STROBE-MR checklist

No.	Section	Checklist item	Position
1	Title and abstract	Indicate MR as the study's design in the title and the abstract as a main purpose of the study	Title, Abstract
2	Background	Explain the scientific background and rationale for the reported study. Explain the exposure and a plausible potential causal relationship between exposure and outcome. Justify why MR is a helpful method to address the study question.	Introduction: paragraphs 1-2 (page 2)
3	Objectives	State specific objectives clearly, including prespecified causal hypotheses. State that MR is a method that intends to estimate causal effects.	Introduction: paragraphs 2-3 (page 2)
4a	Study design and data sourses	Setting: Describe the study design (two-sample MR) and the underlying population . Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection.	Method: Study design
4b		Participants: Report 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.	Method: Data sources, Table S1
4c		Describe measurement, quality control, and selection of genetic variants.	Method: Data extraction
4d		For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases.	Methods: Data sources (page 3)
4e		Provide details of ethics committee approval and participant informed consent, if relevant.	Methods: Ehical approval and consent to participate (page 4)
5	Assumptions	Explicitly state the 3 core instrumental variable (IV) assumptions for the main analysis (relevance, independence, and exclusion restriction), as well assumptions for any additional or sensitivity analysis.	Methods: Study design and Sensitivity analyses (pages 2-4), Figure 1
6a	Statistical methods:	Describe how quantitative variables were handled in the analyses.	Methods: Genetic analyses to elucidate causality (page 3)
6b		Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	
6c		Describe the MR estimator and related statistics. Detail the included covariates and, in case of 2-sample MR, whether the same covariate set was used for adjustment in the 2 samples.	
6d		Explain how missing data were addressed.	Method: Data extraction
6e		Indicate how multiple testing was addressed (false discovery rate method)	Methods: Mediation analyses link "gut microbiota–blood metabolites–stroke", and Sensitivity analyses (page 4)
7	Assessment of assumptions	Describe any methods or prior knowledge used to assess the assumptions or justify their validity	N/A
8	Sensitivity analyses and additional analyses	Describe any sensitivity analyses or additional analyses performed (eg, comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations).	Method: Sensitivity analyses
9a	Software and preregistration	Name statistical software and package(s), including version and settings used.	Methods: Sensitivity analyses (page 4)
9b		State whether the study protocol and details were preregistered (as well as when and where).	N/A
10a	Descriptive data	Report the numbers of individuals at each stage of included studies and reasons for exclusion. Use of a flow diagram.	Methods: Study design and Data sources (pages 2-3)
10b		Report summary statistics for phenotypic exposure , outcomes, and other relevant variables (eg, means, SDs, proportions).	Results: Genetic instruments for exposures (page 4), Table S1
10c		If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies.	N/A
10d		For 2-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.	Results (pages 6-7), Tables S8 and S11
11a	Main results	Report the associations between genetic variant and exposure and between genetic variant and outcome, preferably on an interpretable scale.	Results: Genetic instruments for exposures (page 4), Tables S2-S4
11b		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.	Results (pages 4-8), Tables S5-S6, S9-S10
11c		If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.	N/A
11d		Consider plots to visualize results (eg, forest plot, scatterplot of associations between genetic variants and outcome vs between genetic variants and exposure).	Figures 2-3
12a	Assessment of assumptions	Report the assessment of the validity of the assumptions by removing confounders-related SNPs.	Results (pages 4-8), Tables 1-3, Table S7 and S12
12b		Report any additional statistics (eg, assessments of heterogeneity across genetic variants, such as I^2, Q statistic).	
13a	Sensitivity analyses and additional analyses	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions.	Results (pages 4-8), Tables 1-3, Table S7 and S12
13b		Report results from other sensitivity analyses or additional analyses	
13c		Report any assessment of the direction of the causal relationship.	
13d		When relevant, report and compare with estimates from other RCTs and meta-analyses.	N/A
13e		Consider additional plots to visualize results.	N/A
14	Key results	Summarize key results with reference to study objectives.	Discussion: paragraph 1 (page 8)
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.	Discussion: paragraph 7 (page 9)
16a	Interpretation	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies.	Discussion: paragraphs 2-6 (pages 8-9)
16b		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.	
16c		Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions.	
17		Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure.	
18	Funding	Describe sources of funding and the role of funders in the present study.	Funding statement (page 9)
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.	Methods: Data sources (page 3), Tables S1-S4
20	Conflicts of interest	All authors should declare all potential conflicts of interest.	Conflicts of interest (page 10)

Three fundamental assumptions

a. The instrument variables (IVs) must be associated with exposures;

b. The IVs must not be associated with any confounders, such as age, sex, lifestyle;

c. The IVs must influence the outcomes only through exposures and not through any direct or alternative pathways.