Oxidative stress in chronic diseases: causal inference from observational studies
Luo, J.

CHAPTER 8

Depression and inflammatory bowel disease: a bidirectional two-sample Mendelian randomization study

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Abstract

Background and Aims: Observational studies have suggested a bidirectional association between depression and inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC). However, it remains unclear whether observed associations are casual due to the difficulties of determining sequential temporality. We investigated the association between depression and IBD by using bidirectional two-sample Mendelian randomization (MR).

Methods: Independent genetic variants for depression and IBD were selected as instruments from published genome-wide association studies (GWAS) among individuals of predominantly European ancestry. Summary statistics for instrument-outcome associations were retrieved from three separate databases for both depression (Psychiatric Genomics Consortium, FinnGen, and UK Biobank), and IBD (the largest GWAS meta-analysis, FinnGen, and UK Biobank), respectively. MR analyses included inverse-variance weighted method, weighted-median estimator, MR-Egger regression, and sensitivity analyses of Steiger filtering and MR PRESSO. From either direction, analyses were performed per outcome database and were subsequently meta-analyzed using fixed-effect model.

Results: Genetically predicted depression (per log-odds ratio increase) was associated with a higher risk of IBD; odds ratios (95% confidence interval) for IBD, CD and UC were 1.20 (1.05, 1.36), 1.29 (1.07, 1.56) and 1.22 (1.01, 1.47) in a combined sample size of 693,183 (36,507 IBD cases), 212,172 (13,714 CD cases) and 219,686 (15,691 UC cases) individuals, respectively. In contrast, no association was observed between genetically influenced IBD and depression in 534,635 individuals (71,466 depression cases).

Conclusions: Our findings corroborated a causal association of depression on IBD, which may impact the clinical decision on the management of depression in patients with IBD. Though our results did not support a causal effect of IBD on depression, further investigations are needed to clarify the effect of IBD activity on depression (with different symptomology).

Introduction

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic and progressive inflammation of the gastrointestinal tract and poses a high disease burden worldwide [1]. Despite the substantial progress in the diagnosis and management of IBD to achieve long-term remission, the causes leading to IBD are yet not fully elucidated. The pathogenesis of IBD is considered as a result of an interplay between genetic susceptibility and environmental risk factors, including but not limited to smoking, unfavorable diet, and lifestyle, which subsequently lead to an inappropriate intestinal immune activation and a proinflammatory intestinal microbiome [2-4]. Over the past decade, the association between depression and IBD (and vice versa) gained considerable interest [5,6], in light of the putative pathophysiological mechanism underlying the dysregulation of the brain-gut axis [7].

The estimated prevalence of comorbid depression or depressive symptoms in IBD patients is approximately 25% in a most recent meta-analysis [8], which is significantly higher than the estimated prevalence of 3.4% in the general population [9]. In line with this evidence, the incidence of depression in IBD patients is higher than in a matched population as early as 5 years before IBD diagnosis [10]. Several longitudinal studies suggested an association between depression and an elevated risk of IBD [11-14]. However, a recent population-based nested case-control study considering prodromal gastrointestinal (GI) symptoms prior to the diagnosis of depression showed that depression alone, i.e. in the absence of prior GI symptoms, is not associated with subsequent IBD risks [15]. Since depression and IBD both involve a vague and subtle onset, it is difficult to determine the temporal order of these two conditions. Thus, the associations described previously may have been partly due to reverse causation and/or residual confounding, which are often noted in observational studies. Therefore, the directionality and causality between depression and IBD remain unclear.

Mendelian randomization (MR) utilizes genetic variants identified through genome-wide association studies (GWAS), which are randomly allocated at conception as instrumental variables to investigate whether a lifetime exposure is causally associated with an outcome [16]. The most recent bidirectional MR study using IBD GWAS summary statistics conducted in the UK Biobank (UKB) did not reveal any possible association in either direction between depression and IBD [17]. The limited number of IBD cases in the UKB likely resulted in an underpowered sample size to detect any association. Therefore, in the present study, we aimed to investigate the potential bidirectional causal relationship between depression and IBD (including both UC and CD) by implementing a bidirectional MR study design using the most up-to-date and larger GWAS on IBD and depression.

Methods

We leveraged a bidirectional two-sample MR to assess the causal association between depression and IBD. The schematic overview of the study design and data sources are detailed in **Figure 1**. All data are publicly available GWAS summary statistics, and therefore, no additional ethical approval or informed consent was required. GWAS summary statistics were searched to extract lead-
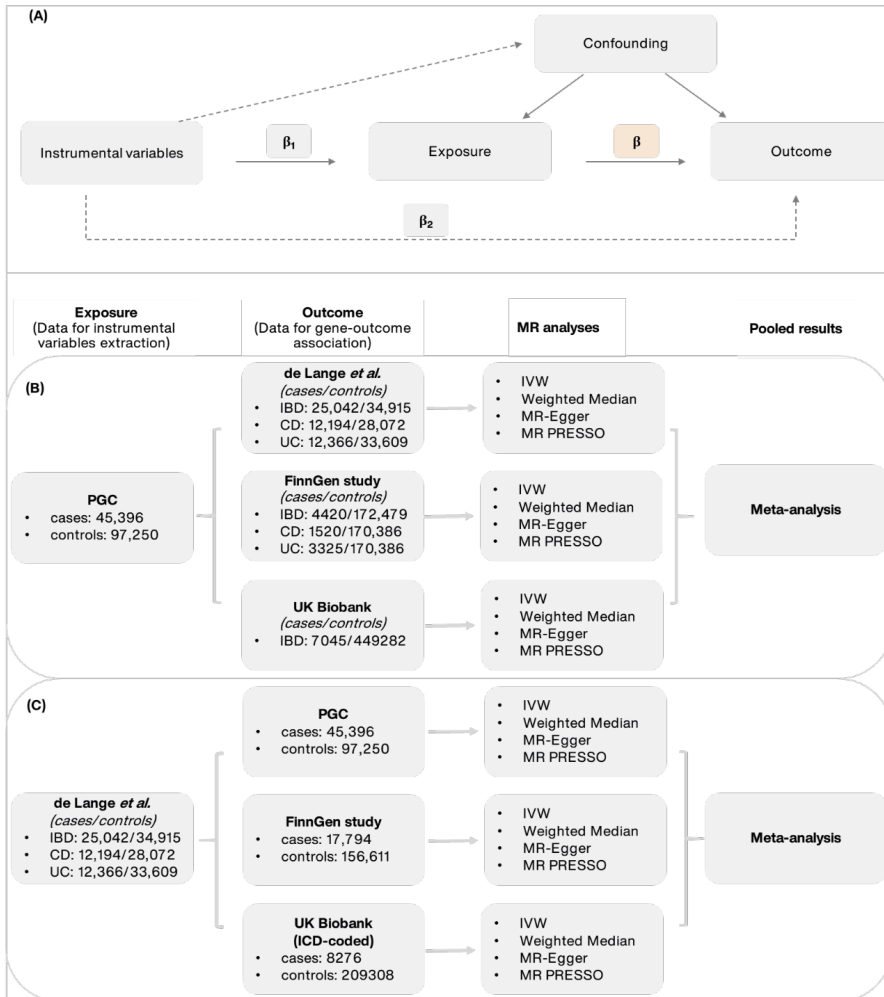
Figure 1 Schematic overview of the study design.

(A) Mendelian Randomization (MR) illustration. There are three principal assumptions in MR design, namely the genetic instrumental variables should 1) be associated with exposure; 2) be associated with outcome only via exposure; 3) not be associated with any measured or unmeasured confounding factors. β_1 and β_2 denote to the gene-exposure and gene-outcome association respectively; β represents the causal association between exposure and outcome, which can be estimated by β_1/β_2. (B) MR study from depression to IBD: independent SNPs for depression were identified as instrumental variables, whereas summary statistics of gene-IBD associations were retrieved separately from the GWAS performed by de Lange et al., FinnGen, and UK Biobank (for IBD only). MR analyses were conducted per outcome database and were subsequently meta-analyzed to generate pooled estimates. (C) MR study from IBD to depression: SNPs for IBD were identified as instrumental variables, whereas summary statistics of gene-depression associations were retrieved separately from PGC, FinnGen study, and UK Biobank (GWAS conducted with ICD-coded depression cases only). MR analyses were performed per outcome database and were subsequently meta-analyzed. IBD: inflammatory bowel disease; PGC: Psychiatric Genomics Consortium. CD: Crohn's Disease; UC: Ulcerative colitis; IVW: Inverse-variance weighted.

ing SNPs (single nucleotide polymorphisms) associated with depression or IBD as genetic instrumental variables. Gene-outcome associations were separately retrieved from three databases for both depression and IBD: 1) large-scale GWAS meta-analysis efforts; 2) FinnGen (data freeze 4); and 3) the UKB. In the FinnGen study, GWAS were performed across a broad spectrum of phenotypes including depression and IBD; the analyses were adjusted for age, sex, principal components, and genotype batch effect. Phenotype definitions were based on the International Statistical Classification of Diseases and Related Health Problems (ICD) coded hospital discharge or death. Detailed information regarding participants, genotype platforms, and statistical analysis protocols are available at the FinnGen website (*https://www.finngen.fi/en/*).

Selection and description of the sources of the genetic instrumental variables

Depression is an exceedingly heterogeneous condition with many different measures for identification in the previous studies, ranging from subjective self-reported symptoms or help-seeking to clinical diagnosis. Genetic variants that were identified from GWAS by using less precisely defined phenotypes including help-seeking, might not be specific to depression per se [18]. Consequently, this may reduce statistical power, particularly when depression is used as an exposure. In addition, diagnosis-based depression, such as depression defined by the International Statistical Classification of Diseases and Related Health Problems (ICD) or Diagnostic and Statistical Manual of Mental Disorders (DSM) clinical guideline, showed statistically significant relation to IBD in the UKB, but not depression defined by other means such as help-seeking behavior or self-reported symptoms [17].

Two GWAS meta-analyses on depression were conducted recently by Howard et al. and Wray et al., respectively [19,20]. Despite the larger total sample size of 807,553 individuals in the study conducted by Howard et al., self-reported help-seeking behavior for mental health difficulties accounted for more than 70% of cases. We therefore used the GWAS summary statistics from Wray et al, which included participants from 29 cohorts in Psychiatric Genomics Consortium (PGC) and 6 additional cohorts including UKB and 23andMe. In each cohort, data were processed following PGC "ricopili" pipeline or using comparable procedures when applicable. Due to the data restriction, in the present study, we used the available GWAS summary statistics data that did not include UKB or 23andMe, consisting of 45,396 cases and 97,250 controls. Individuals who contributed to the data all met the international consensus criteria for a lifetime diagnosis of depression established using structured diagnostic instruments from assessments by trained interviewers, clinician-administered checklists, or medical record review. Since no SNP-depression association reached the genome-wide association threshold (p-value $< 5×10^{-8}$), a suggestive significant level (p-value $< 1×10^{-6}$) was used to extract instrumental variables, as has been adopted in the previous study in disentangling bidirectional relationships between physical activity and depression in MR analyses [21]. Linkage disequilibrium between all SNPs was based on the European 1000 Genome Project reference panel. Independent SNPs were selected by linkage disequilibrium clumping ($r^2 > 0.001$) retaining the one with the smallest P-value.

Summary statistics for IBD was obtained from the latest meta-analysis GWAS (de Lange et al.), which contained a total sample size of 59,957 participants of predominantly European ancestry (cases / controls for IBD: 25,042 / 34,915; UC: 12,366 / 33,609; CD: 12,194 / 28,072) [22]. IBD was diagnosed by accepted radiological, endoscopic, and histopathological evaluation and all included cases fulfilled clinical criteria for IBD. In every single cohort, GWAS was performed using an additive model conditioning on the first ten principal components. We selected SNPs that were significantly associated with IBD (p-value< 5×10^{-8}) and further pruned all SNPs by linkage disequilibrium clumping ($r^2 > 0.001$). To avoid any potential pleiotropic instruments, we excluded 43 SNPs that were associated with more than one phenotype, and 176 SNPs (IBD: 74; UC: 42, CD: 60) remained for the MR analyses.

SNP-outcome data sources

Gene-outcome associations for depression were obtained from three separate databases: 1) the PGC data from Wray et al. as described previously; 2) FinnGen; and 3) the UKB. Depression in FinnGen was defined as depressive episode and recurrent depressive disorder by ICD criteria consisting of 17,794 cases and 156,611 controls. In the UKB, since multiple definitions for depressive disorders were available [18], to minimize the heterogeneity of depression definition, we only considered the ICD-coded (ICD-10 primary and secondary codes for depression) depression as the outcome. GWAS analyses on ICD-coded depression in the UKB included 8,276 cases and 209,308 controls (with a prevalence of 3.80%), adjusted covariates of age, sex, genotyping array, and 8 principal components [23].

Gene-outcome associations for IBD were drawn from three separate databases: 1) latest meta-analysis GWAS by de Lange et al.; 2) FinnGen; and 3) UKB (only IBD data were available). The de Lange et al. study has been described in detail in the previous section. In the FinnGen study, CD and UC cases were defined using their corresponding ICD codes, and IBD is a term comprised of CD, UC, and colitis of indeterminate. In total, the number of cases and controls were 4420 / 172,479 for IBD, 3325 / 170,386 for UC and 1520 / 170,386 for CD, respectively. In the UKB, IBD was composed of CD (UKB data field: 131627) and UC (UKB data field: 131629), which were determined from either a death register, self-reported, hospital admission, or primary care record for the corresponding disease, resulting in a total of 7045 cases and 449,282 controls [17]; GWAS analyses were adjusted for sex, age, and 20 principal components; GWAS on UC and CD were not available in the UKB.

Instrumental strength and power calculation

F-statistics, calculated as $(beta/se)^2$, was computed to quantify the strength of instruments, and a value of above 10 was considered sufficient. The proportions of variance explained by exposures (R^2) were calculated using the Mangrove package in R [24], setting the prevalence of 3.4% for depression and 0.3%, 0.14%, 0.2% respectively for IBD, CD, and UC [1,9]. Statistical power was calculated via the tool for binary outcomes in MR studies (*https://github.com/kn3in/mRnd*) [25], where the alpha level was set to 0.05.

Mendelian Randomization (MR)

Before analysis, we first harmonized exposure and outcome data to make alignments on effect alleles to the forward strand, if it is specified or could be inferred based on the allele frequency. Palindromic genetic variants were discarded for further MR analyses [26]. We used inverse-variance weighted (IVW) meta-analysis as the main analysis to combine the SNP-specific estimates calculated using Wald ratios, assuming no directional pleiotropic effect of each SNP [27]. In addition, we performed several sensitivity analyses to assess pleiotropy and potential genetic outliers. A Weighted-Median estimator can provide a reliable estimate if more than 50% of the instrumental variables are valid [28]. In MR-Egger regression, the slope provides a causal estimate of an exposure on an outcome if the instrument strength independent of direct effect assumption is met; additionally, the intercept of a MR-Egger regression deviates from zero indicates pleiotropy. MR-Egger is statistically less efficient (i.e. with wider confidence intervals) but provides a causal estimate (i.e., the regression slope) that is corrected for directional horizontal pleiotropy [29]. Furthermore, MR Pleiotropy RESidual Sum and Outlier (MR PRESSO) test is applied to detect significant outliers and correct for horizontal pleiotropic effect through outlier removal. The global test evaluates whether horizontal pleiotropy among all instruments is present [30]. We further examined the heterogeneity among all SNPs within each database using Cochran's Q test statistic and generated scatter plots of SNP-exposure association versus SNP-outcome association to visualize MR results. Leave-one-out analysis was performed by excluding each SNP at a time sequentially and an IVW method was performed on the remaining SNPs to assess the potential influence of a particular variant on the estimates.

Additionally, when SNPs are selected from very large GWAS, these instrumental variables may have effects on the other downstream traits of the trait of interest, such as effects directly on the outcome. Thus, determining whether a SNP is primarily associated with the exposure of interest or with the outcome could be challenging. If those variants that show stronger associations to outcomes than to exposures are used in the MR analyses, the result may (erroneously) imply that the exposure and outcome are causally related due to reverse causation. Therefore, we applied MR Steiger filtering to test the direction of causality for each instrumental variable on exposure and outcome. Steiger filtering assumes that a valid instrumental variable should explain more variation in the exposure than in the outcome; if an instrumental variable meets the criterion, the direction of this instrument is "TRUE", while if it does not, it is "FALSE" [31]. After removing those SNPs with "FALSE" direction, we repeated all MR analyses.

MR results were expressed as odds ratios (OR) with the corresponding 95% confidence intervals (CIs) on outcome risk of corresponding unit changes in an exposure. Given that all exposure variables in the current analyses are binary, the final effect estimates were interpreted as OR on the risk of outcome per log-OR change in an exposure. From each direction, MR analyses were firstly performed in each outcome database separately and the individual estimates were subsequently combined using fixed-effects meta-analysis.

All the analyses were performed using R (v3.6.3) statistical software (The R Foundation for Statistical Computing, Vienna, Austria). MR analyses were per-

formed using the R-based package "TwoSampleMR" and meta-analysis were conducted using the "meta" package.

Results

Depression to IBD
Nineteen independent SNPs were identified as genetic instrumental variables for depression, which explained 0.41% of the total variation; the median (minimum, maximum) F statistics were 26.2 (23.9, 37.4) (**Supplementary Table 1**). Detailed information about the 19 genetic variants is listed in **Supplementary Table 2**.

In the meta-analyses of estimates from IVW, pooled ORs for IBD, CD, and UC of genetically predicted per log-OR increase in depression were 1.20 (95% confidence interval CI: 1.05, 1.36), 1.29 (95% CI: 1.07, 1.56), 1.22 (95% CI: 1.01, 1.47), respectively (**Figure 2**).

For each database, in sensitivity analyses (**Supplementary Table 3**), estimates obtained from Weighted-Median did not differ substantially compared to those from IVW (**Supplementary Figure 1**). No pleiotropic effect was detected by MR-Egger intercept. Potential outliers were identified by MR PRESSO for IBD in the de Lange et al. database and UKB, as well as for UC in the de Lange et al. database, which resulted in potential pleiotropy assessed by the global test. However, results remained similar after outlier correction. Heterogeneity of each instrument estimation evaluated by Cochran's Q test statistics was detected, but only in those databases with outliers indicated by MR PRESSO. Individual SNP effects and combined effects from each MR method per outcome database are visualized in scatter plots (**Figure 3**). Leave-one-out plots suggested that the associations were unlikely driven by certain extreme SNPs (**Supplementary Figure 2**). Steiger filtering detected 4 SNPs that were with a "FALSE" direction, which was likely to primarily be associated with IBD rather than depression (**Supplementary Table 4**). These included rs2060886 in the intronic region of TCF4 in all the three IBD-related traits from de Lange data; rs1936365 in the intronic region of PGBD1 in all the three IBD-related traits in FinnGen while only CD in UKB; rs1950829 in LRFN5 for CD in FinnGen; and rs1491473 in the intronic region of LINC00861 for UC in FinnGen. After removing those four SNPs, analyses were repeated for all methods, and estimates were minimally influenced by applying Steiger filtering (**Supplementary Table 5**).

IBD to Depression
A total of 70 independent genetic variants reached a genome-wide significant level with IBD, which explained about 3.4% of the total variation, whereas 55 and 41 genetic instruments were retained for CD and UC, which accounted for 4.9% and 3.0% of the total variation. The summary and detailed information about the variants for each exposure are presented in **Supplementary Table 1 and 6**.

Overall, in the primary analyses using IVW, we found no association between genetically determined IBD (including both CD and UC) and depression in any individual outcome database, except for a minimal effect of IBD on depression

in FinnGen. Combined ORs of IBD, CD, and UC on depression was 1.01 (95% CI: 1.00, 1.03), 1.00 (95% CI: 0.99, 1.01), 1.02 (95% CI: 0.99, 1.05), respectively (**Figure 4**).

In each outcome database, sensitivity analyses using the Weighted-Median estimator were consistently comparable to the estimates from IVW (**Supplementary Figure 3**). While the MR-Egger intercept test suggested no evidence of pleiotropy, MR-PRESSO global test indicated the presence of horizontal pleiotropic outliers from CD to depression in the FinnGen study (p = 0.04) and UC to depression in PGC (p = 0.03); however, after correcting for outlier by MR PRESSO, results remained similar with those estimates from IVW (**Supplementary Table 7**). No notable heterogeneity was detected by Cochran's Q statistics across single instrument effects within each database. Scatter plots (**Figure 5**) present the individual SNP effect and combined effect from each method per outcome dataset. After removing one SNP at a time, the results remained consistent in the leave-one-out analyses (**Supplementary Figure 4**). Steiger filtering indicated that all genetic instrumental variables used for IBD explained more variance in IBD than in depression in any database (**Supplementary Table 6**).

Discussion

In the present study, we evaluated the bidirectional associations between depression and IBD using MR. We found evidence that genetic liability to depression was associated with an increased risk of IBD, CD, and UC, while genetic liability to IBD, or any subtype was not associated with depression.

Previous observational studies have suggested that depression might be a risk factor for IBD. Specifically, in The Health Improvement Network cohort with 403,665 incident IBD cases, depression was associated with a higher risk of both incident CD and UC [13]. Similarly, a study using the data from 152,461 women aged 29 to 72 years in the Nurses' Health Study also found that depressive symptoms were associated with an increased risk of CD, but not UC [14]. On the contrary, depression alone, in the absence of prior GI symptoms, was not associated with subsequent development of IBD in 10,829 UC cases, 4531 CD cases, and 15,360 controls [15]. Up to now, the only MR study conducted to disentangle the bidirectional relationship between depression and IBD used IBD GWAS summary statistics (7045 cases and 449,282 controls) obtained from UKB, which revealed no association in either direction [17]. The small number of IBD cases in the UKB may account for this null association. The same study identified causal associations between depression and other gastrointestinal disorders for which a much larger number of cases were used in the GWAS. This corroborated that the absence of an association between depression and IBD was likely due to a lack of statistical power. Consistent with the previous study, we did not find an association between depression and IBD using only UKB data. However, when the estimates were combined with those from the other two databases in the meta-analysis, we did observe a causal effect of depression on IBD (**Figure 2**).

The biological connection between depression and IBD has not been fully elucidated yet. Currently, intestinal inflammation and its concomitant microbial dysbiosis have been implicated in the etiology of IBD [32,33], which may explain

Depression	No. of SNPs	IBD	Odds Ratio [95% CI]	Weight
IBD				
deLange	13		1.19 [0.94; 1.50]	31.3%
FinnGen	19		1.30 [1.05; 1.61]	37.3%
UK Biobank	18		1.09 [0.86; 1.38]	31.3%
Combined Effect			**1.20 [1.05; 1.36]**	**100.0%**
CD				
deLange	13		1.27 [1.02; 1.58]	74.9%
FinnGen	19		1.36 [0.94; 1.98]	25.1%
Combined Effect			**1.29 [1.07; 1.56]**	**100.0%**
UC				
deLange	13		1.12 [0.85; 1.47]	46.3%
FinnGen	19		1.31 [1.02; 1.69]	53.7%
Combined Effect			**1.22 [1.01; 1.47]**	**100.0%**

0.5   1   2

OR (95% CI)
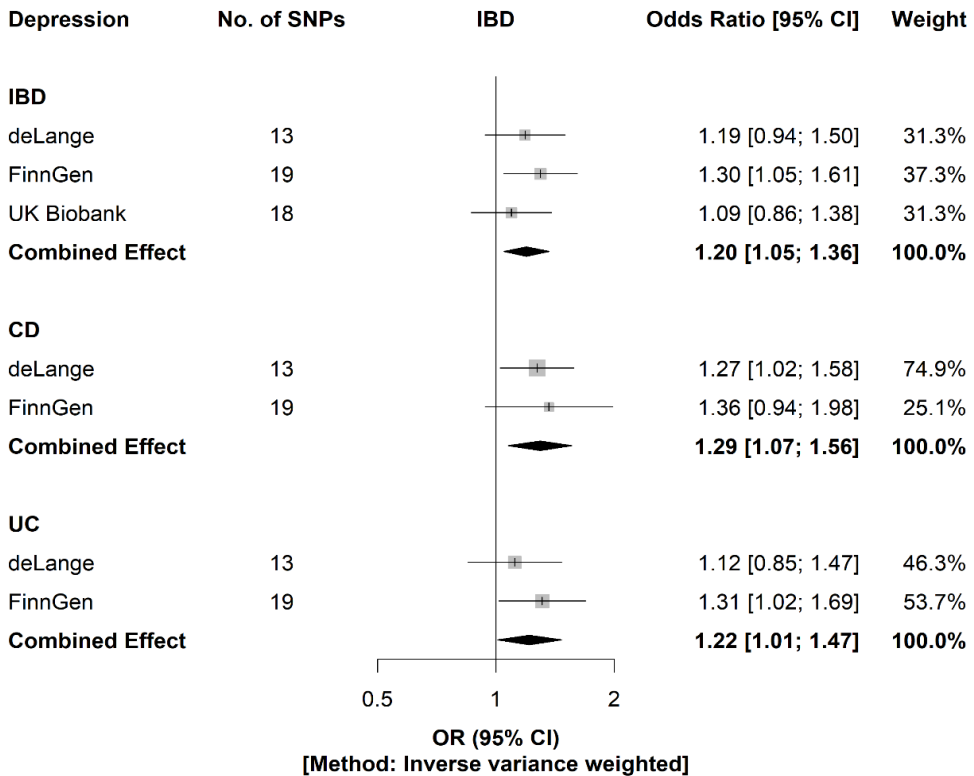**[Method: Inverse variance weighted]**

Figure 2 Association of Depression and IBD in MR analyses

Estimated ORs represent the effect of per log-OR increase in depression on IBD, obtained from an Inverse-variance weighted analysis, per outcome database separately and combined over the three databases for IBD and two databases (data are not available in the UK Biobank) for CD and UC using fixed-effect meta-analyses.

IBD: Inflammatory Bowel Disease; CD: Crohn's Disease; UC: Ulcerative Colitis; OR: Odds Ratio; CI: Confidence Interval.

the causal association between depression and IBD that we observed in the current study. Depression contributes to intestinal inflammation by modulating the psycho-neuro-endocrine-immune system in the brain-gut axis. Numerous complex mechanistic pathways underpinning brain-gut (microbiome) interaction have been extensively explored previously [34-37]. In brief, depression could activate the hypothalamic-pituitary-adrenal axis, which subsequently leads to down-regulation of the corticotropin-releasing factor system, thereby accelerating chronic inflammation and stimulating the immune response. In addition, the autonomic nervous system is also functionally involved in stress-mediated alterations. The activation of the sympathetic nervous system plays a proinflammatory role that could invoke an enhanced secretion of catecholamines. The combination of increased sympathetic outflow and adrenomedullary activity could then stimulate mast cells and macrophages to release inflammatory effectors of cytokines. In parallel, the vagus nerve has an anti-inflammatory function and could be inhibited
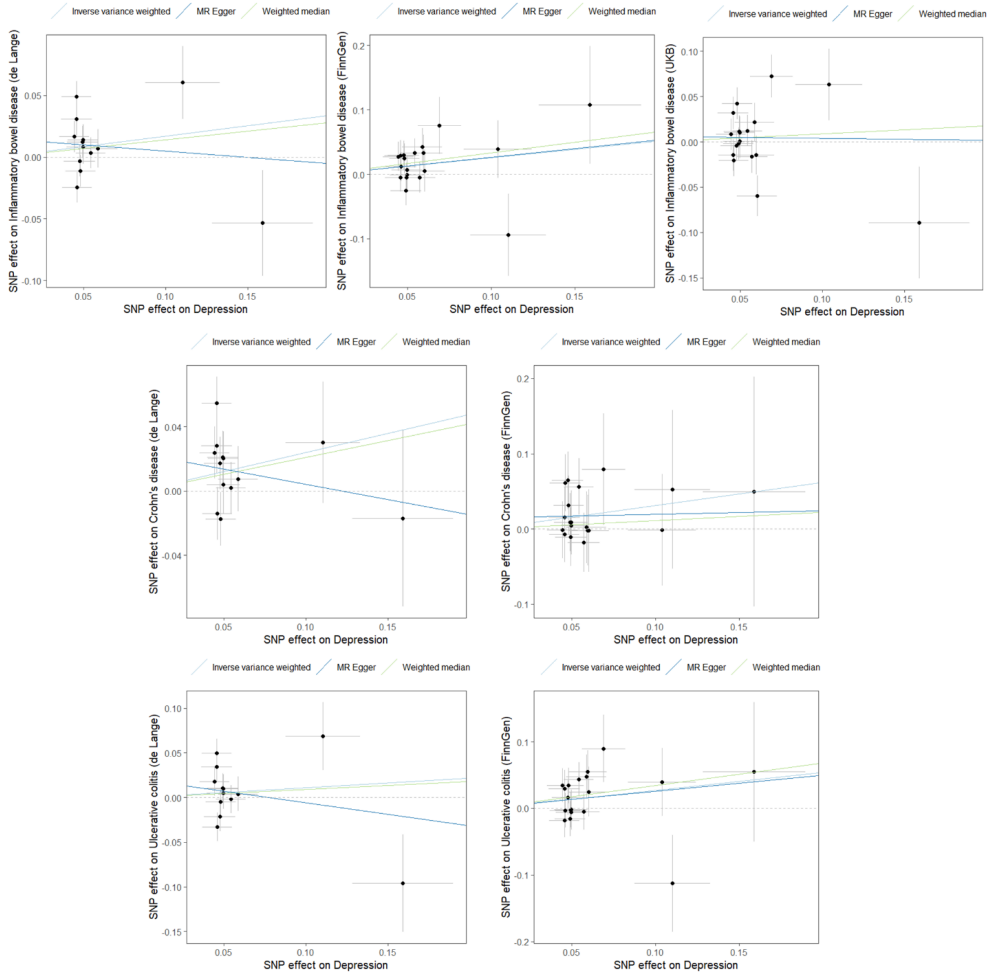
Figure 3 Scatter plot of MR analyses from Depression to Inflammatory Bowel Disease in each database

X axes represent the genetic instruments-depression associations and Y axes represent genetic instruments-IBD associations from different outcome databases. Black dots denote to the genetic instruments included in the primary MR analyses. red: Inverse-variance weighted; blue: Weighted-Median estimator; green: MR Egger. Due to the same estimate from the Inverse-variance weighted and Weighted-Median estimator methods in some analyses, those figures only contain two lines. However, the color of the overlapped lines is darker than the Weighted-Median estimator.
IBD: Inflammatory Bowel Disease; CD: Crohn's Disease; UC: Ulcerative Colitis.

by proinflammatory cytokines (such as IL-1, IL-6, and TNF-α) released from the intestinal mucosa via a decreased efferent outflow. These inflammatory profile changes are related to regional gut motility, luminal secretion, visceral hypersensitivity, and elevated intestinal permeability. As a result of the compromised epithelial barrier, intestinal permeability promotes microbiome translocation and further activates the immune response. All these changes brought about
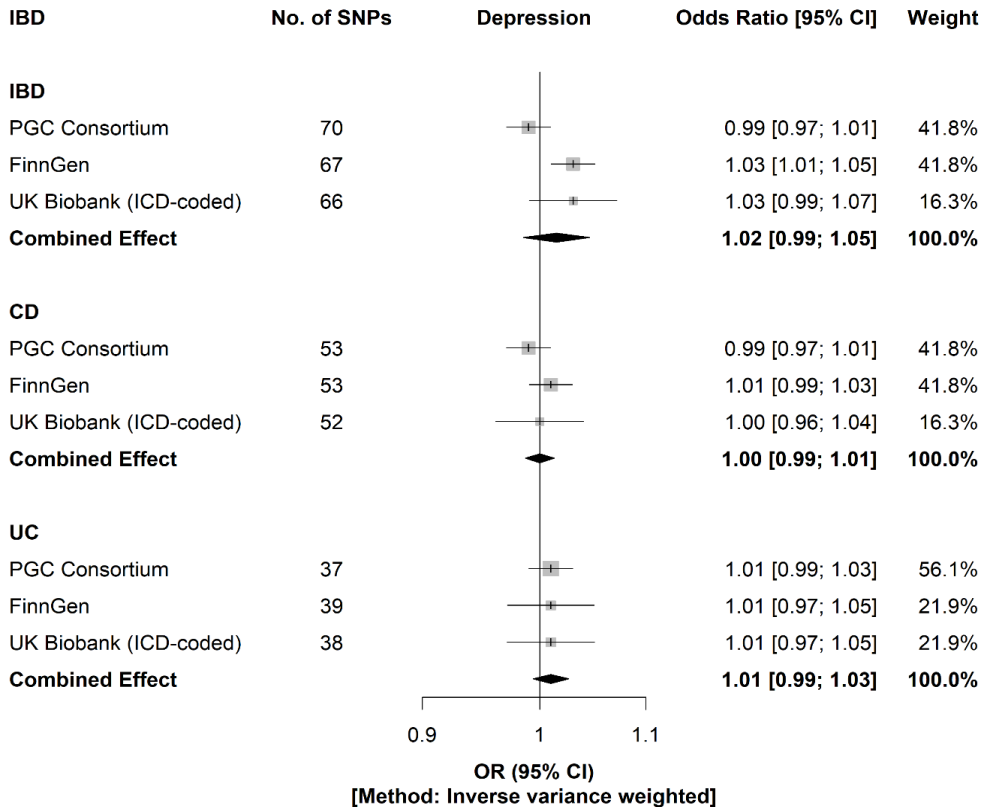
IBD	No. of SNPs	Depression	Odds Ratio [95% CI]	Weight
IBD				
PGC Consortium	70		0.99 [0.97; 1.01]	41.8%
FinnGen	67		1.03 [1.01; 1.05]	41.8%
UK Biobank (ICD-coded)	66		1.03 [0.99; 1.07]	16.3%
Combined Effect			**1.02 [0.99; 1.05]**	**100.0%**
CD				
PGC Consortium	53		0.99 [0.97; 1.01]	41.8%
FinnGen	53		1.01 [0.99; 1.03]	41.8%
UK Biobank (ICD-coded)	52		1.00 [0.96; 1.04]	16.3%
Combined Effect			**1.00 [0.99; 1.01]**	**100.0%**
UC				
PGC Consortium	37		1.01 [0.99; 1.03]	56.1%
FinnGen	39		1.01 [0.97; 1.05]	21.9%
UK Biobank (ICD-coded)	38		1.01 [0.97; 1.05]	21.9%
Combined Effect			**1.01 [0.99; 1.03]**	**100.0%**

0.9   1   1.1

OR (95% CI)
[Method: Inverse variance weighted]

Figure 4 Association of IBD and depression in MR analyses

Estimated ORs represents the effect of per log-OR increase IBD on depression, obtained from an Inverse-variance weighted analysis, per outcome database separately and combined over the three databases using fixed-effect meta-analyses.

IBD: Inflammatory Bowel Disease; CD: Crohn's Disease; UC: Ulcerative Colitis; PGC: Psychiatric Genomics Consortium. OR: Odds Ratio; CI: Confidence Interval.

by depression may disrupt the homeostasis of the gastrointestinal tract and consequently lead to IBD.

Strength and limitations

There are two main strengths worth noting in the current study. First, we extracted gene-outcome associations from three independent sources of GWAS summary statistics, which included large sample sizes, particularly cases, for each exposure, with 534,635 participants (71,466 cases) for depression, 693,183 (36,507 cases) for IBD, 212,172 (13,714 cases) for CD, and 219,686 (15,691 cases) for UC. The effect estimations from various data sources all pointed to the same direction, and meta-analyses further strengthened the estimation. Second, we used clinical diagnosis-based depression as the exposure. A previous study indicated that effect size estimations between depression and IBD varied according to the definition used for depression, and self-reported depression tended to attenuate
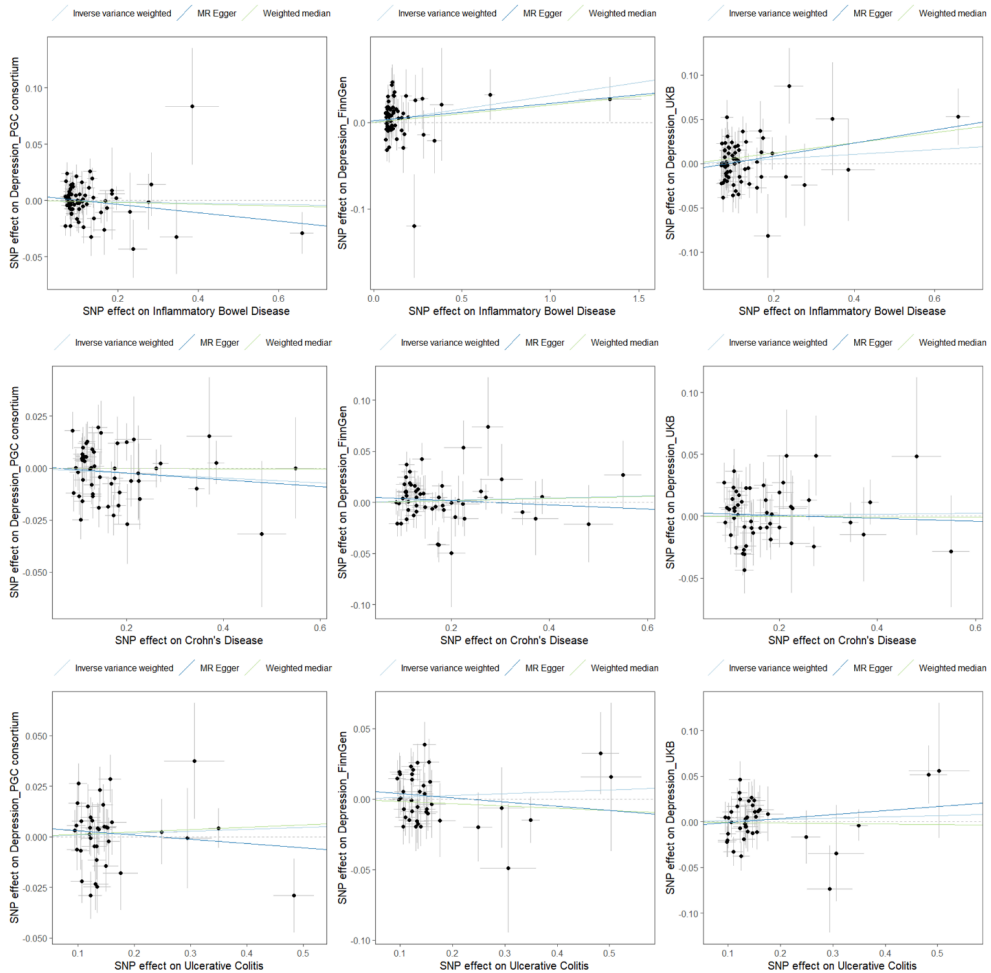
Figure 5 Scatter plot of MR analyses from Inflammatory Bowel Disease to Depression in each database

The figure caption is the same as in Figure 3.

association estimates [17]. Interestingly, 19 SNPs used in the analyses explained 0.41% of the total variation in clinically diagnosed depression, which was comparable to the total variations explained by 102 genetic variants (approximately 0.5%) identified by the GWAS using broad depression [19,38].

Some limitations should be acknowledged. Firstly, all data involved in the analyses were derived primarily from individuals of European ancestry, limiting the generalizability of our findings to other ethnic groups. Secondly, the statistical power may still be insufficient to detect an effect of depression on IBD in a single dataset, as indicated in the FinnGen study (**Supplementary Figure 5**). Thirdly, although we found no causal effect of IBD on depression, we only considered the dichotomous IBD diagnosis, i.e. the incidence, rather than the IBD disease

course. Previous studies showed that both the prevalence and incidence of depression are significantly higher among patients with active IBD than patients with inactive diseases [39,40]. While we acknowledge IBD has a dynamic natural course characterized by alternating periods of remission and relapse, and its episodic flares occur randomly and are largely unpredictable, dissecting the genetic makeup that is associated with IBD activity is still challenging. Due to the lack of GWAS on IBD disease activity to date, we were unable to investigate the effect of IBD activity on depression using the MR approach. However, the genetic determinants of IBD susceptibility may be distinct from mechanisms underlying disease activity. It might not be the disease per se, but rather the disease activity that is pivotal to depression. Therefore, we could not rule out the possibility of disease activity, but not incident IBD, as a causal risk factor for depression. In addition, depression is very heterogeneous and exhibits diverse symptoms. Patients with the same major depressive disorder diagnosis according to DSM-V may experience very different symptom profiles. However, depression is used as a binary variable in the GWAS, without taking symptomology into account. Therefore, it remains possible that IBD would have an effect on specific depression dimensions. We must also keep in mind that, IBD being rare, it can only be causal for a small fraction of patients with depression. Fourthly, when MR analyses were conducted from the direction of IBD to depression, there may be some overlap when depression data from UKB were used as outcome. The IBD GWAS included individuals from UK10K (4,686 IBD cases and 3,781 controls), which may have contributed to the UKB depression data. This accounted for 14% of the total population in the IBD GWAS data and represented the maximum overlap between the two datasets. Nevertheless, with such a low proportion of sample overlap, the bias is expected to be neglectable [41]. Additionally, the results remained consistent if using only PGC and FinnGen data, with an OR (95% CI) of 1.24. (1.06, 1.45). Finally, subgroup analyses are not feasible due to the use of summary statistics, particularly by sex, where previous studies pinpointed a higher prevalence of depressive symptoms in women than men [8], and specifically an increased risk for CD, but not UC, among women [14].

Clinical implications

To date, no consensus has been reached about the role of depression in the counseling and management of IBD. In the American College of Gastroenterology (ACG) clinical guideline for preventive health care, recommendations of screening for depression in patients with IBD is conditional due to a low level of evidence [42]. Meanwhile, in the latest update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE), the absence of health-related anxiety and depression is removed as a therapeutic goal for treat-to-target strategies in IBD given the low (37%) endorsement [43]. Similarly, psychological therapies in IBD patients are rarely recommended to patients with IBD as adjunctive therapy to alleviate symptoms and improve quality of life given the very low quality of evidence by the British Society of Gastroenterology consensus guidelines [44].

The observed causal effect of depression on IBD identified in our study should raise the awareness of the involvement of tackling depression to achieve a better clinical outcome in IBD. On one hand, clinicians should raise the awareness of an index of suspicion about IBD in patients with depression; particularly, gastro-

intestinal symptoms in patients with depression should be spotted, as they are often considered as complaints of depression, such as chronic diarrhea, etc., but not as an early clinical manifestation of IBD and lead to "diagnostic over-shadowing" [45]. On the other hand, the implementation of depression screening and the involvement of appropriate treatment for depression into the routine practice of IBD patients might help optimize the management of IBD and confer possibilities leading to better clinical outcomes for IBD patients.

Conclusion

Our findings corroborated a causal association between depression and IBD, which may have an influence on the clinical decision in the management of depression in patients with IBD. Though our results did not support a causal effect of IBD on depression, further investigations are needed to clarify the effect of IBD activity on depression (with different symptomology).

8

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Supplementary materials

Supplementary Table 1 Summary of Genetic Instruments identified for MR Analyses

Supplementary Table 2 Instrumental variables for depression (significant level of p < 1e-06)

Supplementary Table 3 Effect estimates of the associations of genetic instrumental variables for depression and risk of inflammatory bowel disease

Supplementary Table 4 Steiger filtering results from depression to IBD

Supplementary Table 5 Mendelian randomization results following Steiger filtering of genetic instrumental variables for depression and risk of inflammatory bowel disease

Supplementary Table 6 Instrumental variables for inflammatory bowel disease (significant level of p < 5e-08)

Supplementary Table 7 Effect estimates of the associations of genetic instrumental variables for inflammatory bowel disease and risk of depression

Supplementary Figure 1 Estimated ORs of the effect of per log-OR of depression on IBD, obtained from Weighted-median Estimator per outcome database separately and combined over the three databases for IBD and two databases (data are not available in the UK Biobank) for CD and UC using fixed-effect meta-analyses

Supplementary Figure 2 Leave-one-out plot of MR analyses from Depression to Inflammatory Bowel Disease in each database

Supplementary Figure 3 Estimated ORs for the effect of the effect of log-OR change of IBD on depression, obtained from Weighted-median estimator per outcome database separately and combined over the three databases using fixed-effect meta-analyses.

Supplementary Figure 4 Leave-one-out plot of MR analyses from Inflammatory Bowel Disease to Depression in each database

Supplementary Figure 5 Statistical power of MR analysis; (A) Depression to IBD. Outcome data were from de Lange et al. (black), FinnGen (red), and UK Biobank (blue) for IBD only; (B) IBD to depression. Outcome data were from PGC (black), FinnGen (red), and UK Biobank (blue). Dashed grey line indicates statistical power of 0.8. Depression in the UK Biobank are from GWAS conducted with ICD-coded cases only.

The Supplementary materials for this article can be found online at:

https://academic.oup.com/ecco-jcc/article-lookup/doi/10.1093/ecco-jcc/jjab191

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