Correspondence on '*Prevotella copri* in individuals at risk for rheumatoid arthritis'

We read with great interest the articles by Alpizar-Rodriguez *et al*[1] with regard to the impact of dysbiosis on the risk of rheumatoid arthritis (RA). Meanwhile, we noticed the opposite conclusions of Mendelian randomisation (MR) research works from Inamo[2] and Lee.[3] For obtaining a reliable result, we thought some issues were supposed to pay attention to and illustrate clearly in the MR study. To begin with, single nucleotide polymorphisms (SNPs) associated with gut microbiome at the genome-wide significance level ($p<5\times10^{-8}$) should be derived from a genome-wide association study (GWAS) with the largest sample size or a meta-analysis of GWASs, rather than combining SNPs from different GWASs to analyse directly.[4 5] Initially, we obtained a total of 41 genetic instrumental variables (IVs) from the results of the gut microbiome GWAS carried out with up to 1812 individuals of European ancestry.[6] To avoid the ethnic heterogeneity of genetic associations, the effect size and standard errors for the associations of IVs with RA were extracted from a large RA GWAS, including 14 361 cases and 43 923 controls of European population.[7] Of these IVs, one variant (rs11724031) was absent in the RA GWAS dataset. We replaced it by a suitable proxy (rs11722967), which was in high linkage disequilibrium (LD) with rs11724031 ($r^2=0.88$ in European populations). Moreover, four SNPs (rs12149695, rs17421787, rs34613612 and rs3925158) were excluded for being palindromic with intermediate allele frequencies. LD of all significant SNPs associated with gut microbiome met the condition: $r^2<0.001$. Thus, 37 SNPs were finally incorporated in the MR analysis (online supplemental table 1).

As shown in table 1, in terms of the inverse variance weighted (IVW) method, the OR of gut microbiome on RA was estimated to be 0.98 (95% CI=0.97–1.00, p=0.024). While MR-Egger, weighted median and weighted mode methods represented non-casual associations between gut microbiome and RA (p=0.919, p=0.186, p=0.542, respectively). Horizontal pleiotropy between IVs and outcome was evaluated by MR-Egger regression, and the results indicated no evidence for a significant intercept (intercept=−0.012, p=0.152). Furthermore, no significant heterogeneity was assessed by Cochrane Q statistics across estimates of included SNPs. The leave-one-out analysis showed that two IVs (rs11722967 and rs986417) can influence the estimated causal effect (online supplemental figure 1). Association of p value derived from IVW, MR-Egger, weighted median and weighted mode methods all turned out to be not significant after removing the two IVs. What is more, as mentioned in the GWAS study of gut microbiome, the included SNPs were virtually associated with specific individual bacterial traits.[6] Then, we classified these SNPs into 19 categories and conducted MR analysis in each category separately (online supplemental table 2). Similarly, we did not find that any genetically predicted abundance of bacterial taxon was relevant with RA risk (online supplemental table 3). As demonstrated by Alpizar-Rodriguez *et al*,[1] individuals at risk for RA tend to have an enrichment of *Prevotella spp* compared with first-degree relatives' controls. Even though the genome-wide significant SNPs of genus *Prevotella* were unable to acquire, MR analysis can be performed at the phylum level for genus *Prevotella* belonged to *Bacteroidetes* phylum (online supplemental table 4). A decrease in genetically predicted bacterial traits of *Bacteroidetes* phylum was not significantly associated with RA by IVW method (95% CI: 0.97–1.01, p=0.098). The OR estimates obtained from other methods were also not significant (online supplemental table 5). The above statistical analyses were performed using 'TwoSampleMR' package in R V.3.5.3.

In summary, our results supported that there was no causal link between gut microbiome and RA. Certainly, the lack of genetic power of the limited SNPs may contribute to the potential failure to explore the association between gut microbiome and RA, as the proportion of gut microbiome variation explained by genetic variation among individuals is not estimated,[6] whereas we still need to note that which variable is appropriate to represent intestinal dysbiosis and whether β diversity is a better choice. Further study with updated SNPs of individual bacterial traits or β diversity from GWASs can help to elucidate the potential role of intestinal bacterial traits on RA.

Tianyue Sun,[1] Jing Ni [2]

[1]The First School of Clinical Medicine, Nanjing Medical University, Nanjing, China
[2]Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, China

Correspondence to Dr Jing Ni, Department of Epidemiology and Biostatistics, Anhui Medical University School of Public Health, Hefei, China; nijing@ahmu.edu.cn

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Table 1 Two-sample MR results for causality for gut microbiome influencing RA

Method	nSNPs	OR (95% CI)	P value	Cochrane Q statistic	Heterogeneity p value	nSNPs*	OR (95% CI) *	P value*	Cochrane Q statistic*	Heterogeneity p value*
IVW	37	0.98 (0.97–1.00)	0.024	45.42	0.135	35	0.99 (0.97–1.01)	0.175	37.96	0.294
Weighted median	37	0.99 (0.96–1.01)	0.186	NA	NA	35	0.99 (0.97–1.01)	0.382	NA	NA
Weighted mode	37	0.99 (0.96–1.02)	0.542	NA	NA	35	0.99 (0.96–1.02)	0.604	NA	NA
MR-Egger	37	1.00 (0.97–1.03)	0.919	42.79	0.172	35	1.01 (0.99–1.04)	0.313	33.05	0.465

*Sensitivity analysis without rs1172296 and rs986417.
IVW, inverse variance weighted; MR, Mendelian randomisation; NA, not applicable; nSNPs, number of single nucleotide polymorphisms; RA, rheumatoid arthritis.

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ORCID iD
Jing Ni http://orcid.org/0000-0001-8284-4941

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