

Regional homogeneity alterations in patients with functional constipation and their associations with gene expression profiles

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Functional constipation, a highly prevalent functional gastrointestinal disorder, often accompanies by mental and psychological disorders. Previous neuroimaging studies have demonstrated brain functional and structural alterations in patients with functional constipation. However, little is known about whether and how regional homogeneity is altered in these patients. Moreover, the potential genetic mechanisms associated with these alterations remain largely unknown. The study included 73 patients with functional constipation and 68 healthy controls, and regional homogeneity comparison was conducted to identify the abnormal spontaneous brain activities in patients with functional constipation. Using Allen Human Brain Atlas, we further investigated gene expression profiles associated with regional homogeneity alterations in functional constipation patients with partial least squares regression analysis applied. Compared with healthy controls, functional constipation patients demonstrated significantly decreased regional homogeneity in both bilateral caudate nucleus, putamen, anterior insula, thalamus and right middle cingulate cortex, supplementary motor area, and increased regional homogeneity in the bilateral orbitofrontal cortex. Genes related to synaptic signaling, central nervous system development, fatty acid metabolism, and immunity were spatially correlated with abnormal regional homogeneity patterns. Our findings showed significant regional homogeneity alterations in functional constipation patients, and the changes may be caused by complex polygenetic and poly-pathway mechanisms, which provides a new perspective on functional constipation's pathophysiology.

Key words: Allen Human Brain Atlas; functional constipation; gene expression; regional homogeneity; resting-state functional magnetic resonance imaging.

Introduction

Functional constipation (FCon), defined by Rome IV criteria, is one common type of functional gastrointestinal disorder (FGID) caused by multiple factors and mainly manifests by low frequent of bowel movements, difficulty defecating, miserable feeling of incomplete evacuation and rectal obstruction, hard/big stools, bloating, and abdominal pain (Drossman 2016). The incidence of FCon increases yearly, with a global prevalence of about 0.7% to 79% and an average of 16% (Mugie et al. 2011; Barberio et al. 2021). Moreover, many FCon patients suffer from mental and psychological disorders, including the varying severity of depression, anxiety, sleeplessness, and cognitive dysfunction (Hosseinzadeh et al. 2011). These symptoms seriously affect the FCon patient's health and quality of daily life, imposing huge social and economic burdens on families and countries (Liem et al. 2009). However, the exact pathophysiological mechanism of FCon is still unclear.

Growing evidence has showed that there is an ongoing interaction between the central nervous system and the gastrointestinal tract, which is called the microbiota-gut-brain (MGB) axis (Stilling et al. 2014). It is possible that the FGID may cause

functional/structural anomalies in the brain through the MGB axis, as well as mental and psychological disorders (Mayer et al. 2006). Neuroimaging has thus been used progressively to study the functional and structural alterations of the brains of patients with FGID, including FCon patients (Zhu et al. 2016; Jin et al. 2019; Hu et al. 2020; Cai et al. 2021; Duan et al. 2021; Li et al. 2021; Liu et al. 2021; Peihong et al. 2021; Zhang et al. 2021, 2022a). Zhu et al. first utilized the resting-state functional magnetic resonance imaging (rs-fMRI) with amplitude of low frequency fluctuation (ALFF) to explore the functional abnormalities in patients with FCon and found the altered brain regions mainly involved in somatic and sensory processing, motor control, which includes the precentral gyrus, supplementary motor area (SMA), and emotional process modulation, including dorsal anterior cingulate cortex, orbitofrontal cortex (OFC), anterior insula (aINS), and hippocampus (Zhu et al. 2016). Subsequently, some rs-fMRI studies demonstrated functional impairments in caudate nucleus (Cau), precuneus, and thalamus (THA; Jin et al. 2019; Liu et al. 2021). Moreover, the changes of functional connectivity (FC) of the intrinsic resting brain network were reported

(Jin et al. 2019; Duan et al. 2021; Li et al. 2021), with gender-related difference discovered (Jin et al. 2019). Meanwhile, structural MRI data were also analyzed to reveal the brain abnormalities in FCon patients, including gray matter volume (GMV) and cortical thickness, volume, surface area impairment in various brain regions (Hu et al. 2020; Cai et al. 2021). Besides, several researches employing diffusion tensor imaging (DTI) showed white matter microstructure destruction and alteration of nodal characteristics and small-world-ness of white matter structural networks in patients with FCon (Peihong et al. 2021; Zhang et al. 2021). These findings may partially shed light on FCon's underlying neural mechanisms. Yet, further research is needed to explore the neuropathologic basis of these findings.

Transcription-neuroimaging association analysis could provide us with a deeper understanding of disease-related macroscopic neuroimaging phenotypes (Arnatkeviciute et al. 2023). Over 20,000 genes across 3,702 samples of brain tissue are contained in the Allen Human Brain Atlas (AHBA) data set (Hawrylycz et al. 2012), which has enabled related research into how disease-related gene expression at the micro level influences brain alterations at the macro level in numerous disorders. Based on AHBA database, our previous study conducted transcriptional neuroimaging association and identified the genes involved in the GMV alterations in FCon patients (Cai et al. 2021).

Currently, resting-state spontaneous brain activity alteration can be measured via various approaches, among which regional homogeneity (ReHo) is commonly used because of its efficiency and high reproducibility. As determined by Kendall's coefficient of concordance (KCC), ReHo is a reflection of the degree of local synchronization, which is reflected in the similarity between blood oxygenation level-dependent signals of a given voxel and the 26 nearest neighbors (Zang et al. 2004; Zuo et al. 2013). ReHo abnormalities have been reported in a variety of diseases, such as schizophrenia (Zhao et al. 2019), autism (Ma et al. 2022), depression (Iwabuchi et al. 2015), and inflammatory bowel diseases (Thomann et al. 2021). However, to date, no studies have focused on ReHo alterations and their relevant transcription-neuroimaging association analysis in FCon patients.

In this research, we hypothesized that patients with FCon would have significant ReHo alterations and they would be related to brain gene expression. First, the differential brain regions of ReHo between FC patients and healthy controls (HCs) were compared using rs-fMRI, and then the potential gene expression related to these brain regions was explored through AHBA data set (<http://human.brain-map.org>). Partial least squares (PLS) regression was further used to analyze the correlation between them, and finally, enrichment analysis was conducted on the confirmed genes to explore which metabolic and biological processes these genes are mainly enriched in, thus revealing the potential pathophysiological mechanisms of FCon. The flowchart of handling is illustrated in Fig. 1.

Materials and methods

Subjects

A total of 84 patients with FCon were recruited from Shanghai Tenth People's hospital of Tongji University School of Medicine. All patients were diagnosed by a trained and experienced gastroenterologist using Rome IV criteria. Those patients with congenital giant colon, redundant sigmoid colon, and mental/psychological disorders were excluded. Meanwhile, 72 age- and gender-matched HCs were recruited from the local community. The common exclusion criteria for subjects were listed as follows: (i) MRI

contraindications, (ii) left-handedness, (iii) younger than 18 or older than 70 years, (iv) organic intracranial lesions, (v) history of head trauma, alcoholism, drug abuse, or psychiatric diagnosis, (vi) poor image quality, and (vii) pregnant or lactating women. Eleven FCon patients were excluded from the experiment for the following reasons: (i) 8 subjects with poor image quality, (ii) 1 subject with an intracranial lesion (meningioma), and (iii) 2 subjects with dental implants. In parallel, 4 HC subjects were excluded because of poor image quality (3 subjects) and dental implants (1 subject). Finally, the study included 73 FCon patients and 68 HC subjects. The experiment was approved by the Ethical Committee of Shanghai Tenth People's hospital of Tongji University School of Medicine, and each participant signed an informed consent form before the study.

Clinical assessment

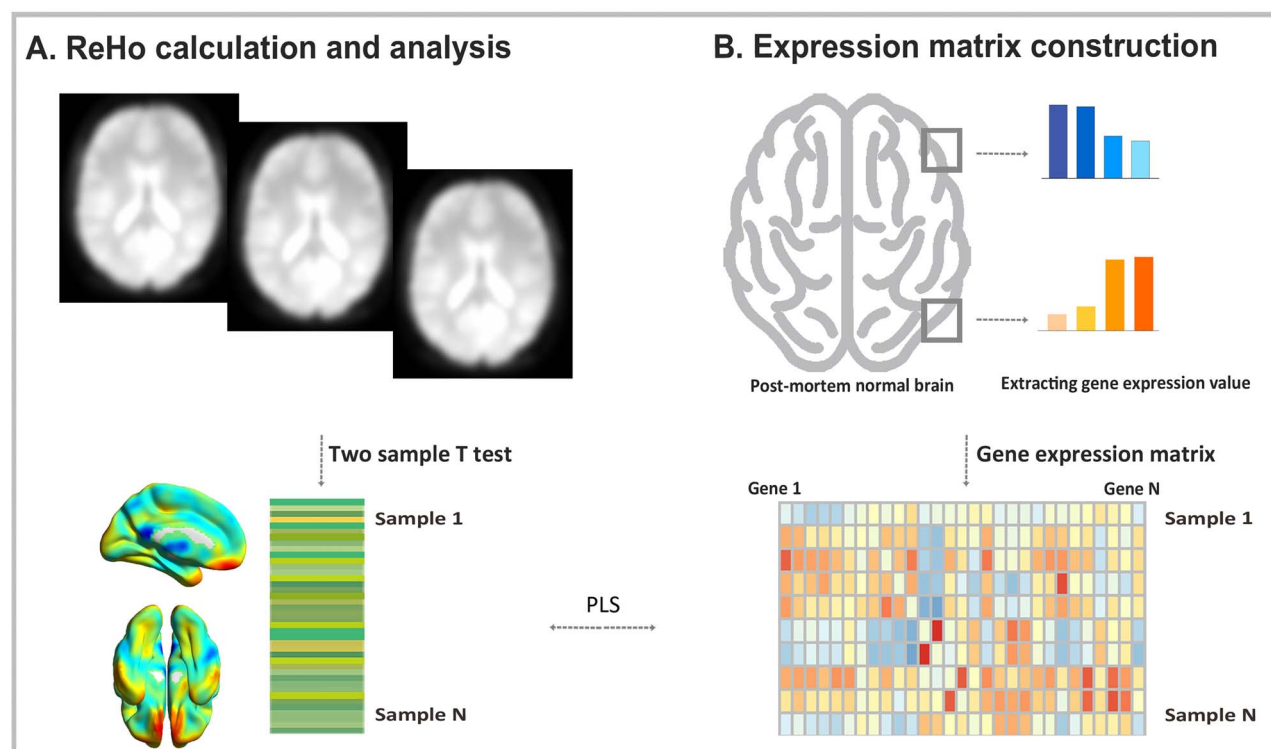
The FCon patients were asked to complete several self-administered surveys, including (i) the Wexner constipation score (Agachan et al. 1996), which is used to assess the severity of patients' constipation symptoms and mainly focuses on frequency of defecation, the sensation of incomplete evacuation, abdominal pain/distension, the difficulty of defecation, and duration of constipation; (ii) patient assessment of constipation quality of life (PAC-QOL; Marquis et al. 2005), which is used to survey the impact of constipation on patients' daily life and work; (iii) the ZUNG Self-rating Anxiety Scale (SAS; Zung 1971) and ZUNG Self-rating Depressive Scale (SDS; Zung 1965) were used to inquire the severity of patients' anxiety and depression. HCs group completed both the SAS and SDS as well.

MRI data acquisition and preprocessing

All MRI scans were conducted on a 3.0-Tesla scanner (Ingenia 3.0, Philips) equipped with a 32-channel head coil. Cotton balls were inserted into the subjects' ears and earplugs were utilized to reduce noise, and foam pads were used to minimize head movement. The turbo field echo sequence was used to obtain sagittal 3D T1-weighted images with the following parameters: repetition time (TR)=7.0 ms; echo time (TE)=3.2 ms; matrix=256 × 256; field of view (FOV)=256 × 256 mm²; flip angle (FA)=12°; slice thickness=1.0 mm, no gap; and 192 slices. The rs-fMRI images were acquired using a gradient-recalled-echo echo-planar-imaging sequence. The parameters are as followed: TR=2000 ms; TE=30 ms; matrix=64 × 64; FOV=220 × 220 mm; FA=90°; slice thickness=3.5 mm, slice gap=0.5 mm; 33 interleaved transverse slices; and 240 volumes. The subjects were asked to remain still, close their eyes, keep their minds blank, and not fall asleep during scans.

Utilizing Statistical Parametric Mapping 12 and Data Processing & Analysis for (Resting-State) Brain Imaging (DPABI) toolbox based on MATLAB, the rs-fMRI data were preprocessed with the steps as followed: (i) eliminating the first 10 volumes to equilibrate the signal; (ii) correcting the remaining 230 volumes for the acquisition time delay between slices and head motion, discarding subjects with maximum rotation > 2.0° and maximum displacement > 2.0 mm from the following analyses, and calculating the mean framewise displacement (Van Dijk et al. 2012); (iii) co-registering the structural and functional images, segmenting the transformed structural images into gray matter, white matter, and cerebrospinal fluid, and then estimating the normalization parameters from individual native space to Montreal Neurological Institute (MNI) space using the DARTEL technique (Ashburner 2007); (iv) normalizing the motion-corrected functional images to standard MNI space with the aforementioned normalization

I. Identification of genes associated with ReHo alteration in FCon Patients



II. Functional annotation of the identified genes

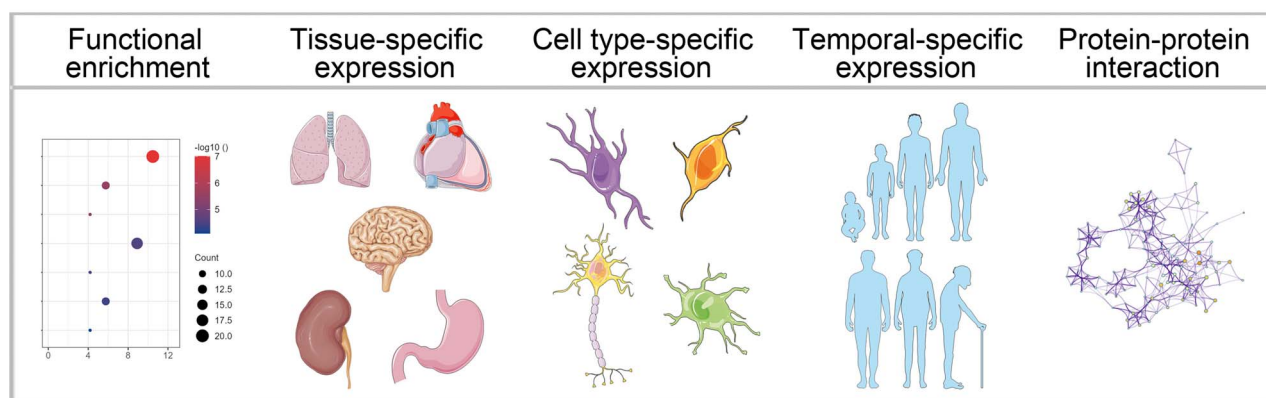


Fig. 1. Schematic overview of the study design. I) Identification of genes associated with ReHo alteration in FCon patients. A) Calculating ReHo difference. B) Constructing the gene expression matrix. Then, PLS regression was applied to explore genes whose expression values were linked to ReHo changes in Fcon patients. II) Functional annotation of the identified genes. The functional annotations included functional enrichment, tissue-specific expression, cell type-specific expression, temporal-specific expression, and PPI.

parameters utilized and then resampling them to 3 mm cubic voxels; (v) removing several spurious variances by regression, including Friston-24 head motion parameters (Friston et al. 1996), linear drift, and mean signals of white matter and cerebrospinal fluid; (vi) applying temporal bandpass filtering in a frequency range of 0.01 to 0.08 Hz to diminish the effects of high-frequency noise and low-frequency drift.

ReHo calculation

The ReHo was calculated using KCC of the time series of a given voxel with those of its 26 nearest neighbors in a voxel-wise analysis. To improve the normality and reliability of the measurement across subjects, the ReHo value of each voxel was

normalized by subtracting the entire brain mean ReHo value and then dividing by the standard deviation (SD) of all brain voxels (Zuo et al. 2013). The standardized ReHo map was then smoothed with a Gaussian kernel of $8 \times 8 \times 8$ mm full width at half maximum.

Statistical analyses

Using a 2-sample *t*-test, we compared the group difference in gender, age, and clinical parameters between FCon patients and NC groups. Between-group comparisons of ReHo maps were conducted via voxel-wise 2-sample *t*-tests, with gender and age controlled. Then, voxel-wise family-wise error (FWE) correction was utilized for multiple comparison correction, and clusters with

corrected $P < 0.05$ and size > 100 were obtained. In addition, mean ReHo values from these regions were extracted based on DPABI toolbox. Finally, partial correlation analyses were performed to test relationships between the acquired mean ReHo values and clinical parameters in FCon patients with age and sex as variables. Statistical significance was set at $P < 0.05$.

Gene expression data preprocessing

Based on the AHBA data set (Hawrylycz et al. 2012), the gene expression data of 6 postmortem human brains (Supplementary Table S1) were acquired, which contains expression data of 20,737 genes in 3,702 brain samples detected by 58,692 probes.

The transcriptomic data was analyzed and mapped using the abagen toolbox (<https://www.github.com/netneurolab/abagen>; Markello et al. 2021). In essence, gene expression data preprocessing consists of updating probe-to-gene annotations, implementing an intensity-based filter, selecting probes, matching samples to regions, handling missing data, normalizing samples, normalizing genes, calculating sample-to-region combination metrics, and selecting stable genes. Ultimately, we obtained 15,633 genes.

Transcription-neuroimaging association analysis

Using PLS regression (Krishnan et al. 2011), the relationships between 15,633 genes' expression values and between-group differences in the mean ReHo values were examined. The independent variable in PLS regression was the z-score normalized gene expression matrix (1,195 regions \times 15,633 genes), and the dependent variable was the z-score normalized ReHo case-control t-values. Then, the explained variances were calculated between independent and dependent variables, which were descending ranked in the PLS components. The component explaining over 10% of the variance and reaching statistical significance above chance levels (permutation $P < 0.05$) was chosen in this study (Romero-Garcia et al. 2019).

To evaluate whether the explained variance of the PLS component was significantly higher than that was expected by chance, we conducted a spatial autocorrelation corrected permutation test (spin test, $n = 1,000$), by utilizing the BrainSMASH (<https://github.com/murraylab/brainsmash>) software. Furthermore, the significance of genes contributing to components was explored using a bootstrapping method. Only significant genes (Bonferroni correction, $P < 0.05$) were selected for the following analyses.

Enrichment analyses

We used Metascape (<http://metascape.org/>; Zhou et al. 2019), a gene function annotation tool based on web version, to conduct enrichment analyses of the above obtained genes. The tool is embedded in the Gene Ontology (GO) database, which was utilized to identify the biological functions of the FCon-related genes (Gene 2021). We adjusted the false discovery rate (FDR) correction method and set a $P < 0.05$ to identify significant enrichment analyses. Next, tissue-specific, cell type-specific, and temporal-specific expression analyses were conducted using online tools (<http://genetics.wustl.edu/jdlab/>). We also applied specificity index probability to identify the likelihood of gene specific expression (Xu et al. 2014). FDR corrected multiple testing was used with a corrected $P < 0.05$. Using STRING v11.0 (<https://string-db.org/>), we performed a protein-protein interaction (PPI) analysis to detect whether the FCon-related genes are capable of creating a PPI network with a highest confidence interaction score of 0.9. A hub gene was defined as one with the highest degree value in the top 10%.

Results

Demographic and clinical characteristics

The demographic and clinical characteristics of FCon patients and HC groups are presented in Table 1. There were significant group differences in body mass index (BMI; $t = 4.533$, $df = 139$, $P < 0.0001$), depression ($t = 4.659$, $df = 139$, $P < 0.0001$), and anxiety ($t = 2.930$, $df = 139$, $P = 0.0019$). Whereas no significant group difference was obtained in age ($t = 0.9401$, $df = 139$, $P = 0.3487$) and gender ($\chi^2 = 0.686$, $df = 1$, $P = 0.4080$).

Group differences in ReHo

Compared with HCs group, significantly decreased ReHo were discovered in bilateral Cau, putamen, aINS, THA (cluster1: size = 1,100 voxels, $P < 0.05$, FWE corrected), and right middle cingulate cortex (MCC), SMA (cluster2: size = 240 voxels, $P < 0.05$, FWE corrected) in FCon patients. While, significant ReHo increase was found in the bilateral Rectus Gyrus (REC), medial part of OFC (cluster3: size = 756 voxels, $P < 0.05$, FWE corrected). They are presented in Fig. 2 and Supplementary Table S2 in detail.

After extracting the mean ReHo values from the above-mentioned clusters in patients with FCon, correlation analyses were performed to analyze the relationship between the ReHo alterations and the clinical parameters including Wexner constipation score, PAC-QOL, SDS, and SAS scores. There were significant positive correlations between PAC-QOL and SAS, SDS scores ($r = 0.722$, $P < 0.001$; $r = 0.743$, $P < 0.001$, respectively), and Wexner constipation score also positively correlated with SAS and SDS scores ($r = 0.400$, $P < 0.001$; $r = 0.326$, $P = 0.002$, respectively; Supplementary Fig. S1), suggesting that behavioral and psychological issues are more likely to manifest in FCon patients when constipation symptoms are more acute. The SAS score of FCon patients was negatively correlated with the ReHo value of right MCC/SMA ($r = -0.197$, $P = 0.047$; Supplementary Fig. S2). However, the result cannot survive after stringent correction. Other significant correlations were not obtained between the ReHo alterations and the clinical parameters ($P > 0.05$).

Transcription-neuroimaging associations

Following the processing of brain gene expression data, we obtained the brain gene expression matrix and used it through PLS regression to explore the gene expression patterns related to ReHo changes in FCon patients. Both the first and second PLS component (PLS1, PLS2) explained $> 10\%$ of the variance in the ReHo case-control differences. However, only the PLS2 score showed positive correlation with the case-control differences t-map ($r = 0.437$, $P < 0.003$, Supplementary Fig. S3). Subsequently, by ranking the normalized weights of PLS2 based on the z-score of each gene, we totally identified 1,634 genes that contributed significantly to PLS2 ($P < 0.05$, Bonferroni corrected). The PLS2 normalized weights of 771 of these genes were positive (PLS2+), and those of 863 genes were negative (PLS2-), indicating either overexpression or underexpression of the obtained gene, which consisting with increased or decreased ReHo alterations in the FCon patients. Detailed information of these genes is shown in Supplementary Tables S3 and S4.

Gene enrichment analyses and specific expression

Using Metascape, a gene function annotation tool, we conducted a GO enrichment analysis to reveal the GO biological processes mediated by the above-obtained genes. It was found that the

Table 1. The demographic and clinical characteristics of FCon and HCs group.

	FCon (n = 73) (mean ± SD)	HCs (n = 68) (mean ± SD)	FCon vs. HCs P-value
Age (years)	51.14 ± 18.03	53.20 ± 12.24	0.3487
Gender	20M/53F	23M/45F	0.4080
BMI (kg/m ²)	21.96 ± 4.10	24.78 ± 3.20	<0.0001
Depression (SDS)	45.54 ± 11.16	37.46 ± 8.15	<0.0001
Anxiety (SAS)	40.51 ± 11.05	35.44 ± 7.52	0.0019
PAC-QOL	45.16 ± 19.07	N/A	N/A
Wexner constipation score	11.71 ± 3.76	N/A	N/A

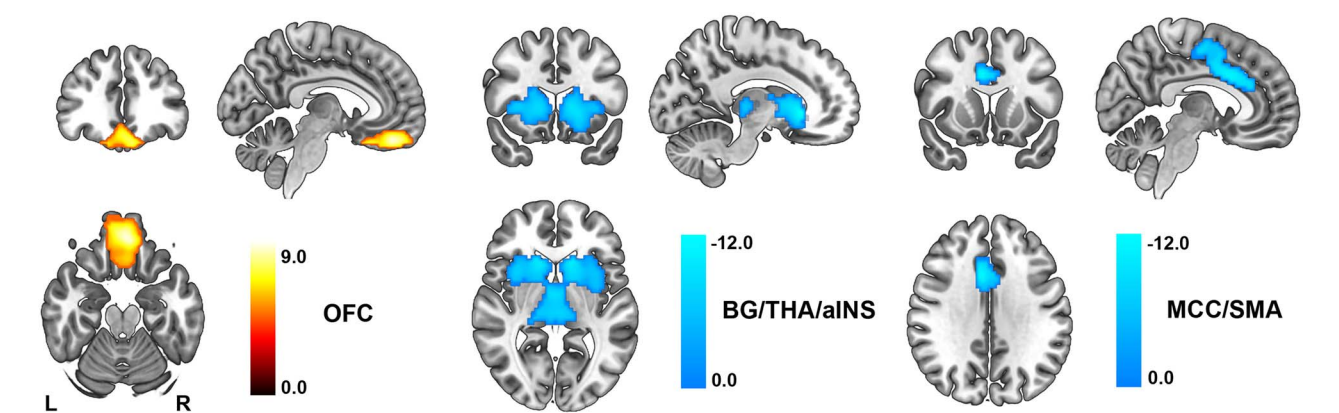


Fig. 2. Brain regions with significant ReHo changes between FCon group and HCs group ($P < 0.05$, FWE correction). The color bar shows the t-value. The significant brain regions with increased ReHo were the bilateral rectus gyrus, OFC (medial part). There was significantly decreased ReHo in the bilateral Cau, putamen, aINS, THA and right MCC, SMA. Abbreviation: L, left; OFC, orbitofrontal cortex; R, right.

PLS2+ gene list was primarily enriched for biological processes involved in various immune responses (Fig. 3A, biological processes). While the PLS2– gene list was predominantly enriched for several GO biological processes related to synaptic signaling, central nervous system development, fatty acid metabolism (Fig. 3B, biological processes). Using tissue-specific expression analysis, we found that the genes associated with ReHo alterations in FCon patients were expressed specifically in the blood in the PLS2+ gene list and the brain in the PLS2– gene list (Fig. 3, tissue-specific expression). Concerning brain cell types, the FCon-related genes were expressed in neurogliaocytes (Myeli), neurons (Cort+) and immune cells in the PLS2+ gene list, and neurogliaocytes (Astro), neurons (Pnoc+, Ntsr+), and immune cells in PLS2– gene list, specifically (Fig. 3, cell type-specific expression). As demonstrated by a temporal-specific analysis of expression, these genes were specially expressed throughout most developmental stages except for early fetal in both PLS2± gene lists (Fig. 3, temporal-specific expression).PPI analysis indicated that the PPI network could be respectively constructed using the FCon-related genes in PLS+ and PLS– genes list with statistical significance ($P = 2.2 \times 10^{-16}$; $P < 1.0 \times 10^{-16}$, respectively; Fig. 3, PPI). Finally, 20 hub genes in PLS+ PPI network and 24 hub genes in PLS– PPI network were identified.

Discussion

To our knowledge, this is the first study to apply rs-fMRI based ReHo value to investigate abnormalities of regional spontaneous brain activity in patients with FCon, and further explore the correlation between case–control ReHo changes and brain gene

expression, in order to determine transcriptional profiles associated with ReHo alterations in FCon patients. Our research findings promote understanding of the pathophysiological mechanisms of FCon and provide potential biomarkers for future FCon treatment strategies.

To date, many approaches have been used to measure resting-state spontaneous brain activity alteration in FCon patients, including ALFF (Zhu et al. 2016), fractional ALFF (fALFF; Li et al. 2021), FC (Duan et al. 2021), and independent component analysis (Zhang et al. 2022b). But, the results of these researches show inconsistent and require further validation. ReHo, as a commonly used and robust metric in rs-MRI (Zang et al. 2004; Zuo et al. 2013), its alterations and their relevant transcription-neuroimaging association analysis were explored in FCon patients in our study.

In this study, significantly decreased ReHo in both bilateral Cau nucleus, putamen, THA, aINS and right MCC, SMA were identified in FCon patients. Among the principal subcortical structures of the basal ganglia (BG), the Cau nucleus, and putamen are considered to be important for processing integrative functions, cognitive functions, and behaviors (Riva et al. 2018). A study using DTI showed a reduced nodal characteristic in the left Cau nucleus in FCon patients (Peihong et al. 2021), followed by an fMRI study reporting decreased brain activities in the bilateral Cau nucleus (Zhang et al. 2022c). These findings confirmed that the Cau nucleus participates in the pathophysiology of FCon. The THA, as a vital relay center, plays an essential role in relaying, integrating, and transmitting numerous sensory inputs and connecting with the widespread cortex (Sherman 2017). Previous neuroimaging research on FCon patients displayed various functional and structural abnormalities in the THA, which revealed its

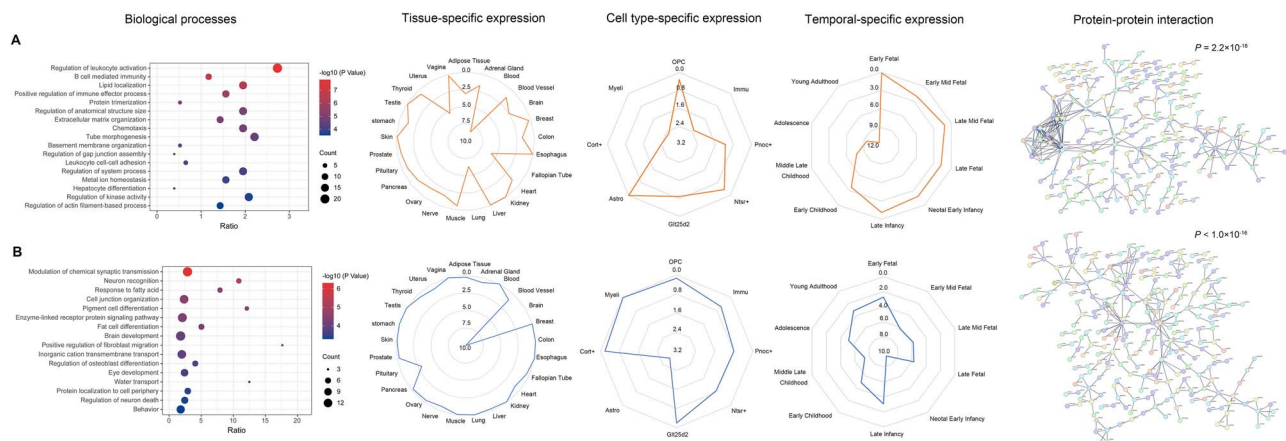


Fig. 3. Functional annotation of the genes related to ReHo changes in FCon patients. A) Functional annotation of the PLS+ genes. B) Functional annotation of the PLS- genes. In biological processes, the bubble size indicates the number of overlapped genes between the PLS ± gene list and GO biological processes (y-axis), and the bubble color indicates the $-\log_{10}(P)$ with the FDR corrected P-value. The x-axis represents the ratio of the number of FCon-related genes annotated to the item to the total number of genes. Statistical significance is higher for tissue-, cellular-, and temporal-specific expression within the inner location ($-\log_{10}(P)$ with the FDR corrected), representing a higher statistical significance. In PPI network, the P-value are used to indicate how likely it is that the proteins encoded by the input genes are interconnected to construct a network. Abbreviation: Astro, astrocytes; Cort+, cortistatin-expressing interneurons; Glt25d2, corticopontine neurons; Immu, immune cells; Myeli, myelinating oligodendrocytes; Nstr+, corticothalamic neurons; OPC, oligodendrocyte progenitor cells; Phoc+, prepronociceptin-expressing neurons.

crucial role in visceral sensory information input and motor controlling (Jin et al. 2019; Cai et al. 2021; Duan et al. 2021; Li et al. 2021; Liu et al. 2021; Peihong et al. 2021; Zhang et al. 2021). Combining rs-fMRI, DTI and graph theory, a decreased nodal degree/efficiency in THA was observed in FCon patients, indicating aberrant transmissions of intestinal sensory signals between the THA and other brain regions, which correlated negatively with the difficulty of defecation (Duan et al. 2021; Liu et al. 2021; Peihong et al. 2021). Additionally, a rectal distension research revealed altered brain activity both in the BG (Cau nucleus, putamen) and THA (Mirbagheri et al. 2017), which is involved in the basal ganglia network, indicating that rectal stimuli transmit visceral sensory information to the BGN. Here, the decreased ReHo in Cau nucleus, putamen, and THA indicated that visceral sensory integration in BGN of FCon patients is impaired, and signal projection to cerebral cortex area is reduced.

The insula, an essential visceral sensorimotor area, is a core node of the salience network and homeostatic visceral network, which is responsible for monitoring homeostatic input and internal reaction to visceral stimulation (Menon and Uddin 2010). The insula can be divided into anterior and posterior regions, each participating in the integration of 2 key complementary networks. Previous studies on FCon discovered the structural and functional alterations of the aINS (Zhu et al. 2016; Duan et al. 2021; Jia et al. 2022), which is known to be involved in the perception and subjective experience of pain by integrating other brain regions linked to sensory and emotional aspects of pain (Berman et al. 2008). In the current study, our result of ReHo alterations in the aINS supports previous studies and reveals the vital role of the aINS in visceral sensory processing and related emotion responses.

The MCC is an interceptor area that collects visceral nociceptive information to mediate nocifensive behaviors, regulate chronic pain, and contribute to decision-making and cognitive control as well (Tolomeo et al. 2016; Tan et al. 2017). It is reported that the activity of MCC is related to the optimally adjusting behavior to uncertainty, negative affect experience, which may cause anxiety (Tolomeo et al. 2016). Furthermore, MCC

abnormalities were also observed in patients with Crohn's disease and irritable bowel syndrome (Elsenbruch et al. 2010; Zhang et al. 2022b), which caused poor localization of sensory stimuli and abnormal chronic pain regulation. Thus, our finding extends the understanding of spontaneous brain activity alterations in FCon patients, and we postulate that abnormal MCC activity may be linked to abnormally consistent abdominal pain/distension and dysfunction of emotional processing and cognitive control in FCon patients. The SMA is a part of the cortical network related to visceral sensory stimuli response and motor behavior regulation (Ruan et al. 2018). It has been found that FCon patients have lower ALFF, impaired cortical thickness of SMA and weaker effective connectivity between the SMA and other brain regions (Zhu et al. 2016; Hu et al. 2020), whereas graph-theory research has revealed a greater nodal degree of SMA (Duan et al. 2021; Liu et al. 2021). These results indicated that motor control and response preparation for bowel movement were impaired, and that functional interaction with more other brain areas was required to complete defecation. Consequently, we hypothesize that reduced ReHo of SMA in FCon contributes to altered bowel movements and visceral responses. The correlation analysis in our study showed that the SAS score was negatively correlated with the ReHo values of right MCC and SMA, suggesting that the more anxious the patient was, the weaker activity of the MCC and SMA, and the worse ability to deal with uncertainty, negative affect experience and the more severity of functional abnormality in control of bowel movements and visceral responses. However, the significance of the result is relatively weak and cannot survive after correction, which may be related to the relatively small sample size and heterogeneous patients with different clinical features. Therefore, we should be cautious to make our interpretation about the result. Future studies with expanded subjects need to validate and clarify the relationship of clinical features with the ReHo alterations in FCon patients.

Our results showed significantly increased ReHo in the bilateral rectus gyrus (REC), medial part of OFC in FCon patients. The REC and OFC belong to the prefrontal lobe, which participates in emotional regulation, cognition function, executive function, and

stimulus response (Rudebeck and Rich 2018). Our previous study depicted a decreased GMV in OFC and its negative correlation with depression in patients with FCon, suggesting that the structural impairment in OFC might associated with abnormal emotional processing (Cai et al. 2021). Based on rs-fMRI, Zhu et al. (2016) found increased baseline ALFF activity in OFC and its positive correlation with incomplete evacuation, indicating that the symptom of FCon might led to dysfunction of emotional processing and sensory integration. While Li et al. (2021) reported decreased baseline fALFF activity in OFC in FCon patients with mental disorders as compared with HCs and FCon patients without mental disorders. These differences may be because of different stages of FCon, metric difference, the population and disease heterogeneity, and the relatively small sample size. Here, we found increased ReHo in OFC, suggesting a compensatory effect for GMV alteration to some extent and associated with sensory and emotional dysfunctions in FCon patients.

Recently, the MGB axis, a consistent crosstalk between the brain and the gut through multiple overlapping pathways, has been discovered to be a potential pathophysiological mechanism of FCon, as well as the cause of mental and psychological disorders among FCon patients (Black et al. 2020; Wang and Yao 2021). Utilizing the transcriptional neuroimaging analysis, several gene profiles with different biological functions related to ReHo changes in FCon were identified, including synaptic signaling, central nervous system development, fatty acid metabolism, immune response and etc., suggesting that FCon may be the result of a complex polygenetic and poly-pathological process. An animal study found that injections of toxin 6-hydroxydopamine into the medial forebrain bundle induced both symptoms of Parkinson's disease and gastrointestinal deficits, implying transsynaptic effects between the brain and the colorectal enteric nervous system (Chai et al. 2020). Our results of gene modules enrichment in synaptic signaling and central nervous system development suggest that synaptic dysfunction and neuronal maldevelopment may be one of the pathophysiological mechanisms of FCon. Short-chain fatty acids (SCFAs), the main metabolites of the anaerobic gut microbiota, play a crucial role in regulating metabolic processes and promoting colon movement (Schroeder and Backhed 2016). Moreover, SCFAs contribute to maintaining immune homeostasis by regulating the maturation and function of microglia, the brain's resident immune cells (Erny et al. 2015). The immune system is one of the main pathways of the MGB axis, and the regulation of gut microbiota on the immune system is mainly accomplished by influencing microglial activation (Erny et al. 2015; Schluter et al. 2020). Among the genes expressed in microglia, ANXA1 is constitutively expressed in cells related to the innate immune stage of the normal brain (Solito et al. 2008), and activated early during inflammatory reactions causing microglia to damage neurons (McArthur et al. 2010). There is evidence that low expression levels of ANXA1 may be associated with inflammation during Crohn's disease and these changes may serve as a biomarker of disease progression, and may act as a predictor of therapeutic effectiveness (Sena et al. 2013). These findings suggest that genes associated with fatty acid metabolism and immune response influence the MGB axis in FCon.

In this study, we observed a specific expression of FCon-related genes in brain and blood tissue, which may help us interpret our findings with greater confidence. In FCon patients, the specific expressions of Myeli, Astro, Ntsr+, Pnoc+, and Cort+ neurons emphasize their importance. Besides, the immune cells, which were also specifically expressed in these genes, play an essential

role in regulation of the MGB axis (Erny et al. 2015; Schluter et al. 2020). In addition, temporal-specific expression analysis revealed specific expression of FCon-related genes almost throughout the development of the cortical cortex, indicating that these genes influence the progression of FCon in an enduring manner. Overall, even though the analysis of transcription-neuroimaging may be a backhanded method for exploring the underlying pathophysiology mechanisms, it still holds promise for exploring the pathophysiology of FCon.

Limitations

The current study has several limitations. Because of various reasons, the sample size of the study is relatively small, including a low proportion of FCon patients who visit hospitals, and some patients refuse to undergo brain MRI examination, which limits its widespread application and weakens its statistical robustness. To enhance generalizability and credibility, further studies involving larger sample sizes are necessary. Second, because of the small sample size, we did not further categorize FCon into more subtypes according to mental status or different categories of FCon. This may lead to bias in the research results. Therefore, it is not possible to categorize and explain the changes in brain function of patients with FCon in different mental states or stages. Third, the results of this study may be influenced by different treatment medications. Some patients only take health care products, and some take laxatives occasionally. In this study, we did not take into account the effect of medicines on the results. In future studies, we may be able to analyze the impact of medicines by expanding the sample size. Finally, the neuroimage data and the gene expression data were obtained from different subjects. Despite the fact that prior research has shown that the gene expression patterns are remarkably preserved across individuals (Zeng et al. 2012), one must exercise caution when interpreting findings from the cross-individual transcription-neuroimaging association analysis.

Conclusion

In conclusion, this study supported our prior hypotheses that significant ReHo alterations existed in FCon patients compared with HCs. These between-group differences were spatially related to gene expression, which implicated in various biological processes that are associated with the MGB axis. In light of these findings, it is possible that FCon develops via complex poly-pathway mechanisms. This perspective presents a novel approach to understanding the pathophysiology of FCon.

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Supplementary material

Supplementary material is available at *Cerebral Cortex* online.

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Data availability

The Allen Human Brain microarray dataset is available at <http://www.human.brain-map.org>.

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