

## Article

# Brain morphometry and cognitive features in prediction of irritable bowel syndrome

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Astri J. Lundervold <sup>1</sup> 0000-0002-6819-6164\*, Ben René Bjørsvik <sup>2</sup>, Julie Billing <sup>1</sup>, Birgitte Berentsen <sup>5,6</sup> 0000-0003-3574-7078, Gülen Arslan Lied <sup>5,6</sup> 0000-0002-1827-5008, Elisabeth K Steinsvik <sup>5</sup> 0000-0002-8371-1988, Trygve Hausken <sup>5</sup> 0000-0001-7080-8396, <sup>4,†</sup>, Daniela M. Pfabigan <sup>1</sup> 0000-0002-4043-1695, Arvid Lundervold <sup>2,3,†</sup> 0000-0002-0032-4182

<sup>1</sup> Department of Biological and Medical Psychology, University of Bergen, Norway<sup>2</sup> Department of Biomedicine, University of Bergen, Bergen, Norway<sup>3</sup> Medical-AI, Mohn Medical Imaging and Visualization Center, Department of Radiology, Haukeland University Hospital, Bergen, Norway<sup>4</sup> Department of Clinical Medicine, University of Bergen, Bergen 5021, Norway<sup>5</sup> National Center for Functional Gastrointestinal Disorders, Department of Medicine, Haukeland University Hospital, Bergen 5021, Norway<sup>6</sup> Department of Biomedicine, University of Bergen, Bergen, Norway<sup>5</sup> Medical-AI, Mohn Medical Imaging and Visualization Center, Department of Radiology, Haukeland University Hospital, Bergen, Norway\* Correspondence: [Astri.Lundervold@uib.no](mailto:Astri.Lundervold@uib.no)

**Abstract:** *Background:* Irritable bowel syndrome (IBS) is a common condition within the spectrum of gut-brain disorders, characterized by abdominal pain, bloating, altered bowel habits, and different patterns of psychological distress. While brain-gut interactions are increasingly recognized in IBS pathophysiology, the relationship between brain morphometry, cognitive function, and clinical presentation remains poorly understood. *Objectives:* To investigate whether multivariate analysis of brain morphometric measures and cognitive test performance can distinguish patients with IBS from healthy controls (HCs), and to evaluate the relative importance of structural and cognitive features in this discrimination. *Methods:* In this cross-sectional study, 49 patients with IBS and 29 HCs underwent structural magnetic resonance imaging (MRI) brain examination and completed the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Brain morphometry was analyzed using two versions of FreeSurfer software (v6.0.1 and v7.4.1). IBS severity was assessed using the IBS-Severity Scoring System (IBS-SSS). We employed both univariate and multivariate statistical and machine learning approaches, including cross-validation, to analyze morphometric and cognitive measures. *Results:* Univariate and multivariate analyses showed limited discrimination between IBS and HC groups using morphometric measures alone. However, when combining morphometric and cognitive measures in a machine learning framework, the model achieved 93% sensitivity in identifying IBS patients, albeit with 78% specificity. Feature importance analysis highlighted the significance of subcortical structures (particularly hippocampus, caudate, and putamen) and two cognitive domains (recall and verbal skills) in group discrimination. Software version comparison revealed substantial impact on morphometric measurements. *Conclusions:* Contributing with a comprehensive open-source framework for data analysis, our findings suggest that the combination of brain morphometry and cognitive measures provides better discrimination between IBS and HC groups than either measure alone. The identified importance of subcortical structures and specific cognitive domains supports a complex brain-gut interaction in IBS. These results emphasize the need for multimodal approaches in IBS research and careful consideration of methodological factors in brain morphometry studies.

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**Keywords:** Irritable bowel syndrome; structural MRI; brain morphometry, cognition; supervised classification; machine learning

## Introduction

Irritable bowel syndrome (IBS) represents a prevalent and complex gastrointestinal (GI) disorder, affecting approximately 10% of the global population [1]. The syndrome is clinically defined by a characteristic symptom pattern: recurrent abdominal pain associated with defecation, accompanied by alterations in bowel habits [2], and can be divided into clinical phenotypes based on predominant bowel patterns [3] and overall symptom severity [4]. The clinical presentation is heterogeneous, with experiences ranging from mild discomfort to severe symptoms that substantially impair quality of life and daily functioning [4]. Notably, women are disproportionately affected, a difference that appears to arise from a complex interplay of biological factors (including hormonal influences), healthcare-seeking behaviors, and sociocultural determinants [5–8]. Such epidemiological patterns highlight the multifactorial nature of IBS and underscore the importance of considering both biological and psychosocial factors in its study and treatment.

A bidirectional relationship between gastrointestinal symptoms of IBS and psychological functioning is well-documented [9]. While gastrointestinal symptoms can trigger or exacerbate psychological distress, anxiety and depression may in turn amplify the intensity and frequency of abdominal pain [10]. Recent research has expanded this psychobiological framework to include cognitive function, revealing a more nuanced picture of brain-gut interactions in IBS. Although cognitive impairments have been demonstrated at the group level [11,12], these deficits seem to characterize specific subgroups rather than being a universal feature of IBS [9,13]. This heterogeneity in psychological and cognitive presentations aligns with contemporary models of the gut-brain axis [14,15], which conceptualize IBS as a disorder of disrupted neural-enteric communication. In these models, the brain serves as the central integration hub for processing and interpreting the complex array of visceral signals, emotional responses, and cognitive processes that may be involved in IBS.

The relationship between brain structure and cognitive function has evolved from simple localization models to more sophisticated network-based frameworks [16,17]. This network perspective gained particular relevance for understanding IBS through Mayer et al.'s [18] seminal paper in 2015, which proposed that alterations in brain networks could directly influence multiple cognitive domains in IBS patients (see also [19]). Recent empirical support for this systems-level approach comes from Li et al. [20], who identified several associations between symptom severity and regional brain volumes, including positive correlations with subcortical structures (globus pallidus, caudate, and putamen) and negative correlations with cortical regions (anterior cingulate, dorsolateral prefrontal cortex, anterior and midcingulate cortices) and subcortical areas (anterior insula, hippocampus, parahippocampal cortex, thalamus). Of special interest to the present study, they also showed that these brain regions were linked to cognitive performance on tests of language skills and memory function.

Studies of abdominal pain and visceral stimulation have consistently demonstrated involvement of distributed brain networks, encompassing both cortical and subcortical structures [21,22]. Building on this network perspective, Skrobisz et al. [23] conducted a comprehensive morphometric analysis in patients with non-specific digestive disorders, including IBS. Using FreeSurfer software (version 6.0.1), they analyzed 36 brain regions, including subcortical, cortical, and global measures derived from structural magnetic resonance imaging (MRI). Their univariate analyses revealed reduced thalamic volume in IBS patients compared to healthy controls, though volumes remained larger than in patients with inflammatory bowel diseases. While these findings suggest structural brain differences in IBS, univariate approaches may not capture the full complexity of brain-gut interactions. Therefore, our study builds upon Skrobisz et al.'s work in two key ways. First, we examine the robustness of their findings by comparing analyses using both FreeSurfer v6.0.1 and a more recent version, allowing us to differentiate between software-dependent and

true biological effects. Second, we extend beyond univariate analyses by implementing multivariate approaches, including supervised machine learning techniques, to capture complex patterns in brain morphometry that might better characterize IBS. This dual approach - methodological validation and advanced pattern analysis - aims to provide a more comprehensive understanding of the structural brain differences associated with IBS. Finally, responding to Skrobisz et al.'s [23] call for integrating clinical measures, we investigated whether combining cognitive performance data with morphometric features would enhance the accuracy of IBS versus HC classification.

Our study has four key aims:

- A To replicate the morphometric differences between IBS patients and HC reported in [23] using the same FreeSurfer software version (FS 6.0.1) and a similar univariate analysis approach as in the original study.
- B To evaluate consistency between FreeSurfer versions by comparing morphometric segmentation outcomes from version 6.0.1 (used in [23]) and version 7.4.1 in our dataset ( $n = 78$ ).
- C To assess whether morphometric features from FS 7.4.1 (both cross-sectional and longitudinal analyses) can differentiate IBS from HC groups through: (i) Univariate group comparisons, (ii) Multivariate analyses incorporating feature covariance, (iii) Machine learning classification, (iv) Feature importance analysis of successful classifications.
- D To determine whether incorporating cognitive performance data enhances the morphometric-based machine learning classification, and if so, identify the most discriminative features between IBS and HC groups.

## Materials and Methods

### Participants

This study is part of the Bergen Brain-Gut project, a prospective clinical investigation conducted at Haukeland University Hospital, Norway (2020–2022; protocol detailed in Berentsen et al. [24]). We enrolled 78 participants (49 IBS patients and 29 healthy controls [HCs]), all  $\geq 18$  years old. Recruitment occurred through media advertisements, informational flyers, and direct referrals from the hospital's outpatient clinic. A trained nurse screened all candidates using standardized inclusion and exclusion criteria (Table 1). Eligible participants underwent comprehensive assessment including gastrointestinal measures, psychometric testing, and multiparametric magnetic resonance imaging (MRI).

Sample size determination balanced multiple considerations. Although we did not conduct an a priori power analysis due to limited effect size data for brain morphometric differences in IBS at study inception, our sample size meets or exceeds comparable neuroimaging studies in functional gastrointestinal disorders [23,25–27]. We included only participants with complete key measures and high-quality MRI scans suitable for automated brain segmentation, optimizing data quality while maximizing sample size.

Inclusion criteria	Exclusion criteria
Rome-IV criteria: Recurrent abdominal pain average at least 1 day/week during the last 3 months, and associated alterations in bowel habits at least 6 months before diagnosis. Other causes are excluded.  Normal diet at least 3 weeks before inclusion IBS score equal to or above 175	Pharmacological treatment affecting GI tract, including medication for anxiety and depression, diabetes, coeliac disease, IBS, Polycystic ovary syndrome, active Helicobacter pylori infection, Parkinson's disease, amyotrophic lateral sclerosis, or Psychiatric disorders.  Treated with antibiotics for the last 3 months Diets such as vegetarian or vegan Use of probiotics or low-FODMAP diet within the last 3 weeks Previous intestinal surgery, except appendectomy Metallic implants, claustrophobia, incompatible with MRI Travel outside Europe last 3 weeks Plan to travel in the near future Pregnancy

**Table 1.** Exclusion and inclusion criteria for the IBS patients. Source: Retrieved from [24].*Measures*

Age and sex (not genetically verified) were self-reported by the participants at baseline. 125 126

## The IBS-Severity Scoring system (IBS-SSS) 127

The IBS-Severity Scoring system is a questionnaire used to assess the severity and frequency of GI-related IBS symptoms [28]. The questionnaire includes five items related to (i) abdominal pain intensity, (ii) abdominal pain frequency, (iii) abdominal distention/bloating, (iv) dissatisfaction with bowel habits, and (v) interference with quality of life – over the past 10 days. The maximum score for each question is 100. A sum of scores < 75 is used to define "no or minimal problems", and the scores in the ranges [75, 175], [175, 300], and > 300 as "mild", "moderate", and "severe" IBS symptoms, respectively [28]. In the present study, an IBS-SSS score  $\geq 175$  was used as the inclusion criteria for the IBS group. Almost all HCs obtained an IBS-SSS score at the lowest level (< 75), with some reporting a score within the mild level ([75, 175]). 128 129 130 131 132 133 134 135 136 137

## Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) 138

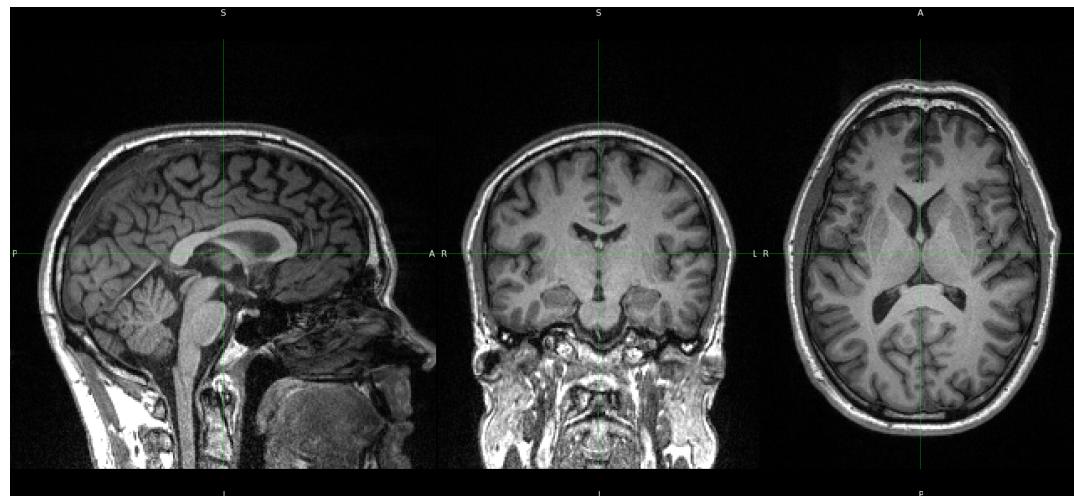
RBANS provides a quick and comprehensive assessment of five key cognitive domains, allowing the identification of specific areas of cognitive impairment, and takes about 30 minutes to administer. RBANS is sensitive to mild cognitive impairment, has good reliability and validity, can track changes over time, and is useful for both screening and detailed assessment. The five cognitive domains are (i) *immediate memory* (e.g., story memory and list learning tasks), (ii) *visuospatial/constructional skills* (e.g., copying geometric designs and identifying line orientation), (iii) *language* (e.g., picture naming and semantic fluency tasks), (iv) *attention* (e.g., digit span and coding tasks), and (v) *delayed memory* (e.g., recall of previously learned stories or lists). All participants performed the Norwegian A version of RBANS, administrated by a nurse trained by a clinical neuropsychologist, following the test manual's instructions [29]. The test battery comprises ten subtests, which are combined into five index scores and a total score. These scores are expressed both as raw and as age-corrected scaled scores. The scaled scores have a mean value of 100 and a standard deviation of 15 and are based on performance in a normative group matched to population statistics of 2012 in Norway, Sweden, and Denmark. We used these scaled scores on each of the five RBANS indices for a pairwise correlation analysis between brain morphometric measures and cognitive performance. 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155

### MRI Data Acquisition

All neuroimaging data were acquired using a 3 Tesla Siemens Biograph mMR PET/MRI scanner equipped with a standard 12-channel head coil. The comprehensive multiparametric imaging protocol consisted of five sequences: a 3D T1-weighted MPRAGE (TA = 5:35), T2-weighted structural imaging (TA = 5:12), gradient echo (GRE) field mapping (TA = 0:54), resting-state functional MRI using echo-planar imaging (EPI) with integrated motion correction (TA = 9:48), and diffusion-weighted imaging with 30 gradient directions and three b-values (TA = 8:34). The total examination time was approximately 45 minutes.

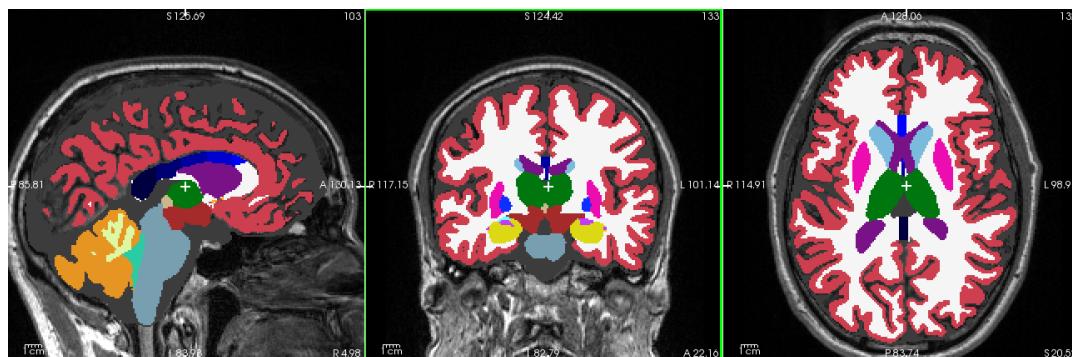
For the current morphometric analyses, we utilized only the high-resolution T1-weighted images, acquired using a 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) sequence. The acquisition parameters included a spatial resolution of 1.0 mm isotropic ( $1 \times 1 \times 1 \text{ mm}^3$ ) across 192 sagittal slices, with repetition time (TR) of 2500 ms, echo time (TE) of 2.26 ms, and inversion time (TI) of 900 ms. The field of view (FOV) was set to  $256 \times 256 \text{ mm}^2$  with a corresponding matrix size of  $256 \times 256$ , and parallel imaging was employed using GRAPPA with an acceleration factor of 2.

Figure 1 shows a representative T1-weighted image from our dataset, demonstrating the high tissue contrast necessary for accurate morphometric analysis. The corresponding FreeSurfer-generated segmentation mask, which forms the basis for our morphometric measurements, is illustrated in Figure 2. These images exemplify the quality standards maintained throughout our dataset.



**Figure 1.** 3D T1-weighted MPRAGE recording from BGA\_046. Panels left to right: Sagittal, Coronal, and Axial section, respectively.

Generated by: <https://github.com/arvid1/ibs-brain/blob/main/notebooks/01-freesurfer-freeview-t1-aseg-bga-046.ipynb>



**Figure 2.** The color-coded *aseg* segmentation mask by FreeSurfer 7.4.1 overlaid on 3D T1-w MPRAGE from *BGA\_046*. Panels left to right: Sagittal, Coronal, Axial section, respectively. The white cross is located in the medial part of left thalamus. Thalamus: green, Hippocampus: yellow, Caudate: light blue, Putamen: pink, Pallidum: purple, Cortex: red, White-Matter: white. See also the Appendix Fig. A2.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/01-freesurfer-freeview-t1-aseg-bga-046.ipynb>

### Brain Morphometry Analysis using FreeSurfer

Image processing and morphometric analyses were performed using FreeSurfer (<https://freesurfer.net>), a widely-validated open-source software suite for analyzing brain MRI data [30]. To address both methodological and biological questions, we conducted parallel analyses using two FreeSurfer versions: version 6.0.1, which was employed in the reference study by Skrobisz et al. [23], and the current version 7.4.1.

The evolution of FreeSurfer's capabilities is particularly relevant to our investigation of brain structure in IBS. Version 7.0 (July 2020) introduced significant improvements in subcortical segmentation accuracy, while version 7.4.1 (June 2023) further enhanced the precision of limbic system structures, notably the hippocampus and amygdala. Additionally, version 7.4.1 provides superior compatibility with multi-modal imaging data and implements refined longitudinal processing algorithms. Since our multimodal MRI examinations were part of a longitudinal IBS intervention study (Berentsen et al. [24]), we also used the longitudinal stream capability of FreeSurfer 7.4.1 to compare baseline longitudinal analysis with a cross-sectional analysis of the first MRI examination.

For both versions, we focused on the automated segmentation of subcortical structures using FreeSurfer's *aseg* pipeline, which identifies and quantifies the volume of distinct brain regions (detailed in Table A.1). This dual-version approach serves two purposes: first, it enables direct comparison with Skrobisz et al.'s [23] findings, and second, it allows us to assess the impact of software evolution on morphometric measurements on a fixed dataset, and differences in cross-sectional and longitudinal stream analysis to discriminate HC and IBS from brain morphometric features. This methodological consideration is crucial, as previous studies have demonstrated that version-dependent variations in automated segmentation can significantly influence morphometric results [31–36]. By analyzing our data with both versions, we can distinguish between genuine biological differences and methodologically-induced variations in brain morphometry.

The enhanced accuracy of version 7.4.1 is particularly relevant for our investigation of IBS, as it provides more reliable quantification of brain regions implicated in visceral sensation, pain processing, emotional regulation, and cognitive function.

We will also like to add that *in vivo* brain segmentation technologies move very fast. Recently (November 2024), the FreeSurfer 8.0.0-beta version enables histological super granularity with identification and volume measurements from more than 300 distinct regions per hemisphere (cf. Fig. A2). The *aseg* mask provides less than 40 brain regions and their volumes within the intracranial space.

### Statistical Analysis and Machine Learning Approaches

All analyses were implemented in Python (version 3.10), with complete computational workflows and reproducibility materials available in our public GitHub repository

(<https://arvidl.github.com/ibs-brain>). Our analytical approach combined traditional statistical methods with advanced machine learning techniques, employing both parametric and non-parametric approaches as appropriate for the data distributions.

For group comparisons, statistical significance was assessed using a threshold of  $p < 0.05$ , with Bonferroni correction applied to control for multiple comparisons. Effect sizes were quantified using Cliff's Delta [37], a robust non-parametric measure particularly suitable for non-normally distributed data [38]. Following established conventions, we interpreted Cliff's Delta (absolute) values as negligible (0.00-0.14), small (0.15-0.33), medium (0.34-0.47), or large (0.48-1.00).

Relationships between variables were evaluated using Spearman's rank correlation coefficient ( $\rho$ ), chosen for its robustness to non-normality and ability to capture monotonic relationships [39]. Correlation strengths were classified as weak (0.20-0.39), moderate (0.40-0.59), strong (0.60-0.79), or very strong (0.80-1.00). Values below 0.20 were considered negligible to minimize the risk of over-interpreting weak associations.

To ensure reproducibility and transparency, all analysis scripts, including data preprocessing steps, statistical analyses, and visualization code, are documented in Jupyter notebooks accessible through our GitHub repository. These notebooks provide detailed documentation of parameter choices, statistical assumptions, and analytical decisions.

Our analysis strategy addressed four interconnected research objectives, progressing from replication to more advanced multivariate approaches:

#### *Research Objectives and Analytical Approach*

##### **A - Replication Analysis :**

Is it possible to replicate the morphometric findings in Skrobisz et al. [23] regarding IBS versus HC discrimination, using the same FreeSurfer-derived features and the same FreeSurfer version?

- (i) By employing a feature-by-feature (univariate) comparison incorporating effect size?
- (ii) By employing a novel consistency score, combining several metrics for replication assessment?

##### **B - Software Version Comparison :**

Are there IBS versus HC disparities in morphometric feature values between FreeSurfer 6.0.1 and FreeSurfer 7.4.1 applied to the same set ( $n = 78$ ) of T1-weighted recordings in our Bergen cohort?

What is the difference in the results between FreeSurfer 7.4.1 cross-sectional analysis versus FS 7.4.1 longitudinal stream?

- (i) By employing a feature-by-feature comparison?
- (ii) Employing a multivariate comparison, incorporating covariance structures in the morphometric features?

##### **C - Morphometric Classification Analysis :**

Is it possible to separate IBS individuals from HC based on morphometric features?

- (i) By employing a feature-by-feature comparison (FS 7.4.1)?
- (ii) Employing a multivariate comparison, incorporating covariance structures in the morphometric features?
- (iii) By predicting IBS versus HC from the morphometric features using a machine learning framework (ML)?
- (iv) Identifying the importance of morphometric measures in the model with the best prediction?

<b>D - Integrated Morphometric-Cognitive Analysis :</b>	262
Would adding cognitive performance as a predictor improve the accuracy of separating IBS from HC?	263
(i) By employing a feature-by-feature comparison?	265
(ii) Employing a multivariate comparison, incorporating covariance structures in the cognitive features?	266
(iii) By predicting IBS versus HC from morphometric and cognitive characteristics using a machine learning framework (ML)?	269
(iv) Identifying the importance of morphometric and cognitive measures included in the model with the best prediction?	270

This hierarchical analytical framework progresses from basic replication to more advanced multivariate approaches, enabling both methodological validation and novel insights into IBS-related brain structure and function.

#### *Statistical Analysis Framework*

Given the complexity of our research questions and the combination of traditional and advanced analytical methods, we implemented a comprehensive statistical framework encompassing both univariate and multivariate approaches. Here we detail our analytical strategy and its methodological justification.

#### *Exploratory and Univariate Analyses*

Initial analyses followed established protocols, as in [23], beginning with exploratory data analysis of numerical features and cross-tabulation of categorical variables (Group: HC/IBS; Sex: F/M). For univariate comparisons (Objectives A-D), we employed both parametric (independent t-tests) and non-parametric (Mann-Whitney U) tests, depending on normality assessments. Multiple comparison correction used the Bonferroni method, and *effect sizes* were quantified using Cohen's d (for parametric tests) and Cliff's delta [37], else. Cliff's delta ( $\delta$ ) between two groups X and Y is defined as  $\delta = \frac{U}{n_x n_y} - 0.5$ , where  $U$  is the Mann-Whitney U statistic,  $n_x$  is the number of observations in group X, and  $n_y$  is the number of observations in group Y. The resulting Cliff's delta ( $\delta$ ) ranges from -1 to +1, where  $\delta = +1$  indicates that all values in group X are greater than all values in group Y,  $\delta = -1$  indicates that all values in group X are less than all values in group Y, and  $\delta = 0$  indicates complete overlap between the two groups.

#### *Permutation Testing*

To address small sample sizes and potential non-normal distributions, we employed permutation testing (1,000 iterations) to assess statistical significance. For each test, we computed an observed test statistic (sum of squared differences between group means) and generated a null distribution by randomly reassigning group labels. The empirical p-value was calculated as the proportion of permuted statistics exceeding the observed value. This non-parametric approach provides robust statistical inference while naturally controlling for multiple comparisons.

#### *Multivariate Approaches - assessing multivariate normality*

For multivariate analyses (Objectives B-D), we first assessed multivariate normality using two complementary methods: Mardia's test and the more comprehensive Henze-Zirkler's test (see Appendix A.2 for details).

#### *Advanced Distance Metrics*

The Mahalanobis distance [40] quantifies the distance between a point  $P$  and a distribution  $D$  while accounting for data correlations [41]. Unlike Euclidean distance, it

incorporates the covariance structure through the formula  $D = \sqrt{(x - \mu)^T \Sigma^{-1} (x - \mu)}$ , where  $x$  represents the data point,  $\mu$  is the mean vector, and  $\Sigma^{-1}$  is the inverse covariance matrix.

*Remark:* While Cohen's  $d$  ( $d = \frac{\mu_1 - \mu_2}{\sigma_{pooled}}$ ) measures standardized univariate group differences, the Mahalanobis distance extends this concept to multivariate space. In comparing IBS and HC groups, the squared Mahalanobis distance relates proportionally to Hotelling's  $T^2$  statistic, a multivariate analog of the squared t-statistic. Unlike Cohen's  $d$ , which has standardized effect size interpretations (small: 0.2, medium: 0.5, large: 0.8), Mahalanobis distance interpretation depends on data dimensionality and covariance structure. To handle outliers and non-normality common in neuroimaging data, we implemented a *robust Mahalanobis distance*. This modification employs winsorization (clipping values at 10th/90th percentiles) and replaces arithmetic means with medians (see Appendix A.3).

### Prediction of class belonging using machine learning

In tasks **C(iii)** and **D(iii)** we applied a comprehensive machine learning framework, utilizing morphometric features derived from FreeSurfer (aseg) to develop predictive models for two distinct classification tasks. We employed *PyCaret* (<https://pycaret.org>), an open-source, low-code machine learning library in Python, to develop and evaluate our classification models.

#### Machine Learning Model Development

Our machine learning approach followed a systematic protocol designed to ensure robust classification while addressing the challenges of limited sample size and potential overfitting. The analysis pipeline consisted of several carefully constructed stages optimized for neuroimaging data classification. Initial data preparation used a stratified sampling approach, partitioning the data set into training (70%) and testing (30%) sets while preserving the distribution of IBS/HC status across both partitions. This stratification was crucial for maintaining representative samples and ensuring valid model evaluation, particularly given our modest sample size and the inherent complexity of neuroimaging data. Model development utilized PyCaret's comprehensive machine learning framework to evaluate multiple classification algorithms, ranging from traditional approaches to advanced ensemble methods. The classifier suite included linear models (Logistic Regression with L1 and L2 regularization), non-linear algorithms (Support Vector Machines [SVM] with various kernels), tree-based methods (Random Forests, Gradient Boosting Machines including XGBoost [42] and LightGBM), and instance-based learners (K-Nearest Neighbors). This diverse algorithm selection allowed exploration of different decision boundaries and pattern of feature interaction.

To ensure robust model assessment and mitigate overfitting risks, we implemented a nested 10-fold cross-validation strategy for model selection. This approach provided unbiased performance estimates while preventing data leakage between model selection and evaluation phases. The final model selection prioritized both predictive performance and model interpretability, considering the clinical relevance of our findings. See Table A1 for an illustration.

#### Model Performance Assessment

To address the class imbalance between IBS and HC groups, we implemented multiple complementary performance metrics. While classification *accuracy* served as a baseline measure, we employed additional metrics such as: the *F1 score* (harmonic mean of precision and recall) to balance false positive and negative rates; the Receiver Operating Characteristic Area Under Curve (ROC-AUC) to assess discrimination ability across classification thresholds; and *Cohen's Kappa* [43] to evaluate classification agreement beyond chance-level performance.

We generated *confusion matrices* to examine error patterns and potential classification biases. For analyses incorporating cognitive function, we used macro-averaged versions of these metrics, ensuring equal weighting across performance levels despite uneven class distributions. Performance assessment followed a dual-track strategy, evaluating models on both cross-validated training data and the held-out test set. This approach enabled us to assess both learning capacity and generalization ability, crucial considerations for clinical applications.

#### *Feature Importance and Model Interpretability Analysis*

To understand how morphometric and cognitive features contribute to classification performance, we implemented two complementary approaches to feature importance analysis: permutation importance and SHAP (SHapley Additive exPlanations) values.

The *permutation importance* [44] analysis quantifies feature relevance by measuring model performance degradation when individual features were randomly permuted. Through multiple iterations per feature, we calculated the mean decrease in model performance, providing a model-agnostic measure of feature importance.

The *SHAP analysis*, grounded in cooperative game theory [45], provided both global and local interpretation frameworks. The global analysis aggregated SHAP values across cases to identify consistently important features, while the local analysis examined feature contributions to individual predictions. We visualized these results using SHAP summary plots, which integrated both magnitude and directionality of feature effects.

By combining permutation importance with SHAP analysis, we gained complementary insights into feature relevance: permutation importance revealed features critical to overall model performance, while SHAP analysis illuminated feature interactions and their contributions to specific predictions. This approach helped identify key neurobiological features distinguishing IBS patients from healthy controls, while exploring relationships between brain structure, sex differences, and cognitive function.

## Results

### *Sample Demographics and Clinical Characteristics*

The study enrolled 78 participants, comprising 49 patients with IBS and 29 healthy controls. Demographic analysis revealed comparable age distributions between groups (median age: IBS = 34 years, controls = 33 years). Female participants predominated in both cohorts, representing 77.6% (38/49) of the IBS group and 69.0% (20/29) of the control group, reflecting the typical gender distribution observed in IBS populations.

Symptom severity, quantified using the IBS Symptom Severity Scale (IBS-SSS), demonstrated clear differentiation between groups. The IBS cohort exhibited predominantly moderate to severe symptomatology, while healthy controls reported minimal gastrointestinal symptoms, aligning with our inclusion criteria. Six participants (three from each group) had missing IBS-SSS data, which we addressed through multiple imputation stratified by group and gender to maintain statistical robustness. Detailed demographic and clinical characteristics are presented in Table 2.

**Table 2.** Demographic and Clinical Characteristics of the Study Sample

Group	Age Median (IQR)	IBS_SSS Median (IQR)	Sex F/M (%)	N	Missing IBS_SSS
HC (N=29)	33.0 (23.0)	21.0 (30.0)	69.0/31.0	29	3
IBS (N=49)	34.0 (14.0)	264.0 (95.0)	77.6/22.4	49	3

Age is reported in years; IBS-SSS scores range from 0-500, with higher scores indicating greater symptom severity. IQR = Interquartile Range; F/M = Female/Male ratio expressed as percentages.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/02-demographics-and-clinical-characteristics.ipynb>

#### Replication analysis of Skrobisz (2022) using the Bergen cohort (with FS 6.0.1)

In our Bergen cohort, we sought to replicate the morphometric findings reported by Skrobisz et al. [23] comparing IBS patients with healthy controls. Table 3 presents our comparative analysis using identical methodological parameters: 35 estimated Total Intracranial Volume (eTIV)-normalized regional brain volumes derived from FreeSurfer 6.0, matching the analytical approach of the original study.

**Table 3.** Comparison of eTIV-normalized regional brain volumes between the two cohorts.

Brain Region	Skrobisz Cohort (FS 6.0)				Bergen Cohort (FS 6.0.1)			
	HC (N=19)	SD	IBS (N=20)	SD	HC (N=29)	SD	IBS (N=49)	SD
Left-Cerebellum-WM	0.00992	0.00113	0.00971	0.00107	0.01050	0.00092	0.01048	0.00092
Left-Cerebellum-Cortex	0.03628	0.00302	0.03553	0.00256	0.03894	0.00344	0.03931	0.00373
Left-Thalamus	0.00511	0.00037	0.00500	0.00024	0.00523	0.00046	0.00514	0.00039
Left-Caudate	0.00239	0.00025	0.00228	0.00021	0.00236	0.00026	0.00236	0.00031
Left-Putamen	0.00336	0.00033	0.00324	0.00028	0.00348	0.00038	0.00344	0.00039
Left-Pallidum	0.00140	0.00012	0.00135	0.00010	0.00140	0.00015	0.00137	0.00011
Left-Hippocampus	0.00270	0.00021	0.00272	0.00020	0.00291	0.00027	0.00290	0.00024
Left-Amygdala	0.00118	0.00013	0.00113	0.00015	0.00122	0.00010	0.00120	0.00010
Left-Accumbens-area	0.00031	0.00005	0.00034	0.00006	0.00043	0.00007	0.00042	0.00006
CSF	0.00061	0.00009	0.00060	0.00012	0.00067	0.00012	0.00070	0.00014
Right-Cerebellum-WM	0.00908	0.00106	0.00922	0.00100	0.00997	0.00089	0.00998	0.00085
Right-Cerebellum-Cortex	0.03652	0.00321	0.03616	0.00264	0.03972	0.00344	0.03998	0.00376
Right-Thalamus	0.00488	0.00030	0.00475	0.00024	0.00512	0.00044	0.00507	0.00036
Right-Caudate	0.00244	0.00024	0.00236	0.00024	0.00244	0.00024	0.00244	0.00030
Right-Putamen	0.00336	0.00030	0.00330	0.00028	0.00351	0.00037	0.00349	0.00035
Right-Pallidum	0.00136	0.00012	0.00133	0.00010	0.00132	0.00013	0.00130	0.00011
Right-Hippocampus	0.00282	0.00022	0.00285	0.00021	0.00301	0.00024	0.00298	0.00023
Right-Amygdala	0.00125	0.00012	0.00120	0.00012	0.00128	0.00009	0.00127	0.00010
Right-Accumbens-area	0.00034	0.00004	0.00036	0.00005	0.00043	0.00005	0.00043	0.00006
WM-hypointensities	0.00047	0.00015	0.00048	0.00013	0.00079	0.00031	0.00069	0.00025
CC_Posterior	0.00065	0.00013	0.00065	0.00010	0.00065	0.00010	0.00070	0.00011
CC_Mid_Posterior	0.00038	0.00007	0.00036	0.00007	0.00037	0.00007	0.00040	0.00007
CC_Central	0.00039	0.00009	0.00043	0.00008	0.00039	0.00009	0.00039	0.00010
CC_Mid_Anterior	0.00041	0.00009	0.00044	0.00013	0.00038	0.00008	0.00041	0.00011
CC_Anterior	0.00062	0.00010	0.00061	0.00008	0.00062	0.00010	0.00065	0.00010
BrainSegVol	0.75340	0.01784	0.74913	0.01647	0.80464	0.02487	0.80558	0.02397
BrainSegVolNotVent	0.74137	0.01880	0.73857	0.01836	0.79224	0.02511	0.79132	0.02490
lhCortexVol	0.15339	0.00620	0.15313	0.00871	0.16670	0.00800	0.16693	0.00951
rhCortexVol	0.15490	0.00690	0.15467	0.00859	0.16614	0.00828	0.16646	0.00939
CortexVol	0.30829	0.01298	0.30780	0.01715	0.33283	0.01611	0.33339	0.01880
lhCerebralWhiteMatterVol	0.15101	0.00748	0.15058	0.00742	0.15990	0.00858	0.15915	0.00876
rhCerebralWhiteMatterVol	0.15103	0.00757	0.15075	0.00727	0.15925	0.00829	0.15827	0.00938
CerebralWhiteMatterVol	0.30205	0.01500	0.30133	0.01461	0.31915	0.01678	0.31742	0.01808
SubCortGrayVol	0.03930	0.00194	0.03855	0.00162	0.04092	0.00258	0.04063	0.00236
TotalGrayVol	0.42105	0.01376	0.41884	0.01868	0.45307	0.02208	0.45396	0.02432

Note: All volumes are normalized to estimated total intracranial volume (eTIV)

HC = Healthy Controls; IBS = Irritable Bowel Syndrome; SD = Standard Deviation; WM = White Matter

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/03-replication-analysis-fs6.ipynb>

The volumetric comparison of brain structures between IBS patients and healthy controls across both cohorts reveals distinct patterns. While the Bergen cohort demonstrates

systematically larger volumes (6–8% for global measures, reaching up to 35% for specific structures such as the *nucleus accumbens*), the within-cohort comparisons between IBS and healthy control groups show remarkable consistency in global brain eTIV-normalized volumes. Specifically, BrainSegVol values remain nearly identical within each cohort (Skrobisz: HC  $0.753 \pm 0.018$ , IBS  $0.749 \pm 0.016$ ; Bergen: HC  $0.805 \pm 0.025$ , IBS  $0.806 \pm 0.024$ ). Cortical measurements demonstrate similar stability, with total cortical volume (CortexVol) showing minimal between-group differences in both cohorts. In subcortical structures, we observed subtle variations, notably a slight trend toward volume reduction in IBS patients' subcortical gray matter (SubCortGrayVol), though these differences remain within standard deviation bounds. White matter volumes maintain consistency between groups within cohorts, with an interesting pattern of white matter hypointensities emerging in the Bergen cohort. Corpus callosum segments exhibit relatively uniform volumes across all groups. Several methodological factors warrant consideration: the disparate cohort sizes (Skrobisz: HC  $n = 19$ , IBS  $n = 20$ ; Bergen: HC  $n = 29$ , IBS  $n = 49$ ), potential variations in FreeSurfer versions (6.0 versus 6.0.1), and differences in operating systems may contribute to the systematic volumetric differences observed between cohorts. While normalization to estimated total intracranial volume (eTIV) facilitates direct comparisons within cohorts by controlling for head size variation, it does not fully account for between-cohort differences.

Figure 3 presents a detailed reproducibility analysis, illustrating the differences in eTIV-normalized brain region volumes between HC and IBS across both cohorts. The plot contrasts effect sizes from the Skrobisz (2022) cohort (*x*-axis) against the Bergen cohort (*y*-axis), with the diagonal line representing perfect agreement. We employed Cohen's *d* values for region-wise effect size calculations, as the availability of only parametric summary statistics from the Skrobisz study precluded non-parametric effect size measures. For each eTIV-normalized brain region volume and cohort, we calculated the pooled standard deviation as:

$$s_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

where  $n_1$  and  $n_2$  are the sample sizes, and  $s_1$  and  $s_2$  are the standard deviations of the two groups, IBS and HC, respectively. Cohen's *d* effect size was then computed as:

$$d = \frac{\bar{x}_1 - \bar{x}_2}{s_p}$$

where  $\bar{x}_1$  and  $\bar{x}_2$  are the means of the two groups. The 95% confidence interval for *d* was calculated using:

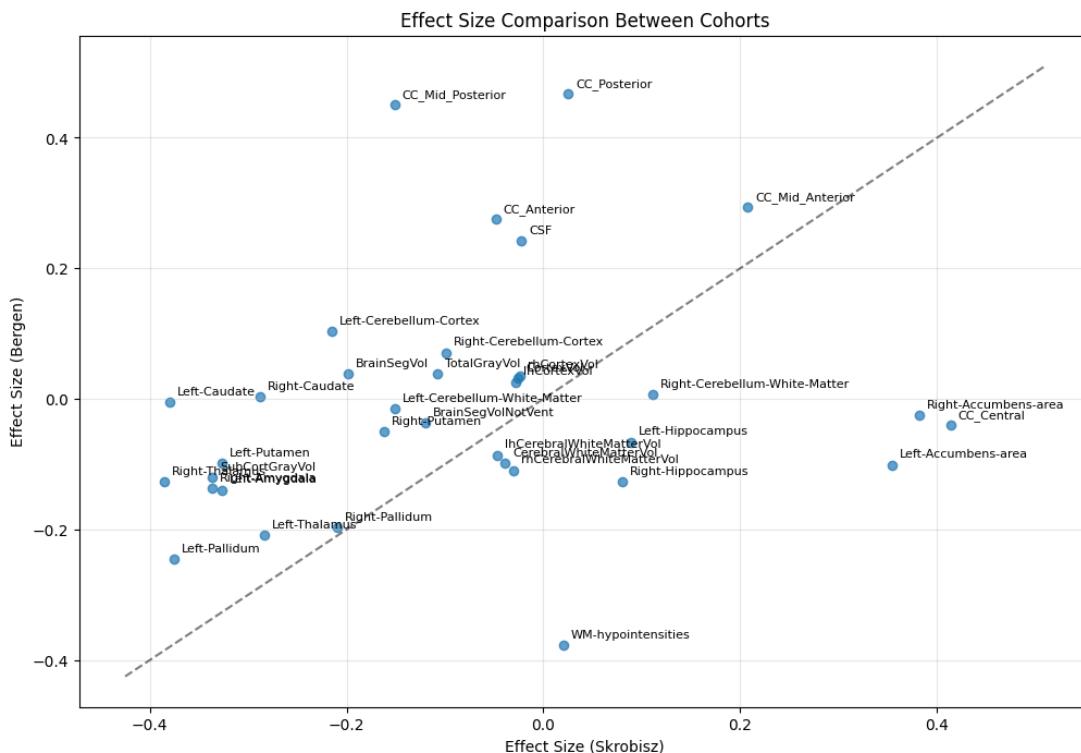
$$CI_{95\%} = d \pm 1.96 \sqrt{\frac{n_1 + n_2}{n_1 n_2} + \frac{d^2}{2(n_1 + n_2)}}$$

where the standard error term accounts for both sampling variance and uncertainty in the effect size estimate.

An overall reproducibility score (*S*) was developed for each brain region to quantify cross-cohort consistency through three complementary metrics: directional consistency ( $\sigma$ ), confidence interval overlap ( $\omega$ ), and effect magnitude ( $\epsilon$ ). The score is computed as:  $S = \sigma + \omega + \epsilon$ , where the binary indicator  $\sigma$  equals 1 if the direction of effect is consistent between cohorts and 0 otherwise, the binary indicator  $\omega$  equals 1 if the 95% confidence intervals overlap and 0 otherwise, and  $\epsilon$  represents the minimum absolute effect size observed across cohorts.

This composite metric prioritizes brain regions exhibiting robust cross-cohort replication, with  $\epsilon$  providing additional weight to stronger effects. Higher scores (*S*) indicate greater reproducibility of morphometric findings across independent study populations

and analysis pipelines, thereby establishing a quantitative framework for identifying the most reliable neuroanatomical alterations in IBS.

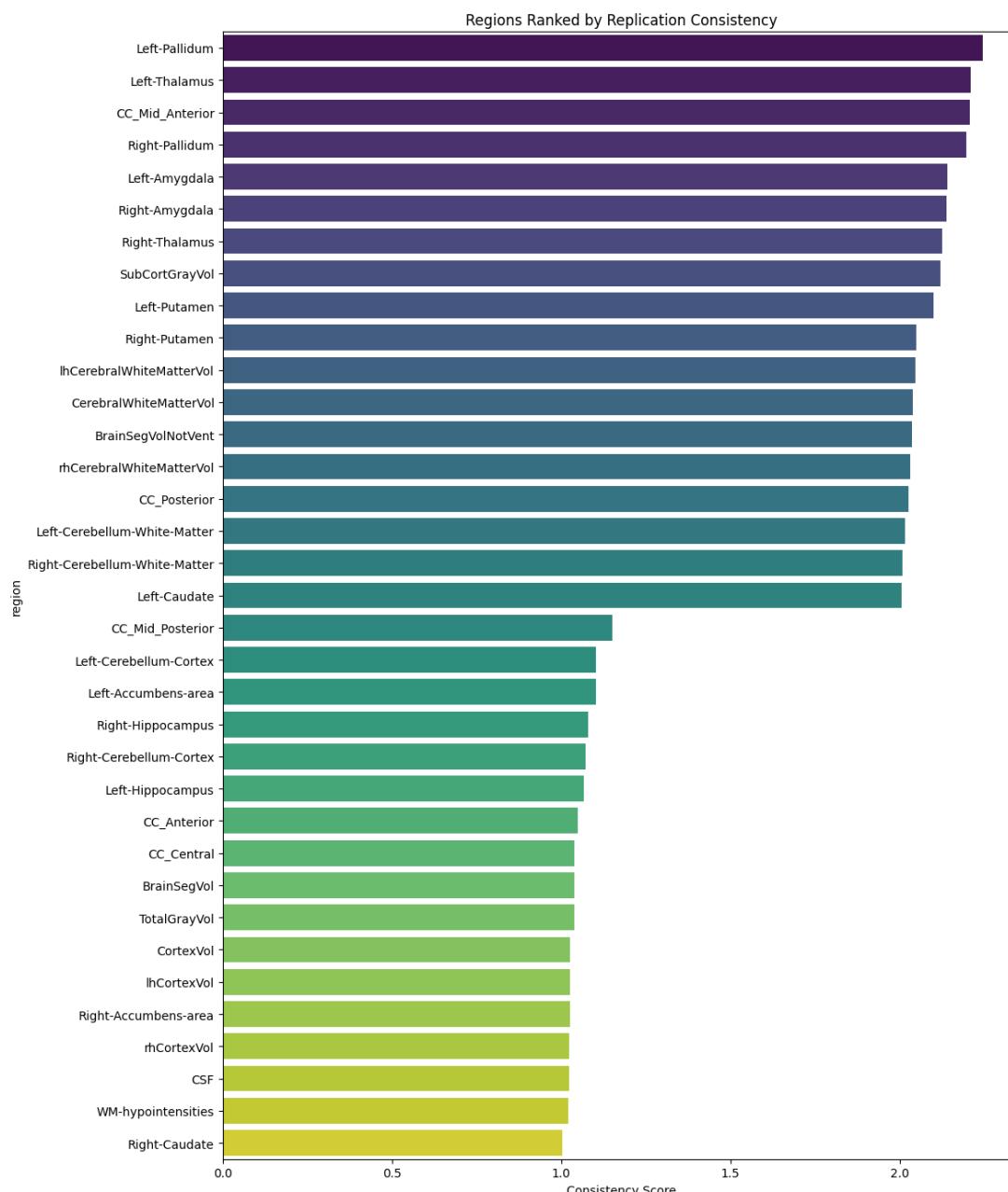


**Figure 3.** The large disparity of region-wise effect size of IBS versus HC in comparison between the Skrobisz (2022) cohort and the Bergen cohort. Scatterplot of calculated Cohen's d effect sizes for each region in both cohorts (see text for details).

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/03-replication-analysis-fs6.ipynb>

The effect size comparison between cohorts revealed moderate correlation ( $r = 0.203$ ,  $p = 0.243$ ). Directional consistency analysis demonstrated that 51.4% of brain regions maintained consistent IBS versus HC differences across cohorts. Notably, all brain regions exhibited overlapping 95% confidence intervals between cohorts, indicating that despite differences in point estimates, the between-cohort variations did not reach statistical significance given measurement uncertainty. Five regions demonstrated particularly strong cross-cohort consistency, achieving the highest overall reproducibility scores ( $S$ ): mid-anterior corpus callosum (CC\_Mid\_Anterior), Left-Pallidum, Left-Thalamus, Right-Pallidum, and Left-Amygdala. These structures showed overall scores ranging from 2.14 to 2.26, suggesting robust replication of IBS-related alterations. Conversely, several regions exhibited marked between-cohort divergence. White matter hypointensities demonstrated particularly discordant effects, while specific corpus callosum segments (CC\_Posterior and CC\_Mid\_Posterior) showed stronger effects in the Bergen cohort. Cerebellar regions clustered near the origin, indicating consistently modest effects across both cohorts. The overall pattern suggests limited agreement between cohorts in IBS-related brain alterations. While specific structures show robust reproducibility, the widespread dispersion around the diagonal reference line, coupled with moderate correlation, indicates substantial heterogeneity in morphometric findings between these independent samples. This variability may reflect genuine biological heterogeneity in IBS-related brain alterations or methodological differences between studies.

Figure 4 plots a ranking of brain regions on how consistently they show similar patterns between the cohorts.



**Figure 4.** Brain regions ranked by cross-cohort reproducibility scores. The composite score ( $S$ ) integrates directional consistency ( $\sigma$ ), confidence interval overlap ( $\omega$ ), and effect magnitude ( $\epsilon$ ) between the Skrobisz and Bergen cohorts. Higher scores indicate greater reproducibility of IBS-related volumetric alterations across independent samples. See text for detailed scoring methodology.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/03-replication-analysis-fs6.ipynb>

The reproducibility analysis revealed varying degrees of cross-cohort consistency in brain structural alterations associated with IBS. Several regions demonstrated robust reproducibility, with the Left-Pallidum, Left-Thalamus, and CC\_Mid\_Anterior achieving overall scores ( $S$ ) exceeding 2.0. These high-scoring regions exhibited both directional consistency and complete confidence interval overlap, coupled with substantial effect magnitudes, suggesting reliable IBS-related volumetric alterations across independent samples. Conversely, regions including the Right-Caudate, Right-Cerebellum-Cortex, Left- and Right-Hippocampus, CC\_Mid\_Posterior, and Left-Cerebellum-Cortex showed lower reproducibility (scores approximately 1.1). While these regions maintained confidence interval overlap, they lacked directional consistency between cohorts, suggesting greater

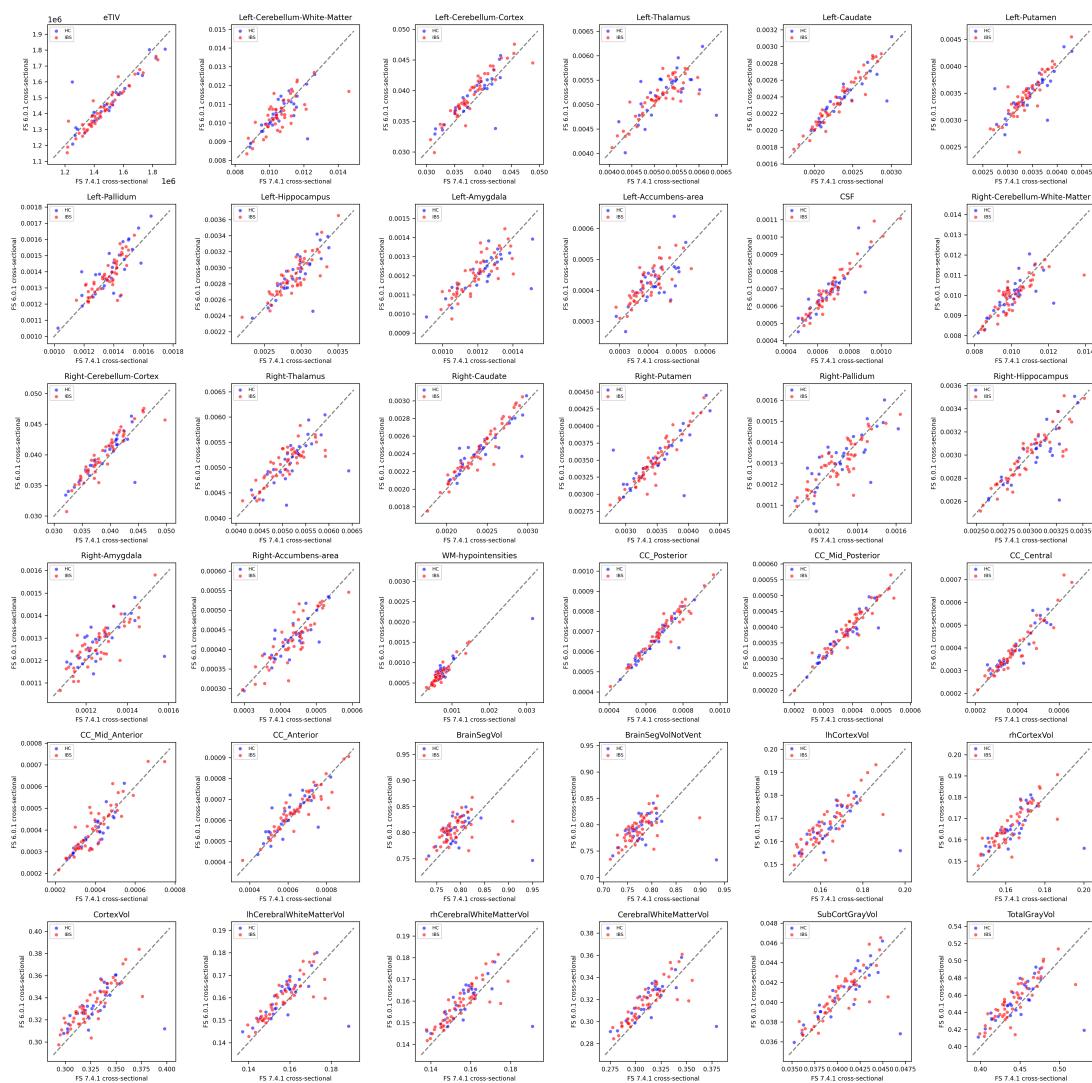
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variability in IBS-related effects. Despite systematic between-cohort differences in eTIV-normalized volumes, certain regions demonstrated consistent relative patterns of alteration. However, our attempt to replicate the specific morphometric differences reported by Skrobisz et al. (2022) yielded limited success. This suggests that structural brain alterations in IBS may be more heterogeneous than previously recognized, potentially reflecting the complex nature of IBS pathophysiology or methodological variations across studies.

To assess the robustness of brain morphometry measurements in IBS research, we conducted a comprehensive analysis of the Bergen cohort data using multiple FreeSurfer processing pipelines. This systematic evaluation examined the stability of morphometric measurements and IBS versus healthy control (HC) group differences across different analytical approaches: FreeSurfer versions (6.0.1 versus 7.4.1) and processing streams within FreeSurfer 7.4.1 (cross-sectional versus longitudinal). Our interventional study design enabled the application of the longitudinal processing stream, providing an additional dimension for assessing measurement reliability. Unlike our previous replication analysis of the Skrobisz (2022) cohort, which relied on summary statistics, this comparison utilized complete morphometric data from all participants, allowing for more detailed assessment of measurement consistency.

#### *Cross-Version Comparison of FreeSurfer Morphometric Measurements*

We examined the consistency of volumetric measurements between FreeSurfer versions 6.0.1 and 7.4.1 (cross-sectional stream) in quantifying brain structural differences between IBS patients and healthy controls (HC). Table A2 in the Appendix presents group-wise summary statistics (mean and standard deviation) for both IBS patients and healthy controls, derived from the `aseg.stats` files generated by each FreeSurfer version. Figure 5 presents a scatter plot matrix illustrating version-wise comparisons for each brain region. Individual plots display FS 6.0.1 volumes against corresponding FS 7.4.1 measurements, with HC and IBS participants distinguished by blue and red markers, respectively. Reference identity lines facilitate direct assessment of cross-version measurement concordance.



**Figure 5.** Comparison of FreeSurfer-derived regional brain volumes across versions 6.0.1 and 7.4.1 (cross-sectional processing). Scatter plots show eTIV-normalized volumes for each brain region, with version 6.0.1 values on the *y*-axis versus version 7.4.1 on the *x*-axis. The eTIV-volume [ $\text{mm}^3$ ] is shown in the upper left panel. Blue and red markers denote healthy controls and IBS patients, respectively. Identity lines indicate perfect cross-version agreement. See text for detailed analysis.

Generated by: <https://github.com/arvid1/ibs-brain/blob/main/notebooks/04-comparing-FS-versions-on-same-dataset.ipynb>

The scatter plot matrix demonstrates varying degrees of consistency between FreeSurfer versions 6.0.1 and 7.4.1 across different brain regions. Subcortical regions, particularly the thalamus, caudate, putamen, and partly the hippocampus, show strong cross-version agreement with minimal deviation from the identity line. However, systematic differences emerge in several structures: the amygdala and the accumbens demonstrate moderate version-dependent variability, with data points showing systematic deviation from perfect concordance. Corpus callosum segments display region-specific variations in cross-version agreement, with CC\_Anterior and CC\_Mid\_Anterior showing more pronounced differences compared to other segments. Importantly, the distribution patterns of IBS (red) and healthy control (blue) groups remain fairly consistent across versions, suggesting that while absolute volume estimates may differ between FreeSurfer versions, the relative group differences are largely preserved.

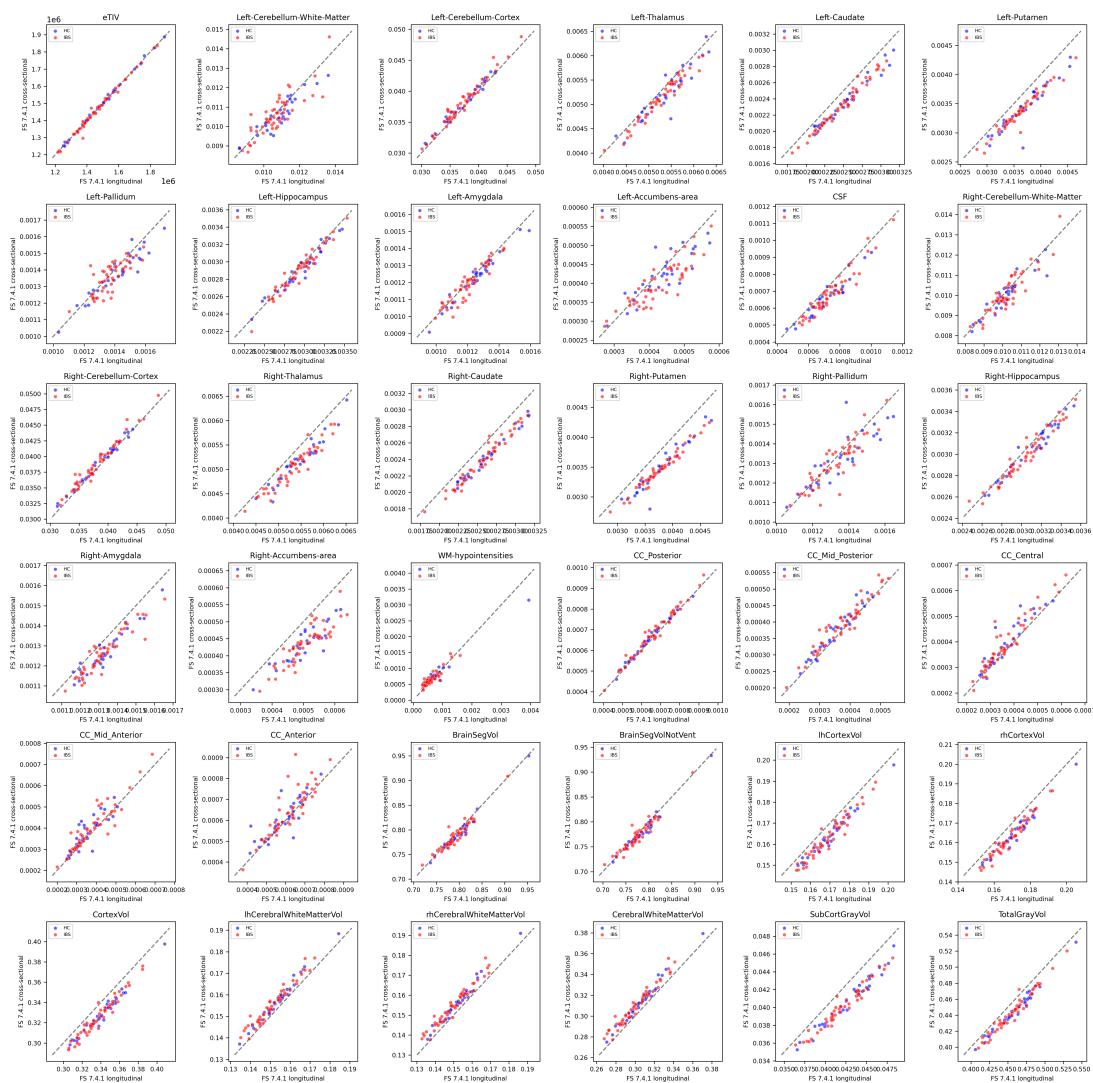
Notably, several regions exhibit strong correlations between versions but with systematic offsets from the identity line, indicating consistent biases between FreeSurfer versions

6.0.1 and 7.4.1. For example: The cortical measurements (lhCortexVol and rhCortexVol) and lh- and rhCerebralWhiteMatterVol and TotalGrayVol show a clear parallel offset above the identity line, indicating that FreeSurfer 6.0.1 consistently produces higher volume estimates compared to version 7.4.1. This systematic bias appears consistent across the full range of eTIV-normalized volumes and both subject groups. Similar parallel offsets are visible in Left- and Right-Cerebellum-Cortex and subcortical structures like the Left-Pallidum and Left-Caudate. Moreover, the eTIV shows systematic higher volumes in version 7.4.1 compared to version 6.0.1 measurements.

Several key structures exhibit individual outliers that warrant attention. In eTIV, a single measurement shows substantial deviation, suggesting potential segmentation challenges in this particular case. The Left- and Right-Hippocampus both show isolated outliers (visible as blue points) significantly deviating from the otherwise tight correlation pattern, indicating potential segmentation inconsistencies between versions for these specific control subjects. The Left-Thalamus displays a particularly notable outlier (blue point) that deviates substantially below the main correlation pattern, suggesting a case where version 7.4.1 produced a markedly lower volume estimate compared to version 6.0.1. Similar isolated discrepancies appear in both Left- and Right-Amygdala measurements, where single data points (again from the control group) deviate notably from the otherwise consistent version correlation. These individual outliers likely represent cases where the segmentation algorithms in the two FreeSurfer versions interpreted the anatomical boundaries differently, possibly due to image quality issues, anatomical variants, or differences in how the versions handle boundary cases. The fact that many of these outliers appear in the control group (blue points) suggests that these discrepancies are not specifically related to IBS pathology but rather to technical aspects of the segmentation process.

These observations underscore the importance of version consistency in morphometry-based classification studies and suggest that meta-analyses or multi-site studies should carefully account for FreeSurfer version effects in their analytical pipelines.

In this context, Figure 6 depicts a scatter plot matrix comparing brain region volumes between two pipelines (cross-sectional and the longitudinal stream) using the *same* FreeSurfer 7.4.1 version, highlighting potential discrepancies.



**Figure 6.** Comparison of regional brain volumes between FreeSurfer 7.4.1 cross-sectional and longitudinal processing streams. Scatter plots show eTIV-normalized volumes [eTIV in  $\text{mm}^3$ ] for each brain region, with cross-sectional values on the y-axis versus longitudinal values on the x-axis. Blue and red markers denote healthy controls and IBS patients, respectively. Identity lines indicate perfect cross-stream agreement. See text for detailed analysis.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/04-comparing-FS-versions-on-same-dataset.ipynb>

The comparison between FreeSurfer 7.4.1's cross-sectional and longitudinal processing streams reveals distinct patterns of agreement and systematic variation across brain regions. Global measurements (BrainSegVol, BrainSegVolNotVent) demonstrate strong cross-stream consistency, with tight clustering along the identity line. However, substantial systematic differences emerge in several key structures. Most notably, cortical volumes (lhCortexVol, rhCortexVol) exhibit a clear systematic bias, with longitudinal processing consistently producing higher volume estimates compared to the cross-sectional stream. This pattern contrasts with Left- and Right-Cerebellum-Cortex, where longitudinal processing yields systematically lower estimates. Subcortical structures display varying degrees of processing stream sensitivity: the putamen and caudate show consistent offsets from the identity line, while pallidum and accumbens measurements demonstrate greater scatter. Corpus callosum segments (CC\_Anterior, CC\_Mid\_Anterior, CC\_Central) reveal processing stream-dependent variations that differ from those observed in other structures. Looking at the eTIV plot in the top-left panel, it shows remarkably high consistency be-

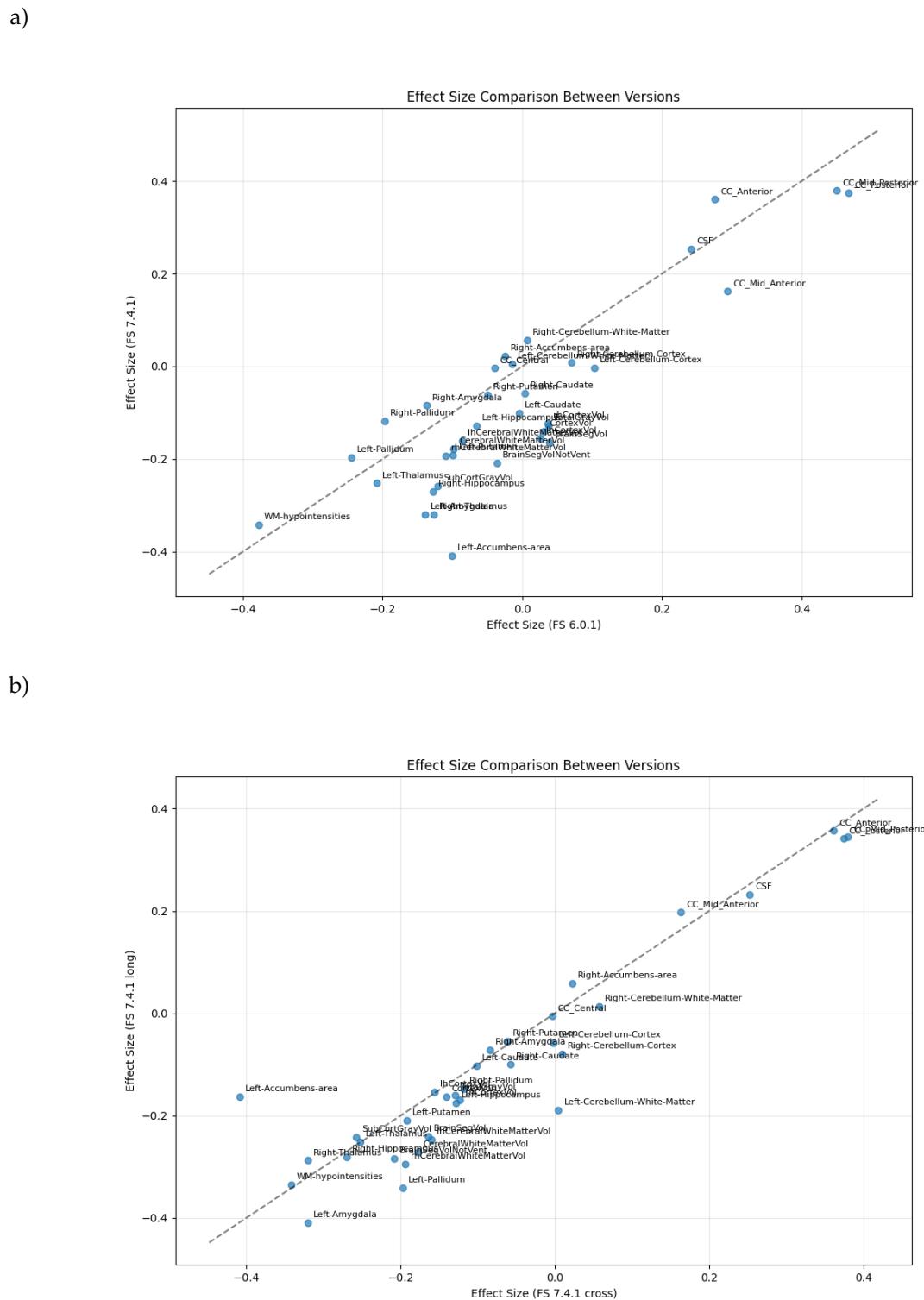
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tween cross-sectional and longitudinal processing streams. The data points cluster tightly along the identity line across the full range of values (approximately  $1.2\text{-}1.8 \times 10^6 \text{ mm}^3$ ), with minimal deviation. This strong agreement in eTIV estimations between processing streams is particularly noteworthy because eTIV serves as the normalization factor for all other volumetric measurements. The consistency suggests that any observed differences in other brain regions are not attributable to variations in total intracranial volume estimation between processing streams, but rather reflect genuine methodological differences in how the two streams segment specific structures.

Importantly, these systematic biases maintain consistency across both IBS and healthy control groups, as evidenced by the parallel patterns of red and blue markers. This indicates that while absolute volume estimates differ between processing streams, the relative group differences remain largely preserved. These findings underscore the critical importance of maintaining consistent processing stream selection when conducting cross-sectional comparisons or longitudinal analyses in clinical studies.

The summary statistics by the mean and standard deviation for Freesurfer v. 7.4.1 cross-sectional and v. 7.4.1 longitudinal stream, respectively, are shown in the Appendix as Table A3.

Figure 7 illustrates the differential impact of FreeSurfer processing choices on IBS versus healthy control effect sizes across brain regions. Panel (a) compares effect sizes between FreeSurfer versions 6.0.1 and 7.4.1 (cross-sectional), while panel (b) contrasts effect sizes derived from FreeSurfer 7.4.1's cross-sectional and longitudinal processing streams, enabling assessment of both version and pipeline-specific influences on group differences.



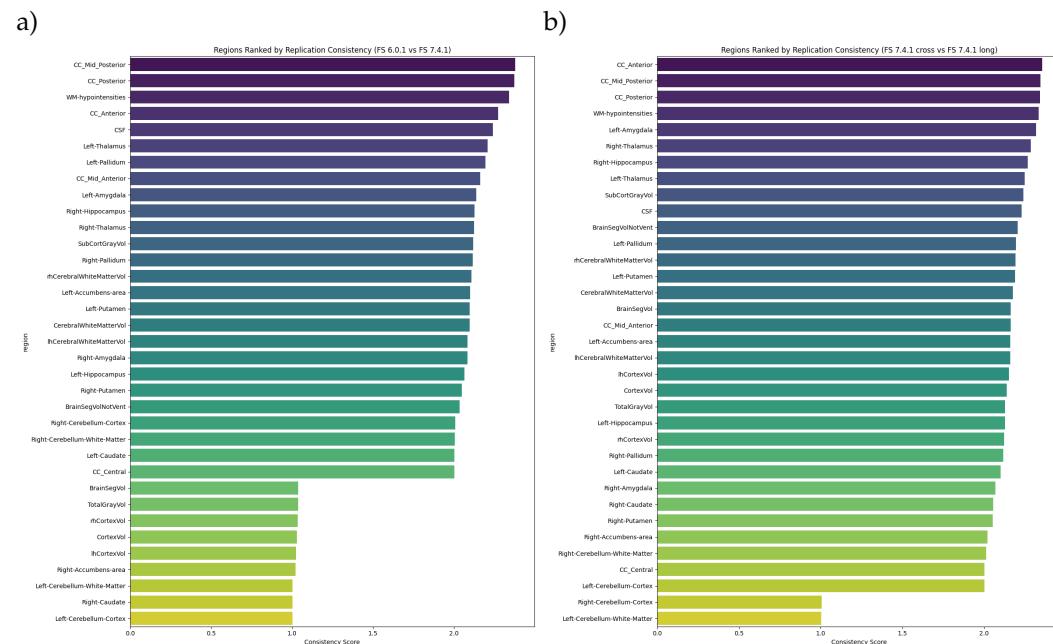
**Figure 7.** Effect sizes (Cohen's  $d$ ) of IBS versus healthy control group differences across brain regions: comparison of FreeSurfer methodological variants. a) Cross-sectional processing stream comparison between FreeSurfer versions 6.0.1 and 7.4.1. b) Processing stream comparison within FreeSurfer 7.4.1 (cross-sectional versus longitudinal). See text for detailed analysis.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/04-computing-FS-versions-on-same-dataset.ipynb>

The scatter plots reveal distinct patterns in how FreeSurfer methodological choices affect IBS versus healthy control effect sizes across brain regions. Panel (a), comparing

FreeSurfer versions 6.0.1 and 7.4.1 (cross-sectional), demonstrates moderate agreement with notable version-specific variations. Key corpus callosum segments (CC\_Anterior, CC\_Mid\_Posterior) show the strongest positive effect sizes (approximately 0.4) and maintain relative consistency across versions. In contrast, the Left-Accumbens-area exhibits the strongest negative effect (approximately -0.4), with its magnitude varying between versions. Panel (b), comparing cross-sectional and longitudinal streams within FreeSurfer 7.4.1, shows that corpus callosum segments maintain their position as regions with the strongest positive effects, while the Left-Amygdala and Left-Accumbens-area show pronounced negative effects. Most subcortical structures cluster more tightly around the diagonal compared to the version comparison in panel (a). The longitudinal versus cross-sectional comparison demonstrates greater overall consistency than the version comparison, as evidenced by tighter clustering along the diagonal reference line. This suggests that processing stream selection within FreeSurfer 7.4.1 introduces less variability in effect size estimates than version changes. However, specific regions, particularly in the limbic system, show sensitivity to processing stream choice. This systematic comparison highlights that while both FreeSurfer version and processing stream selection affect effect size estimates, version differences generally introduce more variability than processing stream choices within the same version.

Figure 8 quantifies the reproducibility of IBS versus healthy control group differences across brain regions under different FreeSurfer methodological variants. Panel (a) ranks regions by their effect size consistency ( $S$ ) between FreeSurfer versions 6.0.1 and 7.4.1 (cross-sectional), while panel (b) presents regional rankings based on effect size stability between cross-sectional and longitudinal processing streams within FreeSurfer 7.4.1, enabling systematic assessment of both version and pipeline-dependent variations.



**Figure 8.** Brain regions ranked by reproducibility of IBS versus healthy control differences across FreeSurfer methodological variants. Composite scores ( $S$ ) combine directional consistency ( $\sigma$ ), confidence interval overlap ( $\omega$ ), and effect magnitude ( $\epsilon$ ). Panel (a) compares FreeSurfer versions 6.0.1 and 7.4.1 (cross-sectional); panel (b) contrasts cross-sectional and longitudinal processing streams within FreeSurfer 7.4.1. See text for detailed analysis.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/04-computing-FS-versions-on-same-dataset.ipynb>

The regional consistency scores reveal distinct patterns in how FreeSurfer methodological choices affect the reproducibility of IBS versus healthy control differences. Panel (a),

comparing FreeSurfer versions 6.0.1 and 7.4.1 (cross-sectional), shows a gradual distribution of consistency scores ranging from 1.0 to 2.5. Corpus callosum regions (CC\_Mid\_Posterior, CC\_Posterior) demonstrate the highest consistency, while cerebellar structures show the lowest. Subcortical regions exhibit intermediate consistency, suggesting moderate stability across FreeSurfer versions. Panel (b), comparing cross-sectional and longitudinal streams within FreeSurfer 7.4.1, reveals a more distinct clustering pattern. The CC\_Anterior and CC\_Mid\_Posterior maintain high consistency, but notably, limbic structures like the Left-Amygdala and Right-Thalamus show improved consistency compared to their version-wise rankings. This suggests that these regions are more sensitive to FreeSurfer version changes than to processing stream selection. The overall pattern indicates stronger methodological stability when varying processing streams within FreeSurfer 7.4.1 compared to cross-version analyses. Importantly, comparing these methodological variations within the same cohort yields higher consistency scores than the previous cross-cohort comparison (Fig. 4), highlighting the substantial impact of cohort-specific factors on brain morphometric findings in IBS research.

#### Multivariate analyses: IBS versus HC

The multivariate normality of brain structural data was assessed across three FreeSurfer processing streams using Mardia's test (examining skewness and kurtosis) and the Henze-Zirkler's test. For FS 6.0.1, Mardia's test revealed significant deviations in both skewness ( $b_{1,p} = 2.33 \times 10^{14}$ ,  $p < 0.001$ ) and kurtosis ( $b_{2,p} = -8.77$ ,  $p < 0.001$ ) for the full sample, with similar patterns in the IBS group but different skewness characteristics in the HC group. For FS 7.4.1 cross-sectional, both groups showed significant non-normality, with particularly extreme values in the IBS group (kurtosis statistic = 153.63,  $p < 0.001$ ). The FS 7.4.1 longitudinal analysis also indicated significant departures from multivariate normality across all groups. The Henze-Zirkler's test showed some numerical instability issues, evidenced by extreme values and negative test statistics, suggesting that its results should be interpreted with caution. Overall, these findings consistently indicate significant departures from multivariate normality across all FreeSurfer versions and subject groups, with particularly pronounced effects in the IBS group. This suggests that robust statistical methods should be employed for subsequent analyses of group differences in brain structure.

In this context, the robust Mahalanobis distance analysis was implemented to quantify the multivariate separation between IBS and HC groups across different FreeSurfer processing streams while accounting for potential outliers and non-normality in the neuroimaging data. The computation employs winsorization at the 10th and 90th percentiles to mitigate the impact of extreme values, followed by robust location estimation using medians instead of means. The analysis revealed decreasing Mahalanobis distances across FreeSurfer versions: FS 6.0.1 showed the largest separation ( $D = 9.348$ ,  $F = 0.598$ ,  $p = 0.939$ ), followed by FS 7.4.1 cross-sectional ( $D = 6.068$ ,  $F = 0.252$ ,  $p \approx 1.000$ ) and FS 7.4.1 longitudinal ( $D = 5.163$ ,  $F = 0.183$ ,  $p \approx 1.000$ ). However, none of these distances reached statistical significance (all  $p > 0.05$ ), suggesting that the multivariate brain volume differences between IBS and HC groups are not statistically meaningful across any of the FreeSurfer processing streams. The consistently high p-values and low F-statistics indicate that, despite the apparent numerical differences in Mahalanobis distances, there is insufficient evidence to conclude that the IBS and HC groups differ significantly in their multivariate brain volume profiles. This analysis, incorporating 35 brain regions and accounting for their covariance structure, suggests that the volumetric differences between IBS and HC groups are not robust enough to clearly distinguish between the groups in a multivariate framework.

To further investigate potential group differences beyond the initial Mahalanobis distance analysis, we employed a machine learning framework with cross-validation to assess IBS versus healthy control discriminability and identify the most diagnostically relevant brain structures. This complementary approach enables systematic evaluation of

multivariate patterns while accounting for potential interactions between brain regions.

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### Machine Learning-Based Classification Using Brain Morphometry

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We evaluated the discriminative power of brain morphometric features for IBS versus healthy control classification using the PyCaret machine learning library. Multiple classification algorithms were trained and compared (Appendix Fig. A1) using FreeSurfer 7.4.1 longitudinal stream measurements from the Bergen cohort (Table 2). We applied a binary classification framework to distinguish between healthy controls (0) and IBS patients (1) based on brain morphometric features. The dataset comprised 78 participants characterized by 37 numerical features, partitioned into training ( $n=54$ ) and test ( $n=24$ ) sets. We employed stratified 10-fold cross-validation to maintain consistent class proportions across folds. Feature preprocessing included mean-based imputation and standardization to zero mean and unit variance, particularly crucial for features with widely differing scales (e.g., raw eTIV values  $> 1.2 \cdot 10^6$  versus eTIV-normalized measures  $< 1$ ). Given the modest dataset size, analyses were performed using CPU computation. All random processes were controlled through a fixed session identifier to ensure reproducibility.

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Model performance evaluation across 15 classification algorithms revealed Extreme Gradient Boosting (XGBoost) as the superior approach for IBS versus healthy control discrimination based on brain morphometry (details in Fig A1). XGBoost achieved the highest performance metrics: accuracy (0.72), AUC (0.68), recall (0.72), precision (0.74), and F1 score (0.71). The model's Cohen's Kappa (0.40) and Matthews Correlation Coefficient (0.42) indicate substantial improvement over chance-level classification. K-Nearest Neighbors demonstrated the second-best performance, while Logistic Regression and Support Vector Machines showed moderate discriminative ability. Several algorithms, including AdaBoost and Linear Discriminant Analysis, performed near chance level, as benchmarked against a dummy classifier baseline. XGBoost's superior performance suggests its ability to capture complex, nonlinear relationships in brain morphometric features that distinguish IBS from healthy controls.

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The best-performing model (XGBoost) demonstrated mixed classification performance on the hold-out test set, as shown in Figure 9a. The model correctly identified 73% of IBS patients (11/15 cases; 8 female, 3 male; IBS-SSS:  $245.7 \pm 60.4$ ; age:  $33.2 \pm 7.6$ ). However, specificity was low at 11%, with 8 of 9 healthy controls misclassified as IBS (3 female, 5 male; IBS-SSS:  $19.2 \pm 19.6$ ; age:  $25.4 \pm 5.7$ ), yielding an overall accuracy of 50% (12/24). This asymmetric performance reveals systematic patterns: correctly classified IBS patients showed higher symptom severity scores (IBS-SSS), female predominance, and higher mean age compared to misclassified controls. The strong bias toward IBS classification suggests that while brain morphometric features contain discriminative information, additional refinement is needed for reliable diagnostic application.

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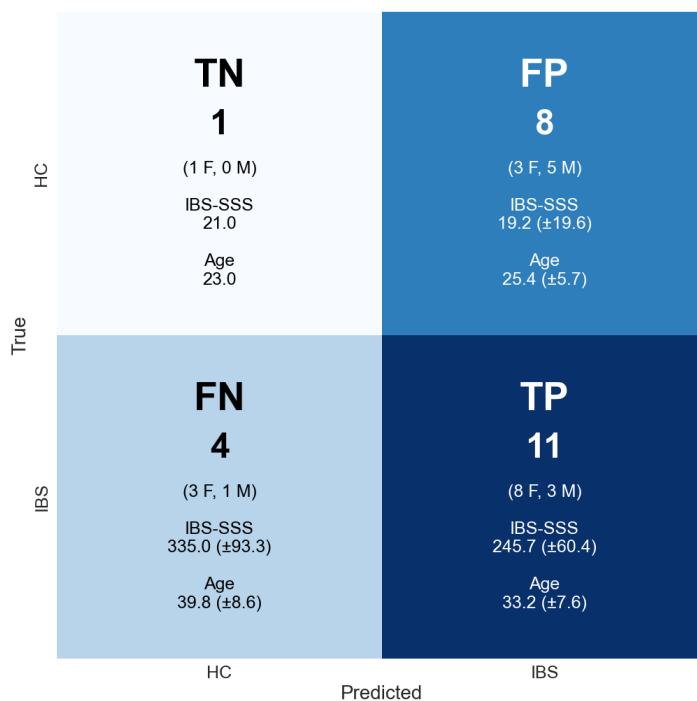
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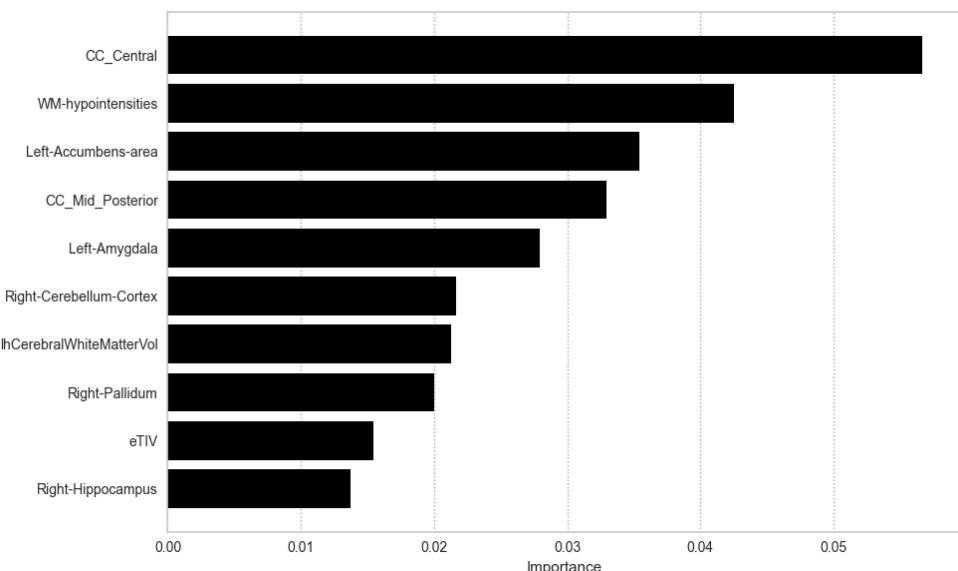
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**Figure 9.** Machine learning-based discrimination between IBS and healthy controls using brain morphometry. a) Confusion matrix showing prediction outcomes from XGBoost classification on the test dataset, with quadrants indicating true negatives (TN), false positives (FP), false negatives (FN), and true positives (TP). b) Ten most discriminative brain regions identified through permutation importance analysis in the XGBoost model.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/05-predicting-IBS-vs-HC-from-morphometric-measures.ipynb>

Permutation importance analysis revealed the relative contribution of brain regions to IBS versus healthy control classification. The central corpus callosum (CC\_Central) 728

emerged as the most discriminative feature ( $\approx 0.057 \pm 0.038$ ), followed by white matter hypointensities ( $\approx 0.043 \pm 0.029$ ) and the left nucleus accumbens ( $\approx 0.035 \pm 0.040$ ). A second tier of discriminative regions includes the mid-posterior corpus callosum ( $\approx 0.033 \pm 0.029$ ) and left amygdala ( $\approx 0.028 \pm 0.045$ ), while cerebellar structures showed moderate importance (right cerebellar cortex ( $\approx 0.022 \pm 0.026$ )). Notably, several traditionally studied regions in IBS, including the hippocampus ( $\approx 0.014 \pm 0.045$ ) and total intracranial volume ( $\approx 0.015 \pm 0.028$ ), demonstrated relatively lower discriminative power. This hierarchy suggests that white matter structures, particularly corpus callosum segments, may play a more prominent role in IBS-related brain alterations than previously recognized. However, the permutation importance ranking should be interpreted cautiously given the large standard deviations and the model's modest classification performance (50% accuracy, 73% sensitivity but only 11% specificity). While the ranking identifies features that contribute most to the model's decisions, these contributions come from a model that shows strong bias toward IBS classification and poor discriminative ability for healthy controls.

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**Figure 10.** SHAP values with morphometry as predictors. The SHAP values (x-axis) indicate the impact each feature (y-axis) has on the model's output, which is the probability of classifying someone as IBS patient or HC. Values to the right of 0 indicate a positive contribution (towards one class, likely HCs in this case). Values to the left indicate a negative contribution (likely towards IBS). Color gradient (Feature Value): This represents the actual feature value: Red/pink indicates a high feature value. Blue/purple indicates a low feature value.

Figure 10 shows SHAP values, which measure how different brain regions impact a predictive model's output. The right-hemispheric structures (caudate, hippocampus) appear most important for whatever outcome the model is predicting, suggesting these areas might deserve particular attention in patient assessment. There is also a pattern suggesting the basal ganglia network as a whole as important for the model's predictions, with right-hemispheric structures (especially caudate) showing stronger effects than left-hemispheric ones. Other subcortical structures like the amygdala and hippocampus also show notable effects, supporting a broader subcortical involvement in predicting IBS versus HC.

#### Univariate analysis of the cognitive features

Analysis of RBANS scores between Healthy Controls (HC) and IBS patients, using Bonferroni-corrected Mann-Whitney U tests ( $\alpha = 0.05$ ), reveals significant differences in cognitive performance across specific domains. Note that Table 4 shows the uncorrected p-values,

where we multiply by 5 to get the corrected values. The Fullscale RBANS demonstrates a significant difference ( $p_{corrected} = 0.012$ ) between HC (median = 103.0, IQR = 93.0-108.0) and IBS patients (median = 91.0, IQR = 85.0-100.0), with a small to moderate effect size (Cliff's  $\delta = 0.213$ ). Similarly, the Recall Index shows a significant difference ( $p_{corrected} = 0.036$ ) between HC (median = 107.0, IQR = 92.0-113.0) and IBS patients (median = 95.0, IQR = 85.0-100.0), also with a small to moderate effect size (Cliff's  $\delta = 0.186$ ). The Memory Index, while showing a small effect size (Cliff's  $\delta = 0.147$ ), does not reach statistical significance after correction ( $p_{corrected} = 0.186$ ). The remaining cognitive domains show minimal differences between groups: Visuospatial Index ( $p_{corrected} = 1.000$ ,  $\delta = 0.021$ ), Verbal skills Index ( $p_{corrected} = 0.522$ ,  $\delta = 0.116$ ), and Attention Index ( $p_{corrected} = 0.708$ ,  $\delta = 0.107$ ). These findings suggest that IBS patients demonstrate significantly lower overall cognitive function and recall abilities compared to healthy controls, while other cognitive domains such as visuospatial, verbal, and attention skills remain relatively preserved. The use of Bonferroni correction strengthens the robustness of these findings by controlling for multiple comparisons, though it may increase the risk of Type II errors.

**Table 4.** A non-parametric analysis comparing cognitive features in the IBS and HC groups

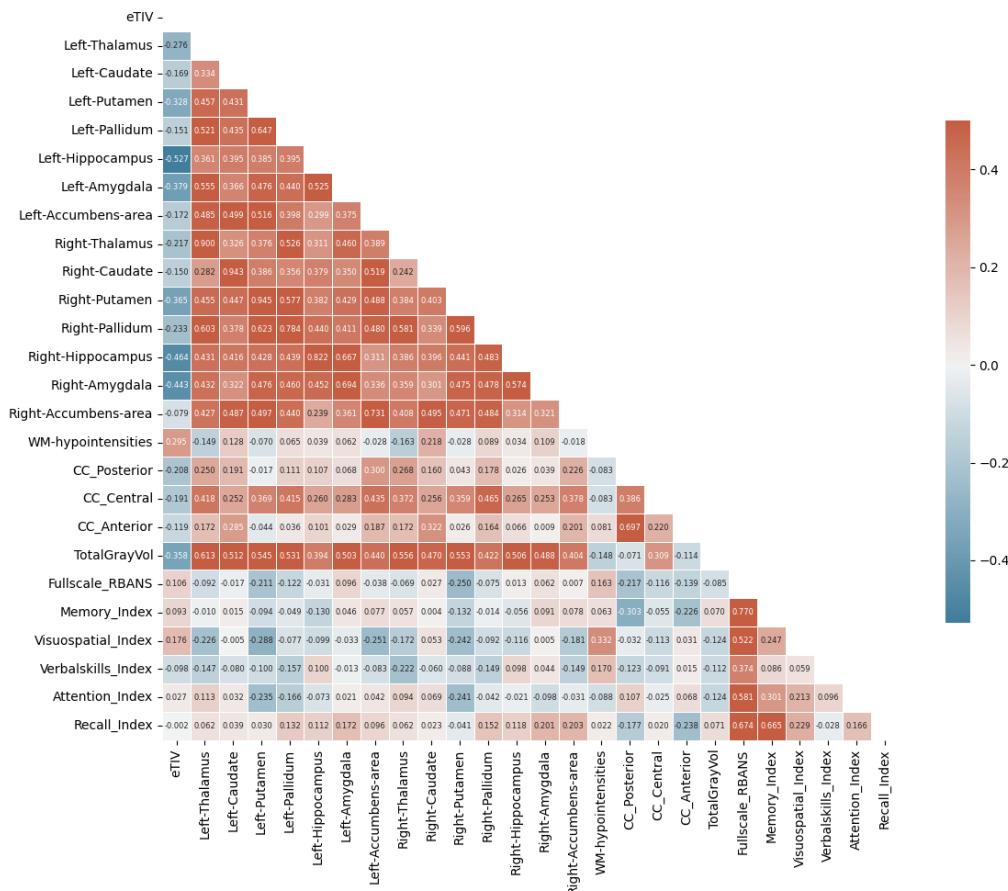
Variable	HC	IBS	p-value	Cliff's delta
Fullscale_RBANS	103.0 (93.0-108.0)	91.0 (85.0-100.0)	0.002	0.213
Memory_Index	100.0 (86.0-109.0)	86.0 (81.0-105.0)	0.031	0.147
Visuospatial_Index	97.0 (90.0-107.0)	96.0 (90.0-105.0)	0.763	0.021
Verbal skills Index	105.0 (95.0-113.0)	95.0 (89.0-111.0)	0.087	0.116
Attention_Index	98.0 (89.0-108.0)	97.0 (83.0-101.0)	0.118	0.107
Recall_Index	107.0 (92.0-113.0)	95.0 (85.0-100.0)	0.006	0.186

Median (IQR) of full-scale RBANS scores, and the index scores of the five cognitive domains. p-values derived from the Mann-Whitney U test are uncorrected. Cliff's delta is used to estimate effect sizes.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/06-morphometry-cognition-exploration.ipynb>

#### Correlations between morphometric and cognitive features

Figure 11 shows a heatmap to illustrate the pairwise Spearman correlations between the included morphometric volume measures, the RBANS indexes, and the Fullscale RBANS score. The correlation matrix reveals several key patterns: Bilateral subcortical structures show moderate to strong correlations with their contralateral counterparts, demonstrating structural symmetry (e.g., left-right hippocampus:  $\rho \approx 0.8$ , bilateral amygdala:  $\rho \approx 0.7$ , bilateral putamen:  $\rho \approx 0.9$ ). TotalGrayVol demonstrates moderate positive correlations with subcortical structures ( $\rho \approx 0.4 - 0.6$ ), reflecting its composite nature. However, the correlations between morphometric measures and cognitive performance indices are notably weak. The Fullscale\_RBANS shows minimal correlations with brain structures ( $|\rho| < 0.25$ ), and Memory\_Index exhibits weak correlations even with medial temporal structures ( $|\rho| < 0.15$ ). The corpus callosum segments (anterior, central, posterior) show a large span of intercorrelations ( $\rho \approx 0.2 - 0.7$ ) but weak associations with cognitive measures. White matter hypointensities (WM-hypointensities) demonstrate particularly weak correlations across all measures ( $|\rho| < 0.15$ ), except for Visiospatial\_Index ( $\rho = 0.33$ ). The correlations between the full scale score and each of the five indexes are moderate to strong for all except for the Verbalskills\_Index ( $\rho = 0.37$ ). Among cognitive indices, moderate intercorrelations exist, with the strongest relationship observed between Recall\_Index and Memory\_Index ( $\rho = 0.67$ ). These findings suggest that while brain structures maintain expected anatomical relationships, their associations with cognitive performance measures are more subtle and complex than might be expected from a simple structure-function relationship model.



**Figure 11. Pairwise Spearman correlations between the morphometric and cognitive variables.**

Generated by: <https://github.com/arvid1/ibs-brain/blob/main/notebooks/06-morphometry-cognition-exploration.ipynb>

#### Prediction of IBS versus HC from morphometric and cognitive measures

The confusion matrix presented in Figure 12a summarizes the performance of an XGBoost model (ranked 2nd best, after knn) predicting irritable bowel syndrome (IBS) versus healthy controls (HC) based on brain morphometry and cognitive features within the test dataset. Each cell provides detailed demographic and clinical information about the participants classified into the corresponding category.

True negatives (TN), representing correctly identified HC participants, include 2 individuals (2 females, 0 males) with an average IBS Severity Scoring System (IBS-SSS) score of  $19.0 \pm 2.8$  and a mean age of  $23.0 \pm 0.0$  years. False positives (FP), where HC participants were misclassified as having IBS, consist of 7 individuals (2 females, 5 males) with an IBS-SSS score of  $19.6 \pm 21.2$  and a mean age of  $25.7 \pm 6.1$  years.

The false negative (FN) category includes one IBS participant (1 male) misclassified as HC, characterized by a high IBS-SSS score of 245.0 and an age of 47.0 years. True positives (TP), indicating IBS participants correctly identified by the model, encompass 14 individuals (11 females, 3 males) with an average IBS-SSS score of  $271.3 \pm 81.0$  and a mean age of  $34.1 \pm 7.7$  years.

This matrix highlights the model's effectiveness, with relatively strong performance in identifying IBS participants ( $TP = 14$ ) compared to HC participants ( $TN = 2$ ). However, the model exhibits a notable rate of false positives ( $FP = 7$ ), indicating misclassification of HC participants as IBS, which could be related to overlapping features between the two groups. The lone false negative suggests a potential challenge in identifying older IBS participants with distinct clinical characteristics, as evidenced by the high IBS-SSS score and advanced age compared to the TP group. This result underscores the complexity of differentiating IBS from HC based on these features and may indicate areas for further optimization of the model, or access to larger cohorts.

To better assess the difference in `xgboost` performance using the morphometric features ( $M$ ), only and using the full dataset, including cognition ( $C$ ), we have produced Table 5.

**Table 5.** Performance Metrics from Confusion Matrices using `xgboost` with different feature sets.

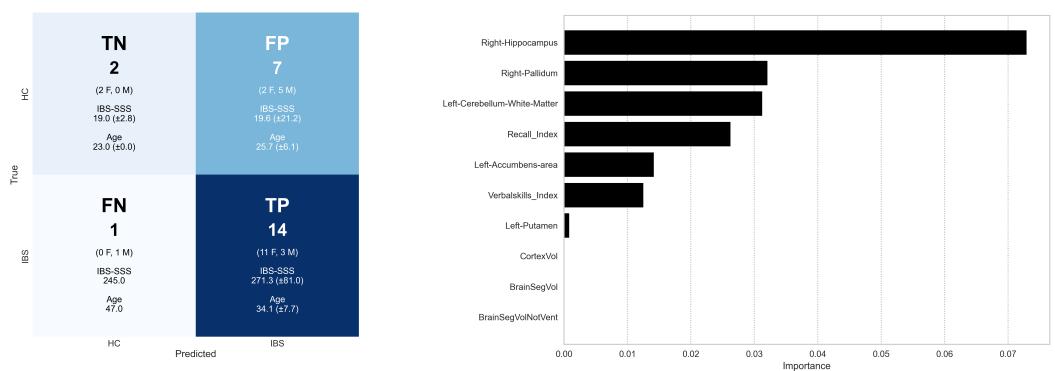
Feature set	TPR	TNR	PPV	NPV	FPR	FNR	FDR	ACC	BACC	F1	MCC
$M$	0.733	0.111	0.579	0.200	0.889	0.267	0.421	0.500	0.422	0.647	-0.185
$M \cup C$	0.933	0.222	0.667	0.667	0.778	0.067	0.333	0.667	0.578	0.778	0.228

M: morphometric features. C: cognitive features. TPR: sensitivity; TNR: specificity; PPV: precision; ACC: accuracy. See list of abbreviations for the rest of column names denoting 11 different metrics.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/07-predicting-IBS-vs-HC-from-morphometry-and-cognition.ipynb>

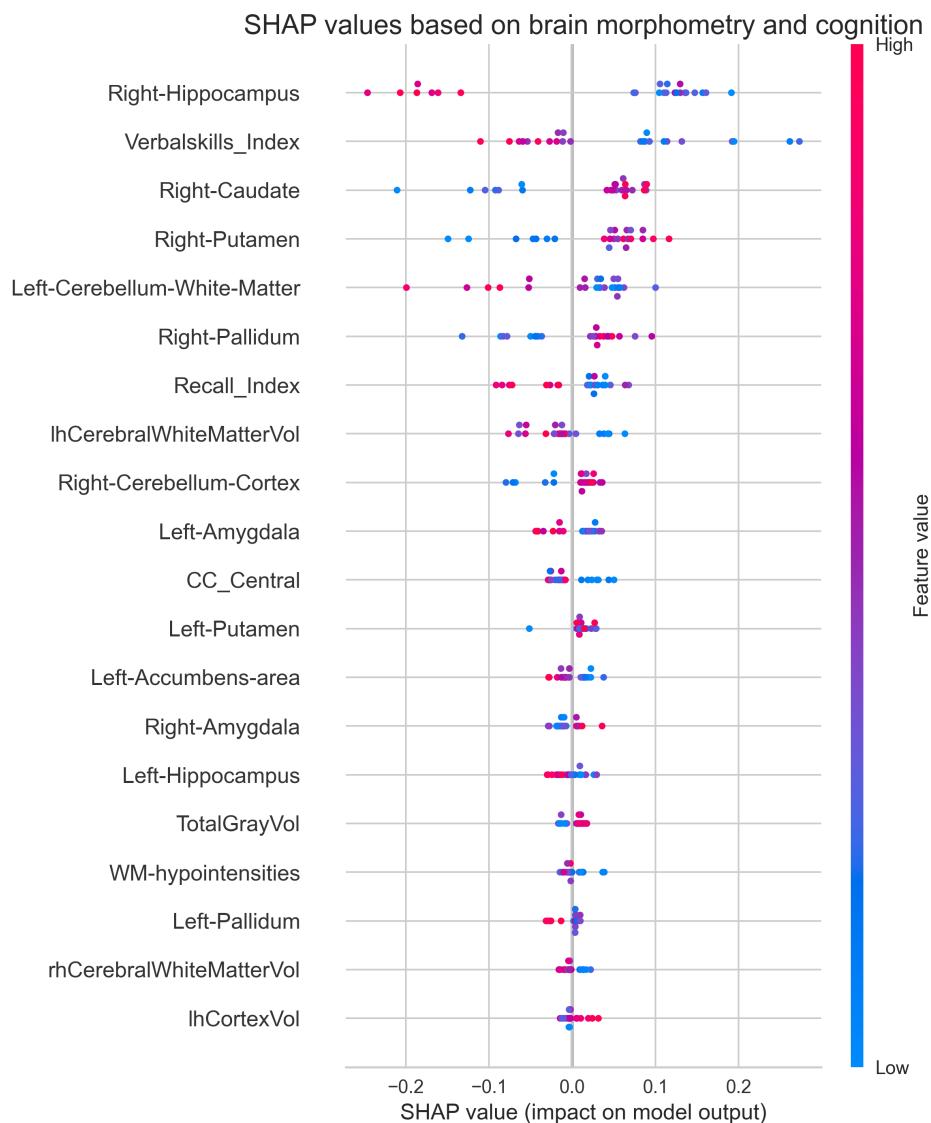
From Tab. 5 we see that using  $M$  alone, the model showed moderate sensitivity (0.733) but struggled with specificity (0.111), resulting in poor overall classification quality ( $MCC = -0.185$ ). In contrast, combining  $M$  and cognitive features ( $M \cup C$ ) substantially improved sensitivity (0.933), specificity (0.222), and overall classification performance ( $MCC = 0.228$ ), demonstrating that integrating cognitive features enhances the model's ability to differentiate IBS from healthy controls.

Figure 12b illustrates how different brain measurements and cognitive abilities influence our model's predictions. Each row shows a different measurement, with the most influential features at the top. The dots represent individual participants, with red dots indicating higher values and blue dots indicating lower values for each measurement. The position of each dot shows whether that particular value pushes the model's prediction higher (towards the right) or lower (towards the left). The Right Hippocampus, shown at the top, has the strongest overall impact: when some people have high values (red dots), this strongly pushes predictions in one direction, while low values (blue dots) push predictions in the other direction. Verbal skills also show important patterns, with a wide spread across different participants, suggesting that both high and low verbal skills can be important indicators, depending on the individual case. Among subcortical regions, Right Caudate and Right Putamen demonstrate particularly notable influences. These patterns reveal meaningful differences between our two groups in both brain structure and cognitive abilities. Particularly important are the variations we see in verbal abilities and in memory-related brain regions like the hippocampus, which could inform how we approach patient care and communication strategies.



**Figure 12.** (a) *Confusion matrix with annotated quadrants*, obtained with xgboost predicting IBS versus HC, jointly from brain morphometry and cognition, in the test dataset. TN = true negative, FP = false positive, FN = false negative, TP = true positive. (b) *Top ten morphometric features in permutation importance* for predicting IBS versus HC in the test dataset, using xgboost.

Generated by: <https://github.com/arvid1/ibs-brain/blob/main/notebooks/07-predicting-IBS-vs-HC-from-morphometry-and-cognition.ipynb>



**Figure 13.** SHAP values with morphometry and cognition as predictors.

Note: X-axis (SHAP values): The SHAP values indicate the impact each feature has on the model's output, which is the probability of classifying someone as IBS patient or HC.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/07-predicting-IBS-vs-HC-from-morphometry-and-cognition.ipynb>

## Discussion

Our study yields two key methodological insights and one substantial findings regarding brain structure and function in IBS. First, we were unable to replicate the morphometric differences between the IBS and healthy control group reported by Skrobisz et al. [23], regardless of whether we used FreeSurfer version 6.0.1 or 7.4.1. Second, we observed substantial discrepancies in morphometric measurements between these software versions, highlighting the critical importance of considering methodological factors in neuroimaging research. Application of advanced multivariate and machine learning techniques to investigate brain-behavior relationships in IBS is another primary contribution. While morphometric features alone proved insufficient for reliable group discrimination, the integration of cognitive performance measures with brain morphometry substantially improved classification accuracy. Specifically, our analyses revealed that two cognitive domain indices, combined with volumetric measures of subcortical structures—particularly the hippocampus and basal ganglia—provided robust discrimination between IBS patients and

healthy controls. The consistency of these findings across different approaches to feature importance analyses strengthens their validity and suggests a fundamental relationship between brain structure, cognitive function, and IBS symptomatology. This observation aligns with views of IBS as a disorder involving complex interactions between central nervous system function and gastrointestinal symptoms, rather than purely peripheral manifestations.

#### *Brain Structures involved in discriminating between IBS and HC*

Our results showed that subcortical structures, particularly within the basal ganglia, played a key role in distinguishing IBS patients from healthy controls. While traditionally associated with motor control, the basal ganglia also critically influence reward processing, habit formation, and pain modulation - functions directly relevant to IBS symptomatology, and the impact on patients' experience of gastrointestinal symptoms. These findings align with recent results from a UK Biobank study [20], which also highlighted the importance of hippocampal and basal ganglia structures, including the Pallidum and Caudate, in IBS. Beyond the basal ganglia, several other subcortical structures relevant to IBS symptomatology emerged as discriminators. The nucleus accumbens, fundamental to reward processing and motivation, may mediate the emotional and motivational aspects of chronic pain in IBS. Dysfunction in this structure could explain the intensified emotional distress and pain sensitivity commonly reported by IBS patients [9]. Similarly, the amygdala appears significant, particularly given its connection to pain-modulation and emotion-processing networks, including the prefrontal cortex and insula. This aligns with previous research [46] demonstrating enhanced amygdala-insula connectivity in IBS patients. Although our results differ from Skrobisz et al.'s [23] findings regarding thalamic involvement, other studies have supported its role in IBS. Diffusion tensor imaging has revealed altered thalamic organization in IBS patients, with reduced fractional anisotropy and increased mean diffusivity [47]. These alterations suggest compromised structural integrity of thalamic circuits, potentially affecting pain processing and sensory integration. The involvement of corpus callosum should also be mentioned, as interhemispheric integration is crucial for visceral sensation processing, pain modulation [48] as well as in mental disorders [49]. Taken together, our findings support that integrated neural signatures are involved in predicting IBS [50].

#### *Integration of Cognitive Performance and Brain Structure in IBS*

The enhanced diagnostic accuracy by including cognitive measures strongly support that IBS should be understood as a disorder of the gut-brain interaction [14,51]. The brain's integral role in cognitive, emotional, and autonomic regulation suggests that these manifestations are fundamentally interconnected rather than merely coincidental. The prominent role of hippocampal volume was a principal finding. The fundamental role of Hippocampus in cognitive processing is well known [52], and was supported by the Recall index being identified as another feature with strong importance. The role of Verbal skills was more surprising. Although research has established connections between memory systems and language processing, particularly in semantic memory organization [53], a negligible correlation between the two indices suggests that IBS affects multiple cognitive domains through independent mechanisms.

Our findings may also have implications for other somatic and psychiatric disorders, like Alzheimer's disease, Parkinson's disease, and major depression. The gut-brain axis are involved in all these diseases, which also are characterized by cognitive impairment. Recent research has identified potential pathways linking gut microbiota alterations to neurological function, particularly through inflammatory responses and tryptophan metabolism [54,55]. The emergence of the microbiota-gut-brain axis as a key framework [56] offers new perspectives on how peripheral inflammation might influence both brain structure

and cognitive function in IBS. This integrated view suggests that cognitive assessment, combined with brain morphometry, might provide valuable insights not only for IBS but for a broader spectrum of gut-brain disorders.

#### *Brain-Gut Axis: Implications for Understanding and Treating IBS*

Our findings should have important implications for clinical practice and treatment development. The observed relationship between brain structure, cognitive function, and IBS symptomatology suggests that effective interventions should target multiple domains simultaneously. Such a multifaceted approach recognizes IBS as a complex disorder requiring coordinated intervention across multiple domains.

Future research directions should expand upon these findings through multimodal investigation. Integration of functional neuroimaging, gut microbiome analysis, and broader clinical assessment [20] could provide a more comprehensive understanding of IBS pathophysiology. Particularly crucial will be longitudinal studies to determine the temporal relationship between brain changes and symptom development. Such studies would allow us to track the evolution of cognitive and structural alterations over time, identify early markers of disease progression, and evaluate the impact of various therapeutic interventions. This temporal perspective is essential for understanding whether observed brain changes represent cause or consequence of IBS symptoms.

This comprehensive approach to understanding IBS aligns with the emerging paradigm of precision medicine. By considering the full spectrum of biological, cognitive, and behavioral manifestations, we may better identify patient subgroups and develop more personalized treatment strategies. The integration of brain structure, cognitive function, and clinical symptoms represents a promising framework for advancing both our understanding and treatment of this complex disorder. Ultimately, this integrated perspective may lead to more effective, personalized interventions that address the full range of IBS manifestations.

#### *Strengths and Limitations: Critical Evaluation and Future Directions*

Although contributing through its multimodal analytical approach, several limitations warrant discussion. The primary limitation concerns sample size, which particularly constrained our ability to conduct robust sex/gender-based analyses. This limitation is especially noteworthy given the evidence for substantial sex/gender differences in IBS presentation, progression, and treatment response [57]. The importance of sex/gender considerations in IBS research has become increasingly apparent. Clinical presentations show clear sex-based patterns, with IBS-C predominating in women and IBS-D in men [58]. These differences reflect complex interactions between biological and environmental factors. Sex hormones, particularly estrogen and progesterone, influence both gastrointestinal function and pain processing in the central nervous system [59]. Recent research has revealed sex-based differences extending to gut microbiota composition [60] and sensory processing. Notably, Labus et al. [21] demonstrated enhanced sensory sensitivity in women with IBS, potentially related to sex-specific morphometric variations in brain structure.

An inability to fully account for IBS symptom severity in our analyses was another limitation. Recent work by Li et al. [20] has demonstrated that symptom severity correlates significantly with both cognitive performance and brain volumetric measures, particularly in regions associated with emotional processing and cognitive control. This finding suggests that future studies should incorporate detailed severity measures to better understand the relationship between symptom intensity and brain-behavior patterns.

The present study's methodological strengths lie in its comprehensive multivariate approach to analyzing brain-behavior relationships. This approach better captures the complex interactions between multiple brain regions and cognitive measures, providing a more nuanced understanding than traditional univariate analyses. However, we acknowledge

important limitations. While our sample exceeds that of many comparable studies, multivariate analyses and machine learning approaches typically benefit from larger datasets. To address this limitation, we implemented robust cross-validation procedures, including 10-fold validation and hold-out test sets, thereby reducing the risk of overfitting and enhancing the generalizability of our findings. Moreover, the results point to several important directions for future research. First, larger-scale studies are needed to validate and extend our multivariate findings. Such studies should maintain rigorous methodological standards while increasing statistical power. Second, standardization of neuroimaging analysis protocols, including careful documentation of software versions and processing parameters, is crucial for reproducibility. Third, the field would benefit from systematic investigation of how different analysis approaches might influence morphometric findings in IBS research. Overall, future studies should consider implementing standardized protocols for both imaging and cognitive assessment, facilitating meta-analyses and enabling more direct comparisons across studies. This standardization, combined with transparent reporting of methodological details, would strengthen the field's ability to build cumulative knowledge about brain-gut interactions in IBS. Longitudinal studies represent a particularly important future direction. Such studies could address crucial questions about the temporal dynamics of brain-gut interactions in IBS, including whether observed structural and cognitive changes precede or follow symptom development. Longitudinal data would also enable better prediction of disease trajectories and treatment responses, potentially informing personalized interventions such as dietary modifications (e.g., Low FODMAP diet) or targeted cognitive interventions. The combination of longitudinal design with multimodal assessment (including brain structure, cognitive function, and clinical symptoms) could provide unprecedented insights into the development and progression of IBS.

## Conclusions and Future Directions

The present study advances our understanding of brain-gut interactions in IBS through several key contributions. First, our comprehensive multivariate analyses reveal the inherent complexity of IBS pathophysiology, demonstrating that single-modality approaches may be insufficient for characterizing this multifaceted disorder. While we did not replicate previously reported volumetric differences in thalamic structure, our machine learning analyses uncovered more subtle and complex patterns of brain-behavior relationships. Particularly noteworthy was the finding that morphometric features gain discriminative power when integrated with cognitive measures, especially in subcortical regions including the hippocampus and basal ganglia. These results strongly support a systems-level conceptualization of IBS, where the condition emerges from complex interactions between neural structure, cognitive function, and gastrointestinal symptoms. This perspective suggests that effective characterization and treatment of IBS requires consideration of multiple biological and cognitive markers rather than focusing on isolated symptoms or structures. The successful integration of structural and functional measures in our analyses points toward more sophisticated approaches for both diagnosis and treatment planning. Moving forward, several research priorities emerge from our findings. Large-scale validation studies are needed to confirm the reliability and generalizability of our brain-cognition relationships across diverse patient populations. Such studies should incorporate standardized protocols for both imaging and cognitive assessment to facilitate cross-study comparisons. Longitudinal investigations are particularly crucial for understanding how these markers evolve over time and relate to treatment response. Additionally, future research should explore how individual differences in brain structure and cognitive function might predict treatment outcomes, potentially enabling more personalized therapeutic approaches. Ultimately, our findings suggest that advancing IBS treatment may require a fundamental shift toward integrated, multimodal assessment approaches that capture both structural and functional aspects of brain-gut interactions. This more comprehensive understanding of IBS pathophysiology could lead to more effective, personalized interven-

tions that address the full spectrum of patient symptoms and experiences.

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## References

1. Black, C.J.; Ford, A.C. Global burden of irritable bowel syndrome: trends, predictions and risk factors. *Nature Reviews Gastroenterology & Hepatology* **2020**, *17*, 473–486. <https://doi.org/10.1038/s41575-020-0286-8>. 1017
2. Lovell, R.M.; Ford, A.C. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clinical Gastroenterology and Hepatology* **2012**, *10*, 712–721. <https://doi.org/10.1016/j.cgh.2012.02.029>. 1018
3. Bonetto, S.; Fagoonee, S.; Battaglia, E.; Grassini, M.; Saracco, G.M.; Pellicano, R. Recent advances in the treatment of irritable bowel syndrome. *Polish Archives of Internal Medicine* **2021**, *131*, 709–715. <https://doi.org/10.20452/pamw.16067>. 1019
4. Drossman, D.A.; Tack, J. Rome Foundation clinical diagnostic criteria for disorders of gut-brain interaction. *Gastroenterology* **2022**, *162*, 675–679. <https://doi.org/10.1053/j.gastro.2021.11.019>. 1020
5. Heitkemper, M.M.; Cain, K.C.; Jarrett, M.E.; Burr, R.L.; Hertig, V.; Bond, E.F. Symptoms across the menstrual cycle in women with irritable bowel syndrome. *Official Journal of the American College of Gastroenterology | ACG* **2003**, *98*, 420–430. <https://doi.org/10.1111/j.1572-0241.2003.07233.x>. 1021
6. Meleine, M.; Matricon, J. Gender-related differences in irritable bowel syndrome: potential mechanisms of sex hormones. *World Journal of Gastroenterology: WJG* **2014**, *20*, 6725. <https://doi.org/10.3748/wjg.v20.i22.6725>. 1022
7. Kim, Y.S.; Kim, N. Sex-gender differences in irritable bowel syndrome. *Journal of Neurogastroenterology and Motility* **2018**, *24*, 544. 1023
8. Toner, B.B.; Akman, D. Gender role and irritable bowel syndrome: literature review and hypothesis. *Official journal of the American College of Gastroenterology | ACG* **2000**, *95*, 11–16. <https://doi.org/10.1111/j.1572-0241.2000.01698.x>. 1024
9. Lundervold, A.J.; Billing, J.E.; Berentsen, B.; Lied, G.A.; Steinsvik, E.K.; Hausken, T.; Lundervold, A. Decoding IBS: a machine learning approach to psychological distress and gut-brain interaction. *BMC Gastroenterology* **2024**, *24*, 267. <https://doi.org/10.1186/s12876-024-03355-z>. 1025
10. Shiha, M.G.; Aziz, I. Physical and psychological comorbidities associated with irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics* **2021**, *54*, S12–S23. <https://doi.org/10.1111/apt.16589>. 1026
11. Lam, N.C.Y.; Yeung, H.Y.; Li, W.K.; Lo, H.Y.; Yuen, C.F.; Chang, R.C.C.; Ho, Y.S. Cognitive impairment in irritable bowel syndrome (IBS): a systematic review. *Brain Research* **2019**, *1719*, 274–284. <https://doi.org/https://doi.org/10.1016/j.brainres.2019.05.036>. 1027
12. Wong, K.M.F.; Mak, A.D.P.; Yuen, S.Y.; Leung, O.N.W.; Ma, D.Y.; Chan, Y.; Cheong, P.K.; Lui, R.; Wong, S.H.; Wu, J.C.Y. Nature and specificity of altered cognitive functioning in IBS. *Neurogastroenterology & Motility* **2019**, *31*, e13696. <https://doi.org/10.1111/nmo.13696>. 1028
13. Billing, J.; Berentsen, B.; Lundervold, A.; Hillestad, E.M.; Lied, G.A.; Hausken, T.; Lundervold, A.J. Cognitive function in patients with irritable bowel syndrome: impairment is common and only weakly correlated with depression/anxiety and severity of gastrointestinal symptoms. *Scandinavian Journal of Gastroenterology* **2023**, pp. 1–9. <https://doi.org/10.1080/00365521.2023.2256916>. 1029
14. Mayer, E.A.; Nance, K.; Chen, S. The Gut-Brain Axis. *Annual Review of Medicine* **2022**, *73*, 439–453. <https://doi.org/10.1146/annurev-med-042320-014032>. 1030
15. Coss-Adame, E.; Rao, S.S. Brain and gut interactions in irritable bowel syndrome: new paradigms and new understandings. *Current Gastroenterology Reports* **2014**, *16*, 1–8. <https://doi.org/10.1007/s11894-014-0379-z>. 1031
16. Lezak, M.D. *Neuropsychological assessment*; Oxford University Press, USA, 2004. 1032
17. Park, H.J.; Friston, K. Structural and functional brain networks: from connections to cognition. *Science* **2013**, *342*, 1238411. <https://doi.org/10.1126/science.1238411>. 1033
18. Mayer, E.A.; Labus, J.S.; Tillisch, K.; Cole, S.W.; Baldi, P. Towards a systems view of IBS. *Nature Reviews Gastroenterology & Hepatology* **2015**, *12*, 592–605. <https://doi.org/10.1038/nrgastro.2015.121>. 1034
19. Mayer, E.A.; Labus, J.; Aziz, Q.; Tracey, I.; Kilpatrick, L.; Elsenbruch, S.; Schweinhardt, P.; Van Oudenhove, L.; Borsook, D. Role of brain imaging in disorders of brain–gut interaction: a Rome Working Team Report. *Gut* **2019**, *68*, 1701–1715. <https://doi.org/10.1136/gutjnl-2019-318308>. 1035
20. Li, Z.; Ma, Q.; Deng, Y.; Rolls, E.T.; Shen, C.; Li, Y.; Zhang, W.; Xiang, S.; Langley, C.; Sahakian, B.J.; et al. Irritable Bowel Syndrome Is Associated With Brain Health by Neuroimaging, 1036

- Behavioral, Biochemical, and Genetic Analyses. *Biological Psychiatry* **2024**, *95*, 1122–1132. <https://doi.org/10.1016/j.biopsych.2023.12.024>. 1075  
1076
21. Labus, J.S.; Wang, C.; Mayer, E.A.; Gupta, A.; Oughourlian, T.; Kilpatrick, L.; Tillisch, K.; Chang, L.; Naliboff, B.; Ellingson, B.M. Sex-specific brain microstructural reorganization in irritable bowel syndrome. *Pain* **2023**, *164*, 292–304. <https://doi.org/10.1097/j.pain.0000000000002699>. 1077  
1078
22. Nan, J.; Yang, W.; Meng, P.; Huang, W.; Zheng, Q.; Xia, Y.; Liu, F. Changes of the postcentral cortex in irritable bowel syndrome patients. *Brain Imaging and Behavior* **2020**, *14*, 1566–1576. 1080  
1081
23. Skrobisz, K.; Piotrowicz, G.; Rudnik, A.; Naumczyk, P.; Sabisz, A.; Markiet, K.; Szurowska, E. Evaluation of subcortical structure volumes in patients with non-specific digestive diseases. *Diagnostics* **2022**, *12*, 2199. <https://doi.org/10.3390/diagnostics12092199>. 1083  
1084
24. Berentsen, B.; Nagaraja, B.H.; Teige, E.P.; Lied, G.A.; Lundervold, A.J.; Lundervold, K.; Steinsvik, E.K.; Hillestad, E.R.; Valeur, J.; Brønstad, I.; et al. Study protocol of the Bergen brain-gut-microbiota-axis study: A prospective case-report characterization and dietary intervention study to evaluate the effects of microbiota alterations on cognition and anatomical and functional brain connectivity in patients with irritable bowel syndrome. *Medicine* **2020**, *99*, e21950. <https://doi.org/doi:10.1097/MD.00000000000021950>. 1085  
1086  
1087  
1088  
1089  
1090
25. Seminowicz, D.A.; Labus, J.S.; Bueller, J.A.; Tillisch, K.; Naliboff, B.D.; Bushnell, M.C.; Mayer, E.A. Regional gray matter density changes in brains of patients with irritable bowel syndrome. *Gastroenterology* **2010**, *139*, 48–57. <https://doi.org/10.1053/j.gastro.2010.03.049>. 1091  
1092
26. Blankstein, U.; Chen, J.; Diamant, N.E.; Davis, K.D. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. *Gastroenterology* **2010**, *138*, 1783–1789. <https://doi.org/10.1053/j.gastro.2009.12.043>. 1094  
1095  
1096
27. Bhatt, R.R.; Gupta, A.; Labus, J.S.; Zeltzer, L.K.; Tsao, J.C.; Shulman, R.J.; Tillisch, K. Altered brain structure and functional connectivity and its relation to pain perception in girls with irritable bowel syndrome. *Psychosomatic medicine* **2019**, *81*, 146–154. <https://doi.org/10.1097/PSY.0000000000000655>. 1099  
1100
28. Francis, C.Y.; Morris, J.; Whorwell, P.J. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Alimentary pharmacology & therapeutics* **1997**, *11*, 395–402. <https://doi.org/10.1046/j.1365-2036.1997.142318000.x>. 1101  
1102
29. Randolph, C. *Repeatable battery for the assessment of neuropsychological status. Norwegian manual*; NL:Pearson, 2013. 1104  
1105
30. Fischl, B. FreeSurfer. *Neuroimage* **2012**, *62*, 774–781. <https://doi.org/10.1016/j.neuroimage.2012.01.021>. 1106  
1107
31. Klauschen, F.; Goldman, A.; Barra, V.; Meyer-Lindenberg, A.; Lundervold, A. Evaluation of automated brain MR image segmentation and volumetry methods. *Human Brain MAPPING* **2009**, *30*, 1310–1327. <https://doi.org/https://doi.org/10.1002/hbm.20599>. 1108  
1109
32. Jovicich, J.; Czanner, S.; Han, X.; Salat, D.; van der Kouwe, A.; Quinn, B.; Pacheco, J.; Albert, M.; Killiany, R.; Blacker, D.; et al. MRI-derived measurements of human subcortical, ventricular and intracranial brain volumes: reliability effects of scan sessions, acquisition sequences, data analyses, scanner upgrade, scanner vendors and field strengths. *Neuroimage* **2009**, *46*, 177–192. <https://doi.org/https://doi.org/10.1016/j.neuroimage.2009.02.010>. 1111  
1112  
1113  
1114  
1115
33. Gronenschild, E.H.; Habets, P.; Jacobs, H.I.; Mengelers, R.; Rozendaal, N.; Van Os, J.; Marcelis, M. The effects of FreeSurfer version, workstation type, and Macintosh operating system version on anatomical volume and cortical thickness measurements. *PLoS One* **2012**, *7*, e38234. <https://doi.org/https://doi.org/10.1371/journal.pone.0038234>. 1116  
1117  
1118  
1119
34. Glatard, T.; Lewis, L.B.; Ferreira da Silva, R.; Adalat, R.; Beck, N.; Lepage, C.; Rioux, P.; Rousseau, M.E.; Sherif, T.; Deelman, E.; et al. Reproducibility of neuroimaging analyses across operating systems. *Frontiers in Neuroinformatics* **2015**, *9*, 12. <https://doi.org/https://doi.org/10.3389/fninf.2015.00012>. 1120  
1121  
1122  
1123
35. Knussmann, G.N.; Anderson, J.S.; Prigge, M.B.; Dean III, D.C.; Lange, N.; Bigler, E.D.; Alexander, A.L.; Lainhart, J.E.; Zielinski, B.A.; King, J.B. Test-retest reliability of FreeSurfer-derived volume, area and cortical thickness from MPAGE and MP2RAGE brain MRI images. *Neuroimage: Reports* **2022**, *2*, 100086. <https://doi.org/10.1016/j.ynirp.2022.100086>. 1124  
1125  
1126  
1127
36. Debiasi, G.; Mazzonetto, I.; Bertoldo, A. The effect of processing pipelines, input images and age on automatic cortical morphology estimates. *Computer Methods and Programs in Biomedicine* **2023**, *242*, 107825. <https://doi.org/https://doi.org/10.1016/j.cmpb.2023.107825>. 1128  
1129  
1130
37. Cliff, N. Dominance statistics: Ordinal analyses to answer ordinal questions. *Psychological bulletin* **1993**, *114*, 494. <https://doi.org/10.1037/0033-2909.114.3.494>. 1131  
1132

38. Meissel, K.; Yao, E.S. Using Cliff's delta as a non-parametric effect size measure: an accessible web app and R tutorial. *Practical Assessment, Research, and Evaluation* **2024**, *29*. <https://doi.org/0.7275/pare.1977>. 1133  
1134  
1135
39. Spearman, C. The proof and measurement of association between two things. *The American Journal of Psychology* **1904**, *15*, 72–101. <https://doi.org/10.2307/1412159>. 1136  
1137
40. Mahalanobis, P.C. On the generalized distance in statistics **1936**, *2*, 49–55. 1138
41. De Maesschalck, R.; Jouan-Rimbaud, D.; Massart, D.L. The Mahalanobis distance. *Chemometrics and intelligent laboratory systems* **2000**, *50*, 1–18. [https://doi.org/10.1016/S0169-7439\(99\)00047-7](https://doi.org/10.1016/S0169-7439(99)00047-7). 1139  
1140
42. Chen, T.; Guestrin, C. Xgboost: A scalable tree boosting system. In Proceedings of the Proceedings of the 22nd ACM SIGKDD international conference on knowledge discovery and data mining, 2016, pp. 785–794. <https://doi.org/10.1145/2939672.2939785>. 1141  
1142  
1143
43. Cohen, J. A coefficient of agreement for nominal scales. *Educational and psychological measurement* **1960**, *20*, 37–46. <https://doi.org/10.1177/0013164460020000>. 1144  
1145
44. Breiman, L. Random forests. *Machine learning* **2001**, *45*, 5–32. <https://doi.org/10.1023/A:1010933404324>. 1146  
1147
45. Lundberg, S.M.; Erion, G.; Chen, H.; DeGrave, A.; Prutkin, J.M.; Nair, B.; Katz, R.; Himmelfarb, J.; Bansal, N.; Lee, S.I. From local explanations to global understanding with explainable AI for trees. *Nature machine intelligence* **2020**, *2*, 56–67. <https://doi.org/10.1038/s42256-019-0138-9>. 1148  
1149  
1150
46. Qi, R.; Liu, C.; Ke, J.; Xu, Q.; Ye, Y.; Jia, L.; Wang, F.; Zhang, L.; Lu, G. Abnormal amygdala resting-state functional connectivity in irritable bowel syndrome. *American Journal of Neuroradiology* **2016**, *37*, 1139–1145. 1151  
1152  
1153
47. Ellingson, B.M.; Mayer, E.; Harris, R.J.; Ashe-McNally, C.; Naliboff, B.D.; Labus, J.S.; Tillisch, K. Diffusion tensor imaging detects microstructural reorganization in the brain associated with chronic irritable bowel syndrome. *PAIN®* **2013**, *154*, 1528–1541. <https://doi.org/10.1016/j.pain.2013.04.010>. 1154  
1155  
1156
48. Ito, A.; Yang, S.; Shinto, E.; Shinto, A.; Toyofuku, A.; Kurata, J. Interhemispheric and Corticothalamic White-Matter Dysfunction Underlies Affective Morbidity and Impaired Pain Modulation in Chronic Pain. *Anesthesia & Analgesia* **2022**, pp. 10–1213. <https://doi.org/10.1213/ANE.0000000006992>. 1157  
1158  
1159  
1160  
1161
49. Piras, F.; Vecchio, D.; Kurth, F.; Piras, F.; Banaj, N.; Ciullo, V.; Luders, E.; Spalletta, G. Corpus callosum morphology in major mental disorders: a magnetic resonance imaging study. *Brain Communications* **2021**, *3*, fcab100. <https://doi.org/10.1093/braincomms/fcab100>. 1162  
1163  
1164
50. Bhatt, R.R.; Gupta, A.; Labus, J.S.; Liu, C.; Vora, P.P.; Stains, J.; Naliboff, B.D.; Mayer, E.A. A neuropsychosocial signature predicts longitudinal symptom changes in women with irritable bowel syndrome. *Molecular Psychiatry* **2022**, *27*, 1774–1791. <https://doi.org/10.1038/s41380-021-01375-9>. 1165  
1166  
1167  
1168
51. Zhao, M.; Hao, Z.; Li, M.; Xi, H.; Hu, S.; Wen, J.; Gao, Y.; Antwi, C.O.; Jia, X.; Yu, Y.; et al. Functional changes of default mode network and structural alterations of gray matter in patients with irritable bowel syndrome: a meta-analysis of whole-brain studies. *Frontiers in Neuroscience* **2023**, *17*, 1236069. <https://doi.org/10.3389/fnins.2023.1236069>. 1169  
1170  
1171  
1172
52. Lisman, J.; Buzsáki, G.; Eichenbaum, H.; Nadel, L.; Ranganath, C.; Redish, A.D. Viewpoints: how the hippocampus contributes to memory, navigation and cognition. *Nature Neuroscience* **2017**, *20*, 1434–1447. 1173  
1174  
1175
53. Burgess, N.; Maguire, E.A.; O'Keefe, J. The human hippocampus and spatial and episodic memory. *Neuron* **2002**, *35*, 625–641. [https://doi.org/10.1016/s0896-6273\(02\)00830-9](https://doi.org/10.1016/s0896-6273(02)00830-9). 1176  
1177
54. Sibelli, A.; Chalder, T.; Everitt, H.; Workman, P.; Windgassen, S.; Moss-Morris, R. A systematic review with meta-analysis of the role of anxiety and depression in irritable bowel syndrome onset. *Psychological Medicine* **2016**, *46*, 3065–3080. <https://doi.org/10.1017/S0033291716001987>. 1178  
1179  
1180
55. Carloni, S.; Rescigno, M. The gut-brain vascular axis in neuroinflammation. In Proceedings of the Seminars in immunology. Elsevier, 2023, Vol. 69, p. 101802. <https://doi.org/10.1016/j.smim.2023.101802>. 1181  
1182  
1183
56. Ishioh, M.; Nozu, T.; Okumura, T. Brain Neuropeptides, Neuroinflammation, and Irritable Bowel Syndrome. *Digestion* **2024**, *105*, 34–39. <https://doi.org/10.1159/000533275>. 1184  
1185
57. van Kessel, L.; Teunissen, D.; Lagro-Janssen, T. Sex-gender differences in the effectiveness of treatment of irritable bowel syndrome: A systematic review. *International Journal of General Medicine*, pp. 867–884. <https://doi.org/10.2147/IJGM.S291964>. 1186  
1187  
1188
58. Lee, O.Y.; Mayer, E.A.; Schmulson, M.; Chang, L.; Naliboff, B. Gender-related differences in IBS symptoms. *Official journal of the American College of Gastroenterology | ACG* **2001**, *96*, 2184–2193. 1189  
1190

59. Chang, L.; Heitkemper, M.M. Gender differences in irritable bowel syndrome. *Gastroenterology* **2002**, *123*, 1686–1701. <https://doi.org/10.1053/gast.2002.36603>. 1191  
1192
60. Vemuri, R.; Sylvia, K.E.; Klein, S.L.; Forster, S.C.; Plebanski, M.; Eri, R.; Flanagan, K.L. The microgenderome revealed: sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility. In Proceedings of the Seminars in immunopathology. Springer, 2019, Vol. 41, pp. 265–275. 1193  
1194  
1195  
1196
61. Casamitjana, A.; Mancini, M.; Robinson, E.; Peter, L.; Annunziata, R.; Althonayan, J.; Crampsie, S.; Blackburn, E.; Billot, B.; Atzeni, A.; et al. A next-generation, histological atlas of the human brain and its application to automated brain MRI segmentation. *bioRxiv* **2024**. <https://doi.org/10.1101/2024.02.05.579016>. 1197  
1198  
1199  
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**Informed Consent Statement:** Written informed consent was obtained from all subjects involved in 1217  
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**Data Availability Statement:** The complete analysis workflow is publicly available at <https://arvidl.github.com/ibs-brain>, 1219  
comprising reproducible Jupyter notebooks containing all analysis 1220  
code and visualizations, cleaned datasets in CSV format, Conda environment configuration for exact 1221  
replication, and source code for generating all tables and figures presented in the Results section. The 1222  
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model integrated within the Cursor (Anysphere) AI code editor and development environment. 1224

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## Abbreviations

The following abbreviations are used in the manuscript:

AUC	Area Under Curve	1229
CM	Confusion matrix	1230
Cohen's d	effect size	
Cliff's delta	non-parametric effect size	
DGBI	Disorders of the gut-brain interaction	
FS	FreeSurfer	
GI	Gastrointestinal	
GitHub	Web-based platform for code sharing with version control	
HC	Healthy Control	
IBS	Irritable bowel syndrome	
IBS-SSS	IBS Severity Scoring System	
IQR	Inter Quartile Range	
ML	Machine learning	
MRI	Magnetic Resonance Imaging	
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status	
RF	Random Forest	
ROC	Receiver Operating Characteristic	
SHAP	SHapley Additive exPlanations	1231
SD	Standard deviation	
SHAP	SHapley Additive exPlanations	
TA	Time of Acquisition	
XGBoost	eXtreme Gradient Boosting	
ML-performance	Definition	
TPR	TP/(TP+FN) (true positive rate, sensitivity, recall)	
TNR	TN/(TN+FP) (true negative rate, specificity)	
PPV	TP/(TP+FP) (positive predictive value, precision)	
NPV	TN/(TN+FN) (negative predictive value)	
FPR	FP/(FP+TN) (false positive rate)	
FNR	FN/(TP+FN) (false negative rate)	
FDR	FP/(TP+FP) (false discovery rate)	
ACC	(TP+TN)/(TP+FP+FN+TN) (accuracy)	
BACC	(Sensitivity + Specificity) / 2 (balanced accuracy)	
F1	1/((1/PPV) + (1/TPR)) (F1-score, harmonic mean of precision and recall)	
MCC	((TP*TN)-(FP*FN))/sqrt((TP+FP)*(TP+FN)*(TN+FP)*(TN+FN)) (Matthews corr.coeff)	



## Appendix A Supplementary definitions, tables, and figures

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### Appendix A.1 FreeSurfer segmented brain regions obtained from aseg

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**Table A1.** FreeSurfer segmented brain regions (aseg) with short descriptions of functional anatomy

Region	Description
eTIV	Estimated Total Intracranial Volume
Left-Cerebellum-White-Matter	White matter in the left cerebellum
Left-Cerebellum-Cortex	Gray matter (cortex) in the left cerebellum
Left-Thalamus	Left thalamus. <i>Thalamus</i> is a relay center for sensory and motor signals. In IBS, thalamic activity will contribute to pain perception and visceral hypersensitivity
Left-Caudate	Left caudate nucleus. <i>Nucleus caudatus</i> is involved in motor and motility control and learning
Left-Putamen	Left putamen. <i>Putamen</i> is part of the basal ganglia involved in motor control and may contribute to habitual responses to gastrointestinal discomfort
Left-Pallidum	Left globus pallidus. <i>Globus pallidus</i> is involved in regulating voluntary movement and gut motility patterns
Left-Hippocampus	Left hippocampus. <i>Hippocampus</i> is crucial for memory formation and spatial navigation, and in IBS, involved in contextual fear learning related to gastrointestinal symptoms
Left-Amygdala	Left amygdala. <i>Amygdala</i> is involved in processing emotions, fear, and anxiety
Left-Accumbens-area	Left nucleus accumbens. <i>Nucleus accumbens</i> is involved in reward and motivation, stress responsivity, and pain modulation
CSF	Cerebrospinal Fluid
Right-Cerebellum-White-Matter	White matter in the right cerebellum
Right-Cerebellum-Cortex	Gray matter (cortex) in the right cerebellum
Right-Thalamus	Right thalamus
Right-Caudate	Right caudate nucleus
Right-Putamen	Right putamen
Right-Pallidum	Right globus pallidus
Right-Hippocampus	Right hippocampus
Right-Amygdala	Right amygdala
Right-Accumbens-area	Right nucleus accumbens
WM-hypointensities	White matter hypointensities (dark on T1-w sequences), can be associated with small vessel disease, demyelination, inflammation, fluid accumulation
CC_Posterior	Posterior part of the corpus callosum
CC_Mid_Posterior	Mid-posterior part of the corpus callosum
CC_Central	Central part of the corpus callosum
CC_Mid_Anterior	Mid-anterior part of the corpus callosum
CC_Anterior	Anterior part of the corpus callosum
BrainSegVol	Total volume of brain segmentation
BrainSegVolNotVent	Brain segmentation volume without ventricles
lhCortexVol	Volume of the left hemisphere cortex
rhCortexVol	Volume of the right hemisphere cortex
CortexVol	Total cortical volume (left + right)
lhCerebralWhiteMatterVol	Volume of left hemisphere cerebral white matter
rhCerebralWhiteMatterVol	Volume of right hemisphere cerebral white matter
CerebralWhiteMatterVol	Total cerebral white matter volume (left + right)
SubCortGrayVol	Volume of subcortical gray matter
TotalGrayVol	Total gray matter volume

### Appendix A.2 Multinormality testing: Mardia's test and Henze-Zirkler test

Mardia's test extends the univariate concepts of skewness and kurtosis to multivariate distributions. For a  $p$ -dimensional random vector  $X$ , multivariate normality implies specific properties of its third and fourth moments. The test examines these moments through multivariate measures of skewness and kurtosis. Given a sample of  $n$  observations,  $X_1, \dots, X_n$ , the sample measures are computed using Mahalanobis distances. The multivariate skewness is defined as  $b_{1,p} = \frac{1}{n^2} \sum_{i,j=1}^n [(X_i - \bar{X})^T S^{-1} (X_j - \bar{X})]^3$ , where  $\bar{X}$  is the sample mean vector and  $S$  is the sample covariance matrix. The multivariate kurtosis is defined as  $b_{2,p} = \frac{1}{n} \sum_{i=1}^n [(X_i - \bar{X})^T S^{-1} (X_i - \bar{X})]^2$ . Under the null hypothesis of multivariate normality,  $nb_{1,p}/6$  follows asymptotically a chi-square distribution with  $\frac{p(p+1)(p+2)}{6}$  degrees of freedom, and  $(b_{2,p} - p(p+2))/\sqrt{8p(p+2)/n}$  follows approximately a standard normal distribution.

The Henze-Zirkler test is based on a non-negative functional distance between two distribution functions, specifically between the empirical characteristic function of the standardized data and the characteristic function of the standard normal distribution. The test statistic is defined as  $HZ_n = n(1 + 2\beta^2)^{p/2} [D_n - (1 + \beta^2)^{-p/2}]$ , where  $\beta = \frac{1}{\sqrt{2}}$  is the smoothing parameter and  $D_n = \frac{1}{n} \sum_{i=1}^n \exp(-\frac{\beta^2}{2} d_i^2)$ , with  $d_i^2$  being the squared Mahalanobis distances  $d_i^2 = (X_i - \bar{X})^T S^{-1} (X_i - \bar{X})$ , with  $\bar{X}$  being the sample mean vector and  $S$  the sample covariance matrix. The test is invariant under affine transformations and has good power against a broad range of alternatives. Under the null hypothesis of multivariate normality, the Henze-Zirkler test statistic  $HZ_n$  follows approximately a lognormal distribution with parameters  $\mu$  and  $\sigma^2$  that depend on the sample size  $n$  and dimension  $p$  as follows:  $\mu = -\frac{1}{2} \log(1 + 2\beta^2) - \frac{p}{2} \log(1 + \beta^2) + \log\left(1 + \frac{p\beta^4}{2(1+2\beta^2)}\right)$   $\sigma^2 = 2\left[-\log\left(1 - \frac{2\beta^4}{(1+2\beta^2)^2}\right) + \frac{p\beta^4}{(1+2\beta^2)(1+\beta^2)}\right]$  where  $\beta = \frac{1}{\sqrt{2}}$  is the smoothing parameter. This means that under  $H_0$ :  $\log(HZ_n) \sim N(\mu + \frac{\log(n)}{2}, \frac{\sigma^2}{n})$ . The test rejects the null hypothesis of multivariate normality for large values of the test statistic.

While both tests assess multivariate normality, they capture different aspects of departure from normality. Mardia's test specifically examines the third and fourth moments of the distribution, making it particularly sensitive to asymmetry and tail behavior. The Henze-Zirkler test, based on characteristic functions, can detect various types of departures from normality, including those that might not be captured by moment-based methods. Using both tests provides a more comprehensive assessment of multivariate normality, though careful attention must be paid to numerical stability, particularly in high-dimensional settings or with small sample sizes.

### Appendix A.3 Robust Mahalanobis distance between IBS and HC

Our computation of a robust Mahalanobis distance method begins with winsorization of the data to reduce the impact of outliers. For each feature  $x_i$ , values are trimmed at the 10th and 90th percentiles such that  $x_{win} = x_{(0.1)}$  if  $x < x_{(0.1)}$ ,  $x$  if  $x_{(0.1)} \leq x \leq x_{(0.9)}$ , and  $x_{(0.9)}$  if  $x > x_{(0.9)}$ , where  $x_{(\alpha)}$  represents the  $\alpha$ -th quantile. Following winsorization, robust location estimation is performed using the median instead of the mean:  $\hat{\mu}_{robust} = \text{median}(X_{win})$ . The pooled covariance matrix is then computed using the winsorized data as  $\hat{\Sigma}_{pooled} = \frac{(n_{HC}-1)\hat{\Sigma}_{HC}+(n_{IBS}-1)\hat{\Sigma}_{IBS}}{n_{HC}+n_{IBS}-2}$ . The robust Mahalanobis distance is calculated as  $D_{robust} = \sqrt{(\hat{\mu}_{IBS} - \hat{\mu}_{HC})^T \hat{\Sigma}_{pooled}^{-1} (\hat{\mu}_{IBS} - \hat{\mu}_{HC})}$ . To assess the statistical significance of this distance, Hotelling's  $T^2$  statistic is transformed to an F-statistic:  $F = \frac{n_{HC}n_{IBS}}{(n_{HC}+n_{IBS})(n_{HC}+n_{IBS}-2)p} D_{robust}^2$ . Under the null hypothesis of no group difference, this follows an F-distribution with degrees of freedom  $p$  and  $n_{HC} + n_{IBS} - p - 1$ , where  $p$  is the number of features. The p-value is computed as  $p\text{-value} = 1 - F_{p,n_{HC}+n_{IBS}-p-1}(F)$ . This robust approach provides a more reliable measure of group separation when the

data contains outliers or deviates from multivariate normality, as is often the case with neuroimaging data. The use of robust estimators (median and winsorized covariance) makes the distance measure less sensitive to extreme values while maintaining the ability to detect genuine multivariate differences between groups.

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#### Appendix A.4 Comparing Freesurfer 6.0.1 and FreeSurferr 7.4.1 cross-sectional

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Table A2 gives the summary statistics, mean and standard deviation from HC and IBS patients in the Bergen cohort on each of the 35 included brain regions (also reported by Skrobisz et al. [23]) derived from the aseg.stats files using cross-sectional Freesurfer 6.0.1 and Freesurfer 7.4.1, respectively. For eTIV [ $\text{mm}^3$ ] computed with each of the two versions, we found the mean (SD) as follows:

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FS6-cross - HC: 1468820 (155501); IBS: 1426237 (136413), and

FS7-cross - HC: 1494273 (171472); IBS: 1462311 (144145), respectively.

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**Table A2.** Comparison of Brain Region Volumes in IBS Patients and Healthy Controls.

Brain Region	Bergen Cohort FS 6.0.1				Bergen cohort FS 7.4.1			
	HC (N=29)		IBS (N=49)		HC (N=29)		IBS (N=49)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Left-Cerebellum-WM	0.010496	0.000915	0.010483	0.000924	0.010603	0.000932	0.010607	0.001027
Left-Cerebellum-Cortex	0.038939	0.003435	0.039314	0.003733	0.038066	0.003526	0.038056	0.003684
Left-Thalamus	0.005232	0.000464	0.005144	0.000393	0.005236	0.000522	0.005114	0.000458
Left-Caudate	0.002356	0.000259	0.002355	0.000306	0.002346	0.000283	0.002317	0.000294
Left-Putamen	0.003479	0.000385	0.003441	0.000386	0.003438	0.000396	0.003370	0.000329
Left-Pallidum	0.001405	0.000154	0.001374	0.000107	0.001380	0.000136	0.001358	0.000095
Left-Hippocampus	0.002913	0.000272	0.002896	0.000242	0.002926	0.000251	0.002895	0.000243
Left-Amygdala	0.001218	0.000097	0.001203	0.000105	0.001228	0.000133	0.001190	0.000111
Left-Accumbens-area	0.000427	0.000069	0.000421	0.000057	0.000424	0.000061	0.000400	0.000057
CSF	0.000670	0.000120	0.000702	0.000141	0.000658	0.000114	0.000689	0.000130
Right-Cerebellum-WM	0.009973	0.000891	0.009979	0.000851	0.010052	0.000934	0.010108	0.001015
Right-Cerebellum-Cortex	0.039719	0.003445	0.039978	0.003760	0.038881	0.003534	0.038912	0.003673
Right-Thalamus	0.005120	0.000438	0.005071	0.000358	0.005190	0.000455	0.005053	0.000413
Right-Caudate	0.002438	0.000240	0.002439	0.000301	0.002418	0.000286	0.002402	0.000285
Right-Putamen	0.003506	0.000366	0.003489	0.000351	0.003487	0.000402	0.003466	0.000322
Right-Pallidum	0.001323	0.000126	0.001301	0.000107	0.001321	0.000137	0.001306	0.000118
Right-Hippocampus	0.003013	0.000240	0.002983	0.000229	0.003049	0.000230	0.002986	0.000235
Right-Amygdala	0.001284	0.000087	0.001271	0.000098	0.001269	0.000107	0.001260	0.000106
Right-Accumbens-area	0.000428	0.000053	0.000427	0.000061	0.000434	0.000054	0.000435	0.000057
WM-hypointensities	0.000791	0.000306	0.000688	0.000253	0.000787	0.000481	0.000667	0.000244
CC_Posterior	0.000652	0.000096	0.000702	0.000113	0.000645	0.000096	0.000685	0.000113
CC_Mid_Posterior	0.000369	0.000067	0.000401	0.000071	0.000366	0.000069	0.000394	0.000073
CC_Central	0.000395	0.000089	0.000391	0.000105	0.000390	0.000091	0.000390	0.000101
CC_Mid_Anterior	0.000379	0.000081	0.000409	0.000113	0.000384	0.000078	0.000400	0.000105
CC_Anterior	0.000623	0.000096	0.000650	0.000101	0.000608	0.000098	0.000646	0.000112
BrainSegVol	0.804644	0.024872	0.805581	0.023967	0.792112	0.037690	0.786845	0.028349
BrainSegVolNotVent	0.792235	0.025106	0.791323	0.024898	0.779857	0.037538	0.772948	0.030305
lhCortexVol	0.166698	0.008003	0.166929	0.009510	0.164771	0.010207	0.163181	0.010196
rhCortexVol	0.166137	0.008276	0.166462	0.009388	0.164149	0.010295	0.162912	0.009834
CortexVol	0.332835	0.016110	0.333391	0.018798	0.328920	0.020369	0.326092	0.019888
lhCerebralWhiteMatterVol	0.159895	0.008578	0.159148	0.008757	0.157377	0.010114	0.155820	0.009472
rhCerebralWhiteMatterVol	0.159252	0.008291	0.158267	0.009384	0.156808	0.010522	0.154840	0.009950
CerebralWhiteMatterVol	0.319147	0.016780	0.317415	0.018079	0.314184	0.020552	0.310659	0.019351
SubCortGrayVol	0.040924	0.002583	0.040629	0.002364	0.040864	0.002871	0.040194	0.002433
TotalGrayVol	0.453068	0.022076	0.453961	0.024324	0.446625	0.027101	0.443252	0.025556
eTIV [ $\text{mm}^3$ ]	1468820.2	155501.4	1426237.4	136412.8	1494273.2	171472.3	1462310.8	144145.1

Note: All volumes are normalized to estimated total intracranial volume (eTIV)

HC = Healthy Controls; IBS = Irritable Bowel Syndrome; SD = Standard Deviation; WM = White Matter

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/04-computing-FS-versions-on-same-dataset.ipynb>

#### Appendix A.5 Comparing FreeSurfer 7.4.1 cross-sectional and longitudinal stream

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Table A3 gives the summary statistics, mean and standard deviation from HC and IBS patients in the Bergen cohort on each of the 35 included brain regions derived from the aseg.stats files using Freesurfer 7.4.1 cross-sectional analysis and Freesurfer 7.4.1 longitudinal stream, respectively. For eTIV [ $\text{mm}^3$ ] computed with each of the two versions, we found the mean (SD) as follows:

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FS7-cross - HC: 1494273 (171472); IBS: 1462311 (144145), and

FS7-long - HC: 1492944 (171478); IBS: 1464197 (143328).

**Table A3.** Comparison of Brain Region Volumes in Bergen cohort, FS 7.4.1 cross-sectional vs. FS 7.4.1 longitudinal stream

Brain Region	FS 7.4.1 cross-sectional				FS 7.4.1 longitudinal stream			
	HC (N=29)		IBS (N=49)		HC (N=29)		IBS (N=49)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Left-Cerebellum-White-Matter	0.010603	0.000932	0.010607	0.001027	0.010888	0.001076	0.010688	0.001044
Left-Cerebellum-Cortex	0.038066	0.003526	0.038056	0.003684	0.037438	0.003633	0.037232	0.003575
Left-Thalamus	0.005236	0.000522	0.005114	0.000458	0.005385	0.000517	0.005262	0.000473
Left-Caudate	0.002346	0.000283	0.002317	0.000294	0.002506	0.000313	0.002474	0.000315
Left-Putamen	0.003438	0.000396	0.003370	0.000329	0.003702	0.000418	0.003621	0.000369
Left-Pallidum	0.001380	0.000136	0.001358	0.000095	0.001415	0.000156	0.001373	0.000098
Left-Hippocampus	0.002926	0.000251	0.002895	0.000243	0.002970	0.000273	0.002925	0.000244
Left-Amygdala	0.001228	0.000133	0.001190	0.000111	0.001253	0.000138	0.001203	0.000111
Left-Accumbens-area	0.000424	0.000061	0.000400	0.000057	0.000440	0.000072	0.000429	0.000070
CSF	0.000658	0.000114	0.000689	0.000130	0.000712	0.000127	0.000742	0.000130
Right-Cerebellum-White-Matter	0.010052	0.000934	0.010108	0.001015	0.010218	0.001008	0.010231	0.000955
Right-Cerebellum-Cortex	0.038881	0.003534	0.038912	0.003673	0.038471	0.003722	0.038176	0.003654
Right-Thalamus	0.005190	0.000455	0.005053	0.000413	0.005475	0.000474	0.005341	0.000456
Right-Caudate	0.002418	0.000286	0.0002402	0.000285	0.002608	0.000310	0.002577	0.000303
Right-Putamen	0.003487	0.000402	0.003466	0.000322	0.003788	0.000418	0.003766	0.000375
Right-Pallidum	0.001321	0.000137	0.001306	0.000118	0.001350	0.000156	0.001330	0.000120
Right-Hippocampus	0.003049	0.000230	0.002986	0.000235	0.003102	0.000245	0.003034	0.000241
Right-Amygdala	0.001269	0.000107	0.001260	0.000106	0.001332	0.000115	0.001323	0.000115
Right-Accumbens-area	0.000434	0.000054	0.000435	0.000057	0.000503	0.000065	0.000507	0.000063
WM-hypointensities	0.000787	0.000481	0.000667	0.000244	0.000757	0.000644	0.000607	0.000274
CC_Posterior	0.000645	0.000096	0.000685	0.000113	0.000632	0.000097	0.000669	0.000112
CC_Mid_Posterior	0.000366	0.000069	0.000394	0.000073	0.000350	0.000066	0.000375	0.000075
CC_Central	0.000390	0.000091	0.000390	0.000101	0.000364	0.000082	0.000363	0.000091
CC_Mid_Anterior	0.000384	0.000078	0.000400	0.000105	0.000361	0.000071	0.000379	0.000101
CC_Anterior	0.000608	0.000098	0.000646	0.000112	0.000587	0.000096	0.000620	0.000093
BrainSegVol	0.792112	0.037690	0.786845	0.028349	0.798892	0.038555	0.790867	0.029736
BrainSegVolNotVent	0.779857	0.037538	0.772948	0.030305	0.785834	0.038695	0.776074	0.031471
lhCortexVol	0.164771	0.010207	0.163181	0.010196	0.170575	0.010496	0.168994	0.010110
rhCortexVol	0.164149	0.010295	0.162912	0.009834	0.170608	0.010675	0.168900	0.009646
CortexVol	0.328920	0.020369	0.326092	0.019888	0.341183	0.021084	0.337894	0.019676
lhCerebralWhiteMatterVol	0.157377	0.010114	0.155820	0.009472	0.153875	0.009612	0.151551	0.009309
rhCerebralWhiteMatterVol	0.156808	0.010522	0.154840	0.009950	0.152915	0.009898	0.150025	0.009752
CerebralWhiteMatterVol	0.314184	0.020552	0.310659	0.019351	0.306790	0.019437	0.301576	0.018991
SubCortGrayVol	0.040864	0.002871	0.040194	0.002433	0.042919	0.003186	0.042213	0.002739
TotalGrayVol	0.446625	0.027101	0.443252	0.025556	0.459994	0.028590	0.455681	0.025742

Note: All volumes are normalized to estimated total intracranial volume (eTIV)

HC = Healthy Controls; IBS = Irritable Bowel Syndrome; SD = Standard Deviation; WM = White Matter

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/04-computing-FS-versions-on-same-dataset.ipynb>

*Appendix A.6 Training 15 binary classifiers and their assessment*

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Model		Accuracy	AUC	Recall	Prec.	F1	Kappa	MCC	TT (Sec)
xgboost	Extreme Gradient Boosting	0.7200	0.6833	0.7200	0.7383	0.7124	0.4031	0.4204	0.0190
knn	K Neighbors Classifier	0.6867	0.6292	0.6867	0.7022	0.6693	0.2982	0.3265	0.1790
lr	Logistic Regression	0.6267	0.5500	0.6267	0.3938	0.4833	0.0000	0.0000	2.7970
svm	SVM - Linear Kernel	0.6267	0.5333	0.6267	0.3938	0.4833	0.0000	0.0000	0.0100
gbc	Gradient Boosting Classifier	0.6267	0.4667	0.6267	0.6544	0.5920	0.1978	0.2357	0.0260
dummy	Dummy Classifier	0.6267	0.5000	0.6267	0.3938	0.4833	0.0000	0.0000	0.0080
dt	Decision Tree Classifier	0.6133	0.6083	0.6133	0.6656	0.6096	0.2039	0.2374	0.1710
nb	Naive Bayes	0.6067	0.5583	0.6067	0.4801	0.5177	0.0450	0.0578	0.1650
ridge	Ridge Classifier	0.6067	0.2917	0.6067	0.3878	0.4726	-0.0364	-0.0408	0.0150
lightgbm	Light Gradient Boosting Machine	0.5867	0.5625	0.5867	0.5003	0.5239	0.0276	0.0270	0.0500
et	Extra Trees Classifier	0.5300	0.5812	0.5300	0.4621	0.4821	-0.0735	-0.0713	0.0300
rf	Random Forest Classifier	0.5267	0.5021	0.5267	0.5167	0.4987	-0.0492	-0.0293	0.0370
qda	Quadratic Discriminant Analysis	0.5133	0.5042	0.5133	0.4528	0.4698	-0.1175	-0.1328	0.0090
lda	Linear Discriminant Analysis	0.5000	0.3792	0.5000	0.5650	0.4773	-0.0224	0.0141	0.0100
ada	Ada Boost Classifier	0.4867	0.3708	0.4867	0.4466	0.4536	-0.0624	-0.0992	0.0210

CPU times: user 5.24 s, sys: 596 ms, total: 5.84 s  
Wall time: 38.1 s

**Figure A1.** *Binary classification models trained using PyCaret.* Based on 36 morphometric features derived from the longitudinal stream of Freesurfer 7.4.1 applied to T1-weighted examinations in the Bergen cohort described in Tab. 2. For each of the 15 models, seven performance metrics on the 0 (HC) versus 1 (IBS) prediction were obtained as the means after stratified 10-fold cross-validation, i.e., for each iteration (out of 10), nine folds are combined to form the training set (90% of data), the remaining fold becomes the validation set (10% of data). The models are ranked according to accuracy (see text for more details).

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/05-predicting-IBS-vs-HC-from-morphometric-measures.ipynb>

*Appendix A.7 High resolution histological atlas segmentation of T1-weighted MPRAGE recording*

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As a proof of concept, Figure A2 displays a high resolution segmentation of the T1-weighted recording from subject BGA\_046 in the Bergen cohort. This is based on the NextBrain project (<https://github-pages.ucl.ac.uk/NextBrain>) described in [61]. The NextBrain project provides a sophisticated brain segmentation module that utilizes a probabilistic atlas to identify 333 distinct regions of interest (ROIs) per hemisphere in *in vivo* brain scans. The segmentation process employs a Bayesian algorithm, making it adaptable to various MRI pulse sequences including T1-weighted, T2-weighted, and FLAIR. The software offers two implementation modes: a comprehensive Bayesian version and a faster alternative. The full version (used in this example), while more computationally intensive, provides detailed segmentation. The faster version utilizes a neural network for pre-computing atlas deformation, significantly reducing processing time to under an hour on standard hardware. Both versions generate outputs including bias-field corrected scans, SynthSeg segmentation, MNI registration, hemisphere-specific segmentations, and volumetric measurements in CSV format. The system employs a sophisticated Gaussian mixture model for tissue classification, with customizable parameters for bias field correction and tissue

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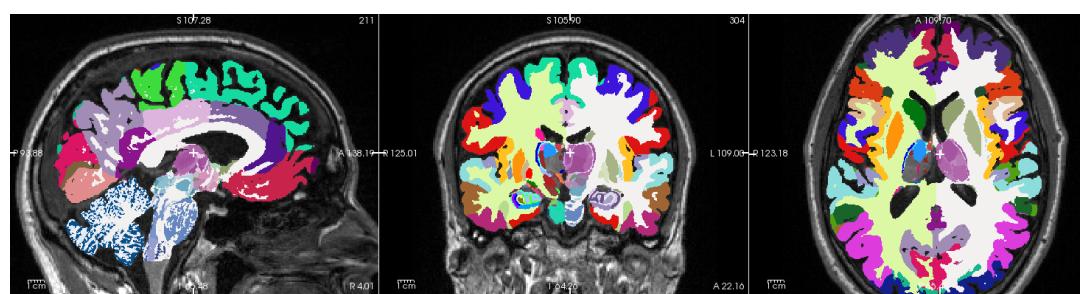
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**Figure A2.** High resolution Histological atlas segmentation, available in FreeSurfer 8.0.0-beta, of 3D T1-weighted MPRAGE recording from BGA\_046. Panels left to right: Sagittal, Coronal, and Axial section, respectively. The white cross-bar in the middle of the brain is located in the *paracentral nucleus* of the *left thalamus* at RAS coordinates 4.03, 22.15, 21.90. Cfr. the much coarser granularity of ASEG segmentation in Fig. 2, with the same positioning of the white cross-bar.

Generated by: <https://github.com/arvidl/ibs-brain/blob/main/notebooks/01-freesurfer-freeview-t1-aseg-bga-046.ipynb>