

# Abnormal neurometabolites in fibromyalgia patients: Magnetic resonance spectroscopy study

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## Abstract

This study aimed to investigate distinct neurometabolites in the anterior cingulate cortex (ACC), right and left thalamus, and insula of patients with fibromyalgia (FM) compared with healthy controls using proton magnetic resonance spectroscopy (MRS). Levels of N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), total NAA (tNAA = NAA + NAAG), myo-inositol (ml), glutamine (Gln), glutamate (Glu), Glx (Glu + Gln), glycerophosphocholine (GPC), total choline (tCho = GPC + phosphocholine) and glutathione (GSH) levels relative to total creatine (tCr) levels including creatine (Cr) and phosphocreatine (PCr) and relative to Cr levels were determined in the ACC, right and left thalamus, and insula in 12 patients with FM and 13 healthy controls using MRS. In the ACC, NAA/tCr ( $P=0.028$ ) and tCho/tCr ( $P=0.047$ ) were higher in patients with FM. In the right and left insula, tNAA/tCr ( $P=0.019$ ,  $P=0.007$ , respectively) was lower in patients with FM. Patients with FM showed lower levels of ml/Cr ( $P=0.037$ ) in the right insula than healthy controls. These findings are paramount to understand decisive pathophysiological mechanisms related to abnormal features in the brain and parasympathetic nervous systems in FM. We suggest that the results presented herein may be essential to understand hidden pathological mechanisms and also life system potential as protective and recovering metabolic strategies in patients with FM.

## Keywords

fibromyalgia, magnetic resonance spectroscopy, neurometabolites, anterior cingulate cortex, thalamus, insula

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## Introduction

Fibromyalgia (FM) is the currently accepted term for chronic widespread musculoskeletal pain.<sup>1</sup> Symptoms commonly observed are fatigue, memory problems, and sleep disturbances.<sup>2</sup> The clinical features of FM are associated with various psychological symptoms related to stress and post-traumatic stress disorder (PTSD).<sup>3,4</sup> In addition, the enhanced nociception was observed in stress-induced chronic widespread pain.<sup>5</sup> Peripheral and central abnormalities of nociception are associated with peripheral and central sensitization in patients with FM.<sup>6</sup> Peripheral, central, cognitive-emotional, and interpersonal sensitization may be responsible for heightened pain sensitivity in patients with FM.<sup>7</sup> Central sensitization in patients with FM is

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revealed using brain imaging, indicating a decreased functional connectivity in the descending pain-modulating system and an increased activity in the pain matrix related to central sensitization.<sup>8</sup>

Localized proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) is a noninvasive tool for measuring *in vivo* neurochemical information, frequently referred to as metabolites in the human brain. Investigating abnormal neurometabolites using MRS may be useful for understanding the neuropathology and metabolic dysfunctions in the specific brain areas in patients with FM. A significant increase was found in the Glx (glutamate [Glu] + glutamine [Gln]) levels in the posterior cingulate cortex (PCC) and right amygdala for patients with FM.<sup>9,10</sup> Glx/creatine (Cr) and Glu/Cr ratios within the ventrolateral prefrontal cortex bilaterally were significantly higher in patients with FM.<sup>11</sup> FM patients had significantly higher levels of Glx/Cr ratios in the posterior gyrus and lower levels of myo-inositol (ml) in the right and left hippocampi.<sup>12</sup> In addition, decreased ml/Cr ratios were found in the left sensorimotor area and left hippocampus, and lower levels of choline (Cho) and total NAA (tNAA) levels including N-acetylaspartate (NAA) + N-acetylaspartylglutamate (NAAG) levels were found in the left hippocampus in FM.<sup>12</sup> In contrast, Cho levels in the right hippocampus were higher in FM.<sup>13</sup> Briefly, previous studies using MRS for FM have mainly reported increases in cerebral Glu levels and reductions in ml and NAA in the hippocampus.<sup>12,14,15</sup>

In this study, we investigated the chemical biomarkers associated with FM. Among a variety of MRS-detectable metabolites, we focused on levels of NAA, NAAG, tNAA (NAA+NAAG), Gln, Glu, Glx (Glu + Gln), ml, glycerophosphocholine (GPC), total Cho (tCho) (GPC + phosphocholine), and glutathione (GSH) levels relative to total creatine (tCr) levels including Cr and phosphocreatine (PCr) and relative to Cr levels in the anterior cingulate cortex (ACC), bilateral thalamus, and insula.

While previous reports using MRS have mainly focused on the hippocampus, amygdala and PCC, we focused on the ACC, bilateral thalamus, and insula in FM. Thus, this study aimed to investigate neurochemical biomarkers and abnormal neurometabolites associated with FM compared with healthy controls. Furthermore, we investigated the relationships of abnormal neurometabolites levels with pain and psychiatric symptoms in patients with FM.

## Methods

### Participants

The present study included 12 patients fulfilling the 2010 American College of Rheumatology criteria for FM,

recruited from the Seoul National University Hospital, while 15 individuals of comparable age and gender, exhibiting no pain or neurological symptoms were recruited using online advertisements. However, MRS data were missing for two patients; thus, 13 subjects were used as healthy controls. The subjects used as the healthy controls were the same used in a previous complex regional pain syndrome study,<sup>16,17</sup> while two additional participants were included in this study. Subjects who exhibited high levels of high-sensitivity C-reactive protein (hs-CRP) or leukocytosis were excluded. The inclusion criteria for FM subjects were as follows: diagnosis of FM; age between 21 and 63 years; and either benzodiazepine not administered or discontinued 2 weeks before the study. Individuals with a major neuropsychiatric disorder before the diagnosis of FM, neurological disease (cerebrovascular disease or brain tumor), history of brain trauma, high levels of hs-CRP or leukocytosis, as well as those who could not undergo magnetic resonance imaging were excluded. Sensory and affective dimensions of current pain were assessed using the McGill pain Questionnaire Short-Form, comprising 11 McGill Pain Questionnaire-Sensory and four McGill Pain Questionnaire-Affective pain items.<sup>18</sup> Moreover, visual analogue scale (VAS) was used for measurement of pain intensity. Stress levels in patients with FM were assessed using the stress response inventor, consisting of 39 items (score range: 0–156) categorized into seven factors: fatigue, tension, frustration, anger, depression, somatization, and aggression.<sup>19</sup> The PTSD checklist assessed the development of PTSD in respondents after exposure to a traumatic event.<sup>20</sup> This study was approved by the Institutional Review Board at the Seoul National University Hospital (Seoul, Korea). All data were obtained under written informed consent granted by all subjects after a full explanation of the experimental methods.

### <sup>1</sup>H-MRS data acquisition and processing

All magnetic resonance (MR) data were collected with a 3.0-T human MR scanner, using a 16-channel head and neck coil (Siemens Trio system; Siemens Medical Solutions, Erlangen, Germany). For <sup>1</sup>H-MRS volume localization, anatomical images were collected using a T2-weighted fast spin echo sequence along the axial (axi), sagittal (sag), and coronal (cor) directions (repetition time [TR]=6090 ms [axi] and, 5910 ms [sag and cor], echo time [TE]=89 ms, flip angle=90°/130°, field of view=220 × 199 mm<sup>2</sup> [axi and cor] and 220 × 220 mm<sup>2</sup> [sag], matrix size=256 × 180, echo train length=5, echo spacing=9.93 ms, receiver bandwidth [BW]=271 Hz/pixel, number of slices=30 [no gap], slice thickness=5 mm, number of signal averages [NSA]=128).

Based on scout images, five volumes of interest (VOIs) were selected in the ACC ( $2 \times 2 \times 2 \text{ cm}^3$ ), right and left thalamus ( $2 \times 2 \times 1.5 \text{ cm}^3$ ) and right and left insula ( $2 \times 1.5 \times 2 \text{ cm}^3$ ) for each subject. The VOI in the right and left thalamus was placed along the axis of the thalamus in order to cover the maximum volume. Following auto shimming over the VOI,  $^1\text{H}$ -MRS data were acquired using a point-resolved spectroscopy pulse sequence (PRESS)<sup>21</sup> with TR/TE = 2000/30 ms, 2048 data points, BW = 2500 Hz, NSA = 128, four dummy scans, and four-step phase cycling. The main PRESS sequence was preceded by water and outer-volume suppression modules. The carrier frequency was adjusted by -2.3 ppm from the water resonance to minimize voxel displacement.

The  $^1\text{H}$ -MRS data were analyzed using LC Model<sup>22</sup> software (ver. 6.3–1J) in the range of 4.2 to 1.0 ppm. The metabolite content was normalized to that of tCr and Cr levels. The final data analysis included only those metabolites with a Cramer–Rao lower bound (CRLB) < 30%. Owing to the selection based on the CRLB < 30%, the number of the samples used in each metabolite was different in each result.

### Statistical analysis

Statistical analyses were performed using SPSS Statistics 21.0 (SPSS, Chicago, IL). The differences in MRS between patients with FM and healthy controls were assessed using a two-tailed student's t-test. After the data were tested for normality, a non-parametric test, the Mann-Whitney U test were used for data that were not normally distributed. The Pearson's correlation analysis was used to evaluate the association between psychological test scores and neurometabolites using MRS. P-values < 0.05 were considered statistically significant without correction for multiple comparisons.

## Results

### Study participants and basic information

A total of 12 FM and 13 healthy control subjects completed the study procedures. Demographic and clinical characteristics of the subjects are presented in Supplementary Table 1. Age, gender ratio, and education levels were not significantly different between the two groups. The average pain duration in patients with FM was 4.4 years.

### Comparison of neurometabolites in ACC, right and left thalamus, and insula between FM patients and healthy control subjects

We investigated neurometabolites levels in the ACC, right and left thalamus, and insula in patients with FM

compared with healthy controls using internal references of tCr (Cr + PCr), and Cr for calculating metabolite ratios.

**Abnormal levels of neurometabolites related to microstructural features in ACC and bilateral insula in FM patients.** In the ACC, NAA/tCr ( $P = 0.028$ ) was higher in patients with FM than in healthy controls (Table 1). In the right and left insula, tNAA/tCr ( $P = 0.019$ ;  $P = 0.007$ , respectively) was lower in FM patients (Table 2). However, there were not found any significant results between FM and healthy controls in the right and left thalamus (Table 3). When the reference marker, Cr levels, was used, patients with FM had lower levels of ml/Cr ( $P = 0.037$ ) in the right insula than healthy controls (Figure 1).

**Higher levels of tCho/tCr in ACC related to parasympathetic nervous system in FM patients.** In the ACC, tCho/tCr was higher ( $P = 0.047$ ) in patients with FM than in healthy controls (Table 1), which may be related to parasympathetic nervous system.

### Correlations between neurometabolite levels and pain severity, PTSD, and stress levels in FM patients

There were positive correlations between Glx/Cr and stress ( $r = 0.779$ ,  $P = 0.005$ ) and PTSD ( $r = 0.786$ ,  $P = 0.004$ ); however, there was a negative correlation

**Table 1.** Comparison of neurometabolites in anterior cingulate cortex between fibromyalgia patients and healthy controls.

Anterior cingulate cortex					
Metabolites	Groups	N	mean	SD	p-value
Gln/tCr	CON	11	0.503	0.227	0.957
	FM	12	0.508	0.150	
Glu/tCr	CON	13	1.379	0.099	0.354
	FM	12	1.431	0.170	
GPC/tCr	CON	12	0.220	0.030	0.194
	FM	12	0.233	0.046	
GSH/tCr	CON	13	0.299	0.078	0.870
	FM	12	0.300	0.058	
ml/tCr	CON	13	0.781	0.080	0.128
	FM	12	0.824	0.074	
NAA/tCr	CON	13	0.822	0.225	<b>0.028</b>
	FM	12	1.029	0.216	
tCho/tCr	CON	13	0.219	0.029	<b>0.047</b>
	FM	12	0.244	0.030	
tNAA/tCr	CON	13	1.201	0.130	0.575
	FM	12	1.171	0.132	
Glx/tCr	CON	13	1.835	0.239	0.189
	FM	12	1.939	0.117	

Gray color: Mann-Whitney U test, White background: Student t-test, bold letter: significant results.

CON; healthy controls, FM; fibromyalgia.

**Table 2.** Comparison of neurometabolites in right and left insula between fibromyalgia patients and healthy controls.

Right insula						Left insula					
Metabolites	Groups	N	mean	SD	p-value	Metabolites	Groups	N	mean	SD	p-value
Gln/tCr	CON	6	0.354	0.060	0.837	Gln/tCr	CON	5	0.496	0.183	0.219
	FM	9	0.344	0.093			FM	5	0.376	0.030	
Glu/tCr	CON	11	1.205	0.181	0.123	Glu/tCr	CON	12	1.109	0.204	0.784
	FM	12	1.296	0.075			FM	12	1.129	0.152	
GPC/tCr	CON	11	0.244	0.037	0.380	GPC/tCr	CON	10	0.268	0.049	0.157
	FM	12	0.256	0.024			FM	12	0.243	0.021	
GSH/tCr	CON	11	0.269	0.053	0.140	GSH/tCr	CON	10	0.301	0.069	0.445
	FM	12	0.298	0.041			FM	11	0.278	0.062	
ml/tCr	CON	11	0.641	0.100	0.626	ml/tCr	CON	11	0.701	0.112	0.095
	FM	12	0.660	0.091			FM	12	0.793	0.137	
NAA/tCr	CON	11	0.993	0.192	0.987	NAA/tCr	CON	10	0.808	0.317	0.742
	FM	12	0.992	0.190			FM	12	0.871	0.264	
NAAG/tCr	CON	8	0.355	0.153	0.751	NAAG/tCr	CON	9	0.662	0.242	0.553
	FM	3	0.388	0.121			FM	3	0.571	0.118	
tCho/tCr	CON	11	0.265	0.054	0.607	tCho/tCr	CON	12	0.276	0.063	0.110
	FM	12	0.256	0.024			FM	12	0.243	0.021	
tNAA/tCr	CON	11	1.275	0.105	0.019	tNAA/tCr	CON	12	1.199	0.137	0.007
	FM	12	1.159	0.111			FM	12	1.044	0.115	
Glx/tCr	CON	11	1.473	0.257	0.085	Glx/tCr	CON	12	1.436	0.307	0.782
	FM	12	1.611	0.111			FM	12	1.403	0.271	

Gray color: Mann-Whitney U test, White background: Student t-test, bold letter: significant results.  
CON; healthy controls, FM; fibromyalgia.

**Table 3.** Comparison of neurometabolites in right and left thalamus between fibromyalgia patients and healthy controls.

Right thalamus						Left thalamus					
Metabolites	Groups	N	mean	SD	p-value	Metabolites	Groups	N	mean	SD	p-value
Gln/tCr	CON	7	0.709	0.390	0.410	Gln/tCr	CON	10	0.604	0.120	0.572
	FM	6	0.554	0.227			FM	4	0.567	0.154	
Glu/tCr	CON	12	1.122	0.259	0.976	Glu/tCr	CON	13	1.153	0.138	0.067
	FM	12	1.125	0.081			FM	12	1.250	0.112	
GPC/tCr	CON	12	0.236	0.037	0.264	GPC/tCr	CON	13	0.257	0.022	0.123
	FM	12	0.258	0.054			FM	12	0.274	0.028	
GSH/tCr	CON	11	0.357	0.176	0.466	GSH/tCr	CON	12	0.343	0.111	0.298
	FM	12	0.316	0.079			FM	12	0.371	0.076	
ml/tCr	CON	12	0.793	0.208	0.887	ml/tCr	CON	12	0.687	0.119	0.509
	FM	12	0.746	0.077			FM	12	0.720	0.126	
NAA/tCr	CON	8	0.731	0.187	0.343	NAA/tCr	CON	12	0.797	0.208	0.308
	FM	12	0.836	0.275			FM	12	0.898	0.260	
NAAG/tCr	CON	12	0.840	0.162	0.074	NAAG/tCr	CON	13	0.692	0.186	0.293
	FM	8	0.663	0.257			FM	10	0.597	0.236	
tNAA/tCr	CON	13	1.335	0.216	0.730	tNAA/tCr	CON	13	1.442	0.150	0.914
	FM	12	1.362	0.159			FM	12	1.436	0.124	
Glx/tCr	CON	13	1.642	0.557	0.747	Glx/tCr	CON	13	1.685	0.213	0.824
	FM	12	1.586	0.203			FM	12	1.667	0.164	
tCho/tCr	CON	12	0.236	0.037	0.264	tCho/tCr	CON	13	0.262	0.031	0.340
	FM	12	0.258	0.054			FM	12	0.274	0.028	

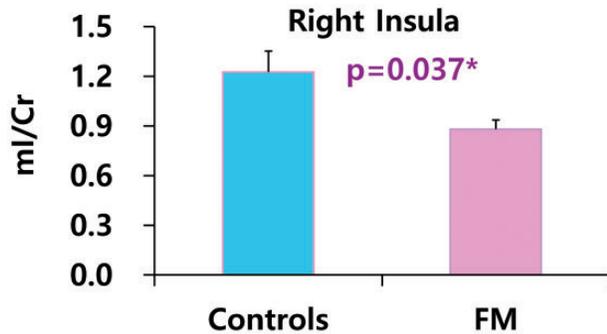
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CON; healthy controls, FM; fibromyalgia.

between GPC/tCr and stress levels ( $r = -0.577$ ,  $P = 0.050$ ) in the right thalamus in patients with FM (Figure 2). There were positive correlations between mI/Cr and VAS pain scores ( $r = 0.602$ ,  $P = 0.038$ ) in the right insula in patients with FM (Figure 3).

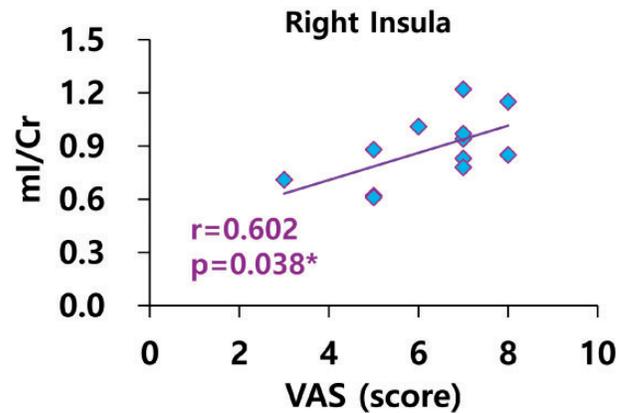
### Discussion

This study revealed abnormal levels of neurometabolites related to microstructural features and associated with

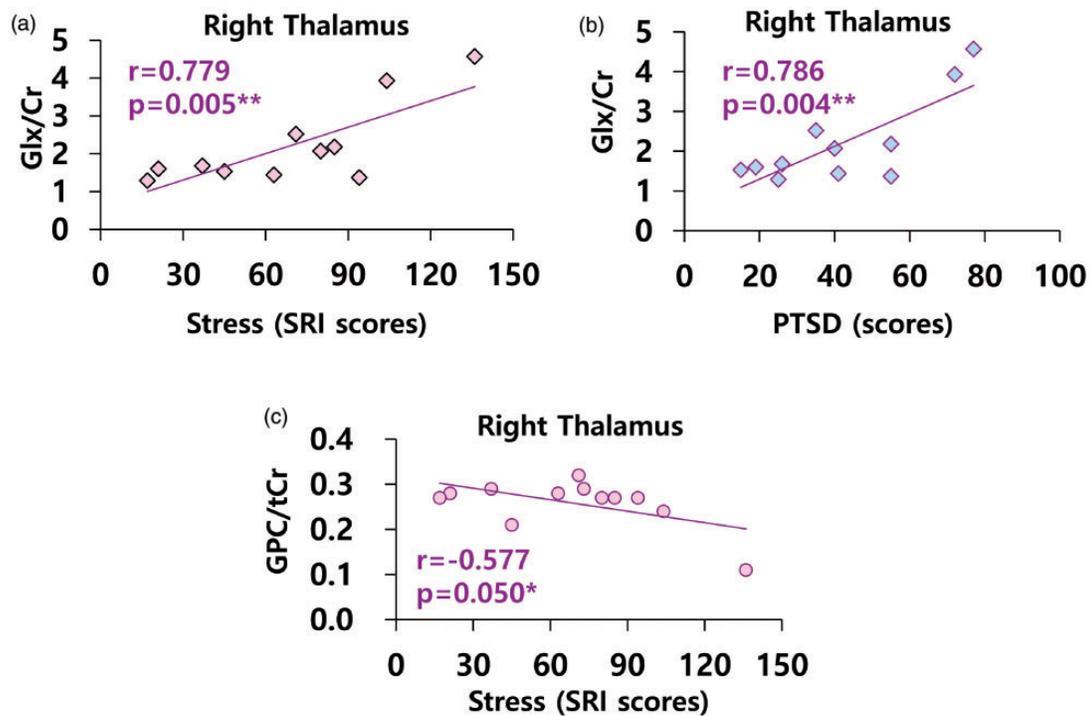
the parasympathetic nervous system in ACC and bilateral insula in patients with FM compared with healthy controls. First, we found abnormal neurometabolites in NAA levels used as a neuronal marker<sup>23</sup> and mI levels used as glial marker,<sup>24</sup> which may indicate microstructural abnormalities in ACC and bilateral insula of FM. Secondly, tCho/tCr were higher in the ACC, which may be associated with recovering life system in the



**Figure 1.** The decreased levels of mI using internal reference marker Cr in right insula of fibromyalgia. Used analysis; Student's t-test. Controls (n = 6), FM; fibromyalgia (n = 12), mI; myo-inositol, Cr; creatine.



**Figure 3.** Correlations between mI levels using internal reference marker Cr and VAS in right insula of fibromyalgia. n = 12, VAS; Visual Analogue Scale, mI; myo-inositol, Cr; creatine.



**Figure 2.** Correlations between neurometabolites and stress and PTSD in right thalamus of fibromyalgia. (a, b) n = 11, Glx; glutamine (Gln) + glutamate (Glu), Cr; creatine. (b) PTSD; post-traumatic stress disorder. (c) n = 12, GPC; glycerophosphocholine, tCr; total creatine levels including creatine (Cr) and phosphocreatine (PCr). (a, c) SRI; Stress Response Inventory.

parasympathetic nervous system in patients with FM. This study elucidated comprehensive pathophysiological mechanisms related to microstructural abnormalities and protective and recovering strategies as life system potential in the brain of patients with FM. Additionally, these pathological abnormalities or recovering strategies were closely associated with stress, PTSD, and pain levels in patients with FM. We emphasized that these abnormalities in the brain metabolites may also exhibit life system potential as protective and recovering metabolic strategies in FM.

The increase of tCho/tCr in ACC may be associated with protective strategies to recover parasympathetic dysfunction in FM. GPC is converted to choline, which is the precursor for the neurotransmitter acetylcholine,<sup>25,26</sup> involved in many functions including cognitive function, memory, and muscle control.<sup>25,27,28</sup> In the parasympathetic division, neurons are cholinergic, and acetylcholine is the primary neurotransmitter responsible for communication between the neurons on the parasympathetic pathway.<sup>29</sup> In addition, the anterior/mid cingulate cortex is critically involved in the response inhibition network in the pain system,<sup>30</sup> and ACC links the processes of cognitive interference and parasympathetic modulation with activation in the ACC, a structure critical for the interface between cognition and emotion.<sup>31</sup> Fortunately, the increase of tCho/tCr in ACC may be associated with protective mechanisms for recovering parasympathetic regulation in patients with FM. However, high stress in patients with FM affects the brain's recovery potential for protecting body disorder, considering that the higher stress levels reduces GPC/tCr levels in the right thalamus in FM in this study. On the other hand, FM is associated with decreased connectivity between pain and the sensorimotor brain areas.<sup>32</sup> Therefore, the increase in tCho/tCr in the ACC may contribute to the pain regulation and protective mechanisms for emotion and cognition in patients with FM. Moreover, the tCho are biosynthetic precursors of acetylcholine for parasympathetic function. Therefore, we can suggest that the increase in tCho/tCr in FM may be more associated with emotional and cognitive regulation for pain modulation in ACC, compensating parasympathetic dysfunction in the body and brain as the brain's protective and recovering strategies.

In addition to the increase in tCho/tCr in the ACC, NAA/tCr levels in ACC increased in patients with FM. Not only is ACC related to parasympathetic modulation,<sup>31</sup> but also the anterior/mid cingulate cortex are related to the response inhibition network in the pain system.<sup>30</sup> Furthermore, NAA is detected in the adult brain in neurons as the neuronal marker.<sup>23</sup> Thus, the increase in NAA/tCr in the ACC may contribute to the increase of neuron and pain inhibition and modulation in FM, showing compensating potential for the

brain's effective recovering system. In addition, as the ACC is critical for the interface between cognition and emotion,<sup>31</sup> the increase in NAA/tCr in ACC may develop cognitive regulation mechanisms for pain inhibition and modulation, enhancing structural recovery by using the increase of neuronal cell in FM. Moreover, reactive neurogenesis occurs in response to naturally occurring apoptosis as well as injury-induced neuronal death in an adult brain.<sup>33</sup> Neurogenesis and morphogenesis were observed through migration and lamination in the regions of the cingulate cortex.<sup>34</sup> Therefore, the increase in the NAA/tCr in ACC may be associated with neurogenesis and migration for natural therapeutic strategies in FM.

The insula is associated with sympathetic dysfunctions and pain perception and processing.<sup>35,36</sup> In addition, mI/Cr levels, known as a glial marker,<sup>24</sup> were lower in the right insula in FM, which may be associated with the structural deficits in the glial cells. Considering that the sympathetic nervous system was involved in trauma stress-induced immune alterations via a mechanism of apoptotic cell death,<sup>37</sup> PTSD and stress may induce glial cell death in the insula of patients with FM. Similarly, these reduced levels of mI/tCr may affect lower pain sensitivity in FM, considering that higher pain intensity (VAS) levels were associated with higher mI/Cr levels in this study. Moreover, these lower levels of mI in the right insula may be related to pathological dysfunction in pain perception and processing<sup>35</sup> and sympathetic dysfunction.<sup>36</sup> Thus, structural abnormalities related to NAA-related neuronal and mI-related glial cells may be a critical pathological factor associated with pain processing in the brain of patients with FM<sup>35</sup> or protective and recovering metabolic strategies. Additionally, considering that abnormal levels of GPC are involved in acetylcholine-related parasympathetic dysfunction,<sup>38</sup> the increased levels of tCho in the ACC may be associated with parasympathetic dysfunction or protective and recovering mechanisms in FM.

The clinical features of FM are associated with various psychological factors, and stress is a necessary link in the pathway between significant psychological factors and key FM symptoms.<sup>3</sup> In addition, FM patients had more PTSD symptoms than controls.<sup>4</sup> Additionally, considering high levels of stress and PTSD scores in patients with FM, long-time stress and longer period of pain may contribute to the severity on pain pathology or stress-induced analgesia with protective and recovering potential of FM. Stress-induced analgesia seems to activate the brain networks involved in sensory, affective, and cognitive modulatory circuits.<sup>39</sup>

This study had a few limitations. Our study examined only a small number of patients with FM. Thus, future studies with larger sample sizes will be required to generalize these findings to such patients.

Altogether, the increase in NAA/tCr, the decrease in mI/Cr, and the increase in tCho/tCr in the ACC and insula may be associated with pathological mechanisms or life system potential for protective and recovering strategies in chronic pain in patients with FM. Moreover, it may be related to stress-induced analgesia, which may contribute to the endogenous protective modulation to regulate stress and pain processing in patients with FM. These findings can suggest extensive and inclusive pathology including structural abnormality, which may affect pathological pain processing and abnormal cognitive and emotional functions. Additionally, these abnormal metabolites in the brain may be associated with protective and recovering strategies as life system potential in chronic pain in patients with FM.

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### Supplemental material

Supplemental material for this article is available online.

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