**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 | 2 | This study employed Bidirectional Mendelian randomization (MR) using publicly available summary statistics from Genome Wide Association Studies (GWAS) and FinnGen Consortium to investigate the causal links between hormonal and reproductive factors on LBP. |
|  | **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 | 2 | The relationship between hormonal fluctuations in the reproductive system and the occurrence of low back pain has been widely observed. However, the causal impact of specific variables that may be indicative of hormonal and reproductive factors, such as age at menopause (ANM), age at menarche (AAM), length of menstrual cycle (LMC), age at first birth (AFB), age at last live birth (ALB) and age first had sexual intercourse (AFS) on low back pain (LBP) remains unclear. |
| 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 | 2 | The relationship between hormonal fluctuations in the reproductive system and the occurrence of low back pain has been widely observed. However, the causal impact of specific variables that may be indicative of hormonal and reproductive factors, such as age at menopause (ANM), age at menarche (AAM), length of menstrual cycle (LMC), age at first birth (AFB), age at last live birth (ALB) and age first had sexual intercourse (AFS) on low back pain (LBP) remains unclear. |
|  | **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. |  | no |
|  | 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 | 3 | Data on LBP was sourced from the FinnGen Consortium ([https://finngen.fi](https://finngen.fi/)). The summary data for the GWAS of LBP from the FinnGen Consortium comprises 177,860 participants of European ancestry (13,178 cases and 164,682 controls). |
|  | c) | Describe measurement, quality control and selection of genetic variants |  | no |
|  | d) | For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases |  | ICD-10 |
|  | e) | Provide details of ethics committee approval and participant informed consent, if relevant |  | no |
| 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 | 4 | Selection of Instrumental Variable |
| 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) | 4 |  |
|  | b) | Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected |  |  |
|  | 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 |  |  |
|  | d) | Explain how missing data were addressed |  |  |
|  | e) | If applicable, indicate how multiple testing was addressed | 4 | Consequently, we conducted MVMR to address this potential confounding factor. |
| 7 | **Assessment of assumptions** | Describe any methods or prior knowledge used to assess the assumptions or justify their validity |  |  |
| 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) | 4 | Cochrane's Q-test was utilized to detect heterogeneity, while funnel plots indicated heterogeneity through symmetry |
| 9 | **Software and pre-registration** |  |  |  |
|  | a) | Name statistical software and package(s), including version and settings used |  |  |
|  | b) | State whether the study protocol and details were pre-registered (as well as when and where) |  |  |
|  | **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 | 5 |  |
|  | b) | Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions) | 5 | The causal association between genetically predicted reproductive and hormonal factors and the risk of LBP is presented in Figure 2. |
|  | c) | If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies |  | no |
|  | 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 |  |  |
| 11 | **Main results** |  |  |  |
|  | a) | Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale | 6 |  |
|  | 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 | 6 |  |
|  | c) | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 6 |  |
|  | d) | Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure) | 6 | The leave-one-out plot reinforces the robustness of our results, indicating that the influence of any individual SNP is unlikely to affect the causal estimate |
| 12 | **Assessment of assumptions** |  |  |  |
|  | a) | Report the assessment of the validity of the assumptions |  |  |
|  | b) | Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as *I2*, Q statistic or E-value) | 6 | Heterogeneity and pleiotropy are depicted in Table 3. |
| 13 | **Sensitivity analyses and additional analyses** |  |  |  |
|  | a) | Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions | 6 | Heterogeneity and pleiotropy are depicted in Table 3. |
|  | b) | Report results from other sensitivity analyses or additional analyses | 6 | Information on pleiotropy and heterogeneity is referred to in |
|  | c) | Report any assessment of direction of causal relationship (e.g., bidirectional MR) | 6 | In the reverse MR analysis, there is a causal negative relationship between LBP and AFB |
|  | d) | When relevant, report and compare with estimates from non-MR analyses | 6 |  |
|  | e) | Consider additional plots to visualize results (e.g., leave-one-out analyses) | 6 | . In addition, funnel plots, scatter plots and leave-one-out plots are shown in the supplementary figures S4-6. |
|  | **DISCUSSION** |  |  |  |
| 14 | **Key results** | Summarize key results with reference to study objectives | 6 |  |
| 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 | 7 | However, the exclusive reliance on European GWAS data may limit the generalizability of our findings to other ethnic or geographic populations. Besides, the inclusion of both genders in the outcome data might also weaken the observed associations. Future MR studies should consider validating these results within female-only samples by appropriate stratification. |
| 16 | **Interpretation** |  |  |  |
|  | a) | Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies |  |  |
|  | 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 |  |  |
|  | c) | Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions | 9 | These insights enhance our understanding of LBP risk factors, offering valuable guidance for screening, prevention, and treatment strategies for at-risk women. |
| 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 | 9 | Our study possesses several strengths. It marks the inaugural application of MR to investigate the causal relationship between hormonal and reproductive factors and LBP. Encompassing six distinct reproductive characteristics, our study offers a comprehensive understanding of the reproductive period. Utilizing data from a diverse range of cohorts enhances the reliability of our findings and minimizes overlap. Employing the principle of random allele assignment, we conducted a Bidirectional MR study to validate the robustness of these results. Furthermore, we corroborated the reliability of our conclusions through MVMR, with adjustments made for BMI. |
|  | **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 | 11 | We express our gratitude for the utilization of the Genome-wide Association Study databases and FinnGen Consortium databases. |
| 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 | 11 | The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors. |
| 20 | **Conflicts of Interest** | All authors should declare all potential conflicts of interest | 11 | The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. |

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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.