REVISITING THE SUBTYPING OF LOWER GASTROINTESTINAL DISORDERS OF GUT-BRAIN INTERACTION PATIENTS USING UNSUPERVISED MACHINE LEARNING

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Background: ROME IV, a set of symptom-based diagnostic criteria for disorders of gut-brain interaction (DGBI), has provided positive diagnoses for patients lacking organic explanations for their gastrointestinal (GI) symptoms. There have been challenges associated with identifying robust diagnostic biomarkers, predicting treatment outcomes, and diagnostic stability within DGBI subtypes. Unsupervised machine learning can be used to discover patterns in unlabeled data and has the potential to reveal alternative patient subtypes unburdened by these limitations. Here, lower GI DGBI and control subjects were clustered using biological and clinical data from a cross-sectional case-control study.

Methods: Patient-reported outcomes (PROMIS, HADS, and SAGIS), as well as fecal and plasma samples, were collected from 315 subjects (128 irritable bowel syndrome (IBS), 42 functional constipation (FC), 16 functional diarrhea (FD), and 129 controls; Fig. 1). Ethical approval was obtained from the Northern A Health and Disability Ethics Committee (16/NTA/21). Fecal samples were analyzed using shotgun sequencing, and taxonomic classifications were determined using Metaxa2 and the SILVA 128 database. Plasma and fecal samples were subjected to biphasic extraction, and global metabolite profiling was performed using polar, semi-polar, and non-polar untargeted liquid chromatography high-resolution mass spectrometry (LC-MS) methods. Fecal bile acids and organic acids were analyzed using LC-MS/MS. Subjects were clustered using merged affinity network association clustering (MANAclust). MANAclust then identified consensus groups, which were groups of clusters within each datatype (*e.g.*, fecal bile acids). Association between categorical variables and cluster/consensus group membership was examined using chi-squared tests, and differences in continuous variables between clusters were tested using pairwise *t*-tests. Statistics were corrected for multiple testing using the Benjamini-Hochberg procedure with a significance threshold of $\alpha = 0.001$.

Results: There were 11 distinct clusters identified (Fig. 2). Using the distribution of DGBI diagnoses present within these clusters, MANAclust identified four diagnosis consensus groups, which could be described as IBS-predominant ($P < 0.001$; Cluster 9), DGBI-predominant ($P < 0.001$; Clusters 1, 4, and

11), control-predominant ($P < 0.001$; Clusters 2, 5, 7, and 10), and mixed ($P < 0.001$; Clusters 3, 6, and 8). Cluster 1 had greater depression scores than the other DGBI-predominant clusters ($P < 0.001$).

Conclusions: Clustering control and lower GI DGBI subjects using biological and clinical measurements revealed clusters that did not align with the underlying ROME IV diagnoses. Additional analysis of the biological characteristics within these clusters may elucidate novel mechanisms driving GI symptom presentation and treatment response.

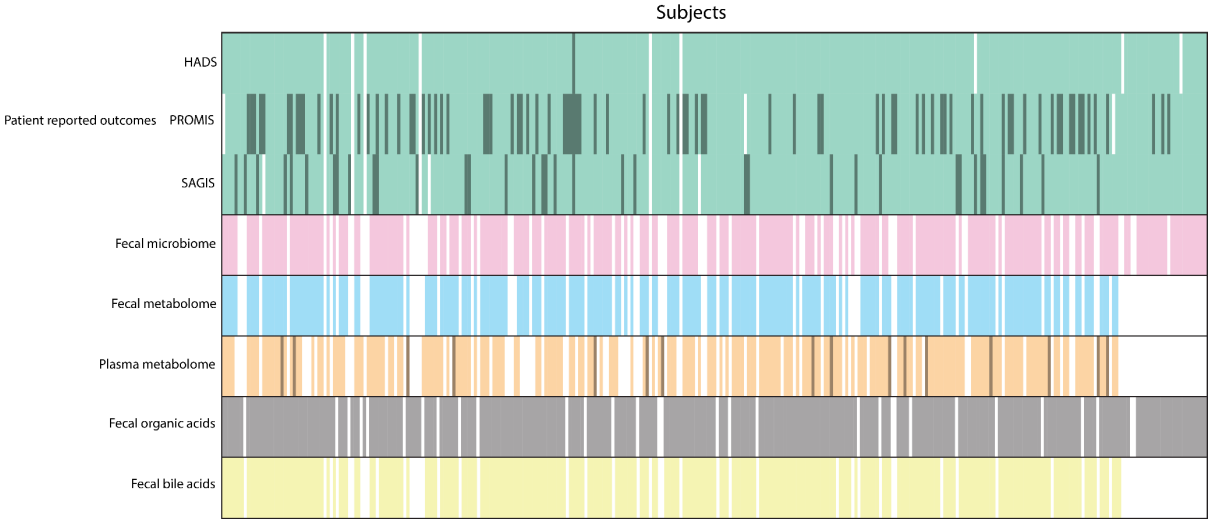

Figure 1: Data provided as input to the merged affinity network association clustering pipeline. Each column is associated with a single subject and each row a different data type. Dark elements represent partial data and white elements represent no data.

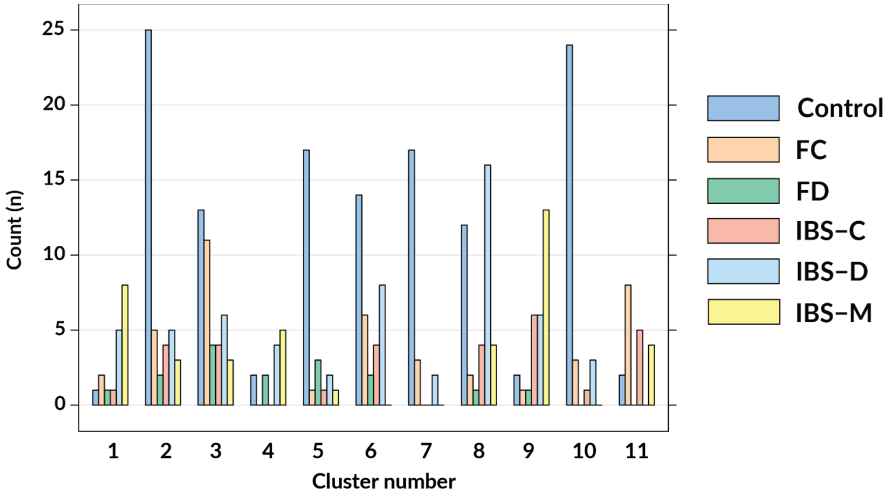

Figure 2: Composition of Rome IV diagnoses present within each cluster.