions of the RA prognostic factors between the RA case groups with high and low abundance in the *Prevotella* spp or *Pg*. They reported that serum LPS-binding protein was positively correlated with activity indices and biomarkers of RA (e.g., Disease Activity Score 28-joint count C reactive protein (DAS28CRP), CRP, rheumatoid factor (RF) and anti-citrullinated peptide antibody (ACPA)).¹ We assessed whether the relative abundance of the *Prevotella* spp and *Pg* showed the correlation with RF, ACPA, DAS28-CRP and the Steinbrocker stage. As for RF and ACPA, the RA cases were compared between the high-level and low-level groups stratified according to the threshold of 15 and 4.5 IU/mL, respectively. While *Prevotella corporis* had nominally significant positive correlation with ACPA (fold change = 3.18, $P = 0.0098$), most of the correlations were not significant (figure 1B). In our study samples, we did not observe positive correlation of the five *Prevotella* spp. and *Pg* with RA activity indices and biomarkers.

In conclusion, our study suggests that *Pg* and the *Prevotella* spp. cooperate in the RA gut microbiome. Further studies focusing on the interaction of these two taxa are warranted to elucidate RA aetiology.

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