Differential Brain Structural and Functional Patterns in Crohn's Disease Patients are Associated with Different Disease Stages

Abstract

Background

Crohn's disease (CD) is an inflammatory, chronic disorder that alternates a quiescent phase to inflammatory flare-ups. Research has begun to elucidate the impact of CD in modulating brain structure and function. The previous neuroimaging studies mainly involved CD patients in remission (CD-R), therefore little is known about how inflammation influences brain-related features in different stages of the disease. We carried out a Magnetic Resonance Imaging (MRI) study to explore whether the different levels of disease activity may differentially affect brain structure and function.

Methods

Fourteen CD-R, 19 with mild to moderate inflammatory activity (CD-A) and 18 healthy controls (HC) underwent an MRI scan including structural and functional sequences.

Results

Between groups comparisons showed morphological and functional brain differences distinctively associated with the stage of disease activity. CD-A patients had reduced gray matter within the posterior cingulate cortex (PCC) relative to CD-R patients. Analysis on resting fMRI data showed the following patterns: A) increased connectivity within the left fronto-parietal network (in the superior parietal lobe) in CD-R patients relative to CD-A patients. B) decreased connectivity in the motor network (in parietal and motor areas) in the CD-A group relative to the HC group. Reduced connectivity in the motor network (C) and in the language network (D) (in parietal areas and in the PCC) in CD-R patients relative to HC.

Conclusions

The present findings represent a further step towards understanding brain morphological and functional changes in the active versus remission stages of CD patients.

Keywords

Crohn's Disease, functional magnetic resonance imaging, voxel based morphometry, resting-state fMRI

Key Messages

What is already known?

- CD has been shown to modulate brain structure and function. However, the previous neuroimaging studies mainly involved CD patients in remission.

What is new here?

- Differential morphological and functional changes are associated with different stages of disease activity in CD and these changes may reflect the neural correlates of fatigue, IBS-like symptoms, cognitive-emotional impairments in CD.

How can this study help patient care?

- This study may help to identify viable neuroimaging markers and putative disease-related pathways, potentially useful for evaluating the progression of the disease, but also to test the effectiveness of treatments aimed at reducing inflammation and inducing and maintaining remission.

1. Introduction

Crohn's disease (CD) is an idiopathic, inflammatory chronic disorder that, together with ulcerative colitis, is referred as to inflammatory bowel disease (IBD). The symptoms of CD may range from mild to severe and essentially include abdominal pain, bloody diarrhea, fever and fatigue, malabsorption with consequent weight loss, and sometimes they cause extraintestinal manifestations such as arthritis, uveitis, or sclerosing cholangitis.

The clinical course of CD typically alternates a quiescent phase, characterized by clinical remission in which patients experience quite or none symptoms, to inflammatory flare-ups. Inflammatory relapses of CD are unpredictable and unexpected; they can be highly debilitating and even lead to complications and surgery. Despite important advances in medical and surgical therapy, CD has a significant negative impact on patients' quality of life [1] and it is associated with psychological stress [2–4] and psychological disorders such as depression and anxiety [5]. CD symptoms are not limited to the gastrointestinal tract, but they can affect other organs, in particular the brain [6]. Research around the gut-brain axis (GBA) [2,7–10] area have just begun to elucidate the role played by CD symptoms in modulating brain structure and function and how these changes may influence the neural substrate of the reciprocal interactions between pain, stress, emotional states in CD patients and the functions of the gastrointestinal tract, including the inflammatory activity. Neuroimaging studies [11] have shown both structural and functional differences in CD patients relative to healthy controls. Voxel based morphometry (VBM) studies have investigated brain morphology in CD patients relative to controls and found reduced gray matter (GM) volume in the frontal lobe [12–14] and in the cingulate cortex (CC) [15]. However, a recent meta-analysis [11] has shown that, when all studies were pooled together, no significant results emerged. Functional magnetic resonance imaging (fMRI) studies, either using task-based paradigms [3,4,16,17] (i.e. stress-evoking tasks,

verbal fluency, implicit association tasks) or a resting-state approach, have shown differential patterns of brain activity between CD patients and controls. Indeed, fMRI studies have shown stress-evoked hyperactivity in the mid-cingulate cortex [4], altered habituation to stress in the amygdala, hippocampus, insula, putamen and cerebellar regions [3], and greater bi-hemispheric activation while performing a cognitive task in CD patients relative to controls [17]. The results of the previous task-based fMRI studies did not reveal common patterns of activation in CD patients probably due to the small number of patients recruited for the studies and the different tasks used. On the other hand, a meta-analysis analyzing resting-state fMRI studies has shown a consistent pattern of reduced resting state brain connectivity mainly localized in the paracental lobule and in the mid- and posterior cingulate cortex in CD patients compared to controls [11]. The brain network that seems to be most commonly affected by CD is the sensorimotor network [18–20]. Brain regions included in this network have been associated with visceral pain sensitivity and abdominal discomfort [21] as well as emotional processing [22]. However, most of the previous neuroimaging studies mainly involved CD patients in clinical remission, therefore little is known about how the inflammatory activity may influence brain structure and function in CD. This is particularly relevant in light of the differences between IBD patients during flares and patients in remission reported by studies using psychometric tests [5,23,24].

Here, we have carried out an MRI study to explore whether the different stage of disease activity may differentially affect brain structure and function. Both CD patients in clinical remission and with mild to moderate inflammatory activity were recruited and compared to a group of controls. This is crucial in order to define the putative pathways affected by the inflammatory activity and to identify imaging markers reflecting pathogenic mechanisms that

could potentially be used as sensitive measures for monitoring the evolution of the disease, but also for defining targets for therapeutic interventions.

2. Materials and Methods

2.1 Participant recruitment

The local Ethics Committee approved this study and all participants provided written informed consent. Fully trained physicians attending the IBD Unit of the S.Orsola-Malpighi University Hospital in Bologna (the national referral centre for research and care of patients with gastrointestinal disorders) examined all participants and enrolled CD patients. In addition, all participants were screened for neurological and psychiatric disorders using a structured interview for the diagnostic and statistical manual of mental disorder IV edition [25]. Handedness was assessed using the Edinburgh handedness inventory [26]. Eligible CD patients were consecutively recruited when they fulfilled the inclusion and exclusion criteria. Hematological and endoscopic data were obtained from routine blood tests and ileo-colonoscopies. All patients had ileo-colonoscopies and blood tests no more than one week before the scan. Fully trained clinicians (FR, PG) used the simple endoscopic score for CD (SES-CD) [27] to evaluate the endoscopic activity, evaluated blood tests and compiled the Crohn's disease activity index (CDAI) [28]. Disease activity was characterized as follow: Remission: CDAI < 150, SES-CD < 3; Mild activity: CDAI between 150 and 250, SES-CD between 3 and 6; Moderate activity: CDAI > 250, SES-CD between 7 and 15. Throughout the manuscript, CD patients with mild-to-moderate inflammatory activity will be defined as CD-A, whereas CD patients in clinical remission will be defined as CD-R. Other inclusion criteria were: range of age between 18 and 55, CD diagnosis performed at least 3 years prior the enrollment and being right-handed. Exclusion criteria included: psychotropic medications in the previous 6 months, corticosteroids in the previous 6 months, pregnancy, clinical indications for forthcoming surgery, current or prior history of neurological or psychiatric disease, claustrophobia, presence of metallic implants in the body. During the recruitment

stage the following information were also collected: date of birth, sex, and educational level. Moreover, according to the Montreal classification [29], were collected age at diagnosis (A), disease location (L), disease behavior (B), and furthermore perianal disease, extra-intestinal manifestations, previous history of intestinal surgery, treatment with biologics, possible maintenance treatments.

A group of 18 right-handed healthy controls (HC) was recruited among the staff of the University of Bologna. They underwent the same screening procedures as the CD patients and the same inclusion and exclusion criteria, except for the CD specific exams.

2.2 Neuroimaging protocol

Scanning was performed at the the Ospedale Civile di Baggiovara, Modena, using a Philips Intera system at 3.0 Tesla equipped with an 8 channels head-coil. The neuroimaging protocol comprised functional and structural sequences as follows.

2.2.1 Structural MRI

3D high-resolution T1-weighted MR images were acquired using a MPRAGE sequence (TR = 9.9 ms, TE = 4.6 ms, 170 sagittal slices, voxel dimension = 1 mm isotropic, acquisition time = 4.6 min).

2.2.2 Resting fMRI

Whole-brain functional imaging was performed using a gradient echo EPI sequence (TR = 2000 ms, TE = 35 ms, field of view = 240 mm, voxel dimension = 3 × 3 × 4 mm, acquisition time = 8 min, number of volumes = 240). For the resting-state scan, subjects were instructed

to lie in dimmed light with their eyes open, think of nothing in particular, and not to fall asleep.

2.3 Image analysis

Data analysis was carried out using FSL tools (FMRIB Software Library, www.fmrib.ox.ac.uk/fsl) [30].

2.3.1 Structural MRI

Pre-processing for structural images included the following steps: a) re-orientating images to the standard (MNI) template, b) bias field correction, c) brain extraction and d) brain tissues segmentation using FMRIB's Automated Segmentation Tool (FAST) that allows extracting global measures of total Gray Matter (GM), White Matter (WM) and cerebrospinal fluid (CSF). Whole brain analysis was carried out using a voxel-based morphometry-style analysis (FSL-VBM) [31], using default settings as described at www.fmrib.ox.ac.uk/fsl/fslvbm/. In brief, brain extraction and tissue-type segmentation were performed and resulting GM partial volume images were aligned to standard space using first linear (FLIRT) and then non-linear (FNIRT) registration tools. The resulting images were averaged, modulated and smoothed with an isotropic Gaussian kernel of 5 mm Full-Width at Half Max (FWHM) to create a study-specific template, and the GM images were re-registered to this, including modulation by the warp field Jacobian. Finally, voxel-wise GLM was applied using permutation-based non-parametric testing (5000 permutations) [32], threshold-free cluster enhancement (TFCE) [33] and then a family-wise-error (FWE) corrected cluster significance threshold of $p < 0.05$ was applied to the suprathreshold clusters.

2.3.2 Functional MRI at rest (rs-fMRI)

fMRI analysis of resting state data was carried out using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components, part of FSL http://www.fmrib.ox.ac.uk/fsl/melodic/) [34]. Individual pre-statistical processing consisted of motion correction, brain extraction, spatial smoothing using a Gaussian kernel of FWHM (full width at half maximum) 5 mm, and high pass temporal filtering with a cut-off of 100 s. (0.01 Hz). FMRI volumes were registered to the individual's structural scan and standard space images using both FLIRT and FNIRT registration tools. As the signal derived from rs-fMRI is affected by the presence of artefacts, many of which have a spatial and/or spectral overlap with resting state networks (RSNs) of interest, here, we have used a tool called FIX [35] to denoise functional images from the spurious signal and increase the possibility of identifying markers of effective connectivity. Pre-processed and denoised functional data containing 240 time-points for each subject were temporally concatenated across subjects in order to create a single 4D dataset. The number of components was fixed to 25 based on an initial analysis of the population using model order estimation. The subject dependent effect sizes identified in the initial analysis suggested that only 25 components were significantly non-zero on average. RSNs of interest covered the entire brain and were selected using spatial correlation against a set of previously defined maps [34]. The between-subject analysis of the resting data was carried out using a regression technique ('dual regression'), which allows for voxel-wise comparisons of resting functional connectivity [36]. For each resting-state network (RSN) identified, a voxelwise GLM, to assess group differences, was applied to the spatial maps using permutation-based non-parametric testing (5000 permutations) [32].

The TFCE method [33] was used to control for voxel-wise multiple comparisons across the whole brain and a FWE corrected cluster significance threshold of $p < 0.05$ was applied to the

suprathreshold clusters. This results in spatial maps characterizing the between-subject/group differences.

2.4. Covariates

To determine whether functional connectivity differences between the 3 groups (HC, CD-A and CD-R) were influenced by underlying structural differences, even at a sub-threshold level, structural images were used as additional covariates on a voxel-by-voxel basis to interrogate fMRI data [37]. GM images of each subject were extracted using FAST, registered to standard space, smoothed to match the intrinsic smoothness of the fMRI data, voxel-wise demeaned across all subjects in both groups together and added as a confound regressor (nuisance) to the GLM design matrix used to analyse fMRI data.

2.5. Statistics

Statistical analyses of derived variables were carried out using SPSS software (SPSS, Inc., Chicago IL). ANOVA test and Post-Hoc Bonferroni correction was used for socio-demographic variables and brain structure volumes. Pearson's test was used to compare sex distribution across the study groups.

3. Results

3.1 Participants

Health controls (HC), CD patients with active disease (CD-A) and CD patients in remission (CD-R) did not differ for age, sex or years of education. Moreover, disease duration was not different between CD-A and CD-R patients (Table 1). The CD-A group consisted of 19 patients (12 female) while the CD-R group consisted of 14 patients (8 female). A detailed description of CD patients' clinical characteristics including: age at diagnosis, disease location, behavior manifestation, extraintestinal manifestation, previous surgery, previous surgery, treatments, Clinical activity [Crohn's disease activity index (CDAI)] and Endoscopic activity [simple endoscopic score for CD (SES-CD)] is reported in Table 2.

--------- TABLES 1,2 ABOUT HERE ----------

3.2 Structural MRI

No differences were observed between the three study groups on global brain measures [i.e. Total brain volume and normalized gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF) volumes] (Table 1). A difference between CD-A relative to CD-R patients was found in the posterior cingulate/retrosplenial cortex (Figure 1). No differences were observed between the two patients' groups and the healthy controls.

---------- FIGURE 1 ABOUT HERE ----------

3.3. Resting state FMRI (rs-fMRI)

Preprocessing results of each of the 51 study participants were visually inspected by a trained neuroscientist (NF) to ensure registration accuracy. All but 1 participant were included in the group analysis. The data were discarded due to signal drop-out. Five random subjects for each of the three study groups (for a total of 15 subjects) were identified to create a study specific training dataset in order to denoise functional images. The optimal threshold for the use of FIX was identified as 30 with a mean (median) true positive rate (TPR) and true negative rate (TNR) of 95.3% (95.8%) and 90.7% (92.5%), respectively. This is in line or above the suggested thresholding (TPR > 95% - TNR > 70%).

ICA defined twenty-five components representing group-averaged networks of brain regions with BOLD fMRI signals that were temporally correlated [33,38]. A total of 9 components were identified as resting state networks (RSNs) (covering the entire brain) and were evaluated further, whereas the remaining components mainly reflected motion and/or physiological artefacts, BOLD signal drift out and were therefore discarded. In detail, the 9 RSNs included the Medial and Lateral Visual Networks (MVN, LVN), the Default Mode Network (DMN), the Auditory Network (AN), the Motor Network (MN), the Language Network (LN), the Executive Function Network (EFN) and the Left and Right Fronto-Parietal Networks (LFPN, RFPN respectively) (Figure 2).

---------- FIGURE 2 ABOUT HERE ----------

Analyses revealed significant group differences in three of the identified RSNs. Here, for each significant difference, we report, 1) the cluster size, expressed in number of voxels, 2) the respective T-max value for the peak significant area within the reported cluster and 3) the coordinates of the peak in MNI space. For the LFPN, increased connectivity was observed in

the superior parietal lobule (65 voxels, T-max: 5.66, MNI coordinates, x-y-z: 57-42-67) of CD-R patients relative to CD-A patients (Figure 3A). For the MN, decreased connectivity was observed in motor-related (525 voxels, 4.63, 56-55-70) areas of CD-A patients relative to HC (Figure 3B1) and decreased connectivity was observed in the cingulate gyrus extending into the precuneus (142 voxels, 5.22, 37-41-52) of CD-R patients relative to HC (Figure 3B2). For the LN, decreased connectivity was observed in the cingulate gyrus (47 voxels, 5.53, 54-30-50) of CD-R patients relative to HC (Figure 3C).

---------- FIGURES 3 ABOUT HERE ----------

It is important to notice that group differences on functional connectivity measures were not influenced by underlying structural differences. Indeed, by adding GM maps as covariates (nuisance variables) to the RSN fMRI analysis models, the related group differences (reported above) survived unchanged.

4. Discussion

In this study, VBM and resting state fMRI were used to investigate potential brain structural and functional alterations across groups of healthy controls (HC), CD patients in remission (CD-R) and CD patients with bowel inflammatory activity (CD-A). Based on the gut-brain axis (GBA) hypothesis, a growing body of evidence [6–8] has begun to delineate the extent of the brain involvement in CD patients. Obviously, the chronic and inflammatory bowel involvement of IBD makes CD and ulcerative colitis the ideal candidates to explore and investigate the GBA hypothesis. Given that CD symptoms causing pain, but also psychological suffering, are differentially influenced by inflammatory exacerbations [5,23,24], in the present neuroimaging study we stratified CD patients based on the level of disease activity. Differential morphological changes and functional alterations associated with different levels of disease activity have emerged in CD patients in several brain areas, notably within the parietal and motor cortices and portions of the posterior cingulate cortex (PCC). Consistent with the results of Yeung's meta-analysis [11], we did not find brain areas with increased resting state brain connectivity in CD patients relative to HC.

4.1 Disease activity-related differences in brain morphology in CD patients

Voxel based morphometry (VBM) results reported here have shown decreased gray matter (GM) volumes in the posterior cingulate/retrosplenial cortex in CD-A compared to CD-R patients (Figure 1). These differences do not relate to comparisons with healthy subjects and may reflect variability related to different stages of disease activity and to differing levels of pain symptom intensity. Indeed, the PCC has a key-role in ongoing self-monitoring of the personal relevance of sensory stimuli [39]. Thanks to its dense interconnections with the anterior cingulate cortex (ACC), the PCC exerts functions in establishing self-relevance and

emotional content of sensory experience likely including visceral pain [40,41]. On the other hand, the retrosplenial PCC is believed to concurrently dominate in connectivity to limbic networks for emotion processing [42]. Further investigations may elucidate whether volumetric changes in the PCC may play a role into impaired mechanisms of central processing of nociceptive inputs in CD patients.

4.2 Differential patterns of functional connectivity are associated with different level of disease activity in CD patients

Functional connectivity alterations in CD-R patients were found within the PCC in two different brain networks. Firstly, decreased connectivity values in CD-R patients relative to HC was found within the PCC in the language network (Figure 3C). Interestingly, previous studies have reported decreased verbal IQ and impairments in verbal learning [43,44] in IBD patients without disease activity characterization. Our neuroimaging results may provide a probable neurophysiological substrate, potentially explaining the observed symptoms of impairments in the language domain in CD patients, beyond the inflammatory stage.

Moreover, decreased connectivity in CD-R patients relative to HC was also found within the PCC in the sensorimotor network (Figure 3B2). Given the integrative functions between visceral sensitivity and affective states of the PCC [39], this result is particularly significant in light of the chronic pain disease that characterizes the clinical course of CD. Indeed, alterations in functional connectivity-derived measures in the PCC have been previously found in CD patients [11] and in patients with irritable bowel syndrome (IBS) [45], a functional gastro enteric disorder characterized by abdominal pain and altered bowel habits. Surprisingly, high percentages of CD patients during quiescent inflammation continue to complain about abdominal pain symptoms. Given the absence of inflammatory activity, these

symptoms are referred as IBS-like symptoms and the neural mechanisms underpinning their occurrence are still unclear [8,46]. Based on our findings, we speculate that in CD the chronic inflammatory condition may cause functional modifications in the sensorimotor network that, in turn, might promote exacerbations of visceral pain and the development of IBS-like symptoms.

Interestingly, a pattern of decreased connectivity in the motor network was also found in CD-A patients compared to HC, although located in different brain structures including motor and parietal areas. Taken together our results suggest that the motor network might be a potentially useful target in CD to evaluate the contribution of disease activity in modulating brain response. Indeed, brain structures included in the motor network are primarily involved in the control and execution of voluntary movements and in the brain emotional processing [22]. In the context of CD, symptoms of emotional disturbances [47] have been consistently reported [3,48–51], often associated with symptoms of fatigue such as exhaustion not proportional to physical effort that is not completely relieved following rest. Fatigue is a common symptom in CD affecting up to 86% of patients with active disease [52,53]. The neural correlates of fatigue in CD are still unclear [52] as well as the role of chronic inflammatory signals ascending from the gut and reaching the brain [9]. The involvement of motor network that we have here shown in CD patients may represent a further step towards understanding the neurological mechanisms promoting symptoms of fatigue and emotional disturbances in CD. The above resting state-fMRI results (Figure 3B1, 3B2, 3C) are in line with the results reported in Yeung's meta-analysis showing a consistent reduction in connectivity in CD patients relative to HC. However, as the respective differences between CD-A and CD-R patients relative to HC are located in different brain areas, the specific spatial localization

identified for the CD-A and CD-R groups may help to define the pathways affected at the different stages of the inflammatory activity.

On the other hand, functional brain changes related to different stages of inflammatory disease activity were highlighted by the result we reported in the left fronto parietal network (LFPN) (Figure 3A) that emerged when we compared CD-A and CD-R patients. Specifically, CD-R patients had increased functional connectivity values in the superior parietal lobe relative to CD-A patients. This result represents a novel finding, given that, to our knowledge, there are no rs-fMRI studies with a stratification of CD patients based on inflammatory activity. The FPN plays a crucial role in attention, execution, adaptive control and emotional processing and it is deemed to provide the rapid adaptive control when endogenous cognition or emotion occur [54]. Elevated inflammation has been associated with cognitive impairment in the general population, in particular with deficits in executive function [55]. In CD patients, reduced cognitive performance [23], as well as psychological stress are relatively common and are negatively influenced by inflammatory exacerbations [56]. The functional differences we found in the LFPN, obtained by stratification of patients based on inflammatory activity, represent a further step towards understanding the complex relationships between inflammatory activity, brain functional changes and symptoms of cognitive and emotional impairment in CD.

Functional brain differences reported here did not highlight a clear-cut dose-response effect in a pattern that mirrors the exposure to gut inflammation (i.e. lack of inflammation in HC, quiescent inflammation in CD-R, ongoing inflammation in CD-A). This could be due to a differential response to the actual state of gut inflammation and resulting in a different effect on the brain. This interpretation seems to be supported by independent studies involving patients with Ulcerative Colitis (UC), which have shown that UC patients in an active stage [57]

and quiescent stage [58] had different patterns of brain connectivity relative to groups of healthy controls. To the best of our knowledge, there are no studies in the literature directly comparing the effect of quiescent and active stages of gut inflammatory activity on brain features and therefore further investigation is warranted to clarify this aspect. Another possible explanation for the lack of a clear-cut dose-response effect might be due to the small number of subjects included in our study, as reported in the "Study limitations" paragraph. Indeed, as shown in Figure 3B1, we have observed a pattern of brain connectivity reduction in motor areas within the Motor Network from HC (no inflammation) to CD-R (quiescent inflammation) to CD-A (active inflammation). Although the step-wise effect reflecting the exposure of gut-inflammation was not significant, the localisation is particularly interesting as the motor network has been shown to be primarily affected by CD [18–20] and associated with symptoms such as visceral pain sensitivity and abdominal discomfort [21]. Future studies with a larger number of patients are needed to elucidate this result.

4.3 Study limitations

The main limitation of this study lies in the lack of measures of cognitive-emotional function and fatigue in patients with CD. This has limited the possibility of establishing correlations between these data and our MRI-related findings. Further studies enrolling patients with different stages of disease activity and with a comprehensive battery of questionnaires investigating perceived pain, fatigue, and cognitive-emotional functions are warranted as they may shed light on the neural correlates of these symptoms that often severely affect the quality of life of patients with CD. Another drawback is the limited number of patients enrolled in this study. This is especially due to the difficulty of recruiting CD-A patients who can undergo an MRI scan and who are not taking corticosteroids, which are known to affect

fMRI results. Studies with more subjects and possibly with patients with moderate to severe disease are needed in the future. Finally, the criteria employed here to categorize the CD patients based on the different disease activity could be expanded. Indeed, a more careful evaluation of the disease activity, implementing serum hematological measures of inflammatory mediators or fecal calprotectin could provide more accurate and quantitative information useful to clarify the relationship between the observed changes in brain activity and the level of disease activity and to further stratify CD patients.

4.4 Conclusion

In the present study, we have shown that different stages of disease activity may differentially influence brain morphology and function in CD patients, potentially reflecting the putative pathways affected by the ongoing inflammatory activity. Although we did not find clear-cut dose-response effect in this study, the present results may represent a step forward in understanding the bidirectional relationship between the effect of gut inflammation on brain function and morphology. Finally, our findings encourage further research on the plausible role of GBA in the occurrence of symptoms such as fatigue, irritable bowel syndrome-like symptoms, and cognitive-emotional impairments.

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6. Ethical Considerations

The submitted manuscript is an original contribution not previously published (also as an abstract or preliminary report) and is not under consideration for publication elsewhere.

All authors contributed to the manuscript and approved the final draft submitted.

The paper is free of plagiarism.

All enrolled patients' anonymity has carefully protected. The local Ethics Committee approved this study and all participants provided written informed consent. The study was conducted following all the guidelines for experimental investigation with human subjects.

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TABLES

Table 1. Socio-demographic and brain features of the three study groups. Values denote mean (± standard deviation) or numbers of subjects. NA (not applicable). CD-A refers to patients with mild-to-moderate inflammatory activity. CD-R refers to patients in remission.

[$]Independent samples T-test was used to compare Disease-Duration between CD-A and CD-R groups. *Values are expressed as percentage of whole-brain volume.

	Controls	**CD-A**	**CD-R**	*p*	*p* Post-Hoc comparisons (Bonferroni)		
	N=18	**N=19**	**N=14**	Anova	Controls *vs* CD-A	Controls *vs* CD-R	CD-A *vs* CD-R
Age (Y)	28.33 (±5.46)	32.84 (±9.75)	29.92 (±6.59)	0.2	0.2	1	0.8
Education (Y)	16.05 (±2.51)	14.05 (±3.15)	15.35 (±2.79)	0.1	0.1	1	0.6
Sex (M/F)	8/10	7/12	6/8	1			
[$]Disease Duration (Y)	N/A	6.89 (±5.14)	10.43 (±6.02)	0.08			
Whole-brain volume (ml)	1476.56 (±111.93)	1436.23 (±113.43)	1427.44 (±178.84)	0.53	1	0.93	1
*Gray Matter	0.42 (±0.01)	0.41 (±0.01)	0.42 (±0.02)	0.75	1	1	1
*White Matter	0.35 (±0.01)	0.35 (±0.01)	0.35 (±0.01)	0.98	1	1	1
*Cerebrospinal fluid	0.22 (±0.01)	0.23 (±0.02)	0.23 (±0.02)	0.79	1	1	1

Table 2. Clinical characteristics of CD patients. Values denote numbers of subjects. CD-A refers to patients with mild-to-moderate inflammatory activity. CD-R refers to patients in remission. CDAI = Crohn's disease activity index. SES-CD = simple endoscopic score for CD. Age at diagnosis: A1, A2, A3 reflect age ≤ 16 years, 17 – 39 years, ≥ 40 years, respectively. Disease location: L1, L2, L3, L4 reflect ileal, colonic, ileal-colonic and isolated upper disease, respectively. Behavior manifestation: B1, B1p B2, B3, B3p reflect non-structuring and no-penetrating, structuring, penetrating, respectively, where p indicates perianal disease.

	CD-A **N=19** (12 female)	**CD-R** **N=14** (8 female)
Montreal classification		
Age at diagnosis	A1 1 A2 15 A3 3	A1 3 A2 11 A3 0
Disease location	L1 8 L2 1 L3 10 L4 0	L1 6 L2 1 L3 4 L4 0
Behavior manifestation	B1 11 B1p 1 B2 3 B3 1 B3p 3	B1 10 B2 2 B3p 2
Extraintestinal manifestations		
Arthralgia Erythema Nodosus	3 1	4
Previous surgery		
Ileal resection Ostomy	3 2	5 0
Treatments		
Biologics (infliximab, adalimumab) 5 Aminosalicylic Azathioprine	9 5 5	4 5 3
Clinical activity (CDAI)		
Remission (CDAI<150) Mild (CDAI 150-250) Moderate (CDAI >250)	 17 2	14
Endoscopic activity (SES-CD)		
Remission (SES-CD<3) Mild (SES-CD 3-6) Moderate (SES-CD 7-15)	 15 4	14

FIGURES LEGENDS

Figure 1. Brain regions where CD-A patients had a reduction in GM volume compared with CD-R patients using a whole brain analysis approach ($p < 0.05$ corrected for multiple comparisons). R refers to right and L to left hemisphere.

Figure 2. Images represent average group maps of the study participants obtained using a data-driven approach. Networks are shown superimposed on the MNI152 standard space template image. All spatial maps are thresholded at z = 3. Red to yellow colours represent z-scores > 3. R refers to right, L to left hemisphere, S to superior and I to inferior.

Figure 3. Group differences in resting fMRI data are reported. Panel A) shows a cluster of regions in the left superior parietal area where increased connectivity in CD-R relative to CD-A patients, within the Left Fronto-Parietal Network (LFPN), was observed. In panel 3B1) we show a cluster of regions in motor areas where increased connectivity in the healthy controls (HC) group relative to CD-A patients was found within the Motor Network (MN). Panel 3B2) shows a pattern of decreased of connectivity in the cingulate gyrus in the CD-R patients relative to the HC group within the MN. Panel 3C) shows a pattern of decreased connectivity in the cingulate areas in CD-R group relative to the healthy controls group within the Language Network (LN). Results reported here are $p < 0.05$ corrected for multiple comparisons. For illustrative purposes, we also report, next to each figure-panel, the respective box-plot with average (and Standard Deviations) of the parameter estimates values (on the Y axes) extracted from brain regions with significant group differences. The groups where brain difference was observed are linked by a black line. Black, red and green colours

in the box-plots define healthy controls, CD-A and CD-R patients respectively. R refers to right and L to left hemisphere.