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Differences in regional homogeneity between patients with Crohn's disease with and without abdominal pain revealed by resting-state functional magnetic resonance imaging

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Abstract

Abnormal pain processing in the central nervous system may be related to abdominal pain in patients with Crohn's disease (CD). The purpose of this study was to investigate changes in resting-state brain activity in CD patients in remission and its relationship with the presence of abdominal pain. Twenty-five CD patients with abdominal pain, 25 CD patients without abdominal pain, and 32 healthy subjects were scanned using a 3.0 T functional magnetic resonance imaging (fMRI) scanner. Regional homogeneity (ReHo) was used to assess resting-state brain activity. Daily pain scores were collected 1 week before fMRI scanning. We found that patients with abdominal pain exhibited lower ReHo values in the insula, middle cingulate cortex (MCC), and supplementary motor area, and higher ReHo values in the temporal pole. In contrast, patients without abdominal pain exhibited lower ReHo values in the hippocampal/parahippocampal cortex

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Conflict of interest statement

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and higher ReHo values in the dorsomedial prefrontal cortex (all $P<0.05$, corrected). The ReHo values of the insula and MCC were significantly negatively correlated with daily pain scores for patients with abdominal pain ($r=-0.53$, $P=0.008$, and $r=-0.61$, $P=0.002$, respectively). These findings suggest that resting-state brain activities are different between remissive CD patients with and without abdominal pain, and that abnormal activities in insula and MCC are closely related to the severity of abdominal pain.

Keywords

Functional magnetic resonance imaging; Neuroplasticity; Regional homogeneity; Pain; Crohn's disease

1. Introduction

Abdominal pain is one of the main complains of CD, which occurs throughout acute inflammation and even in clinical and/or endoscopic remission [9]. Treatment options for this particular symptom are limited due to its obscure mechanism [40]. The potential mechanisms include inflammation, visceral hypersensitivity, brain-gut axis dysfunction and psychological abnormalities, among which dysfunction of brain-gut interactions is considered to be vital [11, 32].

Resting-state functional magnetic resonance imaging (rs-fMRI) detects blood oxygenation level dependent (BOLD) signals, and can be used to explore spontaneous activities of neurons under a resting state. Regional homogeneity (ReHo) is a new analytical method that can be used to characterize the synchronization of fluctuations of BOLD signals among neighboring voxels within a single region. Based on the assumption that temporal patterns in spatially neighboring voxels within a functional region should share similarity, changes in ReHo values suggest possible synchronization and coordination abnormalities of spontaneous neuronal activities in corresponding brain region [45]. Therefore ReHo could be a valuable method complementary to brain structure measurement and could provide a better understanding of pathophysiological changes in brain function. Many recent studies of pain-related diseases, such as irritable bowel syndrome (IBS) [19, 28], migraine [44], and complex regional pain syndrome [10], have adopted rs-fMRI to detect pathophysiological changes in certain brain regions that are related to the recognition and processing of pain signals. In recent years, there has been growing interest in the use of MRI to detect the correlation between brain activities and inflammatory visceral pain in inflammatory bowel diseases (IBD) [1-3, 6, 20, 47]. Previous studies show alternations in gray matter (GM) structures including GM volumes and cortical thickness in multiple brain regions of CD patients, which in certain brain regions are correlated with disease duration [1, 6]. CD patients also have been found to exhibit altered habituation to stress and altered neural activity in the amygdala, hippocampus, insula, putamen, and cerebellar regions when performing stress-evoking tasks [2]. However, to date, there has been no study on resting-state brain activity in CD patients. We believe that rs-fMRI based on ReHo analysis may provide a new insight into the neurophysiology of CD. Given the importance of abdominal pain in CD and the known association between chronic visceral pain and abnormal resting-

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state brain activities [31], it is necessary to understand whether abdominal pain affects resting-state brain activity in CD patients, and whether ReHo values differ between CD patients with and without abdominal pain.

We hypothesized that the resting-state brain activity in CD patients with abdominal pain is different from those without abdominal pain, and that brain regions with abnormal ReHo in patients with abdominal pain are involved in processing visceral pain signals. The aims of the study were (1) to compare ReHo values between CD patients with and without abdominal pain using rs-fMRI;(2) to examine a potential correlation between the ReHo values and daily pain scores in patients with abdominal pain.

2. Methods

The study protocol has been approved by the Ethics Committee of Yueyang Hospital of Integrative Traditional Chinese and Western Medicine affiliated with Shanghai University of Traditional Chinese Medicine.

2.1. Subjects

CD patients: A total of 50 CD patients were included via continuous, non-selective recruitment. There were 25 patients with abdominal pain and 25 without abdominal pain. All patients were recruited from the inflammatory bowel disease specialist outpatient clinic of Shanghai Institute of Acupuncture and Meridian affiliated with Shanghai University of Traditional Chinese Medicine and the Endoscopy Center of Zhongshan Hospital affiliated with Fudan University. All patients received systemic and gastrointestinal examinations (i.e., colonoscopy and pathology biopsy). Laboratory tests and colonoscopy were completed in 2 weeks and 1 month before MRI scanning. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelet (PLT) levels and Crohn's disease endoscopic index of severity (CDEIS) scores were collected from all patients.

Inclusion criteria: (1) age 18–50 years old; (2) education ≥ 6 school years; (3) in remission for 12 months or longer; (4) the Crohn's disease activity index (CDAI) ≤150; and (5) CDEIS<3; (6) right-handed.

Exclusion criteria: (1) abnormal biological disease activity indices (C reactive protein >10 mg/L; erythrocyte sedimentation rate >20 mm/h; platelets $>300\times10^9$/L); (2) having received CD-related abdominal surgery; (3) use of corticosteroids, biologics, psychotropic drugs or opioids in the past 3 months; (4) pregnant or lactating women; (5) current or history of psychiatric and neurological diseases, head trauma, or loss of consciousness; (6) patients with claustrophobia; and (7) patients with metal implants.

In addition, the Pain CD group included CD patients who experienced pain in the periumbilical region, the right lower quadrant or the left lower quadrant in the past 12 months, with the days of pain per week ≥3 days. The Non-pain CD group included CD patients who had not experienced abdominal pain in the past 12 months.

Healthy controls (HCs): A total of 32 right-handed HCs were included in this study. They were recruited with advertisements from Shanghai University of Traditional Chinese

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Medicine. None of the subjects in the HC group were taking any medication, or had gastrointestinal or pain related diseases, or had positive performance in colonoscopic examination.

All participating subjects were assessed by an experienced gastroenterologist in the gastroenterology department of Zhongshan Hospital affiliated with Fudan University, and screened for any neurological and psychiatric disorder by an experienced psychiatrist from Shanghai Mental Health Center according to the structured psychiatric interview for Diagnostic and Statistical Manual of Mental Disorders (4th Edition; DSM-IV).

2.2. Outcome assessment

The abdominal pain of all patients was assessed using a 0–10 cm visual analogue scale (VAS), with 0 representing no pain and 10 representing excruciating pain. The week before the fMRI scan the patients completed a daily pain log, the average pain score was calculated as the sum of the daily pain scores divided by the number of the days with pain. The patient's disease condition was evaluated using CDAI [8]. The quality of life of the patients was evaluated using the inflammatory bowel disease questionnaire (IBDQ) [22]. The psychological conditions of all subjects were evaluated using the Hospital Anxiety and Depression Scale (HADS) [46].

2.3. MRI data acquisition

Functional MRI was performed using a 3.0 Tesla MR scanner (Siemens Medical, Erlangen, Germany) equipped with a 12-channel head coil. Functional images were acquired with a single-shot gradient–recalled echo planar imaging (EPI) sequence (TR/TE: 2,000 ms/30 ms, field of view (FOV): 240 mm×240 mm, matrix size: 64× 64, flip angle: 90°, in-plane resolution: 3.75 mm×3.75 mm and 32 sagittal slices). A set of high-resolution T1-weighted structural images was also collected (TR/TE: 2.3 s/2.98 ms, FOV: 256 mm×256 mm, matrix size: 256×256, flip angle: 9°, in-plane resolution: 1 mm × 1 mm, slice thickness: 1.0 mm with no gaps and 176 slices).

2.4. Imaging data preprocessing

The resting-state fMRI data were pre-processed using SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). The first five images of each functional time series were discarded for the longitudinal magnetization to reach equilibrium. All slices of the remaining images were processed by slice-timing adjustment and realigned to the first volume. Then, the time series of images of each subject were motion-corrected, the head motion parameters were obtained by estimating six parameters capturing translation and angular rotation relative to the first volume. The given data set in which the translation or rotation parameters exceeded 1.5 mm or 1.5 degrees rotation was discarded. The realigned functional images were then spatially normalized to Montreal Neurologic Institute (MNI) space using the normalization parameters estimated by T1 structural image unified segmentation, re-sampled to 3 mm voxels. In order to conserve the fine-grained local pattern and to avoid artificial connections, smoothing was not applied to the normalized data. Several sources of spurious variance, including estimated motion parameters, linear drift, and average BOLD signals in ventricular and white matter regions, were removed from the

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data through linear regression. After that, temporal bandpass filtering (0.01–0.08 Hz) was performed to reduce the effect of low-frequency drifts and high-frequency noise. All of these procedures were performed using DPARSF software (http://www.restfmri.net/forum/DPARSF).

2.5. ReHo analysis

Individual ReHo maps were generated by calculating Kendall's coefficient of concordance (KCC) [45], used to measure the correlation of the time series of a given voxel with the time series of its 26 nearest neighbors, within a GM mask in a voxel-wise manner using REST software (http://restfmri.net/forum/index.php) [43]. When the center cube was on the edge of the GM mask, we only calculated ReHo for a voxel if all of the remaining nearest voxels were within the GM mask. For each participant, the KCC map was normalized by dividing KCC in each voxel by the mean KCC of the total GM again using the DPARSF software.

2.6. Statistical analysis

2.6.1. Behavioral data—Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The mean ± standard deviation was used for normally distributed continuous variables; the median, 25^{th} and 75^{th} percentiles were used for non-normal distributed continuous variables; and frequencies and proportions were used for categorical variables. A two independent samples t test /one way analysis of variance (ANOVA) was used for normally distributed continuous variables. Nonparametric tests (Kruskal- Wallis test) were used for between-group comparisons of non-normal distributed continuous variables. A Pearson's χ^2 test or Fisher's exact test was used for between-group comparisons of categorical variables. All p values were two-sided and $P<0.05$ was considered statistically significant.

2.6.2. Imaging data—At the second level of analysis, to determine the differences in ReHo values among HCs and patients with or without abdominal pain, a one-way analysis of variance (ANOVA) was performed at each voxel to assess the main effect with a significance level of $P<0.05$ (false discovery rate corrected) and cluster size > 5. Age, gender, weight, anxiety and depression were deemed to be insignificant covariates.

Based on the brain regions showing differences in ReHo between the patients and the HCs based on the ANOVA results, regions of interest (ROIs) were selected from the sets of voxels within 6-mm spheres with the centers at the peaks of the clusters. To disclose a possible relationship between the ReHo values and the daily pain scores, a 2-step correlation analysis was conducted in patients with abdominal pain: (1) a Pearson's correlation analysis was applied to detect the relationship between the mean ReHo values in each ROI and the daily pain scores for CD patients with age, gender, weight, anxiety and depression as covariates. The significance level was set at $P<0.05$. Bonferroni test was used to correct for multiple comparisons. (2) whole-brain correlation analysis was applied to disclose potential correlations between brain regions and pain severity. The significance level was set at $P<0.05$. The false discovery rate was corrected with the cluster size exceeded 5.

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Finally, all the ReHo-related maps were superimposed on a template provided by MRIcroN software (http://www.cabiatl.com/mricro/) for display, and the brain regions showing the differences were presented in MNI coordinates.

3. Results

3.1. Clinical characteristics

Among the 50 CD patients, 25 (50.0%) showed the symptom of abdominal pain. The average pain score ranged from 1 to 5.4, with mean and standard deviation values of 3.02±1.52. The Pain CD group, the Non-pain group and the HC group did not differ significantly in demographic characteristics (i.e., gender, age, height, and weight). The Pain CD group and the Non-pain CD group did not differ significantly in disease course, Montreal classification, or concomitant medication. There was a significant difference in the HADS-A and HADS-D scores among the three groups (both $P<0.01$). The HADS-A and HADS-D scores of the patients in the Pain CD group were both significantly higher than that of HCs (both $P<0.01$), and the HADS-A scores of the patients in the Non-pain CD group were significantly higher than those of HCs ($P<0.05$). The HADS-D scores of the patients in the Pain CD group were significantly higher than those of the patients in the Non-pain CD group ($P<0.05$).

The pain group patients yielded higher CDAI scores and lower IBDQ scores than the non-pain group patients (both $P<0.001$). The CRP levels in the pain group were also higher than the non-pain group ($P<0.01$), while the PLT and ESR levels between the two groups were not significantly different. There was no significant difference between the two CD groups in the CDEIS scores (Table 1). The CDAI scores of all patients were correlated with CDEIS scores (r=0.356, $P<0.05$), CRP levels (r=0.423, $P<0.01$), ESR levels (r=0.491, $P<0.001$) and PLT levels (r=0.340, $P<0.05$).

3.2. ReHo value differences among the three groups

A one-way ANOVA including the Pain CD group, the Non-pain CD group and the HC group revealed that the ReHo values differed significantly among the three groups ($P<0.05$, corrected) within six brain regions including: the left insula, the supplementary motor area (SMA), the midcingulate cortex (MCC), the hippocampus (HIPP)/paraHIPP, the temporal pole, and the right dorsomedial prefrontal cortex (dmPFC; Table 2; Figure 1A).

ReHo values extracted from the above six brain regions were subjected to post-hoc analysis. The results showed that the ReHo values of the insula, SMA and MCC in the patients from the Pain CD group were significantly lower compared to the Non-pain CD group and the HC group, whereas the ReHo values of the temporal pole (TP) in patients from the Pain CD group were significantly higher compared to the Non-pain CD group and the HC group ($P<0.05$, corrected). The ReHo values for HIPP/paraHIPP in patients from the Non-pain CD group were significantly lower compared to the Pain CD group and the HC group, whereas the ReHo values of dmPFC in patients from the Non-pain CD group were significantly higher compared to the Pain CD group and the HC group (all $P<0.05$, corrected; Figure 1B).

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The above results revealed that, among the three groups, CD patients with abdominal pain showed the lowest ReHo values in the insula, MCC and SMA, and the highest ReHo values in the TP, whereas CD patients without abdominal pain showed the lowest ReHo values in the HIPP/paraHIPP, and the highest ReHo values in the dmPFC.

3.3. Correlation between ReHo values and daily pain scores

The ReHo values in two brain regions of CD patients with abdominal pain from the group comparison were found to correlate with daily pain scores. The ReHo values in the insula and MCC of CD patients with abdominal pain were significantly negatively correlated with their daily pain scores (r=−0.53, P=0.008; r=−0.61, P=0.002; Figure 1C). The ReHo values in the HIPP and dmPFC of CD patients with abdominal pain were not significantly correlated with their daily pain scores (r=−0.33, P=0.138; r=0.37, P=0.096).

For the whole-brain analysis, the daily pain scores negatively correlated with the ReHo values of the left insula, MCC, right dorsolateral prefrontal cortex (dlPFC), bilateral thalamus, and periaqueductal gray (PAG), and positively correlated with the right orbitofrontal cortex (OFC) (Table 3, Figure 2).

4. Discussion

In this study, we show, for the first time, differences in the spontaneous BOLD signals in several brain areas among HCs and CD patients with and without abdominal pain. In CD patients with abdominal pain, there were substantial decreases of ReHo values in the insula, MCC and SMA, and increases of ReHo values in the TP. The reductions of the ReHo values in the insula and MCC in these patients were negatively correlated with the daily pain score. In CD patients without abdominal pain, there were substantial decreases of ReHo values in the HIPP/paraHIPP and an increase of ReHo values in the dmPFC. These results suggest a different reorganization of resting-state brain activities between CD patients in remission with and without abdominal pain. This may indicate an association between visceral pain and changes in brain activity in IBD patients.

The ReHo values of the insula, MCC, SMA and TP in CD patients with abdominal pain showed abnormal changes. Our previous study also revealed that the GM volumes in these brain regions of CD patients were significantly lower comparing to that of HCs [8]. Agostini et al. [1] reported GM volume decreases in the anterior MCC and part of the dorsolateral PFC of CD patients. Zikou et al. found decreased GM volumes in the right SMA, in the bilateral inferior temporal gyrus and some other brain regions in IBD patients [47]. Taken together, these results suggested abnormalities in both morphology and resting state functional activities of these brain areas in CD patients. Similar changes were also discovered in many other diseases with visceral pain, such as IBS [19, 30], functional dyspepsia [28], painful bladder syndrome/interstitial cystitis [24] and chronic pancreatitis [19]. These brain regions are important components of pain networks. The insula is responsible for the sensation of the body and internal organs, the control of visceral movement and autonomic nerves, and especially critical for pain perception. Functionally, the MCC, hypothalamus and PAG form a descending pain modulatory system, through which some supraspinal areas can directly or indirectly regulate the processing of

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nociceptive stimuli on the dorsal horn of the spinal cord. MCC dysfunction may indicate damages to the endogenous pain suppressing system [17]. The insula and MCC dysfunctions suppress the reaction of the cingulated gyrus-PAG/brainstem descending inhibitory system and the receptive system in the anterior insula to nociceptive stimuli, thereby inhibiting the normal analgesic effect of the body [15]. The SMA is also part of a cortical network involved in the translation of painful cognition. The role of the TP in pain processing is not fully understood, but it may be related to imparting affective tone to short-term memories. In the present study, we observed higher HADS-D scores in CD patients with abdominal pain, suggesting that abdominal pain may aggravate CD patient's depression symptoms. The increase in TP ReHo values in CD patients with abdominal pain suggests increased synchronization of neuronal activities in this region, possibly because of compensatory cortical reorganization in this region.

Abnormal changes in the brain structure and function in patients with chronic visceral pain may be associated with chronic nociceptive stimulation and subsequent reorganization and plasticity of the brain. In CD patients, ascending intestinal inflammation/pain signals are transmitted through the brain–gut axis to cortical and subcortical areas. Through vagal afferent nerve fibers and spinal gastrointestinal afferent fibers, inflammatory signals are transmitted by cytokines and progressively coded in different levels of the CNS and finally projected to related cortical and subcortical regions. Intestinal inflammation signals can induce apoptosis of stellate cells and oligodendrocytes [12, 34]. Inflammatory mediators can also change the excitability of the CNS by activating microglia, macrophages and endothelial cells [13, 36-37]. In acute inflammation, systemic inflammatory stimulation increases the levels of proinflammatory and chemoattractant factors in the brain, and brain microglial cells play an important role in communicating between brain and systemic immune system [38, 42]. Cytokines can be synthesized locally in the brain, and periphery inflammatory cytokines can also reach the brain through active transportation and periventricular organs [25, 36].

The disease activity indices (CDAI scores and CRP levels) of CD patients with abdominal pain were significantly higher than those without abdominal pain, and the health-related quality of life was significantly lower, suggesting that abdominal pain severely affects disease activity and quality of life. In CD patients, abdominal pain during remission is chronic, involving a variety of factors, and is often difficult to accurately quantify and control. The one-week daily pain score log that we used in the current study is considered a rather robust method for assessing the severity of chronic pain [16, 23] and has been applied in a number of studies on chronic pain including abdominal pain in CD patients [4, 39]. Because abdominal pain in CD patients is commonly the most prominent symptom when they are seeking medical help and the patterns of pain remain relatively stable in most patients, the pain score log we used may provide an estimate of pain intensity in these patients that is similar to that experienced during prior time periods. The ReHo values of the insula and MCC were negatively correlated with the daily pain scores in these patients. Reduced ReHo values in the insula and MCC in patients with high abdominal pain scores would suggest reduced synchronization and coordination of neural activities in these brain regions. This negative correlation is opposite to the observation that in patients with chronic pancreatitis, the thicknesses of these two brain areas were positively correlated with daily

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pain scores [17]. The whole-brain analysis further verifies this negative correlation, and improves the reliability of the results. A number pain related brain regions were found in this study. Interestingly, the daily pain scores were negatively correlated with decreased ReHo value of dlPFC, and simultaneously positively correlated with increased ReHo value of OFC, which parallel to the results from cyclic menstrual pain patients [41]. The dlPFC is a region important for top-down pain control, and has been implicated in disinhibition of orbitofrontal networks which may lead to enhanced negative affect [5, 29]. Furthermore, in the present study the ReHo values of the thalamus and PAG were negatively correlated with pain scores. This may be due to sustained abnormal nociceptive input to the brain, rather than with CD pain-specific pathology, because the ReHo values in these regions were not significantly different from patients with abdominal pain or HCs, and these regions are involved in other types of pain conditions [14]. These findings suggest that the dysfunction of dlPFC, OFC, thalamus and PAG may not be central to the pathophysiology of CD pain, but instead either they are adaptations to increased nociceptive input or they represent a preexisting vulnerability to experiencing greater pain [7].

Among the three groups, we found that CD patients without abdominal pain showed the lowest ReHo value of HIPP/paraHIPP and the highest ReHo value of dmPFC. In previous studies, abnormal BOLD signals in IBD patients relative to controls have been found in HIPP and mPFC [25, 30]. The HIPP/paraHIPP is associated with limbic and non-limbic systems. It regulates immune response through the hypothalamic–pituitary–adrenal (HPA) pathway and neurohormonal pathways, and plays an important role in neuroimmune regulation [26]. Studies have shown that experimental colitis/intestinal dysbiosis were associated with aberrant expression of brain-derived neurotrophic factor or its mRNA in the HIPP and abnormal development of anxiety-like behavior [18, 35]. Another study showed increased TNF-α levels in the HIPP and enhanced excitability of the CNS in rats with experimental colitis [4]. The HIPP may also interact with the vagus nerve, which plays a role in neural modulation of intestinal inflammation by releasing acetylcholine to regulate immune cells in the intestinal wall. Although studies of the role of the dmPFC in neuroimmune regulation are rare, it has been reported that the HIPP/paraHIPP is closely connected to the PFC, which forms the hippocampal-prefrontal cortical circuit [27]. The decrease of ReHo values in the HIPP/paraHIPP may cause excessive compensation of the cortical functional reorganization in the PFC, leading to increased ReHo values in the PFC in CD patients. Since the mPFC is known to be involved in regulating chronic pain [21, 33], the increased ReHo value in patients without abdominal pain may suggest enhanced compensation of the mPFC.

Beside these findings, the present study has certain drawbacks. Since it is a cross-sectional study, we could not determine whether the abnormal ReHo changes in CD patients occurred before the disease onset or subsequent to the disease. Additionally, neurotransmitter modulation and receptor binding in the related brain areas and their correlations with the resting-state brain activity could be measured in future studies.

In conclusion, we show different changes in ReHo values between remissive CD patients with and without abdominal pain. The decreased ReHo values of the insula and the MCC and their correlation with pain severity in CD patients with pain suggests the involvement of

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these brain regions in the processing of visceral pain. These findings may help us to better understand the pathophysiology of visceral pain in CD patients and provide an insight to facilitate the development of new therapies in the future.

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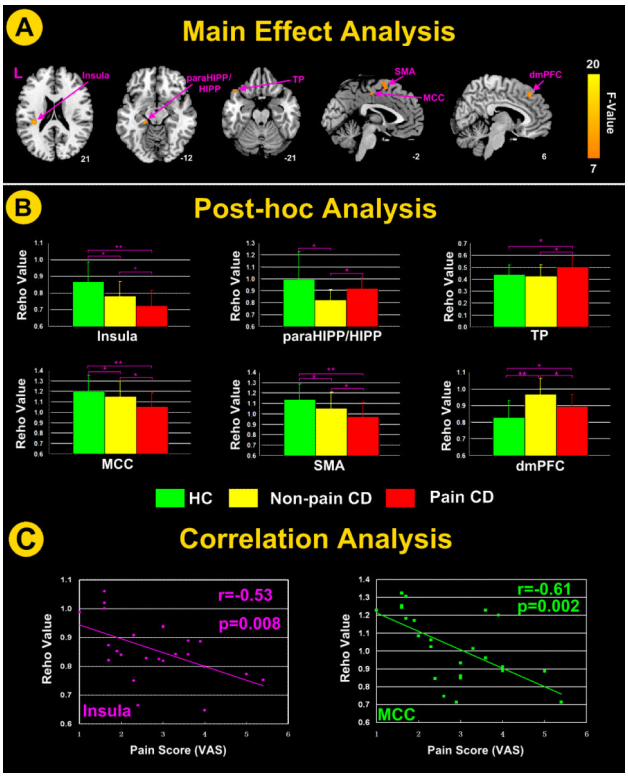

Fig. 1.

Significant differences in ReHo values among the pain CD, non-pain CD, and HC groups with age, gender, weight, anxiety and depression as covariates. (**A**) The main effect analysis revealed significant differences in ReHo values of the insula, hippocampal/parahippocampal cortex (HIPP/paraHIPP), dorsomedial prefrontal cortex (dmPFC), middle cingulate cortex (MCC), supplementary motor area (SMA) and temporal pole (TP) among the three groups. (**B**) The post-hoc analysis showed that the ReHo values of the insula, MCC and SMA in the pain CD group were lower and the TP was higher than the ReHo values of the other two groups. The ReHo values of the HIPP/paraHIPP in the non-pain group were lower and those of the dmPFC were higher than the ReHo values of the other two groups. (**C**) The ReHo values of the insula and MCC in the pain CD group were negatively correlated with the abdominal pain scores. L, left; *r*, correlation coefficient; VAS, visual analogue scale; **P*<0.05, ***P*<0.01.

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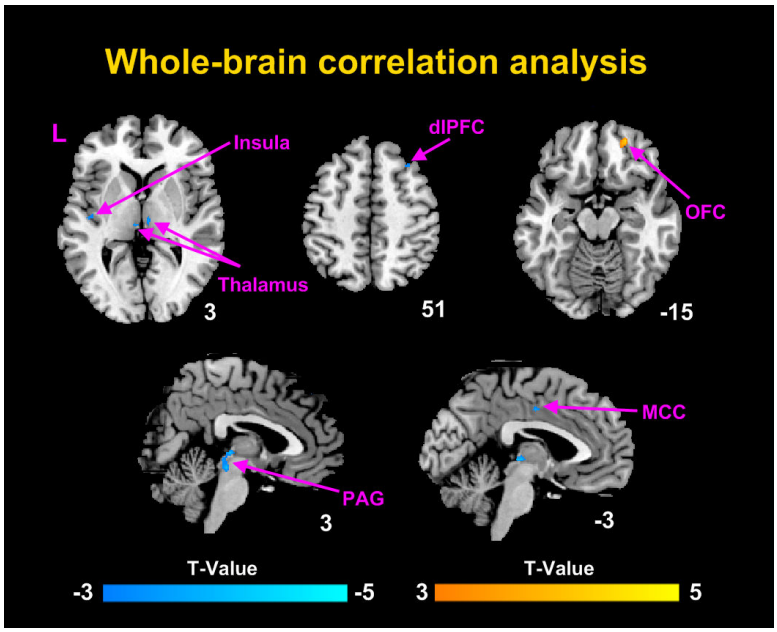

Fig. 2.

Brain regions with significant correlation between the ReHo values and daily pain scores in CD patients with abdominal pain using whole-brain correlation analysis. The daily pain scores correlated negatively with the left insula and MCC, right dorsolateral prefrontal cortex (dlPFC), bilateral thalamus, and periaqueductal gray (PAG), and positively with the right orbitofrontal cortex (OFC).

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Table 1

Demographic and clinical characteristics among pain CD group, non-pain CD group and healthy controls

		Pain Group (n=25)	Non-pain Group (n=25)	HC (n=32)	*P* value
Gender (male/female)		16/9	19/6	21/11	0.605
Age (y)		31.72±8.05	29.24±6.85	30.19±5.85	0.440
Height (cm)		169.72±8.06	170.72±6.08	169.38±7.55	0.781
Weight (kg)		56.28±9.43	57.50±9.54	58.97±6.46	0.488
HAD-A		6.48±3.51**	5.20±2.69*	3.31±1.99	0.000
HAD-D		5.68±3.74**	3.64±2.93#	2.97±1.71	0.014
Duration of illness (y)		6.74±4.40	5.64±3.80	—	0.489
CDAI		97.56±34.93	43.94±31.23###	—	0.000
IBDQ		161.24±27.84	188.56±18.70###	—	0.000
CRP		5.74±2.28	2.99±2.75##	—	0.001
ESR		12.75±4.80	10.01±5.31	—	0.062
PLT		226.76±37.24	210.68±49.90	—	0.203
CDEIS		1.25±0.80	0.90±0.51	—	0.078
Montreal classification					
Age at diagnosis	A1	0	4	—	0.018
	A2	21	21	—	
	A3	4	0	—	
Location	L1	3	7	—	0.344
	L2	6	4	—	
	L3	16	14	—	
	L4	0	0	—	
Behavior	B1	8	8	—	0.556
	B2	1	2	—	
	B3	7	7	—	
	B1P	1	4	—	
	B2P	1	0	—	
	B3P	7	4	—	
Concomitant medication		17	16	—	1.000
5-aminosalicylate		11	11	—	
azathioprine		3	4	—	
5-aminosalicylate & azathioprine		3	1	—	

Compare with HC group

Compare with pain group

CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; HADS-A, Hospital Anxiety and Depression Scale anxiety score; HADS-D, Hospital Anxiety and Depression Scale depression score; HCs, healthy controls; IBDQ, Inflammatory Bowel Disease Questionnaire. Values are presented as mean ± standard deviation.

* for $P<0.05$

** for $P<0.01$

#for $P<0.05$

##for $P<0.01$

###for $P<0.001$.

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Table 2

Brain regions with significant differences in ReHo values among the pain CD, non-pain CD, and HC groups

Regions	Hem	BA	MNI			F value	Voxels
			X	Y	Z		
Insula	L	48	−36	−30	17	10.71	14
MCC	L	23	−2	−18	49	8.01	20
SMA	L	6	−6	15	63	9.27	32
TP	L	38	−48	24	−21	8.89	11
paraHIPP/HIPP	L	30	−19	−31	−12	8.64	13
dmPFC	R	8	6	32	45	13.51	12

The results employed age, gender, weight, anxiety and depression as covariates. The statistical threshold was set at $P<0.05$ (false discovery rate corrected) and cluster size > 5. CD, Crohn's disease; dmPFC, dorsomedial prefrontal cortex; HC, healthy control; HIPP/paraHIPP, hippocampal/parahippocampal cortex; MCC, middle cingulate cortex; SMA, supplementary motor area; TP, temporal pole.

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Table 3

Whole-brain correlation analysis between the ReHo values and daily pain scores in CD patients with abdominal pain.

Regions	Hem	BA	MNI			T Value	Voxels
			X	Y	Z		
Negative correlation							
Insula	L	13	−42	−12	3	−3.61	9
MCC	L	24	−3	−9	42	−3.75	14
dlPFC	R	9	33	24	51	−3.91	8
Thalamus	L	/	−6	−21	3	−4.05	11
	R	/	6	−18	3	−3.71	15
PAG	/	/	3	−28	−9	−4.01	16
Positive correlation							
OFC	R	11	24	51	−15	4.67	18

The results employed age, gender, weight, anxiety and depression as covariates. The statistical threshold was set at $P<0.05$ (false discovery rate corrected) and cluster size > 5. BA, Brodmann area; dlPFC, dorsolateral prefrontal cortex; Hem, hemisphere; MCC, middle cingulated cortex; OFC, orbitofrontal cortex.