**STROBE-MR checklist of recommended items to address in reports of Mendelian randomization studies**12

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| **Item No.** | **Section** | **Checklist item** | **Page No.** | **Relevant text from manuscript** |
| 1 | **TITLE and ABSTRACT** | Indicate Mendelian randomization (MR) as the study’s design in the title and/or the abstract if that is a main purpose of the study | 1 | Unraveling the Molecular Nexus Between Ankylosing Spondylitis and IgA Nephropathy: Insights from Mendelian Randomization |
|  | **INTRODUCTION** |  |  |  |
| 2 | **Background** | Explain the scientific background and rationale for the reported study. What is the exposure? Is a potential causal relationship between exposure and outcome plausible? Justify why MR is a helpful method to address the study question | 4 | To date, no comprehensive study has been conducted to fully unravel the causal relationship between AS and IgAN. In the absence of large-scale and meticulously designed RCTs, Mendelian randomization (MR) has emerged as a promising alternative to explore the causal effects between AS and IgAN. |
| 3 | **Objectives** | State specific objectives clearly, including pre-specified causal hypotheses (if any). State that MR is a method that, under specific assumptions, intends to estimate causal effects | 4,5 | Mendelian randomization (MR) has emerged as a promising alternative to explore the causal effects between AS and IgAN. MR leverages the random allocation of genetic variants at conception, making it less susceptible to confounding factors compared to traditional observational studies. Positioned at the intersection of experimental and observational research, MR provides a robust framework for inferring causal relationships and identifying potential targets for intervention |
|  | **METHODS** |  |  |  |
| 4 | **Study design and data sources** | Present key elements of the study design early in the article. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following: |  |  |
|  | a) | Setting: Describe the study design and the underlying population, if possible. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, when available. | 5 | **Methods**: "GWAS of AS includes 22,647 individuals... FinnGen data from over 500,000 Finnish samples." |
|  | b) | Participants: Give the eligibility criteria, and the sources and methods of selection of participants. Report the sample size, and whether any power or sample size calculations were carried out prior to the main analysis | 5 | **Methods**: "Selection criteria for IgAN cases: biopsy confirmation... AS dataset includes European descent." |
|  | c) | Describe measurement, quality control and selection of genetic variants | 6 | **Methods**: Selection of instrumental variables |
|  | d) | For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases | 4 | **Methods**: "GWAS summary statistics... diagnosis based on clinical criteria." |
|  | e) | Provide details of ethics committee approval and participant informed consent, if relevant | 16 | **Statement of Ethics**: "Original studies contributing to datasets obtained ethical approvals." |
| 5 | **Assumptions** | Explicitly state the three core IV assumptions for the main analysis (relevance, independence and exclusion restriction) as well assumptions for any additional or sensitivity analysis | 6 | **Methods**: "Three critical assumptions... SNPs associated with AS, no confounders, exclusion restriction." |
| 6 | **Statistical methods: main analysis** | Describe statistical methods and statistics used |  |  |
|  | a) | Describe how quantitative variables were handled in the analyses (i.e., scale, units, model) | No | *This study was conducted leveraging data from previous publication or public databank. We applied no particular treatment in analyses concerning GWAS data of quantitative variables* |
|  | b) | Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected | 6 | **Methods**: "To ensure independence between IVs associated with AS... To prevent SNPs from being linked with potential risk factors for outcomes, Phenoscanner was utilized to scrutinize and filter out SNPs associated with potential confounders or risk factors (such as hypertension, diabetes, obesity, etc.)" |
|  | c) | Describe the MR estimator (e.g. two-stage least squares, Wald ratio) and related statistics. Detail the included covariates and, in case of two-sample MR, whether the same covariate set was used for adjustment in the two samples | 6 | **Methods**: "The MR analysis was conducted using the Inverse variance weighted (IVW) method... Data analysis was conducted using R version 4.2.1, incorporating the "TwoSampleMR" package, which facilitates two-sample MR analysis." |
|  | d) | Explain how missing data were addressed | No | *No missing data need to be addressed in this study*. |
|  | e) | If applicable, indicate how multiple testing was addressed | No | Not applicable |
| 7 | **Assessment of assumptions** | Describe any methods or prior knowledge used to assess the assumptions or justify their validity | 7 | **Sensitivity Analysis**: "MR-Egger intercept test... the MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method was used to detect and remove outlier SNPs." |
| 8 | **Sensitivity analyses and additional analyses** | Describe any sensitivity analyses or additional analyses performed (e.g. comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations) | 7 | **Methods**: "Reverse-direction MR analysis... We uesd first-order IVW and MR-Egger methods to perform Cochran’s Q test, which assessed heterogeneity as a potential violation of modeling assumptions." |
| 9 | **Software and pre-registration** |  |  |  |
|  | a) | Name statistical software and package(s), including version and settings used | 6 | **Methods**: "Data analysis was conducted using R version 4.2.1, incorporating the "TwoSampleMR" package, which facilitates two-sample MR analysis. " |
|  | b) | State whether the study protocol and details were pre-registered (as well as when and where) | No | This study was conducted leveraging data from previous publication or public databank, therefore it is not pre-registered. |
|  | **RESULTS** |  |  |  |
| 10 | **Descriptive data** |  |  |  |
|  | a) | Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow diagram | 9 | **Results**: "25 independent SNPs closely associated with AS were identified from the ebi-a-GCST005529 dataset... and all SNPs exhibited F-statistics >10 (Supplementary Table S1). " |
|  | b) | Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions) | 9 | **Results**: "In this study, using the IVW method as the primary approach for causal assessment…Both MR-Egger and WM analyses supported this finding, showing consistent directional trends (Figure 1)." |
|  | c) | If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies | No | Not applicable |
|  | d) | For two-sample MR:  i.  Provide justification of the similarity of the genetic variant-exposure associations between the exposure and outcome samples  ii.  Provide information on the number of individuals who overlap between the exposure and outcome studies | 5 | there was no overlap in samples between the two datasets, ensuring independent analysis |
| 11 | **Main results** |  |  |  |
|  | a) | Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale | 9 | Basic information about these SNPs is presented  in the Supplementary material (Supplementary Table S1). " |
|  | b) | Report MR estimates of the relationship between exposure and outcome, and the measures of uncertainty from the MR analysis, on an interpretable scale, such as odds ratio or relative risk per SD difference | 9 | In this study, using the IVW method as the primary approach for causal assessment, MR analysis revealed a protective causal effect of AS on IgAN [OR=0.552, 95% CI, 0.339–0.900; P = 0.017]. |
|  | c) | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 9 | OR |
|  | d) | Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure) | 9 | Figure 1, Supplementary Figure 1, Supplementary Figure 2 |
| 12 | **Assessment of assumptions** |  |  |  |
|  | a) | Report the assessment of the validity of the assumptions | 9 | **Results**: " these 24 SNPs showed no association with potential confounders in the PhenoScanner, all SNPs exhibited F-statistics >10 (Supplementary Table S1)…The MR-Egger intercept test and MR-PRESSO test (Supplementary Table S3) ." |
|  | b) | Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as *I2*, Q statistic or E-value) | 9 | **Results**: " The MR-Egger intercept test and MR-PRESSO test (Supplementary Table S3)... Cochran’s Q test (Supplementary Table S4) indicated no heterogeneity in the results." |
| 13 | **Sensitivity analyses and additional analyses** |  |  |  |
|  | a) | Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions | 9 | **Results**: "the leave-one-out sensitivity analysis demonstrated that the findings of this study were robust (Supplementary Figure 2). " |
|  | b) | Report results from other sensitivity analyses or additional analyses | 9 | **Results**: "Both MR-Egger and WM analyses supported this finding, showing consistent directional trends (Figure 1). " |
|  | c) | Report any assessment of direction of causal relationship (e.g., bidirectional MR) | 9 | **Results**: "Additionall, reverse MR analysis was performed to examine potential causal relationships between IgAN and AS, but no evidence of reverse causality was observed (Supplementary Table S2). " |
|  | d) | When relevant, report and compare with estimates from non-MR analyses | No |  |
|  | e) | Consider additional plots to visualize results (e.g., leave-one-out analyses) | 9 | **Results**: " the leave-one-out sensitivity analysis demonstrated that the findings of this study were robust (Supplementary Figure 2). " |
|  | **DISCUSSION** |  |  |  |
| 14 | **Key results** | Summarize key results with reference to study objectives | 12 | **Discussion**: " Our MR findings revealed a protective causal link between AS and IgAN " |
| 15 | **Limitations** | Discuss limitations of the study, taking into account the validity of the IV assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias and any efforts to address them | 14 | **Discussion**: " Several limitations of this study should be acknowledged…Furthermore, the genetic analysis was confined to individuals of European ancestry, which may restrict the generalizability of our findings to other racial or ethnic groups." |
| 16 | **Interpretation** |  |  |  |
|  | a) | Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies | 15 | Nonetheless, the findings of our study have significant implications for elucidating the protective causal relationship between AS and IgAN…Additionally, more sophisticated models that account for potential non-linear relationships between exposures and outcomes should be explored. |
|  | b) | Mechanism: Discuss underlying biological mechanisms that could drive a potential causal relationship between the investigated exposure and the outcome, and whether the gene-environment equivalence assumption is reasonable. Use causal language carefully, clarifying that IV estimates may provide causal effects only under certain assumptions | 12,13,14,15 | After confirming the protective causal relationship between AS and IgAN through MR analysis, we investigated the underlying molecular mechanisms using bioinformatics… |
|  | c) | Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions | 12 | A retrospective analysis showed that IgAN-related glomerular damage tended to be less severe in AS patients, with fewer acute tubulointerstitial lesions and distinct immunological characteristics[29]. This aligns closely with our findings…These findings suggest that HLA-B27 may have a protective effect against IgAN, indirectly supporting the potential protective role of AS in IgAN. |
| 17 | **Generalizability** | Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure | 14 | the genetic analysis was confined to individuals of European ancestry, which may restrict the generalizability of our findings to other racial or ethnic groups, particularly those with distinct genetic backgrounds or environmental exposures |
|  | **OTHER INFORMATION** |  |  |  |
| 18 | **Funding** | Describe sources of funding and the role of funders in the present study and, if applicable, sources of funding for the databases and original study or studies on which the present study is based | 16 | Funding Sources |
| 19 | **Data and data sharing** | Provide the data used to perform all analyses or report where and how the data can be accessed, and reference these sources in the article. Provide the statistical code needed to reproduce the results in the article, or report whether the code is publicly accessible and if so, where | 5,17 | Data sources for MR…the raw data supporting the conclusions of this article will be made available by the authors on request. |
| 20 | **Conflicts of Interest** | All authors should declare all potential conflicts of interest | 16 | The authors declare no competing financial interests. |

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1. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) Statement. JAMA. 2021;under review.

2. Skrivankova VW, Richmond RC, Woolf BAR, Davies NM, Swanson SA, VanderWeele TJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomisation (STROBE-MR): Explanation and Elaboration. BMJ. 2021;375:n2233.