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Relationships between brain metabolite levels, functional connectivity, and negative mood in urologic chronic pelvic pain syndrome patients compared to controls: A MAPP research network study

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ABSTRACT

Until recently, the predominant pathology of chronic pelvic pain conditions was thought to reside in the peripheral tissues. However, mounting evidence from neuroimaging studies suggests an important role of the central nervous system in the pathogenesis of these conditions. In the present cross-sectional study, proton magnetic resonance spectroscopy (^{1}H-MRS) of the brain was conducted in female patients with urologic chronic pelvic pain syndrome (UCPPS) to determine if they exhibit abnormal concentrations of brain metabolites (e.g. those indicative of heightened excitatory tone) in regions involved in the processing and modulation of pain, including the anterior cingulate cortex (ACC) and the anterior and posterior insular cortices. Compared to a group of age-matched healthy subjects, there were significantly higher levels of choline ($p = 0.006$, uncorrected) in the ACC of UCPPS patients. ACC choline levels were therefore compared with the region's resting functional connectivity to the rest of the brain. Higher choline was associated with greater ACC-to-limbic system connectivity in UCPPS patients, contrasted with lower connectivity in controls (i.e. an interaction). In patients, ACC choline levels were also positively correlated with negative mood. ACC γ-aminobutyric acid (GABA) levels were lower in UCPPS patients compared with controls ($p = 0.02$, uncorrected), but this did not meet statistical correction for the 4 separate regional comparisons of metabolites. These results are the first to uncover abnormal GABA and choline levels in the brain of UCPPS patients compared to controls. Low GABA levels have been identified in other pain syndromes and might contribute to CNS hyper-excitability in these conditions. The relationships between increased ACC choline levels, ACC-to-limbic connectivity, and negative mood in UCPPS patients suggest that this metabolite could be related to the affective symptomatology of this syndrome.

1. Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) are characterized by chronic pain in the pelvic region often accompanied by increased urinary frequency and urgency to void. These syndromes, referred to collectively here as urologic chronic pelvic pain syndrome (UCPPS) (Clemens et al., 2014; Landis et al., 2014), affect between 3 and 6% of women in the United States (Berry et al., 2011; Mathias et al., 1996) and contribute to a reduced quality of life in patients (Ayorinde et al., 2015). UCPPS was historically thought to be due solely to damage and inflammation in the pelvic region (Janicki, 2003); however, mounting evidence from neuroimaging suggests that for many UCPPS patients the pain may instead be related to alterations in the function and morphology of the central nervous system (CNS) (As-Sanie et al., 2016; Bagarinao et al., 2014; Farmer et al., 2015; Kilpatrick et al., 2014; Kleinhans et al., 2016; Kutch et al., 2017; Kutch et al., 2015; Martucci et al., 2015; Mayer et al., 2015; Woodworth et al., 2015). Similar abnormalities of the CNS occur in other chronic overlapping pain conditions that are frequently comorbid with UCPPS (Kuner and Flor, 2016; Maixner et al., 2016; Schmidt-Wilcke, 2015; Walitt et al., 2016), including fibromyalgia (FM), irritable bowel syndrome, chronic fatigue syndrome, and temporomandibular disorders.

In addition to changes in brain structure and function, neurochemical abnormalities have been identified in chronic pain conditions using proton magnetic resonance spectroscopy (^{1}H-MRS). ^{1}H-MRS is a non-invasive neuroimaging technique that can be used to measure concentrations of brain metabolites in vivo, such as glutamate,

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glutamine, choline, creatine, *N*-acetyl-aspartate (NAA), and γ-aminobutyric acid (GABA). For example, a recent study in a mixed sample of chronic pain patients found significantly increased *Glx* (i.e. glutamate + glutamine) in the anterior cingulate cortex (ACC) compared to pain-free controls (Ito et al., 2017). FM patients show increased Glx in the posterior insular cortex (pIC) (Harris et al., 2009) and decreased GABA (Foerster et al., 2012) in the anterior insular cortex (aIC) compared to healthy controls. Higher levels of Glx and lower levels of GABA were also found to be associated with increased clinical pain intensity and evoked pain sensitivity in FM patients (Foerster et al., 2012; Harris et al., 2009; Harte et al., 2013). As-Sanie et al. recently demonstrated increased insular Glx in patients with painful endometriosis, another form of chronic pelvic pain, compared to pain-free controls (As-Sanie et al., 2016). Furthermore, regional Glx levels were related to the resting functional connectivity between the insula and other brain regions. In chronic migraine patients, elevated GABA levels in the posterior cingulate cortex were associated with increased pain, but patients were studied at times when they were pain-free, so it is unclear whether ACC GABA levels differ in these patients while their pain is occurring (Aguila et al., 2016). In patients with ongoing knee osteoarthritis pain, there were negative correlations between ACC GABA levels and pain severity, but no overall differences between patients and controls (Reckziegel et al., 2016). Overall, these studies suggest that an imbalance of CNS inhibitory (i.e., GABA) and excitatory (i.e., glutamate, Glx) neurotransmission may play an important role in the pathophysiology of chronic pain (Foerster et al., 2012; Harris and Clauw, 2012; Harris et al., 2013; Harris et al., 2009; Harris et al., 2008; Harte et al., 2013; Ichesco et al., 2012; Petrou et al., 2012).

The aim of the present study was to characterize the brain metabolite status of the ACC and insula in patients with UCPPS compared to in healthy controls, and the relationship between these metabolite levels and UCPPS symptoms. In addition, we also examined whether neurochemical tone in these regions was related to resting state functional connectivity with other brain regions. We hypothesized that, similar to other chronic pain conditions, UCPPS patients would show increased Glx and decreased GABA levels in the ACC and/or insula, and that metabolite levels within and connectivity between these regions and others would be related to pain and symptoms.

2. Materials and methods

2.1. Participants

Recruitment was carried out at the University of Michigan (Ann Arbor, MI) Discovery Site of the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network (Clemens et al., 2014; Landis et al., 2014). Eighteen females with UCPPS who met previously described (Landis et al., 2014) criteria to be enrolled in the parent multisite MAPP Network neuroimaging protocol (Alger et al., 2016) agreed to participate in a co-occurring, site-specific study examining the role of brain metabolites in the pathophysiology of UCPPS.

Table 1
Chronic overlapping pain conditions in UCPPS patient sample.

	n (%)
Conditions	
Temporomandibular disorder	6 (33)
Irritable bowel syndrome	4 (22)
Fibromyalgia	2 (11)
Chronic fatigue syndrome	1 (6)
Number per patient	
0	8 (44)
1	6 (33)
2	1 (6)
3	3 (17)

Table 1 shows the frequency of comorbid conditions in our patient sample. An additional 20 females without chronic pelvic pain, who were also enrolled in the broader MAPP protocol, were recruited to undergo spectroscopy and serve as age-matched healthy controls (HC). Study procedures were approved by the University of Michigan Medical School's Institutional Review Board and all subjects gave written informed consent to participate.

2.2. Clinical pain and psychological assessment

Clinical pain was assessed with the short form of the Brief Pain Inventory (BPI) which captures both pain severity and interference due to pain over the course of the previous week (Cleeland and Ryan, 1994). Participants also responded the short form of the McGill Pain Questionnaire (MPQ), which includes subscales of sensory and affective clinical pain levels (Melzack, 1987). The Hospital Anxiety and Depression Scale (HADS) was administered to obtain levels of depression and anxiety (Zigmond and Snaith, 1983). Mood over the previous week was assessed with the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988); to get a more recent indication of mood, subjects responded to a question before the scan asking them to rate their mood over the past 24 h on a scale from 0 to 10, where 0 meant “extremely good mood” and 10 meant “extremely bad mood” (Landis et al., 2014). Finally, genitourinary pain and symptoms were assessed with the Genitourinary Pain Index (GUPI) (Clemens et al., 2009).

2.3. Neuroimaging acquisition and preprocessing

2.3.1. Proton magnetic resonance spectroscopy (^{1}H-MRS)

The neuroimaging scan for each participant was conducted within 48 h of the clinical and psychological visit. Brain imaging was conducted on a 3.0T scanner (Philips Achieva), using an 8-channel receiver head coil. We performed T1-weighted 3-dimensional MPRAGE imaging with an isotropic voxel resolution of 1.0 mm^3. MR spectra were acquired from 3.0 × 2.0 × 3.0 cm voxels placed in the right anterior insula (aIC), right posterior insula (pIC), midline anterior cingulate cortex (ACC), and midline occipital cortex (OC). See Fig. 1. The ACC and insula were chosen for their well-described role in both acute and chronic pain (Bliss et al., 2016), and previous findings from our lab and others showing altered metabolites in these regions in chronic pain patients (As-Sanie et al., 2016; Cagnoli et al., 2013; Cao et al., 2016; Feraco et al., 2011; Foerster et al., 2012; Harris et al., 2013; Harris et al., 2009; Harris et al., 2008; Ito et al., 2017; Petrou et al., 2008; Petrou et al., 2012; Reckziegel et al., 2016; Valdes et al., 2010; Widerstrom-Noga et al., 2013; Zhao et al., 2017). The occipital cortex was selected as a control region. Single-voxel point-resolved spectroscopy (PRESS) spectra (time to recovery [TR]/time to echo [TE] 2000/33 ms) were acquired using VAPOR water suppression with 32 averages and a scan time of 1 min for each voxel. For the quantification of GABA, spectroscopy experiments using the Mescher-Garwood point-resolved spectroscopy (MEGA-PRESS) technique were performed, using the following parameters: TE 68 ms (TE1 15 ms, TE2 53 ms), TR 1.8 s, 256 transients of 2000 data points, spectral width 2 kHz, frequency-selective editing pulses (14 ms) applied at 1.9 ppm (the ON mode) and 7.46 ppm (the OFF mode). Refocusing was performed using the amplitude-modulated pulse GTST1203 (length 7 ms, bandwidth 1.2 kHz). The results from the conventional PRESS technique were analyzed using LCModel (Stephen Provencher, Oakville, Ontario, Canada). The MEGA-PRESS results were analyzed using in-house post-processing software in Matlab (Mathworks, Sherborn, MA, USA), with Gaussian curve fitting to the GABA and inverted *N*-acetylaspartate (NAA) peaks. We measured GABA relative to the NAA signal in the edited spectra (Stagg et al., 2009) to calculate a ratio based on the concentration of NAA, as generated by the MEGA-PRESS technique. After calculating this GABA:NAA ratio, we then multiplied this ratio by the NAA concentration, determined from LCModel analysis of a short-TE PRESS