Opie-Martin, S., Wootton, R. E., Budu-Aggrey, A., Shatunov, A., Jones, A., Lacoangeli, A., Al Khleifat, A., Davey Smith, G., & Al-Chalabi, A. (2020). Relationship between smoking and ALS: Mendelian randomization interrogation of causality. *Journal of Neurology, Neurosurgery, and Psychiatry*.
<https://doi.org/10.1136/jnnp-2020-323316>

Peer reviewed version

Link to published version (if available):
[10.1136/jnnp-2020-323316](https://doi.org/10.1136/jnnp-2020-323316)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Springer Nature at <http://dx.doi.org/10.1136/jnnp-2020-323316> . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Relationship between smoking and ALS: Mendelian randomization interrogation of causality

Sarah Opie-Martin¹, Robyn E Wootton^{2,3,4}, Ashley Budu-Aggrey², Aleksey Shatunov¹, Ashley Jones¹, Alfredo Iacoangeli¹, Ahmad Al Khleifat¹, George Davey-Smith⁴, Ammar Al-Chalabi^{*1}

1) Maurice Wohl Clinical Neuroscience Institute, King's College London, Department of Basic and Clinical Neuroscience, London, UK 2) MRC Integrative Epidemiology Unit at the University of Bristol, University of Bristol, Oakfield House, Oakfield Grove, Bristol BS8 2BN, UK 3) School of Psychological Science, University of Bristol, 12a Priory Road, Bristol, BS8 1TU, UK 4) NIHR Bristol Biomedical Research Centre, University Hospitals Bristol NHS Foundation Trust and University of Bristol, Bristol, UK

*Corresponding author email ammar.al-chalabi@kcl.ac.uk Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Road, London SE5 9RX

Word count: 2255

References: 36

Acceptance date: 11/05/2020 – Journal of Neurology, Neurosurgery and Psychiatry

ABSTRACT

Objective: Smoking has been widely studied as a susceptibility factor for ALS, but results are conflicting and at risk of confounding bias. We used the results of recently published large genome-wide association studies and Mendelian randomisation methods to reduce confounding, to assess the relationship between smoking and ALS.

Methods: Two genome-wide association studies investigating lifetime smoking ($n=463,003$) and ever smoking ($n=1,232,091$) were identified and used to define instrumental variables for smoking. A genome-wide association study of ALS (20,806 cases; 59,804 controls) was used as the outcome for inverse variance weighted Mendelian randomisation, and four other Mendelian randomisation methods, to test whether smoking is causal for ALS. Analyses were bi-directional to assess reverse causality.

Results: There was no strong evidence for a causal or reverse causal relationship between smoking and ALS. The results of Mendelian randomisation using the inverse variance weighted method were: lifetime smoking odds ratio 0.94 (95% confidence intervals 0.74,1.19), p -value 0.59; ever smoking odds ratio 1.10 (95% CI 1,1.23), p -value 0.05.

Conclusions: Using multiple methods, large sample sizes, and sensitivity analyses, we find no evidence with Mendelian randomisation techniques that smoking causes ALS. Other smoking phenotypes, such as current smoking, may be suitable for future Mendelian randomisation studies

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of motor neurons, resulting in progressive paralysis of skeletal and bulbar muscles, with death from neuromuscular respiratory failure typically occurring within two to three years of symptom onset ¹. ALS has an incidence of 1-2 per 100,000 person-years and a lifetime risk of about 1 in 300 ^{2,3}. It has a peak age of onset of 58 and affects men slightly more frequently than women². In 5% there is a family history of ALS in a first degree relative, but twin and other studies have shown that apparently sporadic ALS has a heritability of 60%, leaving the possibility that up to 40% of the contribution could be environmental ⁴. There are currently no agreed environmental risk factors for ALS, although smoking has been widely studied with mixed results ⁵⁻¹⁰.

Summary statistics from genome-wide association studies allow us to use genetic predisposition for environmental risk factors to investigate causality, using Mendelian randomisation ^{11,12}. Mendelian randomisation is based on Mendel's laws of inheritance, allowing genotype to be used as an instrumental variable when studying the effect of an environmental exposure on an outcome ¹³. Genotype is considerably less likely to be confounded with other exposures that may bias the results of observational studies ¹⁴. Mendelian randomisation can also help to reduce bias from reverse causation, because the genetic variants one is born with are unchanged through a lifetime. This means the outcome, for example ALS, cannot change an individual's genetic predisposition for the exposure, for example smoking. In population-based genetic association studies, as opposed to parent-offspring or between-sibling studies, the randomisation is only approximate, and horizontal pleiotropy (where the genetic variants being tested increase the risk of both the environmental exposure and of ALS) can in any setting distort findings. A series of sensitivity analyses are available that can uncover such biases ^{11,15,16}.

Mendelian randomisation analysis has previously been used to assess the causal relationship between smoking and ALS with conflicting results ^{17,18}. Since then, updated genome-wide association studies for smoking and ALS have been published, with much larger numbers, allowing a new analysis with the advantages of sufficient power and updated methods. We performed two-sample Mendelian randomisation analyses to assess whether there was evidence for smoking being causal for ALS and, as a sensitivity analysis, in the other direction to test if ALS liability might be causal for smoking.

METHODS

Two-sample Mendelian randomisation analysis enables the summary statistics of genome-wide association studies to be used to estimate the causal effect of an exposure on an outcome based on the effect sizes of genetic variations on the exposure and on the outcome in the separate samples¹⁹. The effect estimate from a Mendelian randomisation study is an estimation of the true causal effect of an exposure on the outcome of interest. In the case of ALS diagnosis, this will be expressed as an odds ratio.

We defined an instrument for lifetime smoking index, a continuous measure of smoking exposure from a genome-wide association study of 463,033 people, with 126 independently associated single nucleotide polymorphisms (SNPs) of genome-wide significance that explained 0.31% of the variance in lifetime smoking²⁰. We also defined an instrument for 'Ever smoking' a binary measure of smoking exposure from a genome-wide association study of 1,232,091 individuals, with 378 genome-wide significant SNPs accounting for 4% of variance²¹. The variance explained in both studies is in line with genome-wide association studies that have been used to assess the causality of smoking for other conditions.

More details of how the phenotypes were defined can be found in the supplementary file.

We used summary data from the most recently published genome-wide association study for ALS²². The study reported 10 SNPs to be independently associated with risk of ALS, in a population of 80,610 (20,806 cases and 59,804 controls).

Statistical Analyses

To perform the Mendelian randomisation analysis, we used the 'TwoSampleMR' package, an R package and genome-wide association study summary data library, developed as a platform for performing Mendelian randomisation tests and sensitivity analyses²³.

We applied five different Mendelian randomisation methods: inverse-variance weighted, MR Egger, weighted median, weighted mode, and MR using robust associated profile score (MR RAPS). Each of these methods make different assumptions about pleiotropy and instrument strength so a consistent effect across the multiple methods gives us the strongest evidence for causality²⁴. For details of these and other sensitivity analyses, please see supplementary materials.

As an additional, supportive analysis, we defined genetic risk scores for smoking and used the UK Biobank data to test whether genetic risk score for smoking predicts ALS case control status. Details of the methods can be found in the supplementary file under the heading 'Genetic risk score analysis'.

RESULTS

Details of sensitivity analyses performed can be found in supplementary information (tables S1-S5). All instruments passed sensitivity analyses except for: heterogeneity tested using Cochran's Q (table S3); and a minority of SNPs did not pass Steiger filtering analysis (table S4), however re-running the Mendelian randomisation analyses without these SNPs did not change the results (table S5). After quality control, the number of SNPs making up each instrument were $n=119$ for lifetime smoking exposure and $n=353$ for ever smoking. Genome-wide association study summary statistics for each SNP making up the instruments are found in supplementary file 2. Graphs showing scatter plots of effect sizes of SNP on exposure and outcome variable, leave-one-out analyses and single SNP analyses are shown in figures S1-S12.

The results of the Mendelian randomisation analyses for each instrumental variable are shown in figure 1. We did not find strong evidence that lifetime smoking or ever smoking were causal for ALS. The result of the inverse variance weighted method for lifetime smoking index was odds ratio 0.94 (95% confidence intervals 0.74, 1.19) p -value = 0.59, and for ever smoking, OR 1.10 (95%CI 1.00,1.23) p -value=0.05. We did not find that ALS liability was causal of smoking status (table S6). Odds ratios tended to be >1 for the instruments testing if having ever smoked was associated with ALS, and <1 for the lifetime smoking instrument. A forest plot of results is shown in figure 1.

[Insert figure 1 here]

Figure 1: Forest plot of Mendelian randomisation analyses

Using genetic risk score analysis we found no association between smoking and ALS case control status (table S7).

DISCUSSION

Using instruments defined from recently published, large-scale genome-wide association studies, and numerous Mendelian randomisation methodologies and sensitivity analyses, we found no evidence that smoking causes ALS. Our result is supported by a lack of association found when performing genetic risk score analysis. We also found no relationship between genetic liability to ALS and likelihood of smoking, suggesting that reverse causality is not driving the association between smoking and risk of ALS reported in some epidemiological studies.

Two previous studies have used 2-sample Mendelian randomisation analysis to assess the causal relationship between smoking and ALS. One reported a positive association between ever smoking and ALS using inverse variance weighted Mendelian randomisation analysis, but the result was not

replicated with other Mendelian randomisation analyses and no other sensitivity analyses were reported. The ever smoking instrument was defined from (the Social Science Genetic Association Consortium (SSGAC)) genome-wide association study, which has a smaller sample size than GSCAN. The outcome SNPs were from an ALS genome-wide association study published previously to the one used here ²⁵. With a larger genome-wide association study we replicate the inverse variance weighted result with borderline significance, p-value 0.05, (figure 1) but do not consider this evidence of causality in the context of the results of the other Mendelian randomisation analyses presented. The other Mendelian randomisation study found no association using smoking instruments from a smaller smoking genome-wide association study and the same ALS genome-wide association study used here ^{17 18}. Our study is necessary to analyse the larger genome-wide association studies available, and to report all sensitivity analyses needed to interpret Mendelian randomisation results.

Large numbers of SNPs and high F statistic values mean the genetic instruments had enough strength to detect associations using the inverse variance weighted method ²⁶. The I^2 statistic quantifies regression dilution, which can be caused by measurement error ²⁷. When using linear regression analysis (as is the case with MR Egger), measurement error in the exposure variable will cause the effect size to tend to the null, and in the outcome will reduce statistical power ²⁸. All smoking instruments had I^2 values of <0.9 , indicating some regression dilution. In cases where $I^2 > 0.6$ SIMEX modelling was undertaken to estimate regression values and the results support the findings from other Mendelian randomisation models used.

For Mendelian randomisation to be valid, the genetic variants used as instrumental variables must mediate an effect only through the exposure of interest (the risk of ALS is only increased due to smoking, not through another effect of the variants), i.e. there should be no horizontal pleiotropy. A limitation of this study is that we are unable to fully discount this pleiotropy using statistical techniques. All genetic instruments tested positive for heterogeneity using Cochran's Q statistic, which may be caused by pleiotropy ²⁹. MR Egger intercept analysis did not find evidence to support the presence of directional pleiotropy. However, low I^2 values may invalidate the intercept estimation from MR Egger regression. A previous study used linkage disequilibrium regression score analysis and found that exposure to tobacco smoke in the home and being a light smoker (<100 cigarettes in a lifetime) are genetically related to ALS, which may support pleiotropy ¹⁷. Horizontal pleiotropy can cause false positives and false negatives. We used 5 Mendelian randomisation models that vary in their assumptions of pleiotropy to try account for these potential errors, although future models of Mendelian randomisation and how they account for pleiotropy may be better suited to

identifying association between ALS and smoking. Use of a binary outcome measure (which is the case in this study) or otherwise invalidated modelling assumptions may cause heterogeneity tests to produce positive results²⁴.

Inverse variance weighted method will estimate the true causal effect of an exposure if all Mendelian randomisation assumptions hold; if not, other methods have been developed ^{27 30-33}. It is suggested best practice to use multiple Mendelian randomisation methods to check for consistency of estimated effect ²⁴. Following this approach, we find a consistent lack of relationship between smoking and ALS. However, UK Biobank data contribute to the study cohorts of the lifetime smoking index and ever smoking instrument so the exposure phenotype populations are not completely independent.

The advantage of the instrumental variables we used is that they can be used in an unstratified population (we do not need to know the smoking status of people in the outcome genome-wide association study)²⁰. However, ever smoking is not consistently associated with ALS in epidemiology studies. A meta-analysis of case-control and cohort studies did not find strong supportive evidence of risk of ALS in people who had ever smoked (OR 1.12, 95%CI 0.98, 1.27) ⁵. Since then an association has been reported in some studies but not others ⁶⁹. Lifetime smoking index can be used to assess dose-dependency, important contributory evidence to showing causality. Evidence of a dose-dependent effect of smoking on ALS risk is rarely shown, although a dose-dependent effect of reduced risk of ALS with increased time since smoking cessation when comparing former to current smokers has recently been reported ³⁴. The only ALS risk study to use the lifetime smoking index did not find an association (unpublished data).

The most powerful Mendelian randomisation evidence on a potential effect of heaviness of smoking on ALS risk would require individual level data on a large sample, in which the CHRNA5 variant – related to heaviness of smoking amongst smokers – can be related to ALS risk by strata of smoking behaviour ³⁵. There is currently no adequately powered study allowing such analyses.

Triangulation of multiple strands of epidemiological evidence makes findings more robust ³⁶. Since the 1960's, smoking rates in many countries globally have been falling. It may be possible in the future to detect decreased rates of ALS in the population if smoking is a causal risk factor.

Using robust methods to detect association and estimate causal effects with summary statistics from genome-wide association studies we do not find strong evidence to support a relationship between smoking and ALS.

Declaration of interests

There are no conflicts of interest to declare.

Acknowledgements

The project is supported through the following funding organizations under the egis of JPND—www.jpnd.eu (United Kingdom, Medical Research Council [MR/L501529/1] and Economic and Social Research Council [ES/ L008238/1]). The work leading up to this publication was funded by the European Community's Health Seventh Framework Program [FP7/2007–2013; grant agreement number 259867], Horizon 2020 Framework Programme [H2020-PHC-2014-two-stage; grant agreement number 633413] and Programme Grants for Applied Research. This project is also supported by the National Institute for Health Research (NIHR) Maudsley Biomedical Research Centre at South London Maudsley Foundation Trust and King's College London. This research has been conducted using data from the UK Biobank Resource (application number 19278). We have also received funding from the Motor Neurone Disease Association, ALS Association, Patients Like Me and the Psychiatry Research Trust. This research was also supported by the NIHR Bristol Biomedical Research Centre at University Hospitals Bristol NHS Foundation Trust and the University of Bristol. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health and Social Care.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

Contributorship

AA-C and SO-M conceived and planned the study. ABA conducted GRS analysis, S-OM conducted all other statistical analysis supported by RW. AA-C, GDS, ABA and RW provided intellectual input for data interpretation. AA-C and SO-M wrote the first draft of the manuscript. All authors reviewed and approved the final manuscript.

Ethical approval

This research involves analysing publicly available data, with no collection of new data. Ethical approval had been obtained by the original study authors.

1. Brown RH, Al-Chalabi A. Amyotrophic Lateral Sclerosis. *New England Journal of Medicine* 2017;377(2):162-72. doi: 10.1056/NEJMra1603471
2. Chio A, Logroscino G, Traynor BJ, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. *Neuroepidemiology* 2013;41(2):118-30. doi: 10.1159/000351153 [published Online First: 2013/07/19]
3. Johnston CA, Stanton BR, Turner MR, et al. Amyotrophic lateral sclerosis in an urban setting: a population based study of inner city London. *Journal of neurology* 2006;253(12):1642-3. doi: 10.1007/s00415-006-0195-y [published Online First: 2007/01/16]
4. Al-Chalabi A, Fang F, Hanby MF, et al. An estimate of amyotrophic lateral sclerosis heritability using twin data. *Journal of neurology, neurosurgery, and psychiatry* 2010;81(12):1324-6. doi: 10.1136/jnnp.2010.207464 [published Online First: 2010/09/24]
5. Alonso A, Logroscino G, Hernán MA. Smoking and the risk of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *Journal of Neurology, Neurosurgery & Psychiatry* 2010:1249-52.
6. Alonso A, Logroscino G, Jick SS, et al. Association of smoking with amyotrophic lateral sclerosis risk and survival in men and women: a prospective study. *BMC neurology* 2010;10(1):6.
7. de Jong SW, Huisman MHB, Suttedja NA, et al. Smoking, Alcohol Consumption, and the Risk of Amyotrophic Lateral Sclerosis: A Population-based Study. *American Journal of Epidemiology* 2012;176(3):233-39. doi: 10.1093/aje/kws015
8. Pamphlett R, Ward EC. Smoking is not a risk factor for sporadic amyotrophic lateral sclerosis in an Australian population. *Neuroepidemiology* 2012;38(2):106-13. doi: 10.1159/000336013 [published Online First: 2012/03/02]
9. Wang H, O'Reilly ÉJ, Weisskopf MG, et al. Smoking and risk of amyotrophic lateral sclerosis: a pooled analysis of five prospective cohorts. *Archives of Neurology* 2011;68(2):207-13. doi: 10.1001/archneurol.2010.367
10. Yu Y, Su FC, Callaghan BC, et al. Environmental risk factors and amyotrophic lateral sclerosis (ALS): a case-control study of ALS in Michigan. *PloS one* 2014;9(6):e101186. doi: 10.1371/journal.pone.0101186 [published Online First: 2014/07/01]
11. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;32(1):1-22. doi: 10.1093/ije/dyg070 [published Online First: 2003/04/12]
12. Richardson TG, Harrison S, Hemani G, et al. An atlas of polygenic risk score associations to highlight putative causal relationships across the human phenome. *eLife* 2019;8:e43657. doi: 10.7554/eLife.43657
13. Lawlor DA, Harbord RM, Sterne JAC, et al. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. 2008;27(8):1133-63. doi: 10.1002/sim.3034
14. Davey Smith G, Lawlor DA, Harbord R, et al. Clustered environments and randomized genes: a fundamental distinction between conventional and genetic epidemiology. *PLoS medicine* 2007;4(12):e352. doi: 10.1371/journal.pmed.0040352 [published Online First: 2007/12/14]
15. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet* 2014;23(R1):R89-98. doi: 10.1093/hmg/ddu328 [published Online First: 2014/07/30]
16. Davies NM, Howe LJ, Brumpton B, et al. Within family Mendelian randomization studies. *Hum Mol Genet* 2019;28(R2):R170-r79. doi: 10.1093/hmg/ddz204 [published Online First: 2019/10/28]
17. Bandres-Ciga S, Noyce AJ, Hemani G, et al. Shared polygenic risk and causal inferences in amyotrophic lateral sclerosis. *Annals of neurology* 2019;85(4):470-81. doi: 10.1002/ana.25431
18. Zhan Y, Fang F. Smoking and amyotrophic lateral sclerosis: A mendelian randomization study. 2019;85(4):482-84. doi: 10.1002/ana.25443

19. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol* 2013;178(7):1177-84. doi: 10.1093/aje/kwt084 [published Online First: 2013/07/19]
20. Wootton RE, Richmond RC, Stuijzand BG, et al. Evidence for causal effects of lifetime smoking on risk for depression and schizophrenia: a Mendelian randomisation study. *Psychological Medicine* 2018;1-9. doi: 10.1017/S0033291719002678 [published Online First: 2019/11/06]
21. Liu M, Jiang Y, Wedow R, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature Genetics* 2019;51(2):237-44. doi: 10.1038/s41588-018-0307-5
22. Nicolas A, Kenna KP, Renton AE, et al. Genome-wide Analyses Identify KIF5A as a Novel ALS Gene. *Neuron* 2018;97(6):1268-83.e6. doi: 10.1016/j.neuron.2018.02.027 [published Online First: 2018/03/24]
23. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. *eLife* 2018;7:e34408. doi: 10.7554/eLife.34408
24. Hemani G, Bowden J, Davey Smith G. Evaluating the potential role of pleiotropy in Mendelian randomization studies. *Human Molecular Genetics* 2018;27(R2):R195-R208. doi: 10.1093/hmg/ddy163 %J Human Molecular Genetics
25. van Rheenen W, Shatunov A, Dekker AM, et al. Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat Genet* 2016;48(9):1043-8. doi: 10.1038/ng.3622 [published Online First: 2016/07/28]
26. Burgess S, Thompson SG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* 2011;40(3):755-64. doi: 10.1093/ije/dyr036 [published Online First: 2011/03/19]
27. Bowden J, Del Greco MF, Minelli C, et al. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I² statistic. *Int J Epidemiol* 2016;45(6):1961-74. doi: 10.1093/ije/dyw220 [published Online First: 2016/09/13]
28. Liu K. Measurement error and its impact on partial correlation and multiple linear regression analyses. *Am J Epidemiol* 1988;127(4):864-74. doi: 10.1093/oxfordjournals.aje.a114870 [published Online First: 1988/04/01]
29. Bowden J, Hemani G, Davey Smith G. Invited Commentary: Detecting Individual and Global Horizontal Pleiotropy in Mendelian Randomization—A Job for the Humble Heterogeneity Statistic? *American Journal of Epidemiology* 2018;187(12):2681-85. doi: 10.1093/aje/kwy185 %J American Journal of Epidemiology
30. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;44(2):512-25. doi: 10.1093/ije/dyv080 [published Online First: 2015/06/08]
31. Bowden J, Davey Smith G, Haycock PC, et al. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genetic epidemiology* 2016;40(4):304-14. doi: 10.1002/gepi.21965 [published Online First: 2016/04/12]
32. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol* 2017;46(6):1985-98. doi: 10.1093/ije/dyx102 [published Online First: 2017/10/19]
33. Kang H, Zhang A, Cai TT, et al. Instrumental Variables Estimation With Some Invalid Instruments and its Application to Mendelian Randomization. *Journal of the American Statistical Association* 2016;111(513):132-44. doi: 10.1080/01621459.2014.994705
34. Peters S, Visser AE, D'Ovidio F, et al. Effect modification of the association between total cigarette smoking and ALS risk by intensity, duration and time-since-quitting: Euro-MOTOR. 2019:jnnp-2019-320986. doi: 10.1136/jnnp-2019-320986 %J Journal of Neurology, Neurosurgery & Psychiatry

35. Millard LAC, Munafo MR, Tilling K, et al. MR-pheWAS with stratification and interaction: Searching for the causal effects of smoking heaviness identified an effect on facial aging. *PLoS Genet* 2019;15(10):e1008353. doi: 10.1371/journal.pgen.1008353 [published Online First: 2019/11/02]
36. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *International Journal of Epidemiology* 2017;45(6):1866-86. doi: 10.1093/ije/dyw314 %J International Journal of Epidemiology