Response to: 'Can sexual dimorphism in rheumatoid arthritis be attributed to the different abundance of *Gardnerella*?' by Liu *et al*

We thank Liu *et al* for their interest in our paper and for providing their thoughts through correspondence.¹ We agree that gut microbiome studies are promising to identify novel insights into the sexual dimorphism in rheumatoid arthritis (RA). We reported that the genus of *Gardnerella* and the species of *Gardnerella vaginalis* increased in the gut microbiome of patients with RA.² *G. vaginalis* is known as a representative causal bacterium of vaginosis. In our study, *G. vaginalis* was detected in both male and female samples, and there were no significant gender differences in their relative abundance ($P=0.41$). Thus, the increment of *G. vaginalis* was less likely to be contamination from the female genital organ in perineum. We further performed case-control association tests of *G. vaginalis* stratified by gender with age, sequencing groups and the top two principal components as covariates (figure 1A). We found that the effect size was larger in female samples ($\text{beta}=1.14$, $P=4.1\times10^{-4}$) than in male samples

($\text{beta}=0.596$, $P=0.22$). This result suggests that the increment of *Gardnerella* in the gut microbiome of patients with RA is specific to the female samples.

Gardnerella has been detected not only in vaginitis but also in a variety of infections, such as hip arthritis and joint infections.³ However, their biological and pathological roles in gut microbiome have been elusive. *G. vaginalis* was reported to have a symbiotic positive relationship with *Prevotella bivia*.⁴ Considering the association of *Prevotella* with RA aetiology,⁵⁻⁷ Liu *et al* proposed that *G. vaginalis* affects RA aetiology through the symbiotic proliferation of *Prevotella*. In our study, there was no significant positive correlation between the relative abundance of the genus *Prevotella* and that of *G. vaginalis* ($r=0.054$, $P=0.47$; figure 1B). However, when we focused on the total abundance of the five *Prevotella* species with significant RA-control discrepancy (i.e., *P. denticola*, *P. marshii*, *P. disiens*, *P. corporis* and *P. amnii*), significant positive correlation was found ($r=0.20$, $P=0.024$). Among the five RA-associated species, *P. amnii* had nominally significant positive correlation ($r=0.19$, $P=0.035$) with *G. vaginalis*, while the others did not ($P>0.062$). There was no significant correlation between *P. bivia* and *G. vaginalis* either ($P=0.84$). These results demonstrated that *Gardnerella*

Figure 1 Characteristics of the relative abundance of *Gardnerella vaginalis* in RA samples. (A) Boxplots of the relative abundance of *G. vaginalis* in RA and control samples. The y-axes indicate the relative abundance of *G. vaginalis* in a logarithmic scale. The left, centre and right boxplots are for all samples, only female samples and only male samples, respectively. The lower and upper hinges of the boxes indicate the first and third quartiles, respectively. The horizontal lines within the boxes indicate median levels. (B) Correlation of the relative abundance of *G. vaginalis* with that of *Prevotella* spp. The x-axes of the left, centre and right figures indicate the relative abundance of the genus *Prevotella*, the total abundance of the five *Prevotella* species with significant RA-control discrepancy (i.e., *P. denticola*, *P. marshii*, *P. disiens*, *P. corporis* and *P. amnii*) and the relative abundance of *P. amnii* in a logarithmic scale, respectively. The y-axes indicate the relative abundance of *G. vaginalis* in a logarithmic scale. RA, rheumatoid arthritis.

and RA-associated *Prevotella* had a symbiotic relationship in gut microbiome. Further studies are required to reveal the role of the *G. vaginalis* in the gut microbiome of patients with RA.

Liu *et al* also mentioned that female patients with RA confer more severe inflammation profiles (i.e., 28-joint Disease Activity Score (DAS28)) than male patients.⁸ Assessing whether *Gardnerella* is responsible for the sexual difference of RA severity, we performed association tests between the relative abundance of *G. vaginalis* and DAS28-CRP, but no significant association was found ($P=0.91$, $r=-0.013$). This result indicates that *Gardnerella* in gut microbiome may not be related to RA severity in female samples.

We hoped that researches focusing on *G. vaginalis* would lead to further clarification of aetiology of RA.

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