Reply to the comment on: The microbiota in axial spondyloarthritis: what have we learned from Mendelian randomisation studies?

Sirs,

We thank the authors G. Zhong and J. Zhang for their interest in our manuscript entitled "The microbiota in axial spondyloarthritis: what have we learned from Mendelian randomisation studies?" (1) and their insightful comments.

Regarding classification of axial spondyloarthritis (AxSpA), we agree that the diagnosis of AxSpA captures both radiographic forms (previously known as ankylosing spondylitis) and non-radiographic forms. All 8 of the included studies were limited to patients who carried a diagnosis of ankylosing spondylitis (radiographic axSpA). One of them was based upon a genome-wide association study (2) that was limited to patients with confirmed radiographic changes, while the rest were obtained through databases such as FinnGen. This is an important limitation, as there is no easy way to identify non-radiographic axSpA in such a database using ICD-10 codes, and there is furthermore a risk of misclassification bias due to inaccurate interpretation of the radiographs. It is unknown whether the microbiota or genetics in adults with non-radiographic axSpA differs from that of adults with its radiographic counterpart; however, to the extent that it does, Dr Zhong's point is well taken that the inability to be certain regarding the presence or absence of patients with non-radiographic axSpA in the databases could have diluted the findings and resulted in the inconsistent microbial associations.

Regarding the mediation analysis, we thank the authors for their comment and for highlighting the role of mediation Mendelian randomisation (MR) in understanding biological pathways in axSpA. We agree that mediation MR is useful because it allows researchers to examine whether the effect of exposure, such as gut microbiota, operates through intermediate factors like inflammatory cytokines or immune cells (3). This can help move beyond identifying causal relationships to understanding how those relationships occur. However, our review aimed to summarise evidence on the direct causal relationship between gut microbiota and axSpA based on existing MR studies (1). Therefore, our focus was on standard MR analyses rather than mediation MR.

We note that mediation-related analyses were reported in some of the studies we included. Of the eight studies reviewed, three explicitly addressed mediation. Specifically, Du *et al*. and Pan *et al*. employed mediation MR to assess inflammatory cytokines as potential intermediates linking gut microbiota to disease risk (4, 5). Additionally, Lu *et al*. investigated whether gut microbiota mediated the association between inflammatory bowel disease and extraintestinal manifestations, including ankylosing spondylitis. Their analysis found no evidence of a mediating effect, suggesting that, in this context, the observed relationship is unlikely to operate through gut microbiota (6).

We also observed that Tang *et al*. mentioned mediation MR in the conclusion of the abstract as a possible direction for future research, even though mediation was not formally analysed in that study (7).

Taken together, these findings indicate that mediation MR is emerging in this area but remains limited to a small number of studies, with inconsistent results. Although mediation MR offers a valuable framework for elucidating biological pathways, the current body of evidence is insufficient to support definitive conclusions regarding specific mechanisms. Future studies employing robust mediation MR approaches will be essential to clarify the pathways through which gut microbiota may influence axSpA.

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