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Irritable Bowel Syndrome and Migraine: Evidence from Mendelian Randomization Analysis in the UK Biobank

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Abstract

Background: Irritable Bowel Syndrome (IBS) and Migraine are two diseases featuring high prevalence. Previous studies have suggested a relationship between IBS and migraine, although the causal association remains unclear. The authors sought to explore the causal association between IBS and migraine, and to show the importance of migraine prevention in IBS patients.

Methods: This study conducted a Mendelian randomization analysis to explore the association of IBS with migraine. Genetic association with migraine was acquired from the UK Biobank (UKB) genetic databases (cases: 1,072; controls: 360,122). The authors performed estimation using Inverse Variance Weighting (IVW), along with Maximum Likelihood, MR-RAPS, MR-Egger and Weighted Median for sensitivity analysis. Considering possible bias, they also conducted polymorphism, heterogeneity, and directional analysis.

Results: The IVW estimation genetically predicted the causal association between IBS and migraine (OR=1.09, 95%CI 1.01 to 1.17, *p*=0.03). Neither statistical horizontal pleiotropy (MR Egger *p*=0.42; MR-PRESSO *p*=0.78) nor possible heterogeneity (IVW Q = 26.15, *p*=0.80) was found. Reverse causation was also not detected (*p* steiger<0.01).

Conclusion: Mendelian randomization analysis supported a potential causal association between IBS and migraine, providing enlightenment for disease prevention and control.

Keywords: Migraine; Irritable bowel syndrome; Mendelian randomization; Epidemiology; UK Biobank

Article highlights:

- Mendelian randomization analysis is a gene-based approach that can estimate the causal relationship with published Genome-Wide Association Studies summary data.
- Some observational studies have pointed out the association between Irritable Bowel Syndrome and Migraine (IBS).
- This Mendelian randomization firstly established the causal association between the two diseases from a genetic perspective.
- Patients and clinicians should attach importance to migraine-related symptoms in IBS.
- Treatments or palliative measures concerning IBS and migraine should be developed accordingly.

1. Introduction

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal disorder with a pooled prevalence of 11.2% worldwide [1]. Its typical symptoms include abdominal pain and abnormal bowel motility without organic lesion [2]. A prevalence cohort study suggested that IBS patients had a higher risk for experiencing other functional somatic syndromes such as migraine, fibromyalgia, and depression [3]. Migraine is also a chronic disorder, affecting approximately 6% of men and 15 to 17% of women [4, 5]. It features recurrent headache disorder which may be accompanied by various autonomic, affective, cognitive, and sensory symptoms [6]. For young women, migraine is also ranked top in the cause of disability-adjusted life years (DALYs) [7].

IBS and migraine negatively impact the quality of life in a large proportion of the population [1,8-9] and may exert a burden on patients and the health care system. As an important public health problem [10], migraine is even related to a high level of disability [9]. Thus, figuring out the relationship between the two concomitant diseases may pose a vital significance to public health.

Previous studies have observed the association of IBS and migraine [11]. Among IBS patients, approximately 25-50% had migraine or headache compared with only 4-19% among the controls [12]. The Odds Ratio of IBS patients co-existing a migraine or headache reached 2.63 (95%CI: 2.27 to 3.06) [13]. However, a majority of the previous studies examining the relationship between IBS and migraine have been observational, leaving the connection between IBS and migraine to be considered tenuous. Although evidence has indicated that symptoms of the central nervous system (CNS) may occur following the gastrointestinal dysfunction [14], we could not exclude the reverse causality and existing confounding factors.

Mendelian randomization (MR) analysis is a gene-based approach that can estimate the causal relationship between an exposure (such as IBS) and an outcome (for example, migraine). Genetic variants are used as the instrumental variables (IVs) of exposure [15]. The individual genetic variants were pooled for population effect estimation, similar to a random-effects meta-analysis. On the one hand, gametes are formed according to the Mendelian genetic law that parental alleles are randomly assigned to offspring. Therefore, MR can avoid confounding factors such as environmental exposure, socioeconomic status and

behavior. On the other hand, since the genetic variation comes from the parents and remains unchanged after birth, the association between genetic variation and outcome is chronological, overcoming the problem of reverse causation.

We performed Mendelian randomization analysis to infer the causal relationship with no need for individual-level data in the present study. With the available Genome-Wide Association Studies (GWAS) summary data from the UK Biobank cohort and IBS-related single nucleotide polymorphisms (SNPs), we were able to conduct the causal inference testing evaluating the association between irritable bowel syndrome and migraine.

2. Methods

2.1 Mendelian randomization and assumptions

This analysis proceeds from summary data without individual-level resources. We conducted Mendelian randomization analysis using genetic variants as IVs. The SNPs can be considered as valid IVs for the IBS if they satisfied the three core assumptions of MR: (i) genetic variants as IVs are strongly associated with exposures (Correlation hypothesis); (ii) genetic variants are independent of the known or unknown confounders (Independence hypothesis); (iii) genetic variants only associate with outcomes through the exposure (Exclusion hypothesis). (More information is available in Figure 1)

2.2 Data resources

The SNPs associated with IBS were selected from the published open GWAS database up to date, including 1,121 cases and 360,073 population controls [16]. The SNPs associated with migraine were obtained from the UK Biobank (UKB) genetic data (1,072 cases and 360,122 controls). The UKB project is known as a prospective cohort study, recruiting participants ranging from 40 to 69 years old in the United Kingdom [17]. It collected individual genotypes as well as health-related information. UK Biobank has already got ethical approval (REC reference for UK Biobank is 11/NW/0382) (More information about UK Biobank is available on: https://biobank.ndph.ox.ac.uk/showcase/exinfo.cgi?src=UnderstandingUKB). To improve accuracy, our study chose patients with the main diagnosis in hospital as cases according to the International Classification of Diseases version-10 (recorded as “Diagnoses - main ICD10” in UKB), while other individuals as controls.

2.3 SNP selection

Aiming to meet the three core assumptions, standards were set for valid SNPs. We used SNPs at a linkage disequilibrium (LD) threshold $R^2 < 0.001$, with a certain mutation frequency (Minor Allele Frequency (MAF)≥5%) and predicted the exposure significantly at the genetic level ($p<5\times10\text{-}8$). Since no eligible SNPs were identified, we used a higher cut-off ($p<1\times10\text{-}5$) to obtain SNPs related to the exposure. For harmonization, the palindromic SNPs were also excluded to avoid that the direction of the positive and negative chains cannot be determined for the same alleles on both strands.

2.4 Main statistical analysis

We performed Inverse Variance Weighted (IVW) as the main analysis, which is a weighted estimation using the individual ratio from meta-analysis literature. IVW estimate is consistent and efficient if the pleiotropic effects of IVs are zero. Additionally, with a larger sample size, the estimation will be closer to the true value [18].

2.5 Sensitivity analyses

Except for IVW, we also conduct other methods to test the conformity of test results. Maximum Likelihood (ML) is a traditional means with low standard error. It estimates the probability distribution parameters by maximizing the likelihood function. Although it may be biased with limited sample sizes, the bias is so small that can be ignored biologically [19]. Robust analysis method refers to the means that can indicate the causal relationship even under a weak assumption due to pleiotropy or linkage disequilibrium. To provide a more credible estimate, we used robust analyses like MR-Egger, Weighted Median and MR-PRESSO. Originating from assessing the publication bias [20], the MR-Egger analytical approach estimates the effect of gene-outcome on gene-exposure using weighted linear regression. It allows the inclusion of the pleiotropic genetic variants if conform with the InSIDE hypothesis that the pleiotropic effect is independent of the association between IVs and exposure. MR-Egger can assess the pleiotropic effects using the intercept [21]. However, MR-Egger analysis is prone to be affected by outliers, resulting in relatively imprecise and low power [22]. The Weighted Median (WM) can improve the accuracy of the simple median estimator by including the weight of the ordered ratio. WM can

provide a valid and consistent estimate when more than 50% of the involved information comes from valid IVs [18]. MR-PRESSO is a variant of IVW, excluding genetic variants whose causal estimation is different from other IVs significantly. The MR-PRESSO method can provide valid results if the horizontal pleiotropy exists in less than 50% of the IVs [23]. The Robust Adjusted Profile Score (MR-RAPS) features high statistical power, which is robust when systematic pleiotropy exists and unbiases with weak instruments. Considering the measurement error in the association of SNPs and exposure, MR-RAPS can reduce horizontal pleiotropy [24].

To test the conformity of each SNP, we also performed a Leave-one-out analysis by conducting the Mendelian randomization leaving each genetic variant one by one. If the causal relationship is still significant statistically after excluding the none-specific SNP, it provides more credible evidence for the association. Pleiotropy means a genetic variation is associated with multiple risk factors, including horizontal pleiotropy and vertical pleiotropy. Since vertical pleiotropy neither violates the core hypothesis of Mendelian randomization nor causes any bias, we only consider horizontal pleiotropy, which may bias the independence hypothesis and the exclusivity hypothesis. We used MR-Egger intercept and MR-PRESSO analysis to verify and correct for horizontal pleiotropy. As for heterogeneity, Cochran Q statistics were calculated to examine the heterogeneity among different genetic variations. We also drew the funnel plot for visual examination. MR-Steiger can test the confidence of the causal direction of IBS and migraine using summary data [25]. It was also conducted to identify a cause

from a downstream effect. The results of both MR analyses and sensitivity estimates were considered statistically significant at p< 0.05, with two-tailed testing. All estimations were conducted in R Version 4.0.3 with R package "TwoSampleMR" and "MRPRESSO". We used summary data publicly available which needed no ethical approval.

3. Results

According to the selection criteria, we first chose 42 SNPs that were significantly related to IBS ($p<1\times10\text{-}5$) independently ($R^2 < 0.001$). F-statistics ranged from 19.54 to 28.67, indicating the weak instrumental bias can be ignored statistically (Details in Supplementary Tables S1). Given the high comorbidity of IBS and migraine, possible confounders were also considered, such as SNPs related to fibromyalgia, chronic fatigue syndrome and depression. We then excluded 8 SNPs for being palindromic (rs10910535, rs12186442, rs138082719, rs17677265, rs201655992, rs56135098, rs79333841, rs9367125). Finally, a total of 34 SNPs were selected (Supplementary Tables S1 and Figures S1).

The main analysis IVW suggested a causal association of IBS and migraine (OR=1.09, 95%CI 1.01 to 1.17, *p*=0.03). For sensitivity analysis, we also conducted MR-Egger, ML, WM, and MR-RAPS to confirm the conformity of test results. (Figure 2 and Figure 3) The directions of the five models were consistent, ML and MR-RAPS were significant, while MR-Egger and WM were not significant statistically. As for the SNP conformity, we performed a Leave-one-out analysis

and generated a forest map. The forest map showed a stable result (Supplementary Figures S3).

Horizontal polymorphism and heterogeneity analyses were performed as well for sensitivity quality control. MR-Egger intercept and MR-PRESSO analysis identified no horizontal pleiotropy statistically (MR Egger p=0.42; MR-PRESSO p=0.78). Since there was no statistical horizontal pleiotropy, we used a fixed-effect model. To test the heterogeneity, we calculated the Cochran Q statistics, found no heterogeneity statistically between IV estimates (IVW Q = 26.15, p=0.80). The funnel plot also indicated the unlikely heterogeneity among SNPs (Supplementary Figures S2). These results suggested the powerful estimate and weak bias in the MR analysis. Besides, MR-Steiger indicated IBS as the cause while migraine as the downstream consequence (p steiger<0.01, pMR<0.05).

4. Discussion

To our knowledge, this is the first study illustrating the causal association between IBS and migraine from a genetic perspective.

The causal association between IBS and migraine supported the possible common pathogenesis or associated pathways between the two diseases. Previous researchers have proposed several hypotheses which may explain the mechanisms. The leaky gut hypothesis indicated that altered permeability and impaired barrier of the gastrointestinal tract (GIT) may lead to leakage. Bacterial by-products may go across the intestinal mucosa and enter the bloodstream,

thus causing migraine [26, 27]. In addition, the Gut-brain axis is an acknowledged theory, pointing out the bidirectional relationship between the gastrointestinal system and central nervous system (CNS). The CNS affects the gastrointestinal tract by the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic and parasympathetic branches. In turn, the gastrointestinal system is also indicated to influence the CNS, including emotional behavior, pain-modulation systems, and brain neurotransmitter systems [28, 29]. The causal relationship of IBS and migraine may also relate to the GIT-CNS pathways mediated by the Gut-brain axis. Additionally, IBS patients was reported to have significantly difference in fecal microbiota from the controls [30]. The intestinal microbiota is believed to affect neurotransmitter levels, such as serotonin (5-HT) [31], which may activate its respective receptors on spinal afferents. Thus, it may cause dysfunctions in the nervous system [32]. In practice, a therapy regulating serotonin receptors was reported working for both IBS and migraine [33].

Among the five MR methods, IVW, ML and MR-RAPS revealed the causal relationship of IBS and migraine while MR-Egger and WM showed no significance statistically. Considering the pleiotropic effects of the same confounder, MR-Egger has greater error rates than IVW [22]. Since the low statistical power, we stress more importance to the consistency of the direction of the slope rather than the significance [34]. Given that the five methods were in the same direction, we conclude that IBS has potential causal association with migraine.

Clarifying the causal relationship and exploring the mechanism between the two diseases are conducive to improve prevention and control. On the one hand, IBS patients should attach importance to the prevention of migraine. Though frustrating, IBS does not pose a serious threat to a targeted organ, like cancers [35]. A nationwide cohort study in Denmark even suggested that IBS patients had less risk of colorectal cancers with a standardized incidence ratio (SIR) of 0.67 (95% CI: 0.52 to 0.85) [36]. In comparison, migraine may account for more serious consequences and burdens. The severe pain may lead to a higher risk of suicide attempts [37], cardiovascular disease events [38] and stroke [39]. Thus, it is important to prevent migraine among IBS patients from the first beginning. Preventive measures like medication, relaxation training, thermal biofeedback and cognitive behavior therapy are recommended when symptoms occur, such as more than four headaches or at least eight days of headache a month, debilitating headaches, etc [40]. On the other hand, given the connection between these two diseases, treatments or palliative approaches should be developed accordingly. For example, melatonin is a natural hormone in the body maintaining the biological clock. It was also found to reduce the pain of IBS and migraine [41]. A randomized controlled trial showed improvement of symptoms among IBS patients, suggesting a peripheral anti-5-HT-like effect of melatonin [42]. A clinical trial also reported melatonin can decrease the frequency, duration and intensity of a migraine attack [43]. Moreover, neurokinin-1 (NK1) receptor antagonists also play a role in relieving the symptoms of IBS and migraine, as the receptor was found both in CNS and gastrointestinal tract [44]. Further

investigations into the pathogenesis of these diseases to move toward the development of novel therapeutic pharmaceuticals would be valuable for both IBS and migraine.

Admittedly, though we testified the causal association between IBS and migraine, evidence from both preclinical medicine and clinical practice are still needed to explore molecular mechanism and therapies. Despite the Mendelian randomization analysis was less likely to be affected by confounders compared with other observational designs, limitations still exist. First, we used a higher cut-off (p<1e-5) to obtain more SNPs, which may include weak instrumental variables, reducing the effectiveness. However, the F-statistics we calculated varied from 19.54 to 28.67, suggesting the effect of weak IVs was not substantial. Second, MR analysis may underestimate biological effects while overestimating genetic associations, known as the “Beavis Effect”. Concerning the potential association between SNPs and confounding factors, we excluded the weak SNPs using F-statistic and check several reported confounders, excluding related SNPs. Third, it is hard to satisfy the “Exclusion hypothesis” entirely, limiting the association of SNPs to the outcome only through exposure. To detect the bias caused by horizontal pleiotropy, we utilized the MR-Egger intercept and MR-PRESSO analysis, find a minimal pleiotropy effect. Also, we incorporated more IVs, avoiding specific SNPs playing a decisive role. Fourth, due to the data accessibility, we only analyzed the elderly among European populations. Since migraine has a peak prevalence in young people and the association may differ in other populations, further analysis concerning other groups of people would

provide more evidence. Fifth, genetic variants can only explain part of the variation in the risk factor of interest. Further studies with larger sample sizes are needed to evaluate the causal association for more adequate statistical power.

5. Conclusion

In this study, we found that migraine may be a downstream consequence of IBS using Mendelian randomization analysis. These findings serve to remind clinical practitioners of the importance of migraine prevention when patients are diagnosed with IBS.

List of abbreviations

CNS: Central nervous system
GIT: Gastrointestinal tract
GWAS: Genome-Wide Association Studies
HPA: Hypothalamic-pituitary-adrenal
IBS: Irritable bowel syndrome
IVs: Instrumental variables
IVW: Inverse Variance Weighting
LD: Linkage disequilibrium
MAF: Minor Allele Frequency
ML: Maximum Likelihood
MR: Mendelian randomization
NK1: Neurokinin-1
RAPS: Robust Adjusted Profile Score
SIR: Standardized incidence ratio

SNPs: Single nucleotide polymorphisms

UKB: UK Biobank

WM: Weighted Median

Availability of data and materials

All data used for the analysis were derived from published GWAS. Migraine data are available in https://gwas.mrcieu.ac.uk/datasets/ukb-d-G43/

IBS data are available in https://gwas.mrcieu.ac.uk/datasets/ukb-d-K58/

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Declaration of interests

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or conflict with the subject matter or materials discussed in this manuscript apart from those disclosed.

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Author contributions

J Chen, X Chen and Y Xie contributed data acquisition, analysis and writing. Y Sun helped analyze the data. X Wang and T Hesketh contributed to project

design and manuscript revision. All authors approved the final version of the manuscript.

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Figure legends:

Figure 1. Diagram of the Mendelian Randomization Assumptions Supporting the Mendelian Randomization Analysis of the Causal Effect of Irritable Bowel Syndrome on Migraine.

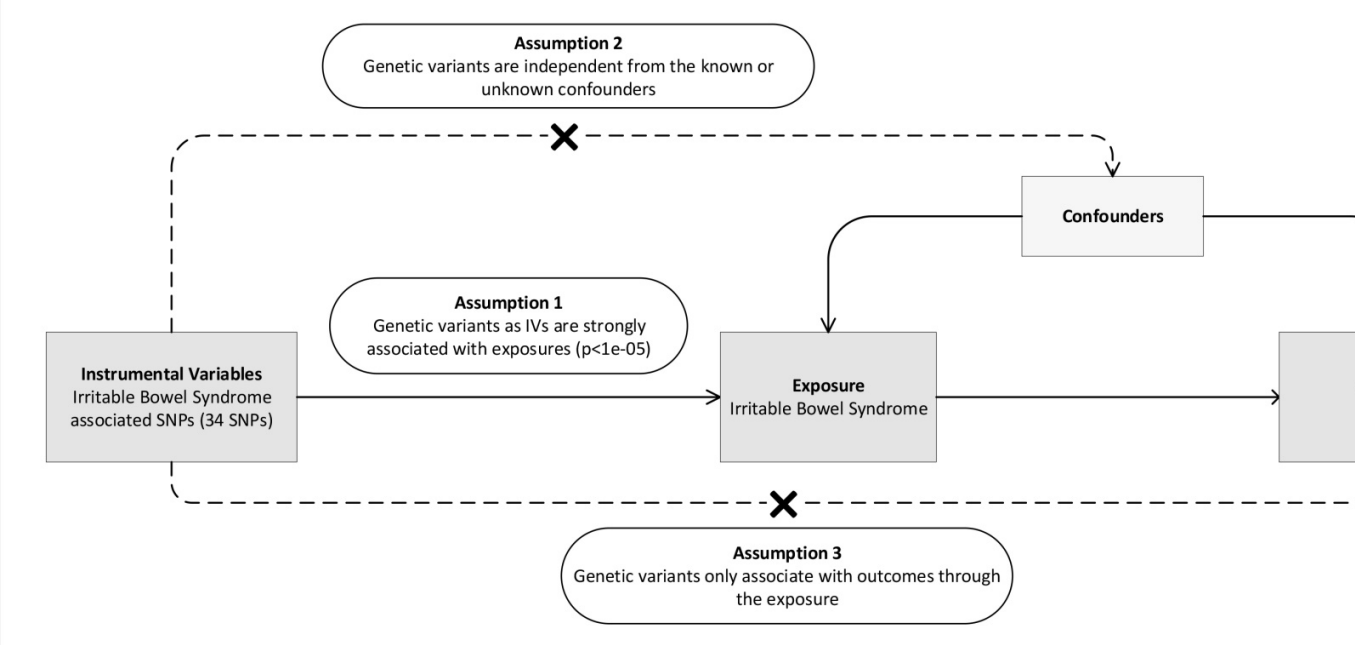

Figure 2. MR Estimates from Each Method of Assessing the Causal Effects of Irritable Bowel Syndrome on Migraine.

Method	nSNP	Odds Ratio(95%CI)	p-value
Inverse variance weighted	34	1.09 (1.01-1.17)	0.03
MR Egger	34	1.14 (1.00-1.30)	0.07
Maximum likelihood	34	1.09 (1.01-1.18)	0.02
Weighted median	34	1.10 (0.99-1.22)	0.08
Robust adjusted profile score (RAPS)	34	1.09 (1.01-1.19)	0.03

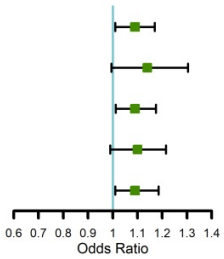

Figure 3. Scatter plots of the genetic causal associations with Irritable Bowel Syndrome against Migraine using different MR methods.