Response to: 'Role of linoleic acid in autoimmune disorders: aMendelian randomisation study' by Lee *et al*

We are pleased that our article on the role of linoleic acid (LA) in autoimmune disorders is of interest to readers. However, regarding the methodological issues raised by Lee,[1] several points need to be considered and clarified.

First, Mendelian randomisation (MR) requires stringent assumptions, that is, the genetic instruments are associated with the exposure, are not linked with the outcomes other than via effects on the exposure and no confounders of the associations of the genetic instruments with the outcome exist.[2] We agree that weak instruments which violate these assumptions would lead to biased associations. As such, we are very cautious in the selection of genetic instruments. Specifically, we used the most significant three uncorrelated ($r^2<0.01$) single-nucleotide polymorphisms (SNPs) from a genome-wide association study (GWAS),[3] as previously,[4] and replicated using uncorrelated SNPs in genes relevant to the metabolism of n−6 PUFA, that is, *FADS1*, *FADS2* and *NTAN1*.[5] To ensure the SNPs predicting LA were not confounded, we assessed their Bonferroni corrected associations with key confounders, that is, socioeconomic position (job and Townsend Index) and lifestyle factors (alcohol and smoking), in the UK Biobank. To ensure the selected SNPs were solely linked with autoimmune disorders via effects on LA (no pleiotropy), we checked using three comprehensive curated genetic cross-reference systems, Ensembl (http://www.ensembl.org/index.html), the GWAS catalogue (https://www.ebi.ac.uk/gwas/) and PhenoScanner (www.phenoscanner.medschl.cam.ac.uk), which provide all well-established known associations of SNPs with their phenotypes, including subgenome-wide associations. We also used MR-PRESSO (MR Egger, Mendelian Randomization Pleiotropy RESidual Sum and Outlier) and multivariable MR to identify and correct for unknown potential pleiotropy. Using these genetic instruments, we validated that the effects on lipid profile were consistent with the well-established cholesterol-lowering effect of LA.[6]

Second, in the letter Lee makes a link between "limited numbers of IVs" and "bias from weak instruments"[1]; however, they are not equivalent. Instead, there is a "bias-variance trade-off for the number of instruments used in IV estimation".[7] Specifically, at a fixed mean F-statistic, increasing the number of instruments will lower the variance of the estimate (increase the precision) but at the same time may increase the possibility of bias from weak instruments.[7] The validity of the instrument is mainly based on the compliance with the MR assumptions rather than the number of instruments available. A single SNP, if validated, can also be used as an instrument in an MR study,[8] as has been the case in previous influential MR studies.[9 10] Lee did not provide any information about checking the instruments for associations with potential confounders, such as socioeconomic position, smoking and alcohol use, or checking for pleiotropic associations, in addition to sensitivity analysis using different analytic methods.[1]

We agree that using more valid instruments could increase the power of an MR study. However, we are unclear as to the validity of the use of 75 SNPs for LA as mentioned by Lee.[1] The 173 SNPs associated with LA at the genome-wide significance are highly correlated.[3] We cannot identify 75 independent SNPs meeting the selection criteria given by Lee ("linkage disequilibrium R^2 of 0.001, clumping distance of 10 000 kb, and a p-value threshold of 5.00E−08")[1]; those criteria only give the three SNPs providing the same information as what we used. However, if we apply a method suitable for correlated SNPs[11] and use all 167 SNPs available at genome-wide significance, we get an estimate very similar to that in our original letter (OR 0.97, 95% CI 0.95 to 0.98, p<0.001).

Jie V Zhao,[1] C Mary Schooling[1,2]

[1]School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China
[2]School of Public Health and Health Policy, City University of New York, New York City, New York, USA

Correspondence to Dr Jie V Zhao, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China; janezhao410@gmail.com

Handling editor Josef S Smolen

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Zhao JV, Schooling CM. *Ann Rheum Dis* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/annrheumdis-2018-214830

Received 5 December 2018
Accepted 5 December 2018

Linked

► https://doi.org/10.1136/annrheumdis-2018-214810

Ann Rheum Dis 2018;**0**:1. doi:10.1136/annrheumdis-2018-214830

REFERENCES

1 Lee YH. Role of linoleic acid in autoimmune disorders: a Mendelian randomisation study. *Ann Rheum Dis* 2018. doi: 10.1136/annrheumdis-2018-214810. [Epub ahead of print]
2 Lawlor DA, Harbord RM, Sterne JA, *et al*. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008;27:1133–63.
3 Guan W, Steffen BT, Lemaitre RN, *et al*. Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. *Circ Cardiovasc Genet* 2014;7:321–31.
4 May-Wilson S, Sud A, Law PJ, *et al*. Pro-inflammatory fatty acid profile and colorectal cancer risk: a Mendelian randomisation analysis. *Eur J Cancer* 2017;84:228–38.
5 Zhao JV, Schooling CM. Role of linoleic acid in autoimmune disorders: a Mendelian randomisation study. *Ann Rheum Dis* 2018. doi: 10.1136/annrheumdis-2018-214519. [Epub ahead of print 8 Nov 2018].
6 Ramsden CE, Zamora D, Majchrzak-Hong S, *et al*. Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968–73). *BMJ* 2016;353:i1246.
7 Burgess S, Thompson SG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* 2011;40:755–64.
8 Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res* 2017;26:2333–55.
9 Tchetgen Tchetgen EJ, Walter S, Glymour MM. Commentary: Building an evidence base for Mendelian randomization studies: assessing the validity and strength of proposed genetic instrumental variables. *Int J Epidemiol* 2013;42:328–31.
10 Au Yeung SL, Jiang C, Cheng KK, *et al*. Is aldehyde dehydrogenase 2 a credible genetic instrument for alcohol use in Mendelian randomization analysis in Southern Chinese men? *Int J Epidemiol* 2013;42:318–28.
11 Burgess S, Zuber V, Valdes-Marquez E, *et al*. Mendelian randomization with fine-mapped genetic data: choosing from large numbers of correlated instrumental variables. *Genet Epidemiol* 2017;41:714–25.