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	2	We integrated two large genome-wide association studies (88,486 patients with asthma and 447,859 controls; 412,024 patients with depression and 1,587,577 controls) with cross-sectional and longitudinal information available from the All of Us Research Program (N=87,167) through polygenic risk scoring (PRS), Cox proportional hazard models, one-sample Mendelian randomization (MR), and gene-set and drug-repurposing analyses.
	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	4	To perform a high-resolution analysis of asthma-depression comorbidity, we integrated genome-wide association statistics generated from large cohorts with newly generated individual-level information available from the All of Us (AoU) Research Program [13, 14, 19].
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	Because we hypothesize that different pleiotropic scenarios contribute to the comorbidity between asthma and depression, we conducted bi-directional analyses to assess whether asthma can cause depression and whether depression has an effect on asthma. Additionally, we investigated biological signatures shared by asthma and depression polygenic risk, also exploring molecular compounds potentially targeting pathogenic processes contributing to their comorbidity.
	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:		
	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	The goal of the present study was to understand dynamics contributing to the asthma-depression comorbidity. Electronic health records (EHR) were the primary data source to derive cross-sectional and longitudinal information regarding these health outcomes, considering the existing literature

			supporting the reliability of EHR-based phenotyping for research purposes [20, 21]. Because different scenarios may be present, we used complementary analytic approaches to integrate genetic and observational data. Polygenic risk scoring (PRS), time-to-event analysis, and Mendelian randomization (MR) were conducted to investigate the bidirectional relationship between asthma and depression. Multiple potential confounders (e.g., age, sex, smoking status, and household income) were considered in a model-based asthma-depression association test. Sensitivity analyses were performed to assess the reliability of the putative causal relationships observed. Evidence consistency across methods was used to assess the robustness of the potential direct effects. To investigate potentially shared processes, we examined the biological signatures overlapping between asthma and depression polygenic risk and conducted a drug-repurposing analysis to identify therapeutic interventions targeting their comorbidity.
	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	Considering AoU participants with WGS data (N=245,394), we excluded individuals who were not of genetically inferred European descent (N=117,201), did not pass the quality control of the WGS data (N=24,982), or did not have complete phenotype data (N=16,044). After applying these exclusion criteria, the final sample included 87,167 AoU participants (Table 1), which comprised 5,013 patients with asthma-depression comorbidity, 6,202 patients with only asthma, 13,550 patients with only depression, and 62,402 controls (AoU participants without asthma and depression diagnoses).
	c) Describe measurement, quality control and selection of genetic variants	6	our analyses, we applied the following quality control criteria: biallelic variants with minor allele frequency>1%, Hardy–Weinberg equilibrium p<10−6, call rate>95%, and per-individual genotyping rate>95%.
	d) For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	7	Leveraging asthma and depression genome-wide association statistics as training dataset and UK Biobank European sample as linkage disequilibrium (LD) reference, posterior variant-level effect sizes were calculated using PRS-continuous shrinkage (PRS-CS) approach and its PRS-CS-auto algorithm to determine the optimal parameters for each

				outcome [28]. PRS-CS approach is a Bayesian regression framework, using priors to allow for marker-specific adaptive shrinkage (i.e., the amount of shrinkage applied to each genetic marker is adaptive to the strength of its association signal in GWAS [28]. Based on the PRS-CS output, we used PLINK1.9 [29] to calculate asthma and depression PRS in AoU participants.
		e) Provide details of ethics committee approval and participant informed consent, if relevant	5	The present study was conducted using individual-level information available from the AoU Research Program. This was approved by the AoU Institutional Review Board. The data used in this study were obtained through an approved data use agreement between the AoU Research Program and Yale University. When enrolled in the AoU cohort, participants provided informed consent and consent for the release of EHRs separately.
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	8-9	To investigate causal effects underlying asthma-depression comorbidity, we employed the one-sample MR design [33]. This permitted us to use asthma and depression PRS as instrumental variables to test in AoU cohort whether one disease has a direct effect on the risk of the other circumventing environmental confounders and reverse causation bias [33]. To ensure the reliability of the MR estimates, we used three different methods that rely on different assumptions: two-stage least-squares (2SLS) approach [34] implemented in R package ivreg (available at https://cran.r-project.org/web/packages/ivreg/) and two-stage predictor substitution (2SPS) and two-stage residual inclusion (2SRI) approaches implemented in the OneSampleMR R package (available at https://cran.r-project.org/web/packages/OneSampleMR/) [35]. One-sample MR covariates included age, sex, annual income, smoking status, and top 10 within-ancestry PCs. To assess the validity of the MR assumptions, we performed multiple sensitivity analyses. Specifically, weak-instrument test was performed to assess possible violations of relevance assumption (i.e., the genetic instrument is robustly associated with the exposure of interest). Wu-Hausman diagnostic was performed to assess possible violations of the exogeneity assumption (i.e., the genetic instrument is independent of

				confounders related to the outcome). Breusch–Pagan test for heteroscedasticity was performed to detect possible violations of the exclusion-restriction assumption (i.e., the genetic instrument is associated with the outcome only through its effect on the exposure).
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 Applicable
	b)	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	7	Leveraging asthma and depression genome-wide association statistics as training dataset and UK Biobank European sample as linkage disequilibrium (LD) reference, posterior variant-level effect sizes were calculated using PRS-continuous shrinkage (PRS-CS) approach and its PRS-CS-auto algorithm to determine the optimal parameters for each outcome [28]. PRS-CS approach is a Bayesian regression framework, using priors to allow for marker-specific adaptive shrinkage (i.e., the amount of shrinkage applied to each genetic marker is adaptive to the strength of its association signal in GWAS [28]. Based on the PRS-CS output, we used PLINK1.9 [29] to calculate asthma and depression PRS in AoU participants.
	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	9	To ensure the reliability of the MR estimates, we used three different methods that rely on different assumptions: two-stage least-squares (2SLS) approach [34] implemented in R package ivreg (available at https://cran.r-project.org/web/packages/ivreg/) and two-stage predictor substitution (2SPS) and two-stage residual inclusion (2SRI) approaches implemented in the OneSampleMR R package (available at https://cran.r-project.org/web/packages/OneSampleMR/) [35]. One-sample MR covariates included age, sex, annual income, smoking status, and top 10 within-ancestry PCs.
	d)	Explain how missing data were addressed	6	Considering AoU participants with WGS data (N=245,394), we excluded individuals who were not of genetically inferred European descent

				(N=117,201), did not pass the quality control of the WGS data (N=24,982), or did not have complete phenotype data (N=16,044). After applying these exclusion criteria, the final sample included 87,167 AoU participants (Table 1), which comprised 5,013 patients with asthma-depression comorbidity, 6,202 patients with only asthma, 13,550 patients with only depression, and 62,402 controls (AoU participants without asthma and depression diagnoses).
		e)	If applicable, indicate how multiple testing was addressed	
7	**Assessment of assumptions**		Describe any methods or prior knowledge used to assess the assumptions or justify their validity	
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)	To assess the validity of the MR assumptions, we performed multiple sensitivity analyses. Specifically, weak-instrument test was performed to assess possible violations of relevance assumption (i.e., the genetic instrument is robustly associated with the exposure of interest). Wu-Hausman diagnostic was performed to assess possible violations of the exogeneity assumption (i.e., the genetic instrument is independent of confounders related to the outcome). Breusch–Pagan test for heteroscedasticity was performed to detect possible violations of the exclusion-restriction assumption (i.e., the genetic instrument is associated with the outcome only through its effect on the exposure).
9	**Software and pre-registration**			
		a)	Name statistical software and package(s), including version and settings used	9 To ensure the reliability of the MR estimates, we used three different methods that rely on different assumptions: two-stage least-squares (2SLS) approach [34] implemented in R package ivreg (available at https://cran.r-project.org/web/packages/ivreg/) and two-stage predictor substitution (2SPS) and two-stage residual inclusion (2SRI) approaches implemented in the OneSampleMR R package (available at https://cran.r-project.org/web/packages/OneSampleMR/) [35].
		b)	State whether the study protocol and details were pre-registered (as well as when and where)	Not Applicable

	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		Considering AoU participants with WGS data (N=245,394), we excluded individuals who were not of genetically inferred European descent (N=117,201), did not pass the quality control of the WGS data (N=24,982), or did not have complete phenotype data (N=16,044). After applying these exclusion criteria, the final sample included 87,167 AoU participants (Table 1), which comprised 5,013 patients with asthma-depression comorbidity, 6,202 patients with only asthma, 13,550 patients with only depression, and 62,402 controls (AoU participants without asthma and depression diagnoses).
	b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	10	The sample investigated included 5,013 patients with asthma-depression comorbidity, 6,202 patients with only asthma, 13,550 patients with only depression, and 62,402 controls (AoU participants without asthma and depression diagnoses). While no major difference was observed in the mean age across these groups, patients with asthma-depression comorbidity included more females (76%) and had lower annual income (≥$50,000, 46%) than the other three groups investigated (Table 1). Conversely, the percentage of lifetime smokers was similar in asthma-depression and depression-only groups (49% and 51%, respectively) and lower in asthma-only patients and controls (38% and 40%, respectively; Table 1).
	c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies		Not Applicable
	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		Not Applicable
11	**Main results**			
	a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	10	In line with the expected generalizability of previously identified genetic effects, one standard deviation increase of asthma PRS was associated

				with a 39% increase in the odds of asthma in the AoU cohort (OR=1.39, 95%CI=1.36-1.42; Nagelkerke's R2=1.83%; Figure 1, Supplemental Table 1). Similarly, depression PRS was associated with AoU depression diagnosis (OR=1.24, 95%CI=1.22-1.26; Nagelkerke's R2=1.04%; Figure 1, Supplemental Table 1).
		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	12	The genetic liability to depression had a statistically significant causal effect on asthma with estimates ranging from 3.21±0.31 (2SPS estimate modeling both linear and non-linear relationship) to 0.36±0.03 (2SLS estimate modeling linear relationship). Conversely, the genetic liability to asthma appeared to affect depression under 2SLS and 2SPS assumptions (0.12±0.04 and 0.76±0.25), but the effect was null in the 2SRI analysis (Table 2).
		c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		Not Applicable
		d)	Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)		
12	**Assessment of assumptions**				
		a)	Report the assessment of the validity of the assumptions	9	To assess the validity of the MR assumptions, we performed multiple sensitivity analyses. Specifically, weak-instrument test was performed to assess possible violations of relevance assumption (i.e., the genetic instrument is robustly associated with the exposure of interest). Wu-Hausman diagnostic was performed to assess possible violations of the exogeneity assumption (i.e., the genetic instrument is independent of confounders related to the outcome). Breusch–Pagan test for heteroscedasticity was performed to detect possible violations of the exclusion-restriction assumption (i.e., the genetic instrument is associated with the outcome only through its effect on the exposure).
		b)	Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)		Not Applicable
13	**Sensitivity analyses and**				

additional analyses			
	a) Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions		Weak-instrument and Wu-Hausman tests supported the strength of the genetic instrument and the robustness of the putative causal effect underlying the effects observed (Supplementary Table 5). Conversely, Breusch–Pagan test detected significant heteroskedasticity in the effects detected between depression and asthma (Supplementary Table 5). This may affect the reliability of the 2SLS estimate. Applying heteroskedasticity-consistent (HC1) robust standard errors, the 2SLS estimates under heteroskedasticity did not change (depression→asthma 0.36±0.03; asthma→depression 0.12±0.04). Because 2SPS and 2SRI approaches are based on the generalized method of moments (GMM), their variance estimator is already heteroskedasticity-robust.
	b) Report results from other sensitivity analyses or additional analyses		
	c) Report any assessment of direction of causal relationship (e.g., bidirectional MR)	12	To assess possible causal effects underlying the associations observed, we performed a bidirectional one-sample MR analysis (Table 2). Applying three methods based on different assumptions (i.e., 2SLS, 2SPS, 2SRI)[34, 35], we aimed to model different causal relationships that may contribute to asthma-depression comorbidity.
	d) When relevant, report and compare with estimates from non-MR analyses	10-12	In line with the expected generalizability of previously identified genetic effects, one standard deviation increase of asthma PRS was associated with a 39% increase in the odds of asthma in the AoU cohort (OR=1.39, 95%CI=1.36-1.42; Nagelkerke's R2=1.83%; Figure 1, Supplemental Table 1). Similarly, depression PRS was associated with AoU depression diagnosis (OR=1.24, 95%CI=1.22-1.26; Nagelkerke's R2=1.04%; Figure 1, Supplemental Table 1). Cross-disorder PRS associations were also observed. Specifically, asthma PRS was associated with depression (OR=1.03, 95%CI=1.01-1.05, Supplemental Table 1), while depression PRS was associated with asthma (OR=1.12, 95%CI=1.09-1.14, Supplemental Table 1). To further investigate whether cross-

disorder PRS associations were fully explained by asthma-depression comorbidity, we also controlled for the co-occurrence of the two disorders. After including comorbidity status as an additional covariate in the model, asthma remained associated with both depression diagnosis and depression PRS (depression-diagnosis OR=3.51; depression-PRS OR=1.06, respectively; Supplemental Table 1). When testing depression as outcome and including comorbidity status in the model, we observed a direct association with asthma diagnosis (OR=3.57; Supplemental Table 1), but an inverse relationship with asthma PRS (OR=0.97; Supplemental Table 1). In all multivariate logistic regression models, there was no evidence of collinearity among the variables (VIF<1.2; Supplementary Table 2).

When including BMI, antidepressants, and asthma medications as additional covariates, the effect of depression PRS on asthma remained significant (OR=1.08, p=6.57□10-11), also when accounting for comorbidity status (OR=1.06, p=2.63□10-7). Similar results were observed when limiting the PRS analysis to AoU participants who are not using antidepressants or asthma medications (Supplementary Table 1). Conversely, the effect of asthma PRS on depression became null when including BMI, antidepressants, and asthma medications as additional covariates, as well as when limiting the PRS analysis to AoU participants who are not using antidepressants or asthma medications (Supplementary Table 1).

Considering the dates of onset for asthma and depressive disorder, we identified 3,185 patients with incident asthma (i.e., asthma occurred after depressive disorder) and 15,679 patients with incident depression (i.e., depressive disorder occurred after asthma). The median follow-up time was 3.45 years for asthma and 3.23 years for depressive disorder. Fitting Cox proportional hazard models, we explored PRS temporal relationships with asthma and depression diagnoses (Figure 2). As expected, asthma PRS was associated with an increased risk of asthma (HR=1.23, 95%CI=1.19-1.28; Supplementary Table 3) and depression PRS

				with depression (HR=1.19, 95%CI=1.16-1.23; Supplementary Table 3) in AoU cohort. In the cross-disorder analysis, asthma PRS was associated with depression risk (HR=1.04, 95%CI=1.01-1.07; Supplementary Table 3), but the effect became null after accounting for asthma comorbidity (Supplementary Table 3). Conversely, depression PRS was associated with asthma risk (HR=1.13, 95%CI=1.09-1.17; Supplementary Table 3), even after accounting for depression comorbidity (HR=1.08, 95%CI=1.04, 1.12; Supplementary Table 3). Accounting for asthma and depression PRS did not reduce the strong association between asthma and depression: asthma remained associated with depression risk (HR=3.94, 95%CI=3.67-4.22) and depression remained associated with asthma risk (HR=3.93, 95%CI=3.61-4.27; Supplementary Table 3). No collinearity was observed in the Cox proportional hazard models (VIF<1.2; Supplementary Table 4).
		e) Consider additional plots to visualize results (e.g., leave-one-out analyses)		
	DISCUSSION			
14	**Key results**	Summarize key results with reference to study objectives	13-14	Depression is a well-known non-respiratory asthma comorbidity contributing to poor disease control and greater risk of exacerbations [42]. As noted above, the analysis of diagnosis records for >151 million US residents highlighted depression as the primary outcome characterizing a distinct subgroup of patients with asthma [10]. Previous studies highlighted the contribution of shared genetic predisposition to asthma-depression comorbidity [16], with a potential causal effect of depression genetic liability on asthma [16-18]. These hypotheses were also supported by studies reporting an association between depression PRS and asthma [43, 44]. Building on these previous studies, the findings generated by our study expanded the understanding of how polygenic risk contributes to asthma-depression comorbidity.

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	First, the data available did not permit us to investigate the comorbidity across asthma and depression symptoms and to investigate heterogeneity across patients affected by both disorders.
16	**Interpretation**			
	a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	15-16	Previous studies applied the two-sample MR framework to investigate asthma-depression causal relationships, observing a possible effect of depression genetic liability on asthma but no reverse relationship [16-18]. Applying one-sample MR approach, we boosted the statistical power of our genetically informed causal inference analysis through the integration of genome-wide association statistics available from large-scale GWAS meta-analyses and individual-level data available from AoU cohort. Additionally, one-sample MR framework permitted us to explore different types of relationships contributing to asthma-depression comorbidities. Our findings highlighted that the effect of depression genetic liability on asthma is much stronger than the effect of asthma genetic risk on depression. Using three one-sample MR designs, we assessed different dynamics. With respect to the effect of depression genetic liability on asthma, all one-sample MR designs confirmed this relationship. However, we observed differences in the effect sizes, with 2SLS method indicating an effect (beta=0.36) much smaller than the ones observed in the 2SPS and 2SRI analyses (beta=3.21 and 2.99, respectively). This could be explained by the fact that 2SLS does not model adequately non-linear relationships as 2SPS and 2SRI [34, 35]. The small reduction in the effect size observed between 2SPS and 2SRI could be due to the fact that the latter uses residuals to account more accurately for the effect of covariates. With respect to the effect of asthma genetic risk on depression, the differences in the estimates observed across one-sample MR methods were less accentuated with 2SLS showing the smallest effect. Importantly, although its direction was consistent with the other methods, 2SRI effect was not statistically different from the null. This suggests that the effect of asthma genetic predisposition on depression may be explained by

				other factors, as also supported by the sensitivity analyses related to the PRS associations observed.
		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	17-18	Our study uncovered how polygenic risk relates to asthma-depression comorbidity through direct effects and shared pathways. In particular, our analyses converged on the potential direct effect of depression genetic liability on asthma, which does not appear to be affected by confounders. Conversely, asthma genetic liability does not seem to be directly related to depression, but the association may be driven by confounders related to their comorbidity. In this context, we also observed shared genetic mechanisms linking asthma and depression to immune system and lung-brain axis, with molecular compounds potentially targeting pathogenic processes contributing to their comorbidity. Overall, these findings can contribute to the development of preventive strategies (e.g., respiratory screening in patients with depression) and approaches designed to treat asthma-depression comorbidity.
		c) Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions		
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	Due to the limited availability of genome-wide data informative of diverse population groups, we investigated only individuals of European descent to avoid interpreting results related to other population groups that may be strongly underpowered. Accordingly, our findings may not be generalizable across other populations, because of potential differences in genetic susceptibility, environmental factors, and clinical assessment. Additionally, because AoU participants are not a representative sample of the US population [60], our results will need to be validated in clinical settings representative of diverse population groups to translate them into preventive strategies to identify individuals vulnerable to asthma-depression comorbidity.
	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	18	This study was supported by a grant from the National Institutes of Mental Health (RF1 MH132337) and One Mind.
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	19	The All of Us Research Program data are available on the All of Us Researcher Workbench.
20	**Conflicts of Interest**	All authors should declare all potential conflicts of interest	19	Dr. Polimanti is paid for his editorial work on the journal Complex Psychiatry. The other authors declare no competing interests.

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;326:1614-21.

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.