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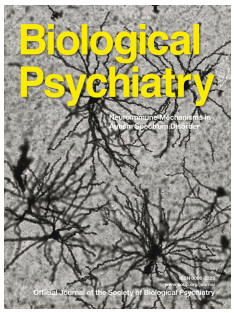

Irritable Bowel Syndrome is Associated with Brain Health by Neuroimaging, Behavioral, Biochemical, and Genetic Analyses

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Irritable Bowel Syndrome is Associated with Brain Health by Neuroimaging, Behavioral, Biochemical, and Genetic Analyses

Running title: Associations between IBS and brain health

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Abstract

BACKGROUND: Irritable bowel syndrome (IBS) interacts with psychopathology in a complex way, yet little is known about the underlying brain, biochemical and genetic mechanisms.

METHODS: To clarify the phenotypic and genetic associations between IBS and brain health, we performed a comprehensive retrospective cohort study on a large population. Our study included 171,104 participants from the UK Biobank who underwent a thorough assessment of the IBS syndrome, with the majority also providing neuroimaging, behavioral, biochemical, and genetic information. Multistage linked analyses were conducted, including phenome-wide association analysis, polygenic risk score calculation, and two-sample Mendelian Randomization (MR) analysis.

RESULTS: The phenome-wide association analysis showed that IBS is linked to brain health problems, including anxiety and depression, and poor cognitive performance. Significantly lower brain volumes associated with more severe IBS were found in key areas related to emotional regulation and higher-order cognition, including the medial orbitofrontal cortex/ventromedial prefrontal cortex, anterior insula, anterior and mid-cingulate cortex, dorsolateral prefrontal cortex, and hippocampus. Higher triglycerides, lower high-intensity lipoprotein, and lower platelets were also related ($p < 1\times10^{-10}$) to more severe IBS. Finally, MR analyses demonstrated potential causal relationships between IBS and brain health, and indicated the possible mediating effects of dislipidemia and inflammation.

CONCLUSIONS: This study, for the first time, provides a comprehensive understanding of the relationship between IBS and brain health phenotypes, integrating perspectives from neuroimaging, behavioral performance, biochemical factors and genetics, which is of great significance for clinical application of brain health impairments in patients with IBS.

Keywords: Brain Health; Neuroimaging; Irritable Bowel Syndrome; Depression; Mendelian Randomization; UK Biobank

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain, bloating and bowel dysfunction, affecting around 1 in 10 people worldwide (1, 2). A growing body of evidence suggests that IBS is associated with brain health. Individuals with IBS have an increased risk of developing mental health conditions, including anxiety disorders, depressive disorders, and somatic symptom disorders (3-5). Primarily because of the lack of diverse data, almost all prior studies on the association between IBS and brain health have focused on one (or a few) specific phenotypes. The overall picture of the phenotypic links between IBS and brain health in the general population remains unclear.

There has been increasing recognition of the potential role of dysfunction of the brain-gut axis (8-10) in the pathogenesis of IBS and its impact on brain health. Brain regions in fronto-limbic and sensorimotor networks showed abnormal functional connectivity in IBS individuals with depressive symptoms (14). Treatments targeting the disordered brain-gut axis in IBS had potentially positive effects, suggesting that maintaining brain health might benefit IBS patients (15). All of these previous studies suggest a potential role for brain structure and function in the pathogenesis of IBS and brain health. However, neuroimaging studies examining the association between the brain and IBS are limited and the sample size is relatively small (usually dozens of participants) (11, 13, 14). Moreover, contemporary researches primarily emphasize brain regions at a broader level, rather than implementing voxel-level analysis which offers higher resolution, enabling the precise identification of specific brain regions linked to IBS. These may limit the ability to capture robust and stable IBS-related brain alterations that may also correlate with brain health.

Emerging evidence suggests an association between IBS and metabolic dysregulation as well as immune system activation, implying that biochemical factors in the peripheral nervous system might play a role in IBS pathophysiology because the brain-gut axis involves a complex network of interactions (16-18). Despite the increasing evidence, previous studies predominantly concentrated on microbiome metabolism, with limited exploration of individual metabolism using blood indicators (12, 19). Meanwhile, to gain a deeper understanding of the inherent biological link between IBS and brain health, a recent study conducted from a genetic perspective has identified shared genes between IBS and mood disorders (20). However, the genetic predisposition between IBS and brain health as well as with biochemical factors remains unknown. Taking all the above together, the neurobiological substrates that link IBS and brain health are complex and multifaceted, encompassing phenotypes, endophenotypes (neuroimaging), biochemical factors, and genetic information. There is a pressing need for an integrated framework that encompasses diverse data modalities, enabling a comprehensive understanding of the neurobiological underpinnings, which will enhance our understanding of the links between IBS and brain health.

In this study, to determine the association between IBS and brain health and correlated biological substrates, we enrolled 171,104 participants from the UK Biobank with various measurements such as brain imaging, behavioral assessments, biochemical markers, and genetic information. Our primary aim was to investigate the association between IBS and brain health, examining their phenotypic and genetic connections. We set three main objectives for this study. First, to explore the relationship between IBS symptoms and brain health measures. Second, to estimate the relationships of IBS symptoms with brain structure and biochemical markers, which may provide great insight into brain-gut interactions in IBS. Finally, to clarify the potential genetic association between IBS symptoms, brain health, and biochemical indicators using Mendelian Randomization (MR) analysis. We hypothesized that IBS would be phenotypically and genetically associated with a broad range

of brain health-related phenotypes and biochemical indicators, such as depression, anxiety, brain structure, dislipidemia and inflammation markers.

METHODS AND MATERIALS

Study Population

We utilized data from the United Kingdom Biobank (UKB) cohort, which included 500,000 participants aged between 38 and 72 years at recruitment. The UKB cohort was approved by the North West Multi-centre Research Ethics Committee (https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us/ethics) and provided oversight for this study. Written informed consent was obtained from all participants. The data utilized in the analyses contained demographic characteristics, assessments of IBS, behavioral assessments, diet assessments, neuroimaging scans, and biochemical markers (Table S1, Supplement). The IBS symptoms were measured by the Digestive Health Questionnaire (DHQ, 2017-2018), which defined the IBS symptom severity score (IBS-SSS) as the total score of five items (Table S2). Three items had a prompt question with a "No" or "Yes" answer, for example "Do you currently (in the last 3 months) suffer from abdominal pain?". Answer "No" would produce a zero score, and "Yes" would result in the participants being given a 0-100 scale to report their severity, such as "How severe is your abdominal pain? (0 meaning no pain and 100 meaning severe pain)". The other two items related to bowel habit satisfaction and life interference, using 0-100 score directly to measure the severity. The IBS-SSS was calculated for 171,104 participants, and a high score indicated severe symptoms. In the current study, we focused on the IBS symptoms rather than the IBS diagnosis (n = 44,993, Supplement) since the timing of collection for IBS-SSS coincides with the data collection time for most brain health phenotypes, ensuring a reasonable association between them as time interval bias was avoided as much as possible. Nevertheless, we further included IBS diagnostic data for validation in our case-control analysis.

Statistical analysis

Association of IBS with behavioural, neuroimaging and biochemical markers

Firstly, we performed a phenome-wide association analysis on the IBS-SSS and the polygenic risk score of IBS-SSS (IBS-SSS PRS). The R package PHESANT (23), an automated rule-based tool, was employed for processing. Phenotypes were categorized into four data formats, including continuous, ordered categorical, unordered categorical and binary. The analysis encompassed phenotypes from various UKB categories following a previous study (24), including population characteristics, health-related outcomes, assessment centers and online follow-up. For specificity and clarity, we selected a finer classification, resulting in 20 categories following the UKB showcase framework (Supplement). Then, we focused the analysis on the associations between IBS symptom and brain health measures (mental health and cognitive function). To further explore the potential influential factors between IBS and brain health, we analyzed the associations of IBS symptoms with diet intake, brain structure (gray matter volume at the voxel level), and biochemical markers, which may play an important role in brain-gut interactions with IBS. Finally, we used clinical IBS diagnosis to replicate identified associations. The following variables were used as covariates: age, sex, body mass index, Townsend deprivation index, educational

qualifications, smoking status, and drinking status. In addition, total intracranial volume and scanning site were added as covariates in the neuroimaging analysis, and genetic principal components were added as covariates in the PRS analysis. FDR correction was performed in the brain structure association analysis, and Bonferroni correction was used in other analyses. A corrected *p* value of less than 0.05 was set as the level for significance.

Functional annotation, neurotransmitter and transcriptomic analyses related to IBS-associated brain map

We performed a voxel-level association analysis between IBS and brain structure, to generate an IBS-associated brain map for subsequent analyses. Firstly, we used Neurosynth (25) to decode functions of brain regions exhibiting associations with IBS. Secondly, we used JuSpace (26) to identify neurotransmitter maps correlated with the IBS-associated brain map (Table S3). Finally, we used a partial least squares (PLS) regression to explore the weighted linear combinations of expression patterns for 15,408 genes from the Allen Human Brain Atlas (AHBA) database (27, 28), and ranked genes according to their associations with the IBS-associated brain map. Then we used Metascape (29) to identify the Gene Ontology (GO) terms that were enriched at the top and bottom of the ranked gene list. A permutation test (n = 10,000) was performed to test the significance (FDR correction, $p < 0.05$), using BrainSMASH (30) to generate surrogate maps. Other details are provided in the Supplement.

Genetic associations between IBS and categories of interest by MR analysis

To further investigate the relationship between IBS and different indicator systems, we investigated potential phenotypic and genetic associations between IBS and typic brain health markers representative of each indicator system. Firstly, phenotypic associations involved 10 categories, including IBS-SSS, IBS-SSS PRS, depression, cognitive function, neuroimaging, immunometabolism, lipoprotein particle concentrations, triglycerides, inflammation and platelets. For categories that had multiple phenotypes, we selected the phenotypes significantly associated with IBS in each category (Table S4), then adjusted directions according to their associations with IBS, and finally normalized and averaged the values to represent the categorical score.

Secondly, genetic associations were investigated using two-sample MR analyses, including inverse-variance weighting (IVW), weighted median (WM) and MR-Egger, implemented in the R package TwoSampleMR (https://mrcieu.github.io/TwoSampleMR/). MR analyses were performed using the same categories as phenotypic associations, excluding IBS-SSS PRS. The genetic instruments were selected at $p < 1\times10^{-6}$ and then we removed correlated SNPs ($r^2 > 0.1$). We performed the heterogeneity test using the IVW (obtain the Q value) and MR-Egger (obtain the Q' value) models. Then Q_R value ($Q_R = Q' / Q$) was calculated to select models, where Q_R close to 1 represents IVW and MR-Egger fitting the model equally well, while Q_R much less than 1 represents the MR-Egger method being better. The pleiotropy test was performed using MR-Egger.

RESULTS

Demographic information

We included 493,865 participants aged 47-84 years (54% female) at the time of completing the DHQ. 171,104 participants aged 47-81 years (57% female) had an IBS-SSS of 82.9±89.9 (mean±s.d., Figure S1), in which females had higher scores than males (Figure S2). Meanwhile, 44,993 participants (34,365 participants with IBS-

SSS) met the diagnostic criteria and served as IBS cases, and there were 301,070 control cases. Compared with controls, IBS cases showed significantly higher IBS-SSS (t = 231, $p < 1\times10^{-300}$, Figure S3). A total of 492,004 participants had an assessment of depressive symptoms (Table S5), and 145,808 participants finished a detailed mental health questionnaire (Table S6). Data on cognitive function were collected through a touchscreen questionnaire (Table S7). We also included T1-weighted structural MRI of 39,578 participants in the analysis. The demographic characteristics of participants with IBS-SSS are shown in Table S8. Figure 1 shows the research approaches of the study.

Phenome-wide association analyses between IBS and brain health

Through the phenome-wide analysis, IBS symptoms showed associations with a wide range of phenotypes across 20 categories (Figure 2A; Table S9). Notably, the brain health-related phenotypes of psychosocial factors and mental health showed the highest t values (Figure 2B). In addition, the polygenic risk score of IBS symptoms also showed associations with psychosocial factors (Figures S4 and S5, Table S10). Specifically, the severity of the IBS symptoms had significant correlations with each domain of mental health (Figure 2C; Table S11), such as depressive symptom (r = 0.229, $p < 1\times10^{-300}$), anxiety symptom (r = 0.235, $p < 1\times10^{-300}$) and wellbeing (r = -0.244, $p < 1\times10^{-300}$). These indicated that more severe IBS symptoms correlated with poorer brain health status, which was further replicated in the IBS cases vs. controls (Table S12).

Meanwhile, lower cognitive function, another important reflector of brain health status, also showed significant associations with the severity of IBS symptoms (Figure 2C; Table S11), such as fluid intelligence (r = -0.060, $p = 1.28\times10^{-50}$), reaction time (r = 0.017, $p = 8.69\times10^{-13}$) and pair matching (r = 0.025, $p = 9.61\times10^{-25}$). These associations of IBS with mental health and cognitive function did not differ by sex (Table S11). Moreover, almost every mental health assessment significantly correlated with IBS-SSS PRS (Tables S13, S14). In addition, we identified the intake of 17 categories of food and drink significantly associated with IBS symptoms (Table S15). Intake of fresh fruit had the highest negative correlation with IBS (r = -0.032, $p = 7.67\times10^{-38}$), while salt-added foods had the highest positive correlation (r = 0.033, $p = 1.63\times10^{-42}$). The analysis also showed less fresh fruit intake among IBS cases (Table S16).

Association of IBS with brain structure and biochemical markers

Next, we estimated cortical and subcortical brain volumes related to IBS utilizing voxel-based morphometry (VBM) analysis. Most significant regional brain volumes showed negative correlations with IBS symptoms (Figure 3; Figure S6 and Table S17). The volumes of brain regions such as the medial orbitofrontal cortex / ventromedial prefrontal cortex (vmPFC) extending into the anterior cingulate cortex, dorsolateral prefrontal cortex, anterior and mid-cingulate cortex, anterior insula, hippocampus, parahippocampal cortex, thalamus, precentral gyrus and supplementary motor area, were negatively associated with the IBS symptoms. Positive associations were found for a few subcortical regions, including the globus pallidus, caudate, and putamen. In a case-control analysis, significantly lower volume was found for the mid-orbitofrontal cortex / vmPFC, parahippocampal gyrus, mid-cingulate cortex, and the triangular part of the inferior frontal gyrus (Figure S7).

Also, we observed a total of 38 blood markers and 83 metabolic markers significantly correlated with IBS (Bonferroni correction, $p < 0.05$, Figure 3C). Triglycerides (r = 0.038, $p = 1.58\times10^{-53}$), high-density lipoprotein (HDL) cholesterol (r = -0.037, $p = 4.27\times10^{-46}$), red blood cell count (r = -0.026, $p = 1.76\times10^{-25}$) and neutrophil

count (r = 0.025, $p = 1.57\times10^{-24}$) were among the most significant in the blood markers. For metabolite markers, cholesterol in very large HDL (r = -0.047, $p = 4.83\times10^{-21}$) and cholesteryl esters in very large HDL (r = -0.046, $p = 8.93\times10^{-21}$) were among the most significant. Consistently, these biomarkers also showed significant differences between IBS cases and controls. For example, the triglyceride level in IBS cases was higher than that in controls (t = 13.94, $p = 4.49\times10^{-44}$). Overall, most markers in the category "immunometabolism" showed high correlations with IBS, and each metabolite in the category "triglycerides" showed a positive correlation with IBS (r values from 0.011 to 0.045). More results are provided in Tables S18 and S19. In addition, we examined the correlations between brain volumes in regions associated with IBS and biochemical markers. Most of the brain regions were associated with at least one biochemical marker (Table S20). For example, the volume of the medial orbital gyrus was associated with the categories "white blood cell", "red blood cell" and "immunometabolism".

Functional annotation, neurotransmitter architecture and transcriptomic profile related to the IBS-associated brain map

Brain volumes in the IBS-associated regions (Figure 3A) presented positive associations with cognitive performance such as language skills, memory processing, and negative associations with emotions such as impulsivity and affective disorders (Figure 4A; Table S21). Among neurotransmitters (26), serotonin had the highest association (r = -0.226, p = 0.001) with the brain regions associated with IBS (Figure 4B; Table S22). Then we performed the spatial association analyses between the IBS-associated brain volumetric map and gene expression profiles (27, 28) using PLS regression (Table S23). The gene expression map of the first PLS (PLS1) component showed significant spatial association with the IBS-associated brain map (r = 0.353, p = 0.005, Figure 4D). Finally, we ranked the genes according to the corrected weight in the PLS regression, to perform GO enrichment analysis. Based on the normalized PLS1 weights, there were 1,574 genes in the PLS1+ gene set (z > 4) and 968 genes in the PLS1- gene set (z < -4). Figure 4E and 4F show the top 20 cellular components, such as postsynapse and presynapse, and 20 biological processes, such as forebrain development and neural crest cell development, which were significantly enriched at the bottom of the gene list (FDR correction, p < 0.05, Tables S24 and S25).

Genetic association between IBS and brain health, brain structure and biochemical markers revealed by MR

As shown in Figure 5A, IBS symptoms had significant associations with each category, including IBS-SSS PRS (r = 0.058, $p = 3.16\times10^{-19}$), depression symptoms (r = 0.229, $p < 1\times10^{-300}$), cognitive function (r = 0.051, $p = 5.42\times10^{-37}$), neuroimaging (r = -0.048, $p = 9.81\times10^{-17}$), immunometabolism (r = 0.047, $p = 7.45\times10^{-70}$), lipoprotein particle concentrations (r = 0.049, $p = 3.35\times10^{-23}$), triglycerides (r = 0.036, $p = 3.43\times10^{-13}$), inflammation (r = 0.041, p = 1.05×10-16) and platelet (r = 0.016, $p = 5.97\times10^{-11}$), among which depression presented the largest effect size. In addition, higher associations were obtained between any two of immunometabolism, lipoprotein particle concentrations, triglycerides and inflammation.

Then we performed two-sample MR analyses in each pair of 9 categories (excluding IBS-SSS PRS) (Figure 5B, C; Table S26). A bidirectional association was observed between depressive symptoms and IBS (IVW: depression to IBS, β = 0.30, $p = 9.4\times10^{-9}$; IBS to depression, β = 0.25, $p = 1.2\times10^{-3}$). Inflammation, measured by glycoprotein acetyls, was more likely to be an 'exposure to' (cause of) IBS than an outcome of depression, serving

as mediators in the depression-to-IBS pathway. Triglycerides and lipoprotein showed bidirectional associations with depression and were an 'an exposure to' IBS, which suggests involvement of both a depression-triglycerides/lipoprotein-IBS pathway and a triglycerides/lipoprotein-depression-IBS pathway. Another finding is the relationship of immunometabolism and IBS, with immunometabolism directly associating with IBS as well as indirectly through the immunometabolism-neuroimaging-depression-IBS pathway. Finally, blood markers including triglycerides, inflammation, lipoprotein and immunometabolism influenced each other.

DISCUSSION

Utilizing a large-scale dataset from the UK Biobank, we elucidated the associations between IBS and brain health by analyzing multidimensional data. Individuals with more severe IBS symptoms exhibited poorer brain health, including higher anxiety and depression levels, poorer cognitive performance and lower brain volumes in regions related to emotion and cognition. Meanwhile, IBS was associated with dysregulated lipid metabolism and altered inflammatory indicators, supporting the hypothesis of a complex and multifactorial pathogenesis of IBS. Finally, MR analysis provides evidence regarding the potential causal relationships between IBS and brain health (specially concerning measures of depression), where IBS is more likely to manifest as an outcome trait, with dysregulated lipid metabolism and inflammation playing mediating roles. Together, these findings suggest several conclusions discussed below.

Consistent with previous findings, phenome-wide association analysis demonstrated a range of phenotypes showing significant association with the severity of IBS, among which mental health and cognitive function showed the greatest effect sizes (3, 31). Cognitive ability (e.g., fluid intelligence) exhibited a weaker effect size of association across the whole population with IBS symptoms compared to mental health, aligning with prior research (31, 32) and supported by indirect genetic association of cognition with IBS symptoms demonstrated in our MR analysis. Contrastingly, patients meeting the diagnostic criteria of IBS showed much lower cognitive ability with a stronger effect size. This difference in effect size could stem from the smaller sample size in the case-control analysis, despite meeting the recommended sample size required for robust results (33). It also raises the possibility that the impact of IBS on cognitive ability may not follow a linear pattern once people met the diagnostic criteria, warranting further investigation. Meanwhile, our findings complement previous neuroimaging studies, demonstrating that more severe IBS is associated with lower brain volumes in regions related to emotional, cognitive and social functions (13, 35). For example, anterior and mid-cingulate cortex are known to be related to affective and interoceptive processing and pain modulation (35, 37, 38), which are associated with IBS symptoms (36). Also, lower brain volume in the hippocampus was observed, which further emphasizes the impairment such as memory processing and language skills in IBS patients (40). Furthermore, IBS-associated brain regions correlated with gene expression profiles enriched in biological processes closely linked to forebrain development, further suggesting the influence of IBS on cognitive performance.

Regarding biochemical mechanisms, our exploratory analysis eventually pinpointed triglyceride, lipoprotein, inflammatory cells, platelets and immunometabolic markers as closely related to IBS. Although less investigated, we identified dyslipidemia (characterized by higher triglycerides and lower HDL) associated with IBS, which has been established as a risk factor for diabetes mellitus (42, 43). As greater signs of future diabetes

were also found in IBS, our findings provided clues that targeting dyslipidemia might prevent future diabetes in IBS patients (42, 43). Moreover, animal studies of diabetes found changes in bowel serotonin receptors, implicated in the pathogenesis of IBS (44, 45). Alterations to serotonin signaling pathways could lead to gastrointestinal dysmotility, visceral hypersensitivity and secretomotor disorders in the gut (46). Since most serotonin is stored in platelets and platelet counts are highly correlated with serum serotonin levels (47, 48), it was not surprising that we found higher platelet counts with more severe IBS symptoms. Together with our findings that the IBS-associated brain map correlated with serotonin architecture, our study demonstrated clear roles for central and peripheral serotonin signaling pathways in IBS. Moreover, as HDL has antioxidative and anti-inflammatory activity (49), together with associations found in inflammatory cells and immunometabolic markers, our results supported the participation of inflammation in the pathophysiology of IBS.

MR analysis suggested that genetically predicted variables, such as depression, metabolites including triglycerides, lipoprotein and glycoprotein acetyls, and immunometabolic indicators, were associated with IBS. We found two possible genetic associations. First, our findings are consistent with the current prevailing view that IBS and mental health (especially depression) have a reciprocal association (9). A recent study using MR analysis for gastrointestinal disorder also demonstrated bidirectional pathways between major depression and IBS (50, 51). We also observed a stronger causal relationship from depression to IBS, indicating that depression affects the development or manifestation of IBS more significantly than the reverse direction. Our second hypothesis is that the relationship between depression and IBS may be genetically influenced by metabolism and inflammatory status. In our study, an increase in immunometabolic dysregulation showed a genetically negative correlation with brain volumes, and lower brain volumes would genetically predict increased depressive symptoms, which finally aggravated IBS. This is supported by a previous finding that immunometabolic dysregulation in the serum was correlated with reduced thickness of the rostral anterior cingulate cortex (53). These potential genetic causalities suggest the potential for future clinical applications aimed at developing treatment options for IBS or mental health through the manipulation of peripheral biochemical markers.

The main strength of the current study lies in the integration of multiple dimensional data with a large sample size, which provides systematic novel insights into the association between IBS and brain health. Moreover, this study systematically described altered brain regions associated with IBS, and explored its underlying molecular architecture and gene expression profile, providing a more detailed and objective basis for the role of the brain-gut axis in IBS. Furthermore, the current study provides a strong research framework applicable to different intestinal diseases and is poised to advance our understanding of the imaging and genetic foundations of various gastrointestinal conditions in future research. Nevertheless, some limitations should be acknowledged. First, the UK Biobank is composed predominantly of Caucasians, limiting the generalizability of the findings to other ethnic groups. Second, while our study utilized various omics data, future research could integrate proteomics and AI algorithms to identify biomarkers specific to IBS with extensive datasets. Third, given the heterogeneity of phenotypes exhibiting diverse symptom profiles, it remains possible that specific IBS symptoms would associate with specific depressive domains, which requires further exploration. Fourth, the main analyses were based on IBS-SSS rather than diagnostic IBS because of the large interval between making diagnoses and brain health-related phenotypes. Nevertheless, our main findings could be replicated for diagnostic IBS patients.

In conclusion, the current study provides a comprehensive understanding of associations between IBS and a wide-range of phenotypes, highlighting the role of brain health-related phenotypes in IBS pathogenesis. More severe IBS symptoms were linked to worse mental health status and cognitive performance. IBS was associated with lower brain volumes in regions involved emotional regulation and higher-order cognition. Biochemical alterations including dyslipidemia and inflammation were also related. Further MR analysis revealed a bidirectional pathway between IBS and brain health, suggesting a dual impact of the brain-gut axis, where brain health may play a more exposure role than IBS. Together, our findings support the hypothesis that IBS is associated with a wide-range of brain health phenotypes, where genetic predisposition towards a poorer mental health associated with more severely IBS syndrome, and dysregulated lipid metabolism and inflammation might play a mediating role in the causal relationship. Further research on whether targeting brain health or the related central and peripheral biochemical mediators will benefit IBS patients is warranted.

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WC, ETR and JF designed the study. ZL and QM conducted main analyses. ZL, QM, and YD wrote the manuscript. QM and YD critically revised the manuscript. CS, YL and WZ contributed to the data collection. CS, WZ and SX contributed to the data analyses. WC, JF, ETR, JTY, CL, BJS and TWR critically supervised improvement of the manuscript. All authors reviewed and approved the final version.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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Figure legends

Figure 1 Guideline of the study. Top, UK Biobank data utilized in the study, including irritable bowel syndrome (IBS), brain imaging, genotype data, behavioral assessments, cellular and molecular data. Middle, association of IBS with behavioral assessments, brain imaging, cellular and molecular data. Bottom left, IBS-associated brain map analyses, including Neurosynth meta-analysis, neurotransmitter analysis and gene ontology enrichment analysis. Bottom right, directional association of IBS with behavioral assessments, brain imaging, cellular and molecular data.

Figure 2 Phenome-wide association of IBS. (**A**) Manhattan plot showing the p values for associations of IBS with phenotypes in 20 categories. The height of each data point denotes the negative logarithm of the univariate correlation *p* value between IBS and one phenotype. The color of the data point denotes different categories. The red dashed horizontal line denotes the Bonferroni threshold for multiple comparisons (α = 0.05). The variables were adjusted for covariates comprising age, sex and assessment center. (**B**) Plot showing the distribution of the absolute *t* value of the phenotypes in each category. (**C**) Density scatter plot and linear-regression line showing the significant associations of IBS with behavioral assessments, including mental health (depressive symptoms, anxiety symptoms, well-being, self-harm, mental distress and trauma) and cognitive function (fluid intelligence, reaction time, pair matching, numeric memory, symbol-digit substitution and matrix pattern completion) after Bonferroni correction (α = 0.05). The variables were adjusted for covariates comprising age, sex, body mass index, Townsend deprivation index, educational qualifications, smoking status and drinking status.

Figure 3 Association of IBS with brain structure and biochemical markers. (**A**) Significant associations between IBS and cortical volumes adjusted for age, sex, body mass index, Townsend deprivation index, educational qualifications, smoking status, drinking status, total intracranial volume and imaging scanning sites after FDR correction (α = 0.05). (**B**) Significant association between IBS and the average brain volume of all negatively associated voxels, adjusted for age, sex, body mass index, Townsend deprivation index, educational qualifications, smoking status, drinking status, total intracranial volume and imaging scanning sites. (**C**) Associations of IBS with biochemical markers, adjusted for age, sex, body mass index, Townsend deprivation index, educational qualifications, smoking status, and drinking status. The height of each data point denotes the negative logarithm of the univariate correlation *p* value between IBS and one marker. The color and size of the data point denote *r* value. The red horizontal line denotes the Bonferroni threshold for multiple comparisons (α = 0.05). IGF, insulin like growth factor; HbA1c, glycated hemoglobin A1c; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate density lipoprotein; HDL, high-density lipoprotein.

Figure 4 Functional annotation, transmitter architecture and transcriptomic profile related to IBS-associated brain map. (**A**) Word-cloud plots showing emotion and cognition terms associated with the IBS-associated brain map. The color of word clouds denotes positive correlation (red) or negative correlation (blue) of the IBS-associated brain map with the meta-analytic map of that term generated by Neurosynth. The font size of a given term corresponds to the spearman correlation coefficient (*r* value). The boxes to the right of word clouds indicate the correspondence between the font size and the correlation coefficient. (**B**) Box-line plot showing the spearman correlation of the brain neurotransmitter distribution map with the IBS-associated brain map (*, p_{FDR} < 0.05; **, p_{FDR} < 0.01; ***, p_{FDR} < 0.005). DAT, dopamine transporter; NAT, noradrenaline transporter; SERT,

serotonin transporter. (**C**) Plot showing the association between the IBS-associated brain map and the AHBA gene expression map using partial least squares (PLS) regression analysis. Brain map showing the spatial distribution of PLS1 score. (**D**) Scatterplot showing the spatial correlation between the IBS brain map and the PLS1 score map. Lines are fitted to linear models, and shaded areas are 95% confidence intervals. (**E**) Significant enrichment of gene ontology terms associated with cellular components was observed for bottom genes with low weights for the PLS1 component. (**F**) Significant enrichment of gene ontology terms associated with biological processes was observed for bottom genes with low weights for the PLS1 component. Terms with a $p < 0.01$, a minimum count of 3, and an enrichment factor > 1.5 (the enrichment factor is the ratio between the observed counts and the counts expected by chance) were collected and grouped into clusters based on their membership similarities. The top 20 clusters with their representative enriched terms (one per cluster) were shown in figure.

Figure 5 Causal link between IBS and brain health revealed by Mendelian Randomization (MR) analyses (**A**) Phenotypic associations between IBS, IBS-SSS PRS, depression, cognition, neuroimaging, immunometabolism, lp (lipoprotein) concentration, triglycerides, inflammation (glycoprotein acetyls) and platelets. (**B**) Genetic association between IBS, depression, cognition, neuroimaging, immunometabolism, lp concentration, triglycerides, inflammation and platelets, using the MR IVW method. The *t* value was calculated by dividing the beta by the standard deviation. The direction of (*i*, *j*) is row *i* to column *j*. (*, $p < 0.05$; **, $p_{FDR} < 0.05$; ***, $p_{Bonferroni} < 0.05$). (**C**) MR with a p_{FDR} value less than 0.01 in (B). The color of the arrow indicates the positive (red) or negative (blue) beta value. The number on the arrow represents the *p* value. In above analyses, cognition included fluid intelligence, reaction time and pairs matching; Neuroimaging included significant voxels associated with IBS; Immunometabolism included C-reactive protein, glucose, HbA1c, apolipoprotein a, apolipoprotein b, cholesterol, HDL cholesterol, LDL direct and triglycerides; Lipoprotein particle concentrations included concentration of chylomicrons and extremely large VLDL, very large VLDL, large VLDL, small VLDL, IDL, very large HDL and large HDL particles; Triglycerides included total triglycerides, triglycerides in VLDL, very large VLDL, large VLDL, medium VLDL, small VLDL, very small VLDL, LDL, large LDL, medium LDL, small LDL, HDL, medium HDL, small HDL and IDL; Inflammation only included glycoprotein acetyls; Platelets included platelet count, mean platelet volume and platelet crit.

UK Biobank data (n = 493,865)

Irritable bowel syndrome

abdominal pain
abdominal distension
bowel habit change

Brain imaging

T1-weighted structural MRI

Genotype data

single nucleotide polymorphisms

Behavioral assessments

Mental health

depression, anxiety, well-being, trauma...

Cognitive function

fluid intelligence, reaction time...

Cellular and molecular

Blood cell count

red blood cell, white blood cell...

Blood biochemistry

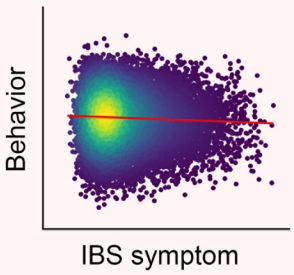
IGF1, HbA1c, apolipoprotein A...

NMR metabolomics

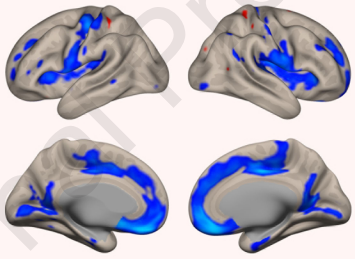
triglycerides, cholesterol lipoprotein...

Phenome-wide association analyses

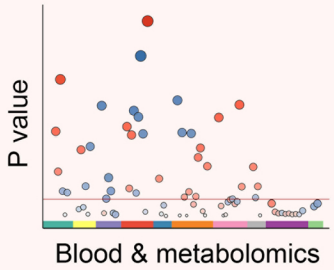
Association between IBS and behaviors



Association between IBS and brain imaging



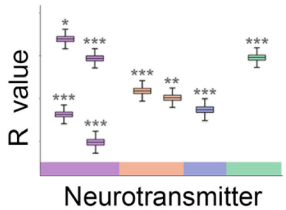
Association between IBS and cellular&molecular



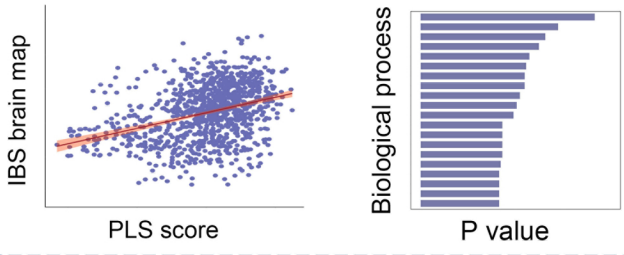
IBS brain map analyses

Neurosynth meta-analysis

Neurotransmitter analysis



Gene Ontology enrichment analysis



Mendelian randomization