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Neurotransmitter alterations in the anterior cingulate cortex in Crohn's disease patients with abdominal pain: A preliminary MR spectroscopy study

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ABSTRACT

Purpose: Crohn's disease (CD) has been known to cause both abdominal pain alongside functional and structural alterations in the central nervous system (CNS) in affected patients. This study seeks to determine the alternations of metabolites in the bilateral anterior cingulate cortex (ACC) of CD patients with abdominal pain by using proton magnetic resonance spectroscopy (^{1}H-MRS) to further explore the neural mechanism.

Methods: Sixteen CD patients with abdominal pain and 13 CD patients without abdominal pain, were recruited alongside 20 healthy controls (HCs) for this study. Clinical evaluations, including the 0–10 Visual Analogue Scale (VAS) of pain, Hospital Anxiety and Depression Scale (HADS) and Crohn's Disease Activity Index (CDAI), were evaluated prior to MR scanning. This study selected the bilateral ACC as the region of interest (ROI). The metabolites of the bilateral ACC were quantitatively analyzed by LCModel and Gannet. A independent sample *t*-test and one-way analysis of variance (ANOVA) were performed for statistical analysis. Spearman correlation analyses were performed to examine the relationship between the metabolite levels and clinical evaluations.

Results: The results indicated that CD patients with abdominal pain exhibited significantly higher levels of Glutamate (Glu)/(creatine + phosphocreatine, total creatine, tCr) over CD patients without abdominal pain, and HCs ($p = 0.003$, 0.009, respectively) in the bilateral ACC. The level of (Glutamate + Glutamine, Glx)/tCr of pain CD group was higher than non-pain CD group ($p = 0.022$). Moreover, within the pain CD group, Glu/tCr and Glx/tCr levels correlated strongly with the VAS scores of pain ($\rho = 0.86$, 0.59 respectively, $p < 0.05$). Meanwhile, the results indicates that CD patients with abdominal pain have significantly lower levels of γ-aminobutyric acid plus (GABA+)/tCr ($p = 0.002$) than HCs. To some extent, CDAI demonstrated a trend of negative correlation with GABA+/tCr levels ($p = 0.088$, $\rho = -0.60$).

Conclusion: The neural mechanism of CD patients with abdominal pain in pain processing is tightly associated with neurochemical metabolites. An imbalance in Glu and GABA may play a key role in abdominal pain processing for patients with CD. This mechanism of pain may associate with the intestinal microbiota on the brain-gut axis.

1. Introduction

Crohn's disease (CD) is a chronic, non-specific granulomatous inflammatory disorder which can affect any part of the digestive tract. Geographically, CD is most prevalent in developed, western countries (Molodecky et al., 2012). Moreover, recent epidemiologic studies (Ng et al., 2013) reveal a rapid increase in incidence over recent years. As the chief complaint of CD, abdominal pain causes discomfort in affected patients and significantly decreased quality of life. Pathogenesis of CD in pain processing has not been fully understood, but is commonly believed to be associated with inflammation, visceral hypersensitivity, brain-gut axis dysfunction and psychological abnormalities (Bonaz and Bernstein, 2013; Mayer and Tillisch, 2011). The brain-gut axis (Bonaz and Bernstein, 2013; Collins et al., 2012; Cryan and O'Mahony, 2011) describes a bidirectional communication system involved between the brain and enteric microbiota. Dysfunction of one or more nodes of the

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brain-gut axis would affect CD, which may be related to abnormal pain processing in the central nervous system (CNS).

With regard to the brain-gut axis; a study (Bao et al., 2016) reported that the differences in regional homogeneity (ReHo) of specific brain regions in CD patients with abdominal pain compared with CD patients in remission and without abdominal pain by using resting-state blood oxygenation level dependent functional magnetic resonance imaging (BOLD-fMRI). The specific brain regions included the cingulate cortex, insula, hippocampus, supplementary motor area, temporal pole, and dorsomedial prefrontal cortex. Similarly, in a previous fMRI study (Bernstein et al., 2002), they reported abnormal activity in the anterior cingulate cortex (ACC) and left somatosensory cortex in CD patients during periods of pain. Therefore, it is evident that functional alterations in neurological processing occur in CD patients with abdominal pain.

BOLD-fMRI (Matthews and Jezzard, 2004; Ogawa et al., 1990) was first reported by Ogawa et al. in 1990 and has become a powerful method for detecting brain activity. BOLD-fMRI functions by detecting a local increase in relative blood oxygenation that results from neurotransmitter activity, and thus, indirectly reflects local neuronal firing rates. Furthermore, studies (Attwell et al., 2010; Donahue et al., 2010) have suggested that changes in brain blood oxygen concentration are typically associated with changes in regional neurotransmitters. The neurotransmitters or metabolites can be detected by proton magnetic resonance spectroscopy (^{1}H-MRS), a non-invasive method for detection of biochemical molecules (Jansen et al., 2006), such as γ-aminobutyric acid (GABA), *N*-acetyl-aspartate (NAA), myo-inositol (mIns), choline (Cho), glutamate (Glu), glutamine (Gln), glutamate + glutamine (Glx), creatine + phosphocreatine (total creatine, tCr), and lactate (Lac) etc. However, detection of GABA using the conventional ^{1}H-MRS is limited due to its relatively low concentration and the spectral overlap of signals from other major metabolites. An advanced MRS method, MEscherGArwood Point RESolved Spectroscopy (MEGA-PRESS) (Mescher et al., 1998; Puts and Edden, 2012) may be used to detect GABA levels in the healthy brain and compare them to levels in various pain and emotional disorders (Gao et al., 2013; Plante et al., 2012; Zunhammer et al., 2016). ^{1}H-MRS studies have revealed altered levels of cerebral neurotransmitters in various chronic pain conditions, such as fibromyalgia (Foerster et al., 2012), chronic back pain (Sharma et al., 2011; Zhao et al., 2017), and neuropathic pain (Widerstrom-Noga et al., 2013). Recently, we reviewed (Lv et al., 2017) the brain changes of CD detected by fMRI and MRS, and observed CD spectrum analysis of metabolites were commonly used in vitro, serum, urine, excreta and tissue samples. However, there have no studies to investigate the brain metabolites in vivo by using MRS in CD patients suffering from abdominal pain.

Based on previous studies, we hypothesized that CD patients with abdominal pain show not only changes in brain structure and function, but also altered neural metabolites levels in ACC. It additionally creates an abnormal rest activity brain region of CD patients with pain, which plays an important role in the perception, formation and regulation of pain. This study is aimed at determining alternations in metabolite levels, especially the neurotransmitters involved with pain, such as Glu and GABA, within the bilateral ACC of CD patients with abdominal pain using ^{1}H-MRS, and determining the relationship between metabolite levels and the clinical scores to further explore the neural mechanism in pain processing.

2. Methods

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University. All participants provided written informed consent.

2.1. Participants and study design

Sixteen CD patients with abdominal pain and 13 CD patients without abdominal pain confirmed by endoscopy or pathology, and were recruited from the First Affiliated Hospital of Zhejiang Chinese Medical University. The pain CD group included CD patients who experienced pain in the past 3 months, with the days of pain per week ≥ 3 days. The non-pain CD group included patients with CD who had not experienced abdominal pain in the past 3 months. Patients suffering from abdominal pain stemming from intestinal diseases other than CD, brain parenchymal lesions and mental or psychological disorders, recent (6 months) use of the CNS drugs, anodyne or recent (6 months) use of antidepressants or opioids, adverse life events resulting in temporarily depressed mood, and patients with claustrophobia or metal implants were excluded. Twenty healthy controls (HCs) were recruited with advertisements from Zhejiang Chinese Medical University. None of the HC subjects were taking any medication, or complained of gastrointestinal pain or pain related diseases. The clinical scores including hospital anxiety and depression scale (HADS), 0–10 Visual Analogue Scale (VAS) of pain and Crohn's disease activity index (CDAI) were evaluated before MR scan. According to the score of VAS, the CD patients divided into pain and non-pain CD groups. In the entire study, only 1 patient was excluded due to noise intolerance during scanning.

2.2. MR image and spectrum acquisition

Imaging of this study was conducted on a small aperture 3.0-Tesla MR scanner (Discovery 750, GE Healthcare, Milwaukee, WI),equipped with an 8-channel head phased array coil. Head motion was restricted by placement of comfortable paddings around the participant's head. A set of high-resolution T1-weighted structural images were obtained using three-dimensional BRAVO (brain volume imaging) sequence: TR = 8.2 ms, TE = 3.2 ms, FA = 12 degrees, matrix = 256 × 256, slice thickness: 1.0 mm with no gaps, was then used for the orientation and positioning of the subsequent ^{1}H-MRS scans. Point resolved spectroscopy (PRESS) was used to obtain single voxel spectrum. The voxel position for the single voxel spectroscopy in bilateral ACC (Fig. 1): TR = 2000 ms, TE = 35 ms, Voxel size = 20 × 20 × 20 mm^3, total number of scans = 64, number of excitations (NEX) = 8, water suppressed, with automatic shimming. Pre-scan requirements were < 8 Hz in automatic shimming at full width at half maximum (FWHM) and larger than 95% in water suppression. After the sequence of MEGA-PRESS was ready by GE Healthcare; nine CD patients with abdominal pain, 2 CD patients without abdominal pain and 8 HCs of those subjects were analyzed with MEGA-PRESS in the bilateral ACC to acquire GABA data: TR = 2000 ms TE = 68 ms, Voxel size = 20 × 20 × 20 mm^3 total number of scans = 64, NEX = 8, water suppressed. The voxel was carefully positioned within the ACC and outer-volume suppression (OVS) pulses around the ACC were facilitated for pre-saturation the lipid signals. Pre-scan requirements were same as PRESS acquisition.

2.3. MRS data processing

The raw P files of MRS were exported to a workstation equipped with linear combination model (LCModel) (Provencher, 1993) and Gannet (GABA analysis tool) (Edden et al., 2014) for processing (Fig. 2). This obtained the relative concentration (/tCr) and absolute concentration (mmol/L) of each metabolite, including Glu, Gln, GABA+, NAA, tCr, mIns etc. Only metabolites/neurochemicals processed by LCModel with Cramer-Rao Lower Bounds < 20% were analyzed. Since the resonances of Glu and Gln are overlapped quite a bit at 3T spectroscopy, they were labeled as Glx. However, Glu was also analyzed independently because it was determined reliably in high quality control of spectra at 3 T with short TE (Barker and Lin, 2006; Provencher, 1993). This study evaluated the relative concentration of metabolites (the relative ratio of tCr), due as tCr values were used as reference

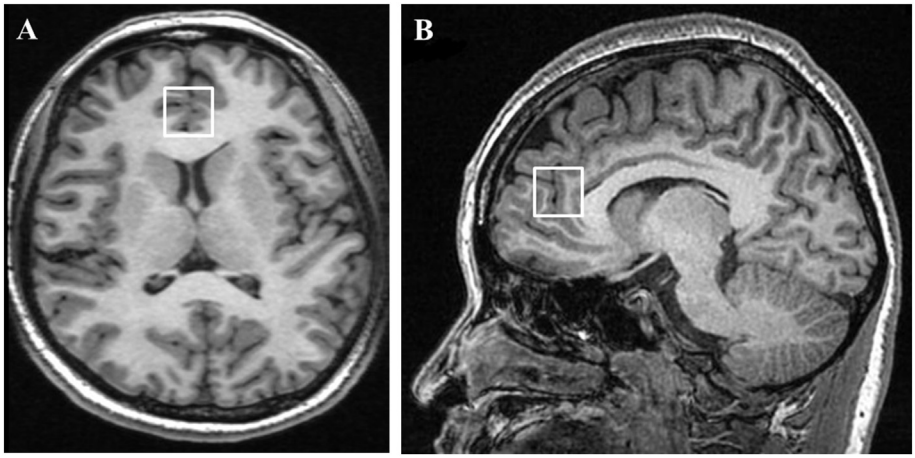

Fig. 1. Voxel placement. Axial (A) and sagittal (B) T1-weighted BRAVO slice showing the voxel position for the single voxel spectroscopy in the bilateral ACC (Voxel size = 20 × 20 × 20 mm^3). ACC, anterior cingulate cortex.

values as the total amount of Cr acted as a measure of general brain metabolism and appeared stable in subjects (Keshavan et al., 1991). Measurements of GABA+ using the MEGA-PRESS sequence are known to contain some contribution from co-edited macromolecule signals. To indicate this fact, GABA plus macromolecule measurements are reported using the notation GABA+ (Edden et al., 2012; Henry et al., 2001).

2.4. Statistical analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences software (SPSS 19.0). A Two-sample *t*-test and one-way analysis of variance (ANOVA) were performed in statistical analyses. Spearman correlation analysis was conducted to examine the relationship between the metabolite levels and clinical scores. In order to determinate the metabolite changes of abdominal pain in patients with CD, the differences in the means of metabolites levels among three groups were estimated after adjustments for age, gender and anxiety score. MRS data were normally distributed and presented as mean ± SD values. A two-tailed P-value of < 0.05 was considered significant. For Glu/tCr, mIns/tCr, Glx/tCr, NAA/tCr, GABA+/tCr Bonferroni-correction was used with a type I error = 0.01 (0.05/5) (5 referring to 5 metabolite ratios).

3. Results

3.1. Clinical characteristics of study subjects

The demographic information and results of all subjects are presented in Table 1. The age of pain CD group have statistically lower than the non-pain CD group. The HADS anxiety score of pain CD group have statistically higher than HC group. There were no statistically differences in gender and HADS depression score (all $p > 0.05$) among pain CD group, non-pain CD group, and HC group. There was no difference in CDAI between pain CD group and non-pain CD group.

3.2. Comparisons of metabolite levels among the 3 groups

One way ANOVA was employed among three groups, and revealed that CD patients with abdominal pain have statistically higher levels of Glu/tCr than CD patients without abdominal pain, and HCs ($p = 0.003$ and $p = 0.009$, respectively) in bilateral ACC (Table 2, Fig. 3A). The levels of Glx/tCr of pain CD group has a higher trend than the non-pain CD group, with no statistical differences compared to the HC group ($p = 0.022$ and $p = 0.639$, respectively). There were no statistical differences in NAA/tCr and mIns/tCr among three groups (all $p > 0.05$). Meanwhile, a two-independent sample *t*-test was conducted between nine CD patients with abdominal pain and 8 HCs. The results (Table 3) indicated that CD patients have significantly lower levels of GABA+/tCr ($p = 0.002$, Fig. 4A) and higher levels of Glu/tCr ($p = 0.014$) than HCs. The GABA+/tCr levels of two CD patients without abdominal pain were excluded in the statistical analyses due to a low number of samples.

3.3. Correlation between the metabolite levels and clinical scores

Within CD group, Glu/tCr (Fig. 3B) and Glx/tCr (Fig. 3C) levels of the pain CD group correlated strongly with the VAS scores of pain ($\rho = 0.86$, 0.59, respectively, $p < 0.05$), and no significant correlation with other clinical scores was found. Additionally, there were no significant correlations between GABA+/tCr levels of CD patients and clinical scores. However, GABA+/tCr levels demonstrated a trend of inverse correlation with CDAI of CD patients ($\rho = -0.60$, $p = 0.088$, Fig. 4B).

4. Discussion

This study was the first to investigate the changes of brain metabolites in patients with CD with abdominal pain using ^{1}H-MRS, a non-invasive measurement. Previous studies (Apkarian et al., 2005; Peyron et al., 2000) have shown that pain signals and psychological information are integrated, and activated during pain perception, attention, and emotional processing in the ACC. This finding was confirmed by the presence of abnormal activity in the ACC revealed by fMRI (Bernstein et al., 2002). Moreover, it has been demonstrated that high density opioid receptor binding sites were found in the ACC of patients with pain and acute pain affects the levels of Glx and GABA in ACC (Cleve et al., 2015; Jones et al., 1991). Therefore, based on the regulation of neurotransmitter activity, the study selected the ACC as a region of interest (ROI), and predicts the neurotransmitters will change in CD patients suffering from abdominal pain.

In the clinical data of this study, results indicated that the age of the non-pain CD group was higher than that of the pain group, presumably because the pain sensitivity of younger patients was higher than older patients (Riley et al., 2014). Meanwhile, HAD anxiety scores in pain group were statistically higher than healthy controls. Modi et al. (2014) found that the levels of Glu, mIns, and Glx within anxious individuals were increased when compared to relaxed individuals using MRS. Previous MRS studies (Mayer et al., 2015; Modi et al., 2014) have provided evidence that emotional involvement modulates brain response, and anxiety is associated with changes in glutamate system. Findings within this study are consistent with previous MRS studies, but

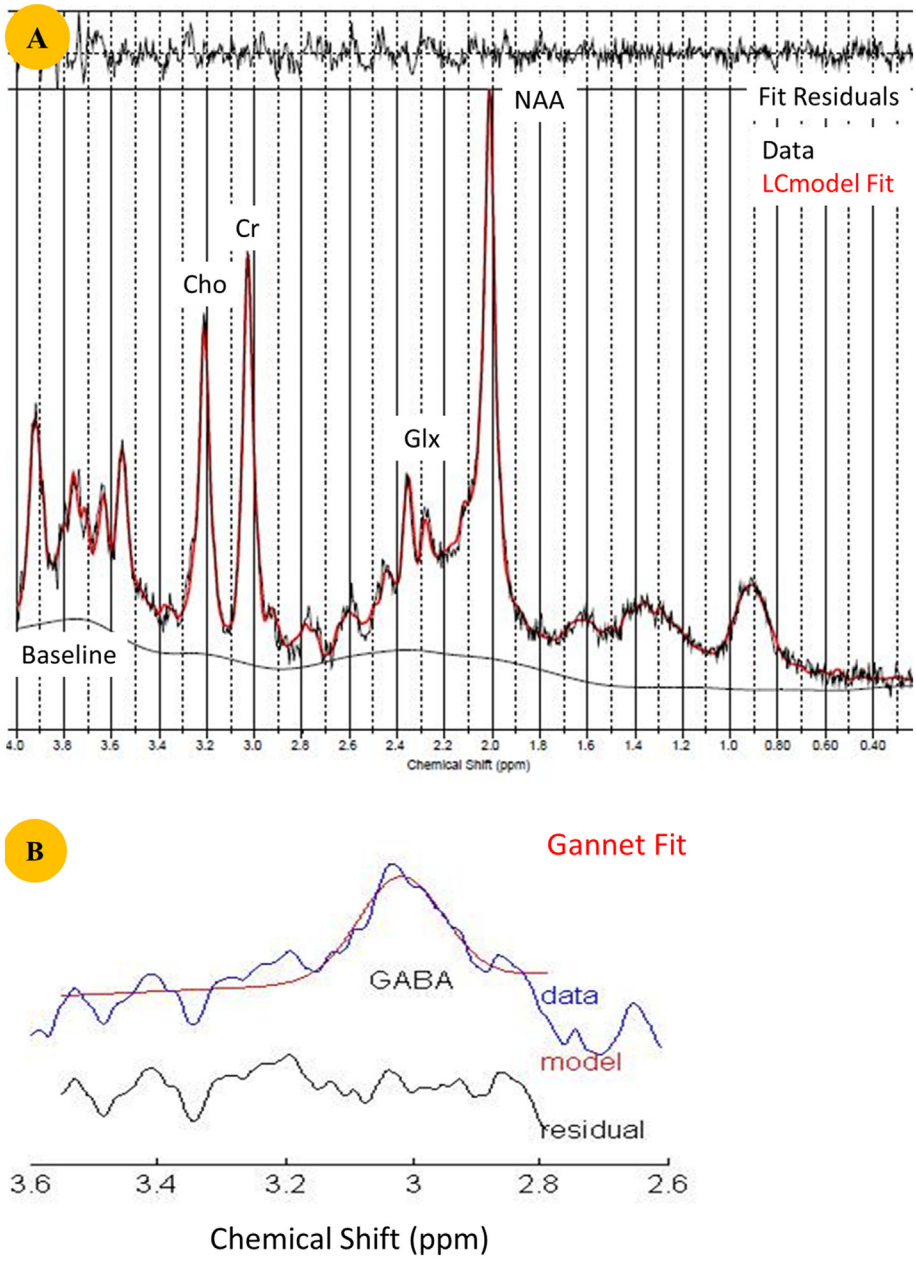

Fig. 2. The corresponding quantification results of LCModel and Gannet. The red lines delineate the spectral regions used for Glx and GABA quantification by LCModel (A) and Gannet (B), respectively. GABA, γ-aminobutyric acid; Glx, Glu + Gln; Cho, choline; Cr, creatine; NAA, *N*-acetyl-aspartate; MEGA-PRESS, Mesher–Garwood point-resolved spectroscopy; ppm, parts per million; PRESS, point-resolved spectroscopy.

Table 1
Demographic and clinical data among three groups.

	Pain group (n = 16)	Non-pain group (n = 13)	HCs (n = 20)	p-value
Age (years)	28.56 ± 7.10*	35.08 ± 8.86	29.35 ± 7.59	0.030
Gender (male/female)	10/6	8/5	12/8	> 0.05
CDAI	185.38 ± 67.63	175.08 ± 109.77	NA	> 0.05
VAS	4.19 ± 2.48	NA	NA	NA
HADS-A	5.31 ± 1.88†	4.77 ± 3.34	3.55 ± 2.08	0.013
HADS-D	3.69 ± 2.08	5.77 ± 4.32	3.75 ± 1.97	> 0.05

Values are presented as mean ± SD.
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; VAS, Visual Analogue Scale; NA, Not available.
* Compared with non-pain group, for $p < 0.05$.
† Compared with HC group, for $p < 0.05$.

Table 2
Mean ratio of metabolites levels in studied region among three groups.

Ratio of metabolites	Pain group (n = 16)	Non-pain group (n = 13)	HCs (n = 20)	p-value	
Glu/tCr	1.57 ± 0.17	1.33 ± 0.24	1.38 ± 0.21	0.003*	0.009†
Glx/tCr	2.10 ± 0.27	1.88 ± 0.28	2.06 ± 0.22	0.022*	0.639†
NAA/tCr	1.27 ± 0.12	1.23 ± 0.13	1.24 ± 0.14	> 0.05	
mIns/tCr	0.83 ± 0.095	0.80 ± 0.15	0.91 ± 0.17	> 0.05	

Values are presented as mean ± SD.
CD, Crohn's disease; HCs, healthy controls; Glu, glutamate; Glx, glutamate (Glu) + glutamine (Gln); NAA, *N*-acetyl-aspartate; mIns, myo-inositol.
* Pain group compared with non-pain CD group.
† Pain group compared with HCs group.

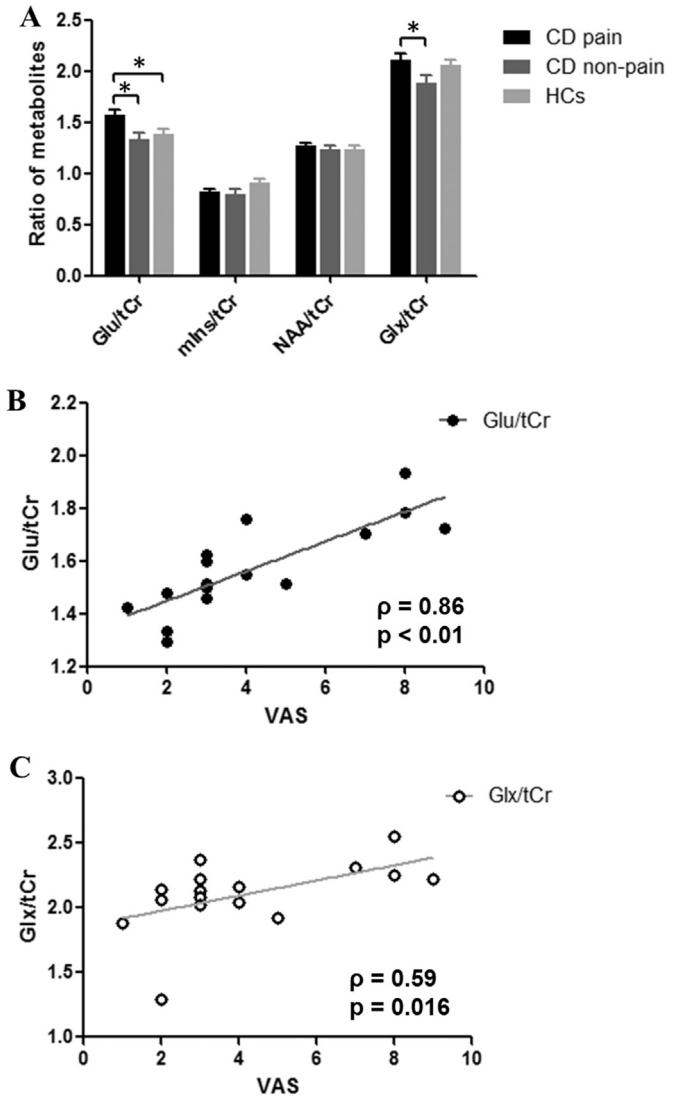

Fig. 3. The mean ratio of brain metabolites of three groups and correlation analysis. The pain CD group has higher levels of Glu/tCr than non-pain CD group, and HC group, respectively (A). The level of Glx/tCr of pain CD group had a higher trend than the non-pain CD group. The levels of Glu/tCr (B) and Glx/tCr (C) of the pain CD group correlated strongly with the VAS score of pain. CD, Crohn's disease; HC, healthy control; tCr, total creatine; Glu, glutamate; Glx, Glu + Gln; mIns, myo-inositol; NAA, *N*-acetylaspartate; VAS, Visual Analogue Scale; *p < 0.05.

Table 3
The smaller MEGA-PRESS study: clinical data and mean ratio of metabolites levels in studied region between nine CD patients with abdominal pain and 8 HCs.

	Pain group (n = 9)	HCs (n = 8)	p-value
Age (years)	31.11 ± 13.13	28.63 ± 3.96	0.615
Gender (male/female)	5/4	5/3	0.788
CDAI	199.89 ± 61.71	NA	NA
VAS	2.67 ± 1.41	NA	NA
HADS-A	5.44 ± 2.60	4.50 ± 2.07	0.425
HADS-D	4.56 ± 2.50	3.50 ± 1.51	0.318
Glu/tCr	1.59 ± 0.20	1.38 ± 0.16	0.014
Glx/tCr	2.11 ± 0.25	2.09 ± 0.22	0.889
NAA/tCr	1.22 ± 0.12	1.18 ± 0.14	0.575
mIns/tCr	0.82 ± 0.07	0.86 ± 0.05	0.209
GABA+/tCr	0.14 ± 0.03	0.25 ± 0.09	0.002

Values are presented as mean ± SD.
CD, Crohn's disease; HCs, healthy controls; HADS-A, hospital anxiety and depression scale, anxiety score; HADS-D, hospital anxiety and depression scale, depression score; Glu, glutamate; Glx, glutamate (Glu) + glutamine (Gln); NAA, *N*-acetyl-aspartate; mIns, myo-inositol, GABA+, γ-aminobutyric acid; NA, Not available.

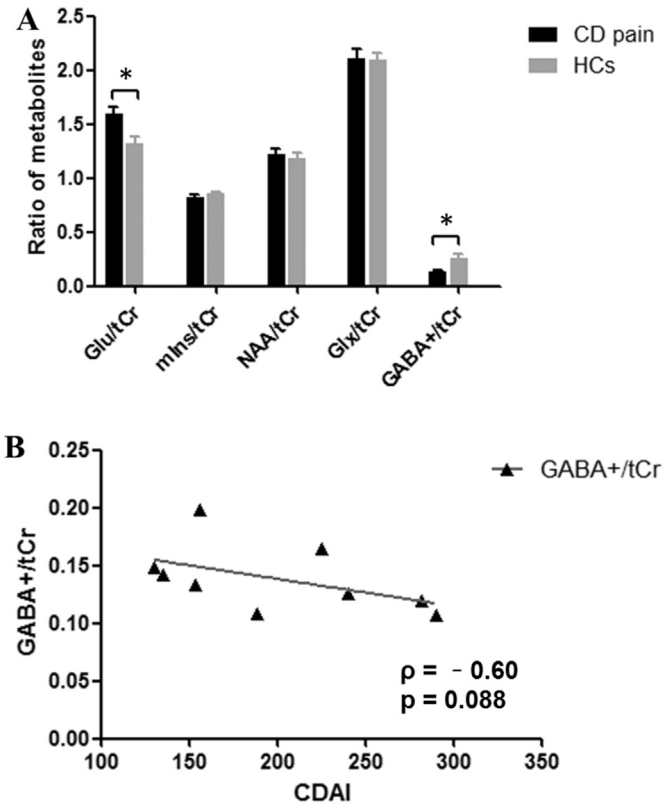

Fig. 4. The mean ratio of brain metabolites of two groups and correlation analysis. The pain CD group has significantly lower levels of GABA+/tCr and higher levels of Glu/tCr than HC group (A). GABA+/tCr levels demonstrated a trend of inverse correlation with CDAI of CD patients (B). CD, Crohn's disease; HC, healthy control; tCr, total creatine; Glu, glutamate; Glx, Glu + Gln; mIns, myo-inositol; NAA, *N*-acetylaspartate; GABA+, γ-aminobutyric acid plus; CDAI, Crohn's Disease Activity Index; *p < 0.05.

should be further validated by a more rigorous experimental design.

The most important finding of this study revealed that Glu/tCr levels within the bilateral ACC of CD patients with abdominal pain were elevated compared to levels in CD patients without abdominal pain, and the HC group. Further, the Glu/tCr levels were positively correlated with pain categorized by the VAS score. These findings are in

agreement with previous studies of pain (Ito et al., 2017; Mullins et al., 2005). Glu is the most abundant amino acid in mammalian brains. As the main excitatory neurotransmitter in the CNS, Glu is involved in many metabolic functions, such as the oxidative energy supply of neurons and astrocytes, and converts into glutamine and GABA synthesis in astrocytes (Mangia et al., 2012). Therefore, it was suggested that the physiological response observed by fMRI is resultant from an increased energy demand of activated neurons directly related to the Glu/Gln circulation (Rothman et al., 2003). ^{1}H-MRS and fMRI studies (Frankenstein et al., 2001; Fulbright et al., 2001) of pain showed increased levels of Glu and Gln in activated cerebral regions. The findings of this study support these hypotheses as well. Meanwhile, the results of this study revealed a trend of increased level of Glx/tCr in the pain CD group over the non-pain CD group, and a positive correlation with the VAS score of pain, which indicated that the Glx may associate with the pain sensitivity. Similarly, Zunhammer et al. showed that Glx levels were positively associated with individual pain sensitivity, indicating that increased levels of excitatory neurotransmitter may be a cause, or consequence of increased pain sensitivity (Zunhammer et al., 2016).

After the sequence of MEGA-PRESS was ready by GE Healthcare; nine CD patients with abdominal pain and 8 HCs were analyzed using MEGA-PRESS, which revealed a lower concentration of GABA+ in the bilateral ACC of CD patients compared to HCs. GABA is the main inhibitory neurotransmitter in the CNS, and mainly localized to inhibitory neurons, critically influencing the prefrontal cortical and ACC function (Buzsaki et al., 2007; Palomero-Gallagher et al., 2008). Therefore, reduced GABA levels could indicate either a loss, or dysfunction of GABAergic neurons in the ACC of CD patients with abdominal pain. In the studies of pain related metabolite alterations (Cleve et al., 2015; Watson, 2016), it was considered that the increase of Glu/tCr and the decrease of GABA+/tCr may be attributed to the increase of glutamate turnover and reflect a decreased activity of inhibitory system, respectively. This finding may be a result of cortical hyperactivity and hyperalgesia/allodynia in some pain conditions. Hyperalgesia is also involved in the spinal cord *N*-methyl-D-aspartate (NMDA) receptor (an ionotropic receptor of Glu).

Separately, results herein showed that levels of GABA+/tCr were negatively correlated with CDAI, indicating that reduced GABA+/tCr levels may be involved in the pathogenesis of CD. CDAI is the most frequently used index for clinical trials and must be considered the gold standard for evaluation of disease activity (Sostegni et al., 2003). Based on brain-gut axis, the GABA+/tCr were found to play a role in the relationship between the intestinal microbiota and GABA (Byrne et al., 2016; Lyte, 2011; Wall et al., 2009). Animal experiments (Bravo et al., 2011; Janik et al., 2016) have shown that the administration of lactobacillus rhamnosus could reduce depression, anxiety, change stress induced plasma cortisol levels and expression levels of GABA receptors in different brain regions. Increases the neurotransmitter concentration, including Glu/Gln and GABA, which implicated the vagus nerve was also reported. Combined with previous animal experiments and the decreased levels of GABA+/tCr, which correlated with CDAI; we speculated disorders of intestinal microbiota in patients with CD. Also, it was suggested that the intestinal microbiota may associated with brain metabolites. Moreover, normalization of GABA+ levels in the brain by treatment with selective serotonin reuptake inhibitors or electroconvulsive therapy was associated with reducing depression (Croarkin et al., 2011; Sanacora et al., 2003; Sanacora et al., 2002), which further implied that restoring GABA+ levels may be helpful for clinical treatment of CD. Furthermore, excessive glutamatergic neurotransmission is implicated in excitotoxic neuron damage. The increase in glutamate activity caused by persistent attacks of pain may lead to neurological dysfunction and death (Bleich et al., 2003; Rothman et al., 1987). On the basis of a variety of animal models and limited human clinical data, glutamate synapses are a potential target for drug intervention in a wide range of neurological and psychiatric disorders (Meldrum, 2000). The clinical trials of NMDA antagonists are limited by side effects, but intrathecal administration may be a useful approach (Kristensen et al., 1992). This may allow for reduced activity of glutamate by treating chronic pain to not only relieves pain and discomfort, but also may be neuroprotective. Therefore, regulating the levels of metabolites in the brain may be helpful for the clinical treatment of CD, and it may play a role in the treatment of inflammatory bowel disease by regulating the intestinal microbiota of clinical fecal microbiota transplantation.

While this study reported many findings, several limitations are worth noting. Firstly, changes in metabolites in regions outside the ACC were not examined due to time limitation. It is possible that changes in metabolite levels in other brain regions, such as the insula, hippocampus, temporal lobe and amygdala etc. that associated with visceral pain, or perception and emotion regulation. Therefore, it is not yet believed that ACC has a specific effect on the pain regulation of CD patients with abdominal pain. Secondly, the sample size of this study was small for a clinical study of this kind, especially for the results of GABA+. Further studies with a larger sample size, and staging including remission and active stages, are needed to further shed light on the neural mechanism of CD. Third, emotional disorders, such as anxiety and depression, whether it will cause changes of brain metabolites levels in CD and its correlations need further study, and further verify the impact of anxiety on Glu in this study. Finally, this study did not use fMRI to study functional changes in the brain of CD patients. MRS combined with fMRI can timely and more accurately detect the change of metabolites in the abnormal activity brain regions of CD patients. This will further verify the relationship between abnormal brain activity and metabolite levels, which will helpful for further clarify the neural mechanism of pain in CD patients.

In conclusion, the neural mechanism of CD in pain processing is an intricate network of signaling cascades. However, this study revealed several aspects of this matrix. Most notably, changes in bilateral ACC metabolite status was found in CD patients suffering from abdominal pain. An imbalance of Glu and GABA may imply disturbances of the intestinal microbiota, and play key role in pain processing for CD patients with abdominal pain. These findings provide new insight into the neural mechanisms of the disease with regard to pain processing, and help us better understand the pathophysiology of visceral pain in patients with CD. Results from this investigation provide insight to facilitate the development of novel therapies in future.

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Presentation

The abstract of this paper has been selected for presentation as a traditional printed poster at the Joint Annual Meeting ISMRM-ESMRMB 2018.

Declarations of interest

None.

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