Non-causal association of gut microbiome on the risk of rheumatoid arthritis: a Mendelian randomisation study

I read with great interest the article by Alpizar-Rodriguez *et al* regarding the risk of intestinal dysbiosis, particularly *Prevotella* spp enrichment, in preclinical rheumatoid arthritis (RA).[1] Immune response in gut is assumed to be one of the triggers of development of RA.[2] However, it is hard to assess causal association by case–control study due to limitations such as latent confounding factors; *dysbiosis first or RA first*. Therefore, to investigate causal effect of gut microbiome on the development of RA, I conducted Mendelian randomisation (MR) analysis.[3] MR is useful to investigate causal association among phenotypes and/or biomarkers because it is based on genetic variation to mimic the design of randomised controlled trials. In MR, single nucleotide polymorphisms (SNP) are expected to be random and causally upstream of the exposure; thus, SNP are used as instrumental variables (IVs) in MR.

I used the publicly available two data sets of genome-wide association studies (GWASs) for gut microbiome (totally 3326 individuals) of European ancestry as the exposure[4 5] and one data set of GWAS for RA (19 234 cases and 61 565 controls) of European and Asian ancestries as the outcome,[6] respectively. To improve inference, selection of genetic variants associated with gut microbiome as IVs was based on linkage disequilibrium R^2 of 0.001, clumping distance of 10 000 kb and p value threshold of 5.00E−08 (genome-wide significance). Then, I examined the association between single SNP and risk of RA. Finally, by combining them using MR analysis, I estimated the causal association between gut microbiome and risk of RA. The effect size was shown by beta coefficient or OR. I assessed heterogeneity across SNPs by Cochran's Q statistics. To explore whether single SNPs drives causal association, I performed a leave-one-out analysis. All MR analyses were performed in MR Base platform (http://www.mrbase.org/; App version: 1.2.2 3a435d) and R V.3.6.1.

I obtained 26 SNPs as IVs from gut microbiome GWASs (online supplementary table 1). Among them, rs1230666 (*MAGI3*) was also strongly associated with the risk of RA (figure 1A, online supplementary table 1), implying this single IV might bias the result of MR. Correspondingly, although the inverse variance weighted (IVW) and MR Egger methods showed decrease in bacterial taxa in gut microbiome reduced the risk of RA, this result might be biased by single rs1230666 according to heterogeneity p value of both IVW and MR Egger methods (<0.05, table 1) and scatter plots of genetic associations with gut microbiome against the genetic associations with RA (figure 1B). Indeed, leave-one-out sensitivity analysis demonstrated IVW method without rs1230666 lost significance (figure 1C).

Therefore, I conducted sensitivity analysis without rs1230666. As a result, association p value derived from IVW, MR Egger and weighted median methods were not significant (p = 0.286, p = 0.057, p = 0.166, respectively, table 1) with no evidence of heterogeneity (heterogeneity p value>0.05, table 1), implying gut microbiome might not have causal effect for risk of RA. According to other sensitivity analysis to assess violations of assumptions, test for directional horizontal pleiotropy by the MR-Egger regression showed that directional pleiotropy was unlikely to bias the results of both the former and later analysis using 26 and 25 IVs, respectively (intercept=0.009, p=0.614; intercept=−0.003, p=0.548; respectively), indicating no evidence of pleiotropy.

The current study suggested that dysbiosis might be secondary phenomenon rather than triggers in the pathogenesis of RA. Even after taking into consideration of limitation of MR analysis that power of the test could be insufficient when SNPs have weak association with exposure, the impact of gut microbiome as triggers of the development in RA might be small.

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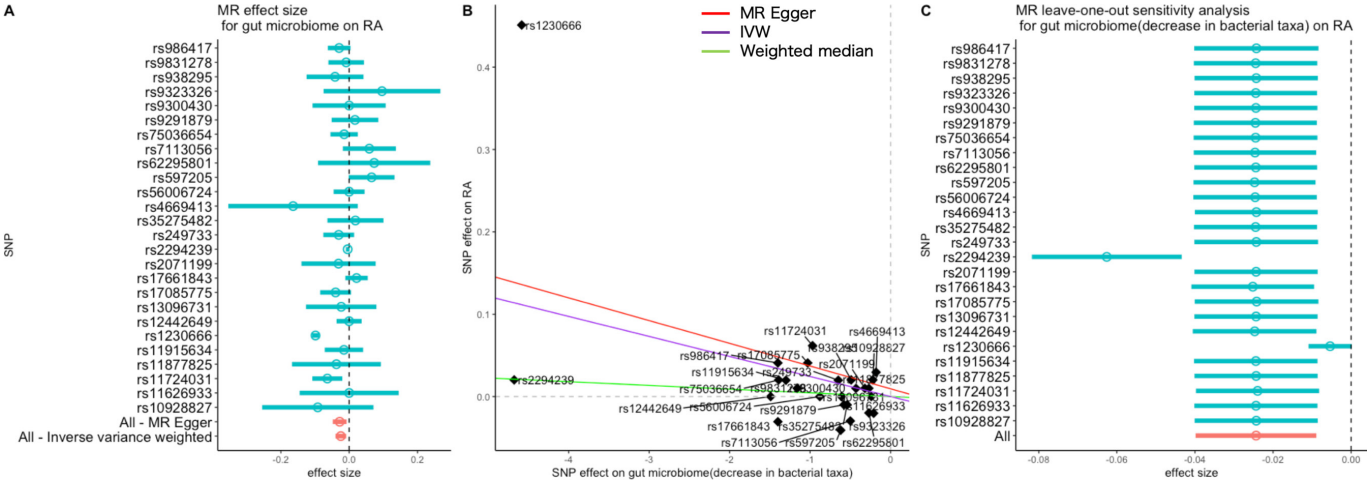

Figure 1 MR of the causal effect of gut microbiome and risk of RA. (A) Forest plot of the causal effects of gut microbiome (decrease in bacterial taxa) SNPs on RA. The causal effect of gut microbiome on RA is estimated using each SNP singly using the Wald ratio, and represented in a forest plot. The MR estimate using all SNPs using the MR Egger and IVW methods are also shown. Each point represents effect estimates and bar represents 95% CI. (B) Scatter plots of genetic associations with gut microbiome against the genetic associations with RA. SNP effects on the RA are plotted against SNP effects on the gut microbiome. The slope of the line represents the causal association, and each method has a different line. (C) Leave-one-out sensitivity analysis is performed to ascertain if an association is being disproportionately influenced by a single SNP. Each turquoise point in the forest plot represents the MR analysis (using IVW) excluding that particular SNP. The overall analysis including all SNPs is also shown for comparison. IVW, inverse variance weighted; MR, Mendelian randomisation; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism.

Table 1 The MR estimates from each method of the causal effect of gut microbiome on RA risk

Method	Number of SNPs	OR (95% CI)	Association p value	Cochrane Q statistic	Heterogeneity p value	*Number of SNPs	*OR (95% CI)	*Association p value	*Cochrane Q statistic	*Heterogeneity p value
MR Egger	26	0.97 (0.95 to 0.99)	0.013	306.4	8.78E–51	25	1.00 (0.99 to 1.00)	0.286	29.8	1.54E–01
IVW	26	0.98 (0.96 to 0.99)	0.002	309.7	6.79E–51	25	0.99 (0.99 to 1.00)	0.057	30.3	1.75E–01
Weighted median	26	1.00 (0.98 to 1.00)	0.143	N/A	N/A	25	1.00 (0.99 to 1.00)	0.166	N/A	N/A

*Sensitivity analysis without rs1230666.
IVW, inverse variance weighted; MR, Mendelian randomisation; N/A, not applicable; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism.

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