

SUPPLEMENTARY INFORMATION

Irritable Bowel Syndrome Is Associated With Brain Health by Neuroimaging, Behavioral, Biochemical, and Genetic Analyses

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Supplementary Methods

Data

Irritable bowel syndrome

For replication, we also conducted the same analysis for patients diagnosed with IBS. IBS cases in the UK Biobank were identified as one or more of the following four criteria (1): a) Participants who completed the DHQ and whose abdominal symptoms were consistent with a diagnosis of IBS based on the Rome III definition (Table S27); b) Participants who answered “Yes” to the question “Have you ever been diagnosed with IBS?” (field 21024); c) Participants indicated a previous diagnosis of IBS and were given diagnosis code 1154 (“irritable bowel syndrome”) in the non-cancer illness field (field 20002); d) Participants diagnosed with IBS during a hospitalization and coded in ICD-10 diagnosis as K58 (field 41270). We also excluded cases with a potentially confounding condition. Excluded conditions included inflammatory bowel disease, GI malignancy, malabsorption, celiac or gluten sensitivity based on a blood test or endoscopy, and a number of abdominal surgeries (Table S28). IBS controls in the UK Biobank were known to lack significant abdominal symptoms according to the DHQ response: a) less than one day per month of abdominal pain (field 21025); b) hard/lumpy stools or loose/watery stools either ‘never’ or at most ‘sometimes’ in the last three months (fields 21033 and 21034). For DHQ non-responder controls, we didn’t apply this exclusion. In addition, we also excluded controls with a potentially confounding condition (Table S29).

Behavioural and diet assessments

The factors used in the phenome-wide analysis contained 20 categories (4981 variables), including baseline characteristics, cognitive function, diet by 24-hour recall, digestive health, early life factors, employment, family history, food (and other) preferences, health and medical history, health-related outcomes, lifestyle and environment, medical conditions, medications, mental health, operations, physical measures, psychosocial factors, sex-specific factors, social demographics and work environment. These variables were from four categories in UKB, including population characteristics, health-related outcomes, assessment centers and online follow-up, and we re-categorized them based on the framework of UKB showcase. More details are provided in Table S9.

Mental health and cognitive function data were utilized to perform the following analyses. In particular, the Patient Health Questionnaire-4 (PHQ-4), which was assessed in the UKB center, was used to measure depressive symptoms among participants (2006-2010, $n = 492,004$). The PHQ-4 total score was defined as the average of four subscores, for example “Frequency of depressed mood in the last 2 weeks”, in which high values indicated severe symptoms. The details about the PHQ-4 questionnaire were provided in Table S5. The other mental health assessment questions used in the analyses include anxiety, mania, wellbeing, subjective experience, self-harm, mental distress and trauma, which were measured through a detailed online mental health questionnaire (2015-2018, $n = 145,808$). The subscores of each category of the questionnaire were adjusted in the same direction such that a high value indicated a severe symptom (except for wellbeing, where a higher value indicates better wellbeing). Then we normalized the subscores for each category and averaged them as the total value. More information about each category is provided in Table S6. In addition, data on cognitive function were collected through the touchscreen questionnaire from the UKB assessment center. Seven cognitive tests were utilized in the following analyses, including fluid intelligence, reaction time, pairs matching, numeric memory, Tower re-arrangement solutions, symbol digit substitution and matrix pattern completion. More details about each cognitive test and sample sizes were provided in Table S7.

Dietary data were utilized to calculate the association with IBS. The dietary category (ID 100052) contains data from the touchscreen questionnaire on the reported frequency of intake of a range of common food and drink items. Eighteen items from nearly 500,000 participants (2006-2010) were selected to calculate the association.

Neuroimaging

T1-weighted structural MRI (sMRI) data ($n = 39,578$) measured at the UKB assessment center (2014), were utilized in the analyses. The scanner was a standard Siemens Skyra 3T with a standard Siemens 32-channel RF receive head coil. The details of the image acquisition are provided at the UKB website (<http://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=2367>). Structural MRI data were preprocessed with the Statistical Parametric Mapping software version 12 (<http://www.fil.ion.ucl.ac.uk/spm>) using the CAT12 toolbox (<http://dbm.neuro.uni-jena.de/cat>) with default settings. All images were normalized with an integrated Dartel template in Montreal Neurological Institute (MNI) space, subjected to nonlinear modulations and corrected for individual head size. We utilized an 8-mm full-width at half-maximum Gaussian kernel to smooth images and finally obtained a voxel size of 1.5 mm^3 . We used brain cortical and

subcortical regions to perform the following analyses, excluding the cerebellum. The distribution of the individuals with IBS symptom score in neuroimaging association analysis was shown in Figure S8.

Biochemical markers

Blood biochemistry (ID 17518) included about 480,000 participant samples from the recruitment visit (2006-2010), and biomarker assay quality procedures are provided in the open-source document (https://biobank.ndph.ox.ac.uk/showcase/ukb/docs/biomarker_issues.pdf). Blood counts (ID 100081) were also collected from 480,000 participants at the first visit, using Beckman Coulter LH750 instruments to analyze samples collected in 4ml EDTA (ethylenediaminetetraacetic acid) vacutainers. More information about haematology analysis is provided in the document (<https://biobank.ndph.ox.ac.uk/showcase/ukb/docs/haematology.pdf>). 30 blood biochemistry markers and 31 blood cell counts are available from UKB category of blood biochemistry and blood count. In the analyses, we used 59 markers out of 61, excluding nucleated red cell count and percentage, and stratified testosterone, estradiol and sex hormone-binding globulin by sex. Then we categorized blood biochemistry markers as “liver function”, “renal function”, “endocrine”, “immunometabolism” and “bone and joint” according to their function. Blood count were categorized as “white blood cells”, “red blood cells” and “platelet”. More details about each item in the categories and sample sizes were provided in Table S30.

Metabolic markers were measured from randomly selected EDTA plasma samples using a high-throughput NMR-based metabolic biomarker profiling platform, and it included about 120,000 UKB participants at the first assessment. The process and calculation of metabolic markers can be found on the website (<https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=220>). NMR metabolomics (ID 220) included 249 metabolic markers (168 directly-measured and 81 ratios of these). We used 168 directly-measured metabolic markers in the analyses and categorized metabolic markers as “Amino acids”, “Apolipoproteins”, “Lipoprotein particle sizes”, “Lipoprotein particle concentrations”, “Fatty acids”, “Triglycerides”, “Phospholipids”, “Cholesteryl esters”, “Free cholesterol”, “Cholesterol”, “Other lipids”, “Total lipids”, “Ketone bodies”, “Glycolysis related metabolites”, “Fluid balance” and “Inflammation”. More details about each category can be found in Table S31.

Statistical analysis

Polygenic risk score for IBS symptoms

Genotype data from about 500,000 participants was extracted from UKB v3 imputation. We performed quality control for genotype data, excluding single nucleotide polymorphisms (SNPs) with call rate < 95%, minor allele frequency < 0.1% and deviation from Hardy-Weinberg equilibrium ($p < 1 \times 10^{-10}$). Subjects who were estimated to have recent British ancestry and no more than ten putative third-degree relatives were included in the analyses. Finally, we obtained 337,199 participants with 8,894,431 SNPs. In order to avoid circular analysis, we excluded participants who had MRI data from the genome-wide association study (GWAS) sample, to calculate the polygenic risk score.

We used PLINK 2.0 (<https://www.cog-genomics.org/plink/2.0/>) to perform genome-wide association analyses on the IBS-SSS, adjusting for age, sex and the first 20 ancestry principal components. We removed participants who had MRI data to avoid overlap bias and finally obtained 8,894,431 SNPs from 121,089 participants with IBS-SSS. We used PRSice-2 (<http://www.prsice.info>) to calculate the polygenic risk score (PRS) for the IBS-SSS, which only contained participants with MRI data. First, SNPs were clumped ($r^2 = 0.1$, physical distance = 250kb) so that only the most strongly associated SNP in a region was retained. Second, we used various thresholds from 0.005 to 0.5 with an interval of 0.005 to estimate PRS for each participant.

Functional annotation and neurotransmitter analysis related to IBS-associated brain map

Firstly, a meta-analysis was performed using Neurosynth (2) to decode functions of brain regions exhibiting associations with IBS. The spatial correlation was calculated between the IBS-associated brain map (voxels negatively correlated with IBS after FDR correction in the whole brain) and meta-analytic brain maps of 122 terms, which were associated with emotion or cognitive function. Second, spatial correlations between the IBS-associated brain map (voxels in the whole brain) and neurotransmitter maps were investigated using Spearman correlation. Neurotransmitter maps were obtained from the toolbox JuSpace (3) (Table S3). In both analyses, we used the toolbox BrainSMASH (4) to generate 10,000 surrogate maps and performed the permutation test ($n = 10,000$) to test the significance (FDR correction, $p < 0.05$).

Transcriptomic analysis related to IBS-associated brain map

We used gene expression data from six human brains from the Allen Human Brain Atlas (AHBA) database (5, 6) (<https://portal.brain-map.org/>) to identify the genes associated with the IBS-associated brain map. We

used gene expression data from the left hemisphere because six donors were available for the left hemisphere but only two donors for the right hemisphere. Then we used a partial least squares (PLS) regression analysis to explore the weighted linear combinations of expression patterns for 15,408 genes, to identify the association between genes and the IBS-associated brain map (left hemisphere cortex). The response variable was calculated by the average r value of a spherical region with a radius of 4 mm, centered by the MNI coordinates of each gene expression sampling site. We used a permutation analysis ($n = 10,000$) to test the significance of the variance explained by the PLS components. Finally, we calculated the z score of each gene by dividing the PLS weight by the standard deviation in bootstrapping ($n = 1,000$), and ranked the genes according to their corrected weights to perform the following genetic enrichment analyses.

References

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Supplementary Figures

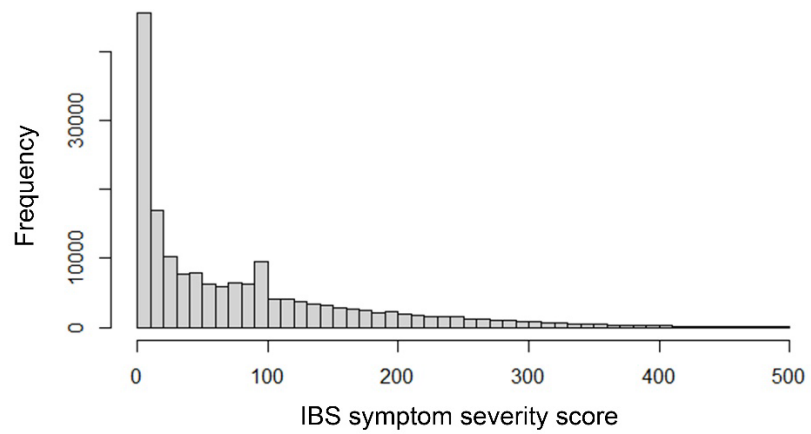


Figure S1. Distribution of the individuals with IBS symptom score. The x-axis represents the IBS score, and the y-axis represents the number of individuals.

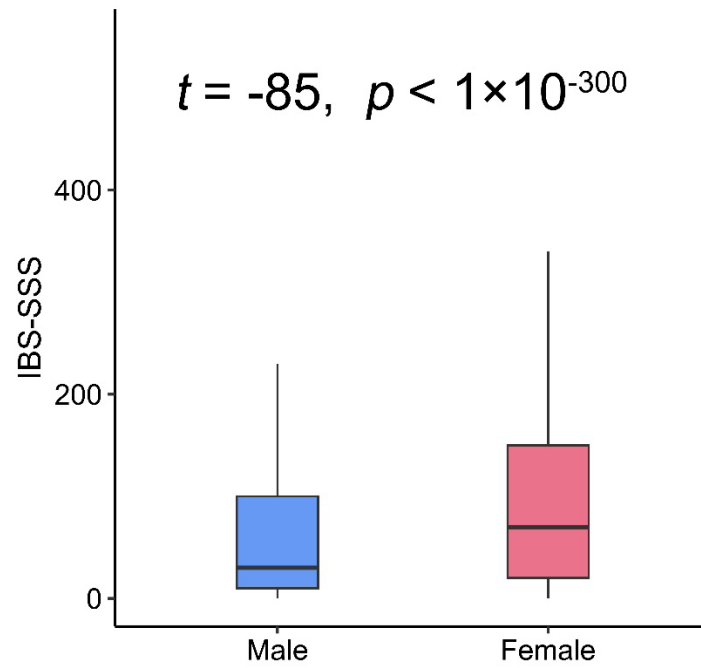


Figure S2. Gender distribution of the IBS symptom. The boxplot showed the distribution of the data. T-test was used to find gender difference in IBS-SSS, adjusting for age, body mass index, Townsend deprivation index, educational qualifications, smoking status and drinking status

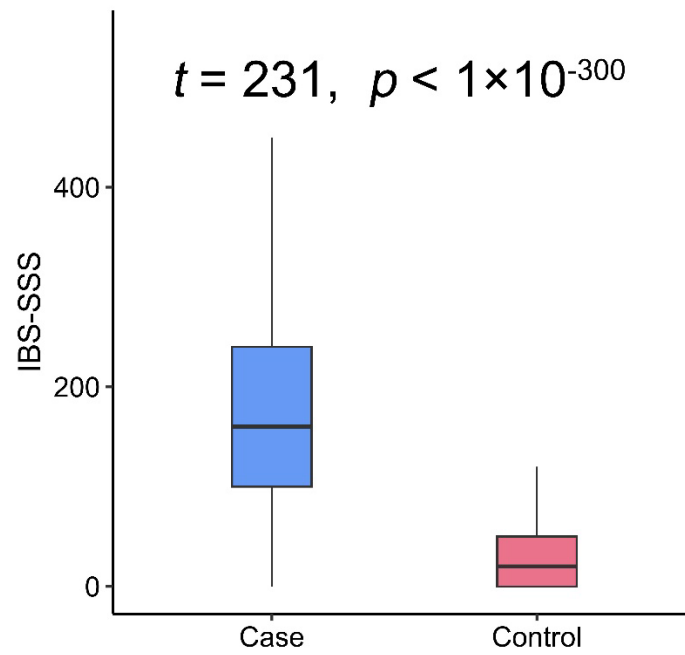


Figure S3. Distribution of the IBS symptom in IBS case and control groups. The boxplot showed the distribution of the data. T-test was used to find difference of IBS-SSS in IBS case and control groups, adjusting for age, sex, body mass index, Townsend deprivation index, educational qualifications, smoking status and drinking status

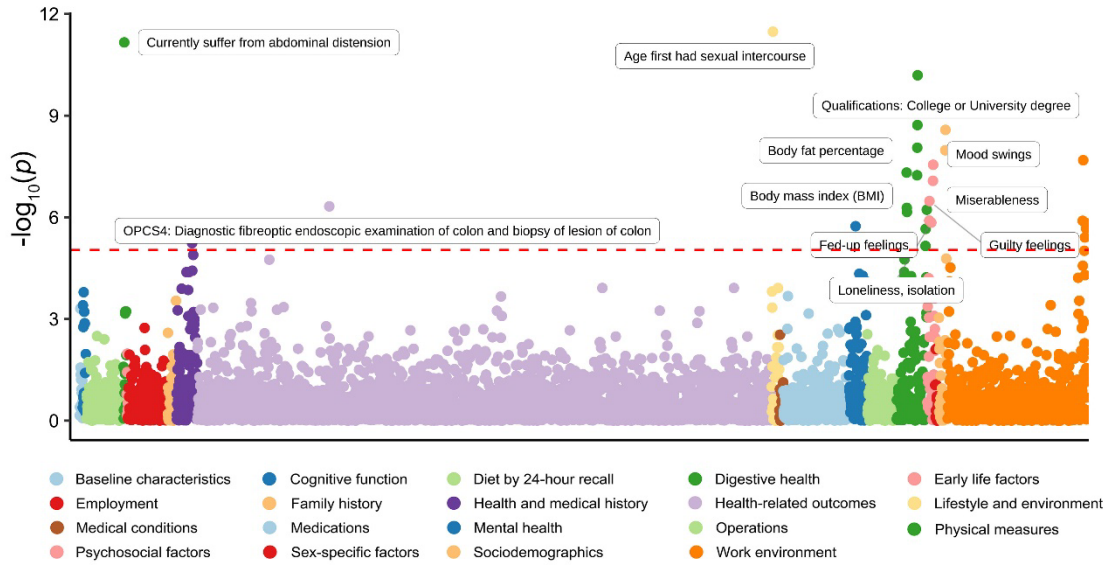


Figure S4. Phenome-wide association analysis on PRS (threshold=0.5) of IBS symptom. Manhattan plot showing the p values for associations of PRS with phenotypes in 19 categories (The number of individuals in category “Food (and other) preferences” was insufficient for analysis). The height of each data point denotes the negative logarithm of the univariate correlation p value between PRS and one phenotype. The color of the data point denotes different categories. The red dashed horizontal line denotes the Bonferroni threshold for multiple comparisons ($\alpha = 0.05$). The variables were adjusted for covariates comprising age, sex, assessment center and genetic principal components.

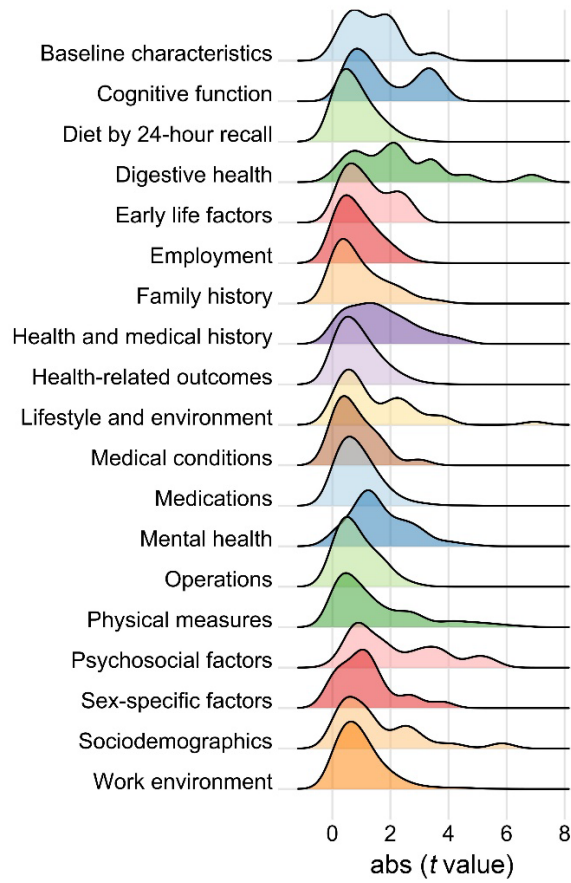


Figure S5. Distribution of the absolute t value in each category in phenome-wide association analysis of PRS (threshold=0.5).

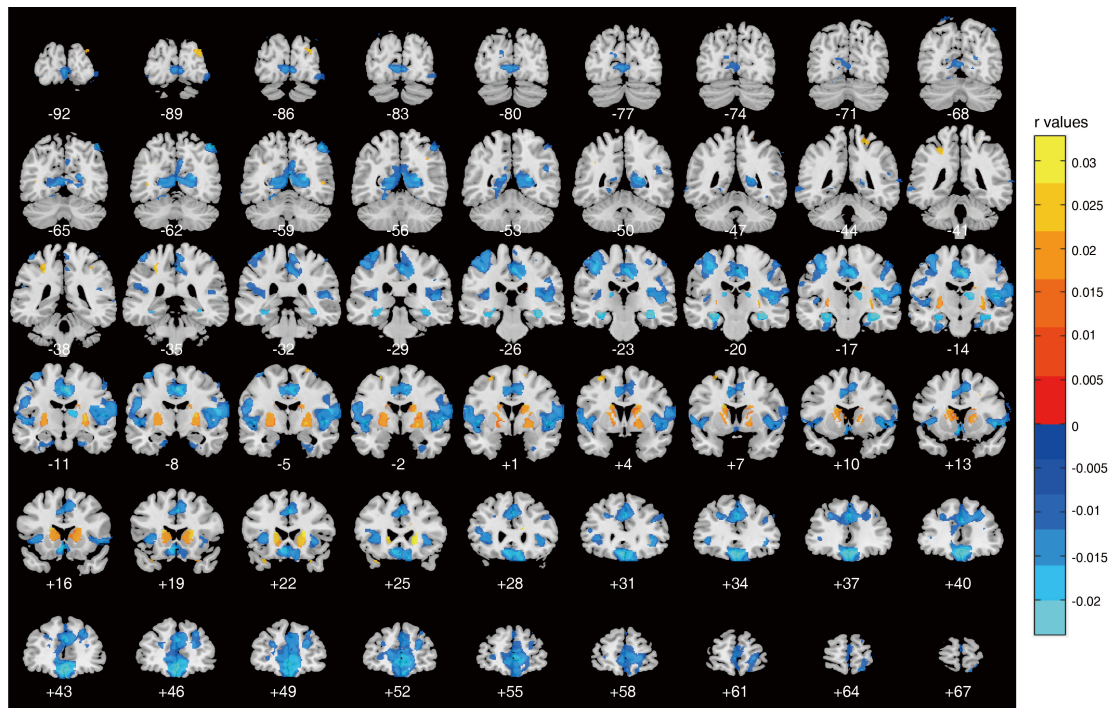


Figure S6. Association between IBS and brain regions after multiple comparison correction at the voxel level (FDR correction, $p < 0.05$).

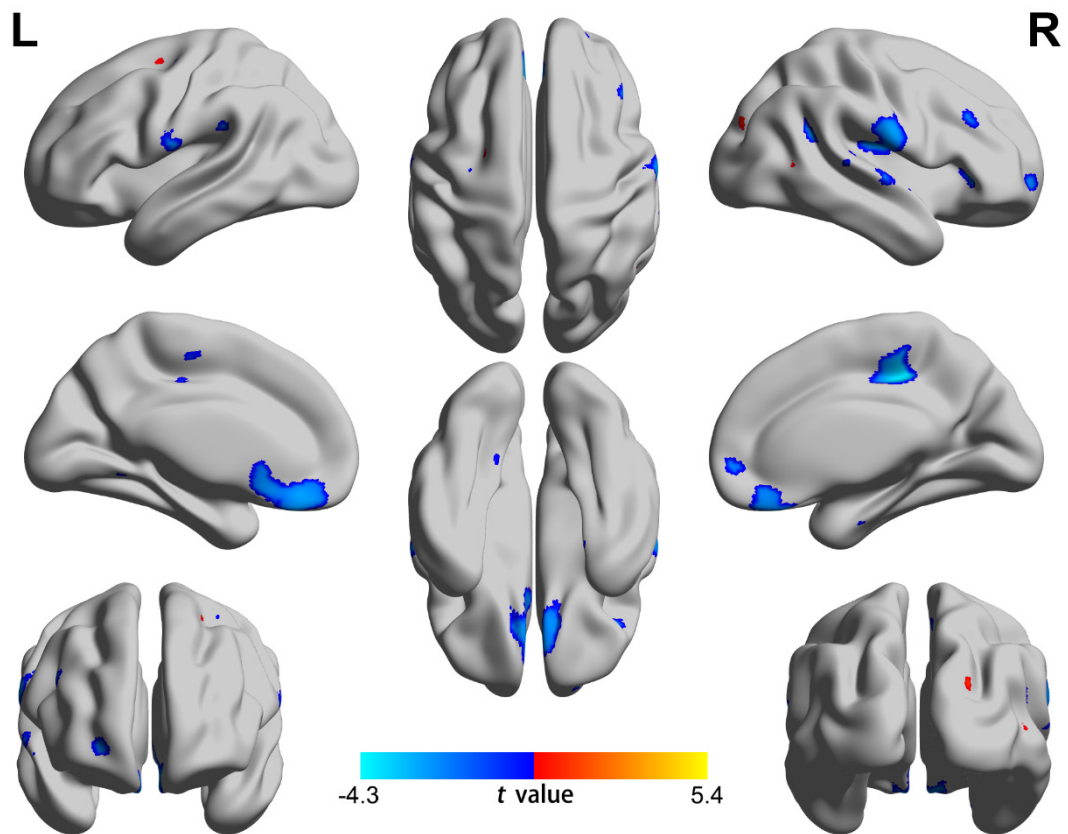


Figure S7. The difference of brain regions which were significant in IBS-SSS association analysis, between IBS case and control group, adjusting for age, sex, body mass index, Townsend deprivation index, educational qualifications, smoking status, drinking status, total intracranial volume (TIV) and scanning site of neuroimaging. Figure showed voxels with $p < 0.05$.

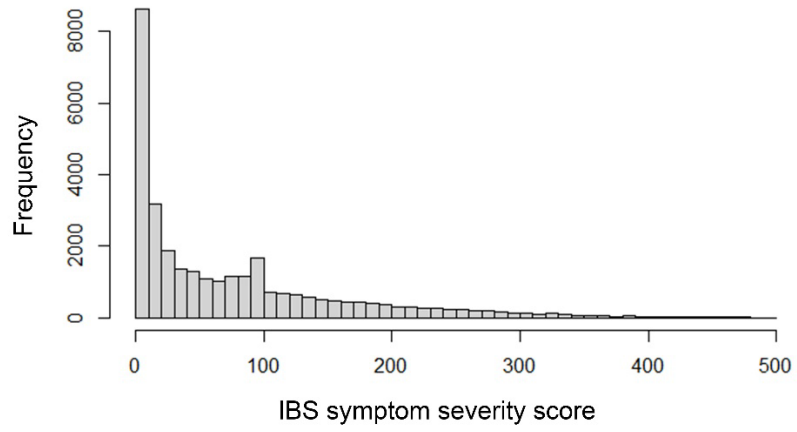


Figure S8. Distribution of the individuals with IBS symptom score in neuroimaging association analysis. The x-axis represents the IBS score, and the y-axis represents the number of individuals.