Supplementary Table SVI. 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	**Title:** The Causal Effects of Brain Structure and Gene Expression on Neurodegenerative Diseases: A Mendelian Randomization Study **Abstract:** "This study employs Mendelian randomization (MR) to investigate potential causal relationships..."
	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	1-4	**Background & Rationale:** Describes NDDs as a global health threat, limitations of traditional observational studies (confounding, reverse causation) in establishing causality between brain structure and NDDs. (p.1-3). **Exposure:** Brain structural characteristics (512 UDIPs) and region-specific gene expression profiles (13 brain regions). (p.2, p.5-6). **Outcome:** Four NDDs: Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS). (p.1). **Justify MR:** "Mendelian randomization (MR) emerges as a paradigm-shifting methodological framework... This approach fundamentally circumvents the persistent confounding biases and reverse causation..." (p.4).
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	4	**Objectives:** "This study employs MR analysis to assess potential causal associations among brain structure, region-specific gene expression patterns, and four major categories of NDDs." (p.4). **MR as causal method:** "MR employs heritable genetic variants as instrumental variables to establish causal inference models." (p.4).
	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:	4-6, 21	**Study Design:** Figure 1 (p.21) shows the study flowchart. Section 2.1 (p.4) describes the two-phase MR design. **Data Sources Table:** Table 1 (p.21) provides outcome GWAS source information. Section 2.2 (p.5-6) describes exposure (UDIPs, eQTLs) and outcome data sources.
	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.	5-6	**Population & Setting:** Exposure and outcome datasets were primarily derived from European ancestry populations (p.6). Data are from large, publicly available GWAS/eQTL consortia (Patel et al. 2024, GTEx, IEU OpenGWAS). Details and PMIDs are provided.
	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	6,21	**Participants & Sample Size:** Case-control counts: AD (21,982 vs. 41,944), PD (33,674 vs. 449,056), ALS (12,577 vs. 23,475), MS (47,429 vs. 68,374) (p.6; Table 1, p.21). "Case ascertainment and diagnosis were performed in accordance with the study-specific clinical criteria and protocols detailed in the respective original publications (14–17)..." (p.6). No prior power calculation mentioned.
	c)	Describe measurement, quality control and selection of genetic variants	6-7	**Variant Selection & QC:** SNP selection: $p \leq 5\times10^{-8}$, $r^2 < 0.001$, LD clustering, 100 kb window. F-statistic calculated. (p.6-7). For SMR/eQTLs: cis-eQTLs with MAF >1% and p < 5e-08 were retained. (p.8).

		d) For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	5-6, 21	**Exposure Assessment:** UDIPs from Patel et al.: "512 unsupervised deep-learning imaging phenotypes (UDIPs)... through unsupervised deep learning of brain MRI scans." (p.5). Gene expression from GTEx: "eQTL summary statistics for the 13 brain regions were obtained from the GTEx Consortium database." (p.6). **Outcome Assessment:** "Case ascertainment and diagnosis were performed in accordance with the study-specific clinical criteria and protocols detailed in the respective original publications (14–17) and summarized in Table 1." (p.6).
		e) Provide details of ethics committee approval and participant informed consent, if relevant	8-9	**Ethical Statement:** "The study utilized publicly available GWAS summary statistics databases... all participants having provided informed consent. Thus, no additional ethical approval was required..." (p.8-9).
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	6	**Core IV Assumptions:** "To ensure the validity of MR analysis, adherence to three key criteria is critical: (1) single-nucleotide polymorphisms (SNPs) should exclusively influence exposure traits...; (2) SNPs should not be correlated with confounding factors...; and (3) SNPs should affect outcome traits solely through their impact on exposure traits." (p.6).
6	**Statistical methods: main analysis**	Describe statistical methods and statistics used	6-8	**MR Estimators:** Inverse variance weighted (IVW), MR-Egger, weighted median, weighted mode, Wald ratio. (Abstract, p.6). Summary-data-based MR (SMR) also used. (Abstract, p.8). **Model Selection:** Wald ratio (1 SNP), fixed-effects IVW (2-3 SNPs), random-effects IVW (>3 SNPs). (p.7). **Effect Scale:** "Odds ratios (OR) represent the relative risk of the outcome per standard deviation (SD) increase in the exposure phenotype." (p.7). **Multiple Testing:** Benjamini-Hochberg FDR correction applied independently per outcome and exposure category. Associations with FDR q-value < 0.05 considered robust. (p.7).
		a) Describe how quantitative variables were handled in the analyses (i.e., scale, units, model)	7	**Handling of Variables:** "Since the exposure phenotypes (UDIPs) are latent features... all effect sizes were standardized. Consequently, the reported Odds Ratios (ORs) represent the relative risk of the outcome per standard deviation (SD) increase in the exposure phenotype." (p.7).
		b) Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	6-7	**Variant Handling & Weighting:** Multiple MR methods used (IVW, MR-Egger, etc.). Model choice (Wald ratio, fixed/random effects IVW) based on number of valid SNPs. (p.6-7).
		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	6-7	**MR Estimators:** Listed in Item 6 above. **Covariates:** For outcome GWAS: "Genetic associations were adjusted for genomic principal components to account for population stratification." (p.6). Two-sample MR using summary statistics from different consortia.
		d) Explain how missing data were addressed	-	Not explicitly discussed for missing data in summary statistics.
		e) If applicable, indicate how multiple testing was addressed	7	**Multiple Testing Correction:** "To account for multiple comparisons... we implemented the Benjamini–Hochberg (BH) procedure... We defined robust causal associations as those with an FDR-adjusted P-value (q-value) < 0.05." (p.7).
7	**Assessment of assumptions**	Describe any methods or prior knowledge used to assess the assumptions or justify their validity	6-7, 8	**Instrument Strength:** F-statistic calculated (p.7). **Pleiotropy & Validity Assessment:** Sensitivity analyses described in Section 2.5 (heterogeneity I^2, Cochrane's Q, Egger intercept, MR-PRESSO, leave-one-out) are used to assess assumptions. (p.8). For SMR, HEIDI test used to distinguish causality from linkage (p.8).
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)	8	**Sensitivity Analyses:** "Sensitivity analyses included heterogeneity I^2 statistics, Cochrane's Q test, Egger intercept test, MR-PRESSO, and leave-one-out validation." (Abstract, p.8). **Additional Analysis:** Summary-data-based MR (SMR) with HEIDI test applied to gene expression data. (Abstract, p.8).

9	**Software and pre-registration**		8	**Software:** "All statistical analyses were performed using R software (version 4.2.1) with relevant statistical packages." (p.8). **Pre-registration:** Not mentioned.
	a)	Name statistical software and package(s), including version and settings used	8	**Software:** "All statistical analyses were performed using R software (version 4.2.1) with relevant statistical packages." (p.8).
	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	5-6, 21	**Sample Sizes:** Provided in Section 2.2 (p.6) and Table 1 (p.21) for outcomes. Descriptions of exposure data (UDIPs, brain regions) in Section 2.2 (p.5-6). **Two-sample MR overlap:** Not explicitly stated. Both exposure and outcome samples are of European ancestry, justifying comparability. (p.6).
	b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	5-6, 21	**outcome Summary:** Case and control counts provided (p.6, Table 1 p.21). **Exposure Summary:** Descriptions of UDIPs (512 features, heritability) and brain regions (13 regions) provided (p.5-6).
	c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	-	The GWAS sources are themselves large meta-analyses. Heterogeneity assessments (Cochran's Q) are reported as part of the MR sensitivity analysis results (see Item 13), not for the original constituent studies.
	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	**i. Sample similarity:** Both exposure and outcome datasets are stated to be from European-ancestry populations (p.6). **ii. Overlap:** Not explicitly stated. Use of summary statistics from different consortia suggests minimal or unknown overlap.
11	**Main results**		9-11, 22-27	**MR estimates (OR with 95% CI)** are extensively reported in Section 3.1 and 3.2 (p.9-11), and visualized in Figures 2-8 (p.22-27). **Example UDIP:** "T2-58 demonstrated a positive association with AD risk (OR = 1.012, 95% CI: 1.002–1.02)" (p.9). **Example Gene:** "elevated HLA-DRB1 expression was associated with increased disease risk for AD (OR = 1.142, 95% CI: 1.032–1.262)..." (p.10).
	a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	9-11	Associations are reported as MR estimates (OR per SD increase in exposure). Detailed SNP-level associations (beta, SE) are noted to be in supplementary files.
	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	9-11, 22-27	MR estimates (OR with 95% CI) are reported throughout Section 3.1 and 3.2 and in Figures 2-8.

		c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-	Not done.
		d) Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)	22-27, 28-29	Multiple forest plots (Figures 2-8, p.22-27) visualize MR estimates for UDIPs and genes. Venn diagrams (Supplementary Figures 1 & 2, p.28-29) show overlap of significant exposures across diseases.
12	**Assessment of assumptions**		9, 11	**Heterogeneity (Cochran's Q):** "94.6% of the significant multi-SNP associations exhibited no evidence of heterogeneity (P > 0.05, see Supplementary Table S2)." (p.9). **HEIDI Test (for SMR):** For HLA genes in MS, significant heterogeneity (P_HEIDI < 0.05) suggested potential LD confounding. For AD, PD, ALS, associations passed HEIDI test (P_HEIDI > 0.05), supporting causality. (p.11).
		a) Report the assessment of the validity of the assumptions	9, 11	Reported via heterogeneity tests (Cochran's Q, HEIDI) and mention of F-statistic calculation.
		b) Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)	9, 11	Cochran's Q test and HEIDI test p-values are reported as mentioned above.
13	**Sensitivity analyses and additional analyses**		9, 11	**Sensitivity Results:** Low heterogeneity for most multi-SNP associations confirmed by post-hoc Cochran's Q test (p.9). HEIDI test results for SMR analyses are reported, distinguishing signals for MS from AD/PD/ALS (p.11). Detailed results for Egger intercept, MR-PRESSO, leave-one-out are implied but not fully detailed in main text. **Additional SMR Results:** Causal associations for specific genes (HLA-DRB1, HLA-DQA2, etc.) across brain regions and NDDs are reported, with interpretations informed by HEIDI test. (p.10-11).
		a) Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	9	Post-hoc Cochran's Q test showing low heterogeneity for most associations supports robustness against pleiotropy for the main MR analyses. (p.9).
		b) Report results from other sensitivity analyses or additional analyses	11	HEIDI test results from the SMR analysis are reported, providing nuance on the nature of HLA gene associations (causal vs. potentially linked) across different diseases. (p.11).
		c) Report any assessment of direction of causal relationship (e.g., bidirectional MR)	-	Not performed. The study is unidirectional (brain features/gene expression -> disease).
		d) When relevant, report and compare with estimates from non-MR analyses	-	Not done.
		e) Consider additional plots to visualize results (e.g., leave-one-out analyses)	-	Leave-one-out analysis was a performed method (p.8), but specific plots are not included in the provided figures.
	DISCUSSION			
14	**Key results**	Summarize key results with reference to study objectives	11-12	Start of Discussion (p.11-12) summarizes key findings: causal effects of multiple UDIPs and brain-region genes (especially HLA genes) on four NDDs, identification of shared and distinct genetic factors.
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	17	**Major Limitation:** Reliance on European-ancestry data limits generalizability, especially for HLA/MHC findings which may be population-specific due to complex LD. (p.17). **Addressing LD:** Use of HEIDI test to assess robustness in MHC region. (p.17). Acknowledges challenge of LD in MHC despite using standard 100 kb window. (p.17).

16	**Interpretation**		11-17	**Meaning:** Results are interpreted cautiously in context of limitations and prior literature. Language includes "provides genetic evidence suggestive of potential causal links" (Abstract), "lending support to the potential universality" (p.16). **Mechanism:** Extensive discussion on potential biological mechanisms, especially for HLA genes (five immune-mediated mechanisms detailed on p.14-16). Causal language is used but clarified by reliance on MR/HEIDI assumptions. **Clinical Relevance:** Discussed implicitly by linking findings to disease pathogenesis and identifying potential therapeutic targets (e.g., "providing a rationale for prioritizing these immune pathways in future experimental studies", p.16). Direct clinical translation is not specified.
		a) Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	11-17	As above. Comparisons with previous imaging and genetic studies are made throughout the 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	13-16	Detailed discussion of potential HLA-mediated immune mechanisms (antigen presentation, immune cell dysfunction, etc.) linking brain gene expression to NDDs (p.13-16). The gene-environment equivalence assumption is inherent to MR and is not explicitly discussed.
		c) Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	16	Clinical relevance is framed in terms of informing future mechanistic and experimental research priorities rather than direct clinical application or intervention effect sizes.
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	17,18	**a) Other populations:** Explicitly discussed as a limitation. "Future research should expand sample populations to include diverse ethnic groups..." (Conclusion, p.18). **(b) Exposure periods/timings and (c) other exposure levels:** Not specifically discussed. Genetic instruments represent lifelong predisposition.
	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	20	**Funding:** "This study has received the funding by grants from the National Nature Science Foundation of China (8200184)." (p.20). Sources for original datasets are acknowledged via citations.
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	20	**Data Availability:** "All data sources used in this article have been explained in the article. Further inquiries can be directed to the corresponding author." (p.20). Original public data sources are referenced (e.g., URLs/DOIs in Section 2.2 and Table 1). Code availability is not explicitly stated.
20	**Conflicts of Interest**	All authors should declare all potential conflicts of interest	19	**Conflict of Interest:** "The authors declare that there is no conflict of interest." (p.19).

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.