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	Page 1 - 2	Title:Gut microbiota, disorders of gut–brain interaction and psychiatric disorders: A Mendelian randomisation study Abstract: We adopted a Mendelian Randomization (MR) approach to investigate the causal relationship among the abundances of several gut microbiota and the risk of developing disorders of gut-brain interaction (DGBIs) and psychiatric disorders.
	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	Page 2 - 3	Observational studies suggest that there are associations among gut microbiota, disorders of gut-brain interaction (DGBIs) and psychiatric disorders. Nonetheless, the precise biological mechanisms underlying this association remain unclear. The aim of this study was to use Mendelian randomisation (MR) to systematically identify the causality of the associations among the abundances of several gut microbiota and the risk of developing DGBIs and psychiatric disorders.
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	Page 3	Mendelian randomisation (MR) is a statistical method that can be used to estimate the causal association between exposure and disease outcome utilizing single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs). We used the bidirectional MR approach to explore the causal relationships between the abundances of several gut microbiota and the risk of developing 3 common DGBIs, between the abundances of several gut microbiota and the risk of developing 7 kinds of psychiatric disorders, and between the risk of developing 3 common DGBIs and the risk of developing 7 kinds of psychiatric disorders.
	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:	Page 3	Figure 1 displays the study design.

	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.	Not mentioned	
	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	Page 3 - 4 Supplementary file 1: Table S1	Data on genetic variants related to the human gut microbiota were obtained from the MiBioGen study, including 18,340 individuals from 24 cohorts. Genetic associations with 3 DGBIs, including irritable bowel syndrome (IBS), functional dyspepsia (FD) and functional constipation (FC), were obtained from the FinnGen. The summary GWAS statistics of psychiatric disorders were obtained from the Psychiatric Genomics Consortium (PGC) based on participants of European ancestry, including anxiety disorder (anxiety), autism spectrum disorder (ASD), bipolar disorder (BIP), anorexia nervosa (ED), major depressive disorder (MDD), posttraumatic stress disorder (PTSD), and schizophrenia(SCZ). Detailed descriptions about the sources and sample size are presented in Supplementary file 1: Table S1.
	c)	Describe measurement, quality control and selection of genetic variants	Page 3	To ensure the accuracy and authenticity of the conclusions, a series of quality control steps was used to select the optimal IVs. First, when gut microbiota served as the exposure, potential IVs for each feature were selected at $p < 1.0 \times 10^{-5}$ according to the criteria proposed in the study by Sanna et al.. Second, when the exposure was DGBIs or psychiatric disorders, when the selected IVs had to meet the genome-wide statistical significance threshold (5×10^{-8}), unfortunately, only a small number of SNPs were eligible to be selected as IVs. To obtain more comprehensive results, we used the locus-wide significance level (5×10^{-6}) to identify SNPs as the candidate IV set. Third, the linkage disequilibrium (LD) threshold was set as $r^2 < 0.01$ with a clumping window of 10,000 kb, and 1000 Genomes Project European sample data were used as the reference panel to calculate LD. Fourth, palindromic, ambiguous and duplicated SNPs were excluded. Finally, F statistics were calculated to assess the strength of the selected SNPs via the following equation: $F = [R^2/(1-R^2)] \times [(n-1-k)/k]$, where R^2 represents the proportion of exposure variance

				explained by the IVs, n is the sample size, and k is the number of IVs. A corresponding F-statistic ≥10 indicated that there was no significant weak instrumental bias.
		d) For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	Page 3 - 4 Supplementary file 1: Table S1	The MiBioGen study coordinated 16S rRNA gene sequencing profiles, and Spearman's correlation analysis was performed to identify genetic loci associated with the abundance levels of bacterial taxa. A total of 211 bacterial taxa were grouped into five taxonomic levels (phylum, class, order, family, and genus). The classification code of IBS was K57 in the International Classification of Diseases-Tenth Revision (ICD-10). The classification code of FD was ICD-10 K30, and the classification code of FC was ICD-10 K59.0. The summary GWAS statistics of psychiatric disorders were obtained from the Psychiatric Genomics Consortium (PGC), and the assessment and diagnostic criteria for diseases of psychiatric disorders is not mentioned in this study. Detailed descriptions are presented in Supplementary file 1: Table S1.
		e) Provide details of ethics committee approval and participant informed consent, if relevant	Page 15	This research has been conducted using publicly available GWAS summary data, and the Ethics approval and consent to participants could be obtained in the original GWAS. In addition, no individual-level data was used in this study. Therefore, no new ethical review board approval was required
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	Page 4-5	For causal estimates from MR studies to be valid, three assumptions must be ad here to: 1) the genetic variants are highly associated with the exposure, 2) the genetic variants are not associated with any potential confounder of the exposure-outcome association, and 3) the variants exclusively affects the outcome through the exposure. The core assumptions are reflected in the analysis method. The methods of sensitivity analysis and horizontal pleiotropy testing are also described: MR-PRESSO, Cochran's Q test, MR-Egger intercept test, scatter plots, funnel plot and leave-one-out analysis.
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)	Not mentioned	

		b) Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	Not mentioned	
		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	Page 4 - 5	Three different MR methods [random-effects inverse-variance weighting (IVW), MR-Egger, and weighted median] were used to obtain the MR estimates. The IVW method is reported to be slightly more powerful than the other methods under certain conditions. Therefore, IVW was used as the major outcome, whereas MR-Egger and the weighted median were used to improve the IVW estimates, as they could provide more robust estimates in a broader set of scenarios, despite being less efficient.
		d) Explain how missing data were addressed	Not mentioned	
		e) If applicable, indicate how multiple testing was addressed	Page 5	Multiple comparisons were performed. The significance thresholds corrected by Bonferroni correction were as follows: phylum $p = 5.56 \times 10^{-3}$ (0.05/9), class $p = 3.13 \times 10^{-3}$ (0.05/16), order $p = 2.5 \times 10^{-3}$ (0.05/20), family $p = 1.56 \times 10^{-3}$ (0.05/32), genus $p = 4.20 \times 10^{-4}$ (0.05/119), DGBIs and psychiatric disorders $p = 2.38 \times 10^{-3}$ (0.05/21). p-values between the Bonferroni-corrected significance level and 0.05 were considered to indicate potential associations.
7	**Assessment of assumptions**	Describe any methods or prior knowledge used to assess the assumptions or justify their validity	Page 4	Only independent SNPs ($r^2 < 0.01$ within 10,000 kb windows) were used. F-statistic of all SNPs was more than 10 indicated that there was no significant weak instrumental bias. When gut microbiota served as the exposure, potential IVs for each feature were selected at $p < 1.0 \times 10^{-5}$. when the exposure was DGBIs or psychiatric disorders, the locus-wide significance level (5×10^{-6}) was used to identify SNPs as the candidate IV set.
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)	Page 5	The MR-pleiotropy residual sum and outlier (MR-PRESSO) test and Cochran's Q test were used to calculate the potential heterogeneity, and the list of the remaining SNPs after removing outliers was used for subsequent MR analysis. The MR-Egger intercept test was employed to address horizontal pleiotropy, and the intercept term was used to evaluate the existence of pleiotropy. Scatter plots are used to observe the consistent effects estimated by the three methods. As a complement, a funnel plot and leave-one-out analysis

				were used to determine whether there was bias in the individual SNPs.
9	**Software and pre-registration**			
	a)	Name statistical software and package(s), including version and settings used	Page 5	All two-sample MR analyses were performed using the TwoSampleMR and MR-PRESSO R packages, and for multivariate MR analysis, we utilized the MendelianRandomization R package. All the statistical analyses were performed using R software version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).
	b)	State whether the study protocol and details were pre-registered (as well as when and where)	Not mentioned	
	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	Supplementary file 1: Table S1	The numbers of individuals included study is already provided in the supplementary data: Supplementary file 1: Table S1.
	b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	Supplementary file 1: Table S2 - 11	Summary statistics are shown in Supplementary file 1: Table S2 - 11
	c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	Not mentioned	
	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	Page 4 - 5	Genome-wide significant single-nucleotide polymorphisms (SNPs) were extracted from the GWAS summary data, and those with a longer physical distance (> 10,000 kb) and less possibility of linkage disequilibrium ($r^2 < 0.01$) were retained. Besides, the Cochran's Q-test was used to assess heterogeneity across the cohorts. All summary statistics used were GWAS analyses and no sample overlap was observed
11	**Main results**			
	a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	Supplementary file 1: Table S2 - 4	The associations are shown in Supplementary file 1: Table S2 - 4

		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	Page 6 - 12 Supplementary file 1: Table S6 - 11	Our MR analysis revealed 44 causal relationships between the abundances of several gut microbiota and the risk of developing DGBIs and 66 causal relationships between the abundances of several gut microbiota and the risk of developing psychiatric disorders. In addition, in the reverse-MR analysis, 15 causal relationships between the risk of developing DGBIs and the abundances of several gut microbiota and 47 causal relationships between the risk of developing psychiatric disorders and the abundances of several gut microbiota were explored. Our results showed that the abundances of some microbiota and their child taxa might be closely associated with the risk of developing certain diseases. Moreover, we observed 1 causal relationship between the risk of developing DGBIs and the risk of developing psychiatric disorders and 7 causal relationships between the risk of developing psychiatric disorders and the risk of developing DGBIs. Compared with the causal effect of the risk of developing DGBIs on the risk of developing psychiatric disorders, the risk of developing psychiatric disorders was more likely to causally influence the risk of developing DGBIs. The details of the MR estimates are shown in Page 6 - 12 and Supplementary file 1: Table S6 - 11.
		c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not mentioned	
		d) Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)	Figure 2 - 4, Supplementary file 2: Figure S1 - 5	Plots are shown in Figure 2 - 4, Supplementary file 2: Figure S1 - 5
12	**Assessment of assumptions**			
		a) Report the assessment of the validity of the assumptions	Page 5 - 6 Supplementary file 1: Table S2 - 11	Supplementary file 1: Table S2 - 4 reported the F-statistics for SNP. The F statistics of the IVs were all substantially > 10, indicating no evidence of weak instrument bias. Supplementary file 1: Table S6 - 11 reported Q-statistics for assessment of heterogeneity.
		b) Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)	Page 5 - 6	Supplementary file 1: Table S2 - 4 reported the F-statistics for SNP. The F statistics of the IVs were all substantially > 10, indicating no evidence of weak

			Supplementary file 1: Table S2 - 11	instrument bias. Supplementary file 1: Table S6 - 11 reported Q-statistics for assessment of heterogeneity.
13	**Sensitivity analyses and additional analyses**			
	a)	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	Page 5 - 6 Supplementary file 1: Table S5 - 11	After removing outliers identified by the MR-PRESSO test, most of the Cochrane Q statistic outcomes showed no significant heterogeneity. In addition, if directional horizontal pleiotropy was observed and uncorrectable according to the results of the MR-Egger regression intercept analysis, the associated component of the MR analysis was removed for violation of MR assumptions. Funnel plots exhibited a symmetrical distribution of effect points corresponding to causal associations, suggesting that IVs did not have a significant impact on the results. The leave-one-out sensitivity also confirmed the above conclusion. Supplementary file 1: Table S5 - 11
	b)	Report results from other sensitivity analyses or additional analyses	Page 5 - 6 Supplementary file 1: Table S5 - 11	After removing outliers identified by the MR-PRESSO test, most of the Cochrane Q statistic outcomes showed no significant heterogeneity. In addition, if directional horizontal pleiotropy was observed and uncorrectable according to the results of the MR-Egger regression intercept analysis, the associated component of the MR analysis was removed for violation of MR assumptions. Funnel plots exhibited a symmetrical distribution of effect points corresponding to causal associations, suggesting that IVs did not have a significant impact on the results. The leave-one-out sensitivity also confirmed the above conclusion. Supplementary file 1: Table S5 - 11
	c)	Report any assessment of direction of causal relationship (e.g., bidirectional MR)	Page 6 - 10 Supplementary file 1: Table S6 - 11	The details of the assessment of direction of causal relationship are shown in Page 6 - 10 and Supplementary file 1: Table S6 - 11.
	d)	When relevant, report and compare with estimates from non-MR analyses	Not mentioned	
	e)	Consider additional plots to visualize results (e.g., leave-one-out analyses)	Supplementary file 2: Figure S1 - 5	Leave-one-out results are presented in Supplementary file 2: Figure S1 - 5
	DISCUSSION			

14	**Key results**		Summarize key results with reference to study objectives	Page 12	Gut microbiota, DGBIs and psychiatric disorders are the key regulators of the microbiota-gut-brain axis, and in our study, we comprehensively assessed the causal relationships and potential mediators among those three regulators.
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	Page 14	Some limitations of this study should be noted. First, since the majority of participants were of European ancestry, caution should be taken when applying these MR results to people of other races. Then, to obtain a sufficient number of SNPs, the p threshold was relaxed, which might increase the risk of violating the first assumption of MR analysis. However, the F statistic for each SNP was greater than 10, indicating that there were no weak SNPs. Finally, considering the biological plausibility and the multistage statistical process, the use of Bonferroni correction may produce false-negative results. Therefore, we did not always use a significance threshold that was corrected for multiple comparisons to assess the outcome.
16	**Interpretation**				
		a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	Page 12 - 14	In this manuscript, the content of this item is discussed a lot, and the MR results are reasonably interpreted by comparing them with several published 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	Page 13 - 14	We observed that the abundances of most of the studied gut microbiota, which have causal effects on the risk of developing DGBIs and psychiatric disorders, are closely related to short-chain fatty acid (SCFA) concentrations. SCFA might be the key underlying biological mechanisms of causality of those three regulators. Besides, the abundances of some microbiota and their child taxa might be closely associated with the risk of developing certain diseases. Moreover, compared with the causal effect of the risk of developing DGBIs on the risk of developing psychiatric disorders, the risk of developing psychiatric disorders was more likely to causally influence the risk of developing DGBIs.
		c)	Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	Page 14	These findings may be useful in elucidating the underlying mechanisms and providing novel insights into microbiome-based preventive and therapeutic strategies for bidirectional dysregulation of brain-gut interactions.

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	Page 14	Due to the absence of extensive GWAS studies conducted in non-European ancestries, we were compelled to rely solely on GWAS conducted in persons of European ancestry to estimate the causal effects.
	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	Page 15	This work was supported by the Science and Technology Benefiting People Demonstration Project of Qingdao (No. 2428SMJK12NSH).
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	Page 15	The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material. The data generated by our study can be obtained from the corresponding authors.
20	**Conflicts of Interest**	All authors should declare all potential conflicts of interest	Page 15	The authors declare no 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.