Hidden link in gut–joint axis: gut microbes promote rheumatoid arthritis at early stage by enhancing ascorbate degradation

With great interest, we read the review article by Agus *et al*, which suggested that gut microbiome alterations could affect metabolic homeostasis.[1] Moreover, gut microbiome alterations in concert with metabolites perturbation could contribute to the early development of rheumatoid arthritis (RA).[2] We thus conducted a three-pronged association study[3] on multiomics datasets to detect the potential microbiome–metabolites–arthritis link.

We integrated multiomics datasets including gut metagenomics, clinical phenotypes and metabolites of blood and knee-joint synovial fluid from 122 participants in the healthy group (n=27), osteoarthritis (OA) group (n=19) and RA group (n=76), using a three-pronged association framework (figure 1, online supplemental material).[3] Metagenomic genes were collapsed into metagenomic species (MGS)[3 4] and grouped into KEGG functional modules (figure 1A).[3] Additionally, the co-abundant metabolites were categorised into metabolite clusters using WGCNA framework (figure 1A).[3] The functional modules associated with clinical phenotypes (eg, types of arthritis and levels of cytokines) were further identified and the cross-domain associations between these modules and metabolite clusters were assessed (figure 1B).[3] Furthermore, the leave-one-out analysis was performed to determine the MGS that particularly contributed to the observed linkage between functional modules and clinical phenotypes (figure 1C).[3]

We found that gut microbial functionality in ascorbate degradation (KEGG module: M00550) was positively correlated with the types of arthritis (healthy=0, OA=1, RA=2, $p_{Wilcox}=2.15\times10^{-4}$) and the levels of proinflammatory cytokines TNF-α (tumour necrosis factor-α, $p_{Wilcox}=6.59\times10^{-4}$) and IL-6 (interleukin-6, $p_{Wilcox}=1.12\times10^{-3}$). Ascorbate (vitamin C) was previously reported to prevent the development of inflammatory arthritis,[5] possibly through facilitating collagen synthesis, moderating autoimmune responses and ameliorating inflammation.[6] Additionally, the patients with RA are usually ascorbate deficient and require high-dose supplementation to maintain an acceptable plasma level of ascorbate.[7] In this study, the functional module of ascorbate degradation was observed to positively correlate with the blood metabolite cluster MB02 ($p_{Wilcox}=6.90\times10^{-3}$), which was represented by the level of palmitic acid (kME (eigengene-based connectivity) =0.911, kIN (intramodular connectivity) =3.46, online supplemental table 1) that acts as a proinflammatory factor, upregulating IL-6 secretion by human chondrocytes and fibroblast-like synovial cells in inflammatory arthritis.[8] Furthermore, we found that *Escherichia coli* and *Streptococcus bovis* were the driving species for the observed linkage between ascorbate

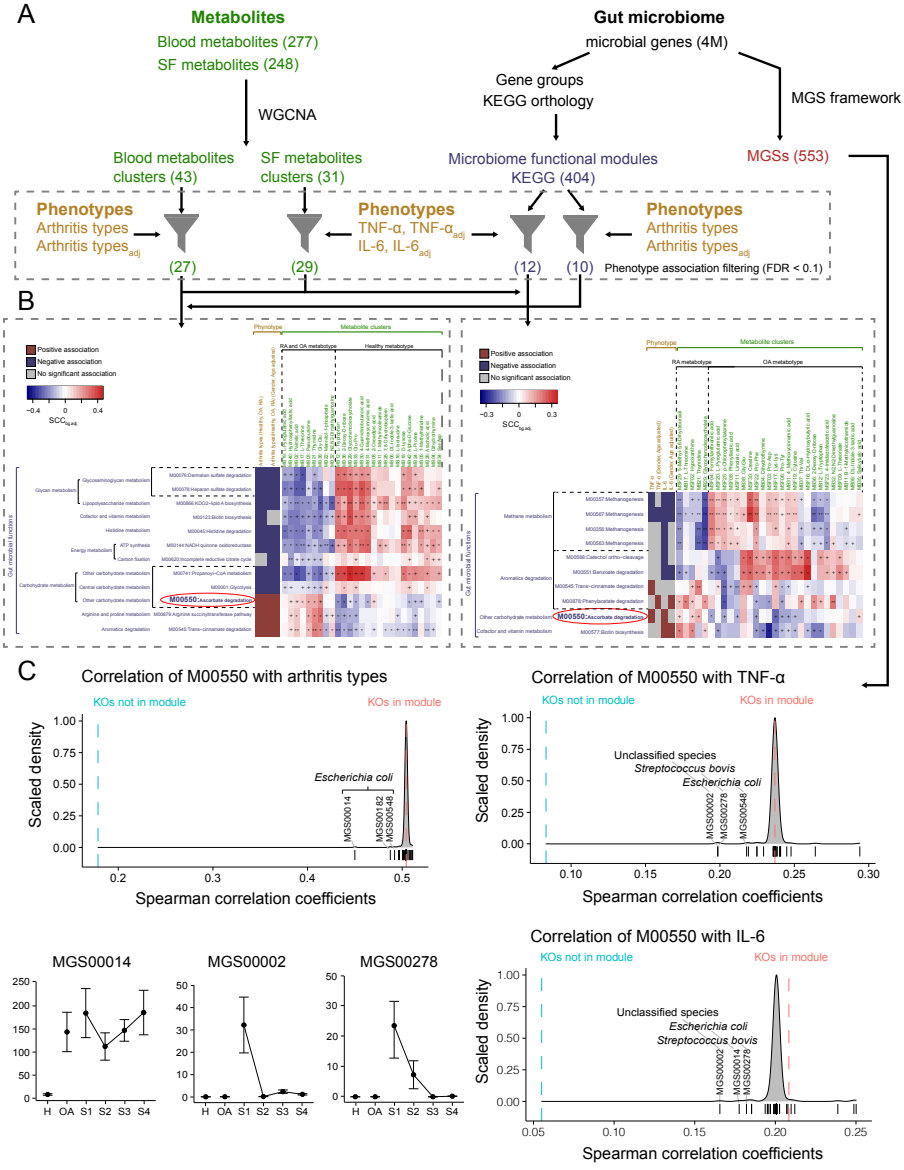

Figure 1 Overview of the three-pronged association framework integrating multiomics datasets. (A) Metabolites are summarised as co-abundance clusters, and microbial genes are grouped into KEGG modules and MGS, which are further filtered for statistically positive or negative associations (based on Spearman correlation) with the clinical phenotypes. The association analyses were divided by using healthy, OA and RA samples for arthritis types and using OA and RA samples for cytokine levels. The number in brackets represent the number of metabolites/metabolite clusters/microbial genes/KEGG modules/MGS in each analytical module. (B) The filtered features are further used for cross-domain association analyses. For each analysis, the left panel shows the significant associations (Mann-Whitney U test FDR<0.1) between KEGG modules and clinical phenotypes, and colour indicates significantly positive association (red), significantly negative association (blue) or insignificant association (grey). The right panel shows the associations between KEGG modules and metabolite clusters, and the colour represents the median Spearman correlation coefficient (SCC) of metabolite clusters with KEGG orthologies (KOs) in KEGG module minus those with KOs not in KEGG module. Mann-Whitney U test FDRs are denoted: $^{+}$FDR<0.1; *FDR<0.01; **FDR<0.001. (C) The MGS that particularly contributed to the observed linkage between functional modules and clinical phenotypes. Three density plots: Dashed line represents the median SCC of the phenotypes with KOs in M00550 (red) and all other KOs (blue). Density plot shows the median SCC of the phenotypes with KOs in M00550, when a given MGS (indicated by short vertical lines) has been excluded from the analysis. The bottom-left dot plots show the mean±SEM of the top three driving MGS abundances among patients at each stage of disease development, with the four RA stages connected to display the variance. FDR, false discovery rate; IL-6, interleukin-6; MGS, metagenomic species; OA, osteoarthritis; RA, rheumatoid arthritis; TNF-α, tumour necrosis factor-α.

degradation[9] and the arthritis types or the cytokines levels of TNF-α and IL-6 (figure 1C). Subsequently, we grouped patients with RA by four stages according to the comprehensive scores in rheumatoid diagnostic criteria,[10] as RASI: 6–7, RASII: 8, RASIII: 9 and RASIV: 10 (online supplemental table 2). We observed that both *E. coli* and *S. bovis* were prevalent at RA stage I (RASI), while *S. bovis* was depleted after RASI or in the OA group. It suggested *S. bovis* mainly functioned at the early stage of RA, while *E. coli* might be crucial throughout the entire developmental stages of RA and OA. Taken together, we speculate that *E. coli* and *S. bovis* could facilitate ascorbate degradation and thus promote proinflammatory responses that facilitate the development of inflammatory arthritis.

Overall, we demonstrate that gut microbiota could promote RA progression via enhancing ascorbate degradation and provide a potential approach to prevent the development of arthritis through interfering gut–joint axis. The results of this study could be prospected in following contexts: First, our study provides a reservoir of the potential microbiome–metabolites–arthritis links as a reference of gut–joint axis for future studies. Second, the findings supplement the potential mechanisms related to metabolic perturbation through which gut microbiome promotes arthritis.[1 2] Third, considering the inflammatory pathways of arthritis were revisited in COVID-19,[11] it deserves further investigations whether microbiome–ascorbate–inflammation link of this study could contribute to the treatment of COVID-19.

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