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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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 | Genetic Evidence for the Causal Association Between Myocardial Infarction and Urinary system cancers Risk |
|  | **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 | 2-3 | The relationship between myocardial infarction (MI) and urinary system cancers has gained increasing attention in recent years, particularly due to the cardiovascular risks associated with cancer treatments rather than the malignancies themselves. Emerging evidence suggests that therapeutic interventions for prostate cancer (PCa), bladder cancer (BCa), and malignant neoplasm of kidney (MRN) may substantially increase cardiovascular event risks, creating complex bidirectional associations that extend beyond shared risk factors..... |
| 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 | 3 | We conducted a bidirectional two-sample MR analysis following STROBE-MR guidelines to investigate potential causal relationships between MI and urinary system cancers, specifically PCa, BCa, and MRN. |
|  | **METHODS** |  | 3-4 | Our analytical framework adhered to three fundamental MR assumptions: relevance (genome-wide significance P < 5×10^-8), independence (absence of confounding factors), and exclusion restriction (no horizontal pleiotropy). s..... |
| 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. | 4 | All MR analyses utilized publicly available genome-wide association study (GWAS) summary statistics accessed through the IEU Open GWAS database. We selected MI phenotype (ID: ebi-a-GCST011364) as exposure data... |
|  | 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 | 4 | PCa discovery data originated from UK Biobank with PRACTICAL consortium providing replication samples; BCa discovery cohort was sourced from MRC-IEU with UK Biobank (ID: ieu-b-4874) serving as validation; MRN utilized FinnGen\_R12... |
|  | c) | Describe measurement, quality control and selection of genetic variants | 4 | Single nucleotide polymorphisms (SNPs) served as genetic instrumental variables in our MR framework through a rigorous multi-stage selection protocol. We initially extracted genome-wide significant variants (P < 5×10^-8) from exposure GWAS data, maintaining this threshold consistently across forward and reverse MR analyses..... |
|  | d) | For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases | 4 | Detailed information regarding data sources is provided in Supplementary Table 1 and Table 1 |
|  | e) | Provide details of ethics committee approval and participant informed consent, if relevant |  | NA |
| 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 | 11 | he directed acyclic graph (DAG) illustrates the three fundamental assumptions of Mendelian randomization:. |
| 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 | We initially extracted genome-wide significant variants (P < 5×10^-8) from exposure GWAS data, maintaining this threshold consistently across forward and reverse MR analysess |
|  | b) | Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected | 4 | subsequently applied linkage disequilibrium filtering (r² < 0.001, clumping distance = 10,000 kb) to eliminate correlated variants that could introduce analytical bias; harmonized effect estimates and allele frequencies between exposure and outcome datasets to ensure analytical consistency. Instrumental variable strength was evaluated using F-statistics calculated as F = R²(n-k-1)/k(1-R²), with variants showing F < 10 excluded to maintain adequate statistical power... |
|  | 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 | 4-5 | subsequently applied linkage disequilibrium filtering (r² < 0.001, clumping distance = 10,000 kb) to eliminate correlated variants that could introduce analytical bias; harmonized effect estimates and allele frequencies between exposure and outcome datasets to ensure analytical consistency. Instrumental variable strength was evaluated using F-statistics calculated as F = R²(n-k-1)/k(1-R²), with variants showing F < 10 excluded to maintain adequate statistical power.... |
|  | d) | Explain how missing data were addressed |  | na |
|  | e) | If applicable, indicate how multiple testing was addressed |  | NA |
| 7 | **Assessment of assumptions** | Describe any methods or prior knowledge used to assess the assumptions or justify their validity | 2 | Emerging evidence suggests that therapeutic interventions for prostate cancer (PCa), bladder cancer (BCa), and malignant neoplasm of kidney (MRN) may substantially increase cardiovascular event risks, creating complex bidirectional associations that extend beyond shared risk factors. In PCa management, androgen deprivation therapy (ADT) has been identified as a major contributor to cardiovascular morbidity, with contemporary meta-analyses demonstrating increased risks of MI and stroke among patients receiving ADT.... |
| 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) | 5 | Secondary analyses comprehensively evaluated heterogeneity, pleiotropy, and sensitivity through MR-Egger intercept analysis, where near-zero intercepts suggested low pleiotropy probability, combined with MR-PRESSO global testing for systematic outlier detection and management..... |
| 9 | **Software and pre-registration** |  |  |  |
|  | a) | Name statistical software and package(s), including version and settings used | 2-3 | Bidirectional causality was assessed through reverse MR analysis, with all computations performed using R software (version 4.2.1) utilizing TwoSampleMR and MR-PRESSO packages. |
|  | b) | State whether the study protocol and details were pre-registered (as well as when and where) |  | NA |
|  | **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 | 3 | The comprehensive study design and three core MR assumptions are illustrated in Figure 1. |
|  | b) | Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions) | 5 | Following the systematic selection process, we identified genetic variants as instrumental variables for MI across different analyses. For the forward MR analyses examining MI as exposure, 19 SNPs were selected for both PCa and MRN analyses, while 6 SNPs were used for BCa discovery cohort and 21 SNPs for BCa replication cohort. The variation in SNP numbers reflected the availability of harmonized variants across different outcome datasets. |
|  | c) | If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies |  | NA |
|  | 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 |  | NA |
| 11 | **Main results** |  |  |  |
|  | a) | Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale | 6-7 | MR analysis revealed no significant causal effect of genetically predicted MI on the risk of any examined urinary system cancer. For PCa, the primary IVW analysis showed null associations in both discovery (OR = 1.000, 95% CI: 0.995-1.004, p = 0.885) and replication (OR = 0.964, 95% CI: 0.904-1.027, p = 0.257) cohorts... |
|  | 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 | 6-7 | Consistent conclusions were obtained through additional statistical methods, with MR-Egger yielding ORs of 1.000 (95% CI: 0.989-1.010, p = 0.963) and 0.998 (95% CI: 0.860-1.158, p = 0.980) for discovery and replication analyses, respectively. Similarly, weighted median and weighted mode approaches produced comparable results, with all confidence intervals encompassing the null value and p-values exceeding 0.05.  BCa analysis demonstrated remarkably consistent null findings across all methodological approaches. In the discovery cohort, IVW analysis yielded an OR of 1.000 (95% CI: 0.999-1.001, p = 0.795), with MR-Egger (OR = 1.000, 95% CI: 0.998-1.002, p = 0.787), weighted median.... |
|  | c) | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |  | NA |
|  | d) | Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure) | 6-7 | The comprehensive forest plot summarizing these null associations for forward MR analyses is presented in Figure 2. |
| 12 | **Assessment of assumptions** |  |  |  |
|  | a) | Report the assessment of the validity of the assumptions | 7 | Comprehensive sensitivity analyses confirmed the robustness of our findings and absence of methodological violations. MR-Egger intercept analyses revealed no evidence of directional pleiotropy across most comparisons, with intercept values close to zero and non-significant p-values (Supplementary Table 5). |
|  | b) | Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as *I2*, Q statistic or E-value) | 7 | Heterogeneity assessment using Cochran's Q statistic demonstrated acceptable levels of between-SNP heterogeneity for most analyses (Supplementary Table 6) |
| 13 | **Sensitivity analyses and additional analyses** |  |  |  |
|  | a) | Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions | 7 | sensitivity analyses confirmed the robustness of our findings and absence of methodological violations.... |
|  | b) | Report results from other sensitivity analyses or additional analyses | 7 | the use of random-effects IVW models in these instances ensured appropriate handling of heterogeneity without compromising causal estimates.. |
|  | c) | Report any assessment of direction of causal relationship (e.g., bidirectional MR) | 7 | Steiger testing confirmed correct causal directionality for all analyses, with genetic instruments demonstrating stronger associations with their respective exposure variables compared to outcome variables (all Steiger test p-values < 0.001 and direction = TRUE; Supplementary Table 7) |
|  | d) | When relevant, report and compare with estimates from non-MR analyses |  | NA |
|  | e) | Consider additional plots to visualize results (e.g., leave-one-out analyses) | 7 | LOO sensitivity analysis showed that no single SNP substantially influenced the overall causal estimates, confirming the stability of our findings across all exposure-outcome pairs |
|  | **DISCUSSION** |  | 7-9 | This comprehensive bidirectional MR analysis provides robust evidence against causal relationships between MI and three major urinary system cancers—PCa, BCa, and MRN. Our findings consistently demonstrate null associations across multiple analytical approaches, discovery and replication cohorts, and both forward and reverse causal directions. ... |
| 14 | **Key results** | Summarize key results with reference to study objectives | 7-8 | bidirectional MR analysis provides robust evidence against causal relationships between MI and three major urinary system cancers—PCa, BCa, and MRN. ... |
| 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 | 9 | Several limitations merit consideration when interpreting our results. The restriction to individuals of European ancestry, while necessary to minimize population stratification bias, potentially limits generalizability to other ethnic populations where different genetic architectures or environmental interactions might exist.... |
| 16 | **Interpretation** |  |  |  |
|  | a) | Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies | 9 | This is especially relevant for the marginally significant results observed in the kidney cancer replication analysis, which, while not reaching conventional significance thresholds, suggest the possibility of very weak causal effects that require cautious interpretation. Additionally, while MR analysis effectively controls for measured and unmeasured confounding through genetic randomization, the potential for genetic pleiotropy or linkage disequilibrium with unmeasured variants could introduce bias, though our extensive pleiotropy testing provides reassurance against systematic violations. |
|  | 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 | 7-8 | The absence of causal associations in our MR analysis contrasts with several observational studies that have reported both positive and negative correlations between cardiovascular disease and various cancers... |
|  | 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 | The clinical implications of our findings are substantial for both cardiovascular and oncological practice. Our results do not support intensified cancer surveillance protocols based solely on MI history, as the absence of causal relationships suggests that increased screening would not yield proportional benefits compared to standard risk-stratified approaches.. |
| 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 | The restriction to individuals of European ancestry, while necessary to minimize population stratification bias, potentially limits generalizability to other ethnic populations where different genetic architectures or environmental interactions might exist. |
|  | **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 | 10 | This study was funded by the ..... |
| 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 | 10 | All data generated or analyzed in this study are included in this published article [and its supplementary information files] and are available from the corresponding author upon request. |
| 20 | **Conflicts of Interest** | All authors should declare all potential conflicts of interest | 10 | The authors declare no competing interests. |

This checklist is copyrighted by the Equator Network under the Creative Commons Attribution 3.0 Unported (CC BY 3.0) license.

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.