STROBE-MR checklist of recommended items to address in reports of Mendelian randomization studies[1 2]

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	Abstract
	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	1.1 Background
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	1.2 Objectives
	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:	3-4	2.1. Study Design 2.2. Data Sources and Study Population
	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.	3	2.1. Study Design
	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	Data sources of gut microbiota and gut microbial metabolites/Data sources of pain
	c)	Describe measurement, quality control and selection of genetic variants	4	2.3. Selection of IVs
	d)	For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	4	2.3. Selection of IVs

		e)	Provide details of ethics committee approval and participant informed consent, if relevant	3	Ethical considerations
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	3	Fig. 1 Study design
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)	5	genome-wide statistical significance threshold ($p<5 \times 10{-}8$)
		b)	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	5	2.3. Selection of IVs
		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	5	2.3. Selection of IVs
		d)	Explain how missing data were addressed	NA	
		e)	If applicable, indicate how multiple testing was addressed	5	Benjamini-Hochberg false discovery rate (FDR)
7	**Assessment of assumptions**		Describe any methods or prior knowledge used to assess the assumptions or justify their validity	5	inverse-variance weighted (IVW)
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	MR-Egger, weighted median, weighted mode, and simple mode
9	**Software and pre-registration**				
		a)	Name statistical software and package(s), including version and settings used	5	R version 4.3.0 2023-04-21 ucrt
		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	6	3.1.IVs selection
		b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	6-23	3.2.The Causal Associations between Gut Microbiota and pain\3.3 The Causal Associations between gut microbial metabolites and pain
		c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	23	Q statistics from the IVW test indicated no significant heterogeneity
		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	6-23	Supplementary Table8-19
11	**Main results**				
		a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	6-23	3.2.The Causal Associations between Gut Microbiota and pain\3.3 The Causal Associations between gut microbial metabolites and pain
		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	6-23	3.2.The Causal Associations between Gut Microbiota and pain\3.3 The Causal Associations between gut microbial metabolites and pain
		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)	6-23	Fig. 2-13
12	**Assessment of assumptions**				

		a)	Report the assessment of the validity of the assumptions	23	3.5 Sensitivity analysis, Benjamini–Hochberg corrected test, Steigher test（reverse analysis）
		b)	Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)	23	3.5 Sensitivity analysis, Benjamini–Hochberg corrected test, Steigher test（reverse analysis）
13	**Sensitivity analyses and additional analyses**				
		a)	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	23	3.5 Sensitivity analysis, Benjamini–Hochberg corrected test, Steigher test（reverse analysis）
		b)	Report results from other sensitivity analyses or additional analyses	23	3.5 Sensitivity analysis, Benjamini–Hochberg corrected test, Steigher test（reverse analysis）
		c)	Report any assessment of direction of causal relationship (e.g., bidirectional MR)	23	3.5 Sensitivity analysis, Benjamini–Hochberg corrected test, Steigher test（reverse analysis）
		d)	When relevant, report and compare with estimates from non-MR analyses	26	Fig.15
		e)	Consider additional plots to visualize results (e.g., leave-one-out analyses)	24-26	Fig.14,15
	DISCUSSION				
14	**Key results**		Summarize key results with reference to study objectives	24	Fig.14
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	26	However, it is also important to acknowledge the limitations of our study.

16	**Interpretation**				
		a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	24-27	4.Discussion
		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	25-27	4.Discussion:More and more research findings have provided possible biological explanations
		c)	Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	24-27	4.Discussion
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	27	5.Conclusion
	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	27	Funding
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	27	Acknowledgments
20	**Conflicts of Interest**		All authors should declare all potential conflicts of interest	27	Conflict of interest

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.