


Aberrant interactions of peripheral measures and neurometabolites with lipids in complex regional pain syndrome using magnetic resonance spectroscopy: A pilot study

Molecular Pain
Volume 14: 1–9
© The Author(s) 2018
Reprints and permissions:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1744806917751323
journals.sagepub.com/home/mpx


Ye-Ha Jung¹, Hyeonjin Kim², So Yeon Jeon¹, Jeong Min Kwon³,
Dasom Lee¹, Soo-Hee Choi¹ and Do-Hyung Kang¹

Abstract

Background: The aim of this study was to assess peripheral measures and central metabolites associated with lipids using magnetic resonance spectroscopy.

Results: Twelve patients with complex regional pain syndrome (CRPS) and 11 healthy controls participated. Using magnetic resonance spectroscopy, we measured the levels of lipid 13a (Lip13a) and lipid 09 (Lip09) relative to total creatine (tCr) levels in the right and left thalamus. We found negative correlations of Lip13a/tCr in the right thalamus with red blood cells or neutrophils, but a positive correlation between Lip13a/tCr and lymphocytes in the controls. We found negative correlations between Lip09/tCr and peripheral pH or platelets in the controls. There were positive correlations between Lip09a/tCr and myo-inositol/tCr, between Lip13a/tCr and N-acetylaspartate (NAA)/tCr, and between Lip09/tCr and NAA/tCr in healthy controls. On the other hand, there were positive correlations between Lip13a/tCr and Lip09/tCr and urine pH in CRPS patients. There were significant correlations between Lip13a/tCr or Lip09/tCr and different peripheral measures depending on the side of the thalamus (right or left) in CRPS patients.

Conclusion: This is the first report indicating that abnormal interactions of Lip13a and Lip09 in the thalamus with peripheral measures and central metabolites may mediate the complex pathophysiological mechanisms underlying CRPS.

Keywords

Complex regional pain syndrome, lipid 13a, lipid 09, thalamus, pH

Date Received: 1 November 2017; accepted: 2 December 2017

Introduction

Clinical features of complex regional pain syndrome (CRPS) include neurogenic inflammation, nociceptive sensitization, vasomotor dysfunction, and maladaptive neuroplasticity,^{1,2} as well as neuroinflammation.³ In our previous study under revision, we found that neuroinflammation is associated with lipids in the thalamus and insula of CRPS using magnetic resonance spectroscopy (MRS). So, this study is to make a further study to elucidate the association of lipids with other peripheral measures and central metabolites, based on our new finding between neuroinflammation and lipids in CRPS study. Mobile lipids that are detectable *in vivo* based on

¹Department of Neuropsychiatry, Seoul National University Hospital, Seoul, Korea

²Department of Radiology, Seoul National University Hospital, Seoul, Korea

³Department of Experimental Animal Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea
Do-Hyung Kang and Soo-Hee Choi contributed equally to this work.

Corresponding Author:

Do-Hyung Kang, Department of Neuropsychiatry, Seoul National University Hospital, 101 Daehak-no, Chongno-gu, Seoul 03080, Republic of Korea.

Email: basuare@daum.net



proton magnetic resonance spectroscopy ($^1\text{H-MRS}$) arise primarily from lipid droplets following necrosis⁴ and are seen when cells are exposed to injurious extracellular events, such as hypothermia, hypoxia, or inflammation, which damages the plasma membrane.⁵ Thus, the detection of mobile lipids with $^1\text{H-MRS}$ may be associated with neuroinflammation in CRPS. It has been suggested that an increased input from peripheral nociceptors alters the central processing mechanisms.⁶ Inflammatory cytokines and other mediators, in both the central and peripheral nervous systems, contribute to the development and progression of CRPS.⁷ Thus, it is important to identify peripheral measures, such as lymphocytes, platelets, and basophils^{8,9} that may affect inflammation and the immune system, as well as central neuroinflammation in CRPS. In particular, there may be specific peripheral measures that contribute to lipid death signals, such as lipid 13a (Lip13a) and lipid 09 (Lip09), which are associated with neuroinflammation and neuronal cells in CRPS. Only mobile lipids in neutral lipid droplets have sufficient rotational freedom to generate a signal on spectroscopy; these signals are detected as peaks at 1.3 ppm, originating from the methylene groups in the fatty acid chain, and at 0.9 ppm, originating from distal methyl groups.¹⁰ We reported increased neuroinflammation throughout the basal ganglia and some cortical regions in CRPS patients in a previous study³ and found an association between lipids in the brain and neuroinflammation. Considering that the somatosensory system involved in pain perception includes the thalamus, the primary somatosensory cortex, and the insula¹¹ and that the thalamus is the region that connects peripheral regions directly, we focused on the right and left thalamic regions. Thus, the aim of this study was to use MRS to assess the peripheral measures and central metabolites that affect lipids in the thalamus in CRPS patients and controls.

Methods

Participants

A total of 15 subjects were enrolled in each group, but 12 participants with CRPS and 11 healthy control subjects completed all study procedures. Twelve patients who fulfilled the International Association for the Study of Pain criteria for CRPS I were recruited from Seoul National University Hospital (Seoul, Korea). Eleven participants (recruited by Internet advertisement) who were of comparable age and gender to the CRPS patients and had no pain or neurological symptoms were used in this study as healthy controls. All participants underwent a detailed neuropsychiatric assessment completed at baseline screening that included any history of accidents, pain symptoms, routine blood analyses,

an electrocardiogram, and urine analysis. Subjects who had high hs-CRP (high-sensitivity C-reactive protein) or leukocytosis were excluded.

The inclusion criteria for CRPS subjects were a diagnosis of CRPS type I, age between 25 and 55 years, and not taking benzodiazepines or could stop benzodiazepine medication two weeks prior to the study. All eligible patients were approached in the clinic and the project research goals were explained to them. Exclusion criteria were a major neuropsychiatric disorder before the diagnosis of CRPS, a neurological disease (cerebrovascular disease or brain tumor), a history of brain trauma, high hs-CRP or leukocytosis, and unable to undergo the MRS process.

This study was approved by the institutional review board at 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.

From routine blood analyses and urine analysis, we can get the peripheral data. White blood cell (WBC), red blood cell (RBC), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), platelet, lymphocyte, monocyte, eosinophil, plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), and basophil were measured using EDTA whole blood. Calcium, phosphorus, glucose, uric acid, cholesterol, serum total protein, albumin, alkaline phosphatase, creatinine, Na, K, Cl, total CO_2 , and hs-CRP were measured from blood serum which does not contain WBCs or RBCs. And pH was measured using urine analysis.

$^1\text{H-MRS}$ data acquisition and processing

All 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 $^1\text{H-MRS}$ 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 = $90^\circ/130^\circ$, field of view = $220 \times 199 \text{ mm}^2$ (axi and cor) and $220 \times 220 \text{ mm}^2$ (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, two volumes of interest (VOIs) were selected in the right and left thalamus ($2 \times 2 \times 1.5 \text{ cm}^3$) for each subject. The VOI in the right and left thalamus was placed along the axis of the thalamus to cover most of the volume. Following autoshimming over the VOI, $^1\text{H-MRS}$ data were acquired using a point-resolved spectroscopy pulse sequence (PRESS (Bottomley)) 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 ^1H -MRS data were analyzed using LCModel (Provencher) software (ver. 6.3-1J) in the range 4.2 to 1.0 ppm. The metabolite content was normalized to that of total creatine (tCr) including creatine (Cr) and phosphocreatine (PCr). The final data analysis included only those metabolites with a Cramer-Rao lower bound (CRLB) < 30%.

Statistical analysis

The statistical analyses were performed using SPSS Statistics 21.0 (SPSS, Chicago, IL). Pearson's correlation analyses were conducted to evaluate the associations between the variables. P -values < 0.05 were considered to indicate statistical significance.

Results

Demographic characteristic of participants

Age, gender, and education level were not significantly different between the groups. The average age of the

12 CRPS patients (nine males and three females) was 41.4 ± 8.6 (range: 30 to 55) years, the average education level was 14.9 ± 2.3 years, and the average duration of illness was 8.5 ± 5.3 years. The average age of the 11 controls (nine males and two females) was 40.2 ± 6.8 (range: 27 to 48) years and the average education level was 16.1 ± 2.3 years.

Correlations between Lip13a or Lip09 in the right and left thalamus and peripheral measures in healthy controls

We found negative correlations between Lip13a/tCr in the right thalamus and RBC of controls ($r = -0.904$, $P = 0.035$; Figure 1(a)). There were negative correlations between Lip13a/tCr in the right thalamus and segmented neutrophils in of controls ($r = -0.886$, $P = 0.046$; Figure 1(b)). In addition, there were positive correlations between Lip13a/tCr and lymphocytes of controls ($r = 0.913$, $P = 0.030$; Figure 1(c)). We found negative correlations between Lip09/tCr in the left thalamus and peripheral pH on urinalysis or platelets of controls ($r = -0.686$, $P = 0.020$ and $r = -0.639$, $P = 0.034$, respectively; Figure 2(a) and (b)). Otherwise, there were no significant correlations between lipid metabolites with peripheral measures.

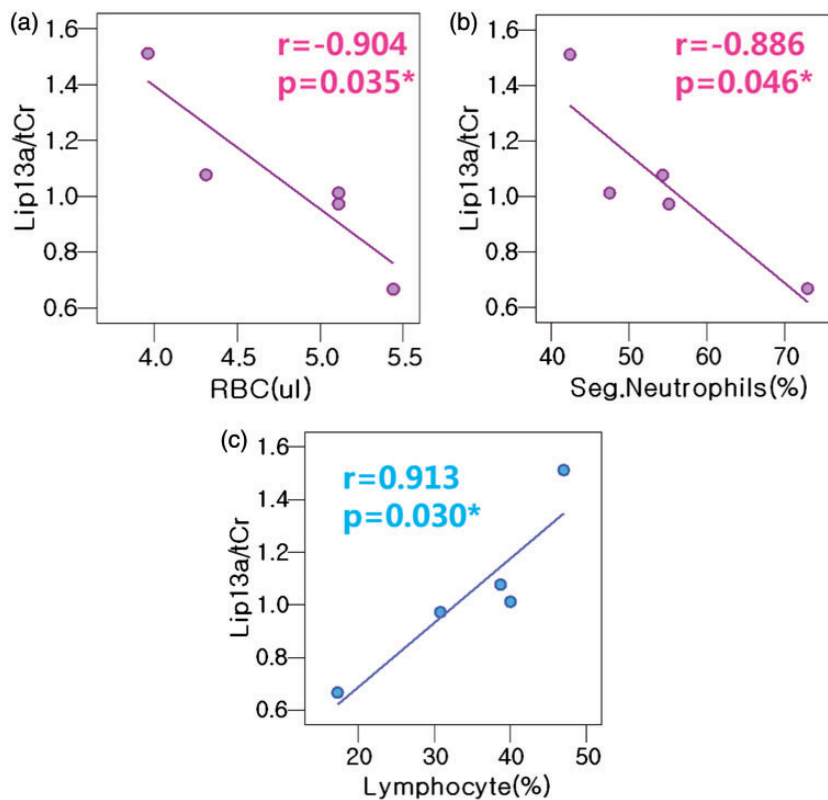


Figure 1. Correlations between peripheral measures and brain Lip13a/tCr in the right thalamus of controls. * $P < 0.05$. Lip13a: lipid 13a; tCr: total creatine; RBC: red blood cell; Seg. Neutrophils; segmented neutrophils.

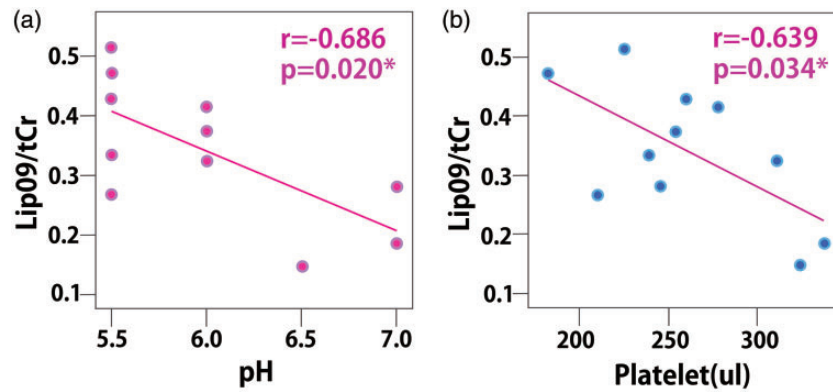


Figure 2. Correlations between peripheral measures and brain Lip09/tCr in the left thalamus of controls. * $P < 0.05$. Lip09: lipid 09; tCr: total creatine.

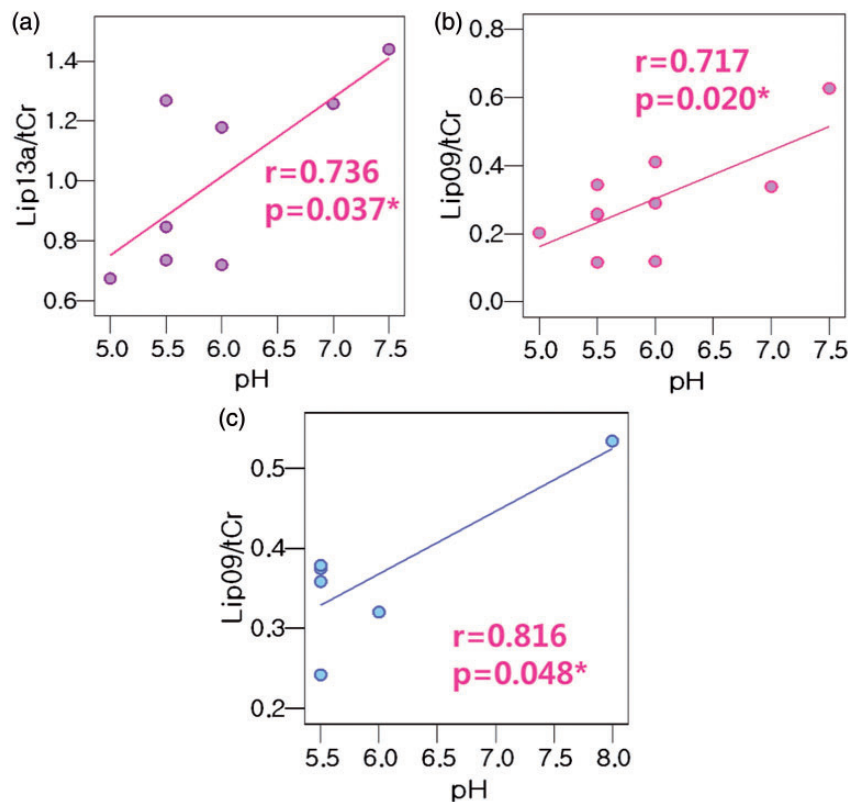


Figure 3. Correlations between peripheral pH and brain Lipids in the right and left thalamus in CRPS patients. (a-b) Left thalamus. (c) Right thalamus. * $P < 0.05$. Lip13a: lipid 13a; Lip09: lipid 09; tCr: total creatine.

Correlations between Lip13a or Lip09 in the right and left thalamus and peripheral measures in CRPS patients

There were positive correlations between Lip13a/tCr in the left thalamus and urine pH in CRPS patients ($r = 0.736$, $P = 0.037$; Figure 3(a)). There were positive correlations between Lip09/tCr in the left thalamus and urine pH in CRPS patients ($r = 0.717$, $P = 0.020$; Figure 3(b)). There were positive correlations between

Lip09/tCr in the right thalamus and urine pH in CRPS patients ($r = 0.816$, $P = 0.048$; Figure 3(c)). In addition, there were negative correlations between Lip13a/tCr in the right thalamus and WBC, total CO_2 , and basophils in peripheral blood in CRPS patients (Table 1). There were negative correlations between Lip09/tCr in the right thalamus and basophil, uric acid, total protein, and albumin in peripheral blood in CRPS patients (Table 1). There were positive correlations between Lip13a/tCr in the left thalamus and calcium, MCH,

Table 1. Correlations between peripheral measures and brain lipid metabolites in right and left thalamus in CRPS patients.

Metabolites	Right thalamus (CRPS)						Left thalamus (CRPS)					
	Lip13a			Lip09			Lip13a			Lip09		
	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>p</i>	<i>N</i>
WBC	-0.921	0.026*	5									
Total CO ₂	-0.882	0.048*	5									
Basophil	-0.970	0.030*	4	-0.913	0.030*	5						
Uric Acid				-0.877	0.022*	6						
Total Protein				-0.884	0.019*	6						
Albumin				-0.916	0.010*	6						
Calcium							0.852	0.007**	8	0.665	0.036	10
Na							-0.712	0.047*	8			
MCH							0.772	0.025*	8			
PCT							-0.845	0.008**	8			
MPV							-0.904	0.002**	8	-0.772	0.009**	10
PDW							-0.952	0.000***	8	-0.849	0.002**	10
Seg.Neut							0.869	0.005**	8			
Lymphocyte							-0.892	0.003**	8			

WBC: white blood cell, MCH: mean corpuscular hemoglobin, PCT: platelet count, MPV: mean platelet volume, PDW: platelet distribution width, Seg.Neut: segment of neutrophil. $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$; Bold: positive correlations.

and neutrophils in peripheral blood in CRPS patients (Table 1). There were negative correlations between Lip13a/tCr in the left thalamus with Na, PCT, MPV, PDW, and lymphocytes in peripheral blood in CRPS patients (Table 1). There were positive correlations between Lip09/tCr in the left thalamus with calcium in peripheral blood in CRPS patients (Table 1). There were negative correlations between Lip09/tCr in the left thalamus with MPV and PDW in peripheral blood in CRPS patients (Table 1).

Correlations between Lip13a or Lip09 in the right and left thalamus and neurometabolites

There were positive correlations between Lip09a/tCr and myo-inositol (mI)/tCr in the right thalamus of healthy controls ($r = 0.838$, $P = 0.037$; Figure 4(a)). There were positive correlations between Lip13a/tCr and N-acetylaspartate (NAA)/tCr in the left thalamus of healthy controls ($r = 0.927$, $P < 0.001$; Figure 4(b)). There were positive correlations between Lip09/tCr and NAA/tCr in the left thalamus of healthy controls ($r = 0.723$, $P = 0.028$; Figure 4(c)). However, these correlations were not found in CRPS patients.

Discussion

This is the first report indicating that abnormal interactions of Lip13a and Lip09 in the thalamus with peripheral measures and central metabolites may mediate the complex pathophysiological mechanisms underlying CRPS, including involvement of the central nervous

system and peripheral nervous system. Furthermore, Lip13a and Lip09 levels in the thalamus may be differently associated with peripheral measures, depending on the side of the thalamus involved (right or left), in healthy controls, and CRPS patients. Although Lip13a and Lip09 are identical lipids and the increase in necrosis-dependent lipid signals⁴ may be associated with CRPS, the differences between Lip13a and Lip09 in terms of the methylene and methyl groups¹⁰ may differently be associated with neuroinflammation in the right and left thalamus in CRPS patients. Thus, the different associations between Lip13a and Lip09 with complex pathological mechanisms in CRPS need to be confirmed according to their activities in the right and left thalamus and their interactions with peripheral measures. The main spectroscopy peaks at 1.3 ppm originate from the methylene groups in the fatty acid chain, while those at 0.9 ppm originate from distal methyl groups.¹⁰ We reported elevated neuroinflammation throughout the basal ganglia and some cortical regions in CRPS patients in a previous study³ and found an association between lipids in brains and neuroinflammation. Mobile lipids visible on MRS can be seen in various acquired disorders, such as areas of necrosis within brain tumors,¹² and demyelinating diseases, such as multiple sclerosis.¹³ Functional differentiation of the Lip13a-dependent right thalamus and Lip09-dependent left thalamus may represent the first step toward understanding abnormal interactions between lipids in the central thalamus and peripheral measures in CRPS patients.

Platelets play a role in hemostasis, innate immunity, and inflammation,¹⁴ and neutrophils comprise an

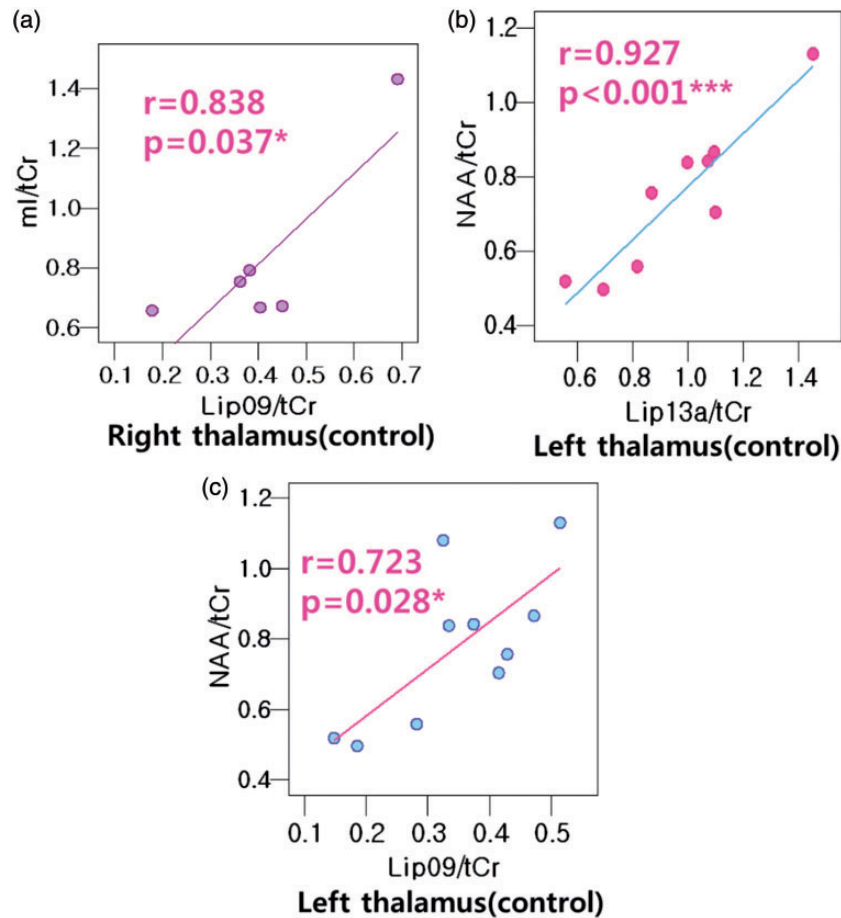


Figure 4. Correlations between NAA/tCr or ml/tCr and Lip13a/tCr or Lip09/tCr in the right and left thalamus of controls. ml: myo-inositol, NAA: N-acetylaspartate, Lip13a: lipid 13a, Lip09: lipid 09, tCr: total creatine.

essential part of the innate immune system. We discovered beneficial relationships of RBC and neutrophils in terms of low Lip13a signals, depending on the right thalamus-dependent interactions between central lipids and peripheral measures in healthy controls. In addition, we can determine the normal beneficial mechanisms of higher pH and platelets with respect to low Lip09 signaling, depending on the left thalamus-dependent interactions between central lipids and peripheral measures in healthy controls. For healthy controls, normal beneficial mechanisms against lipid signal-associated neuronal cell death were detected, depending on the right and left thalamus-dependent mechanisms and functional interactions. However, CRPS patients did not show these normal beneficial interactions between lipids in the thalamus and peripheral measures, but rather showed abnormal interactions between lipids in the thalamus and peripheral measures. While a higher pH was associated lower Lip09 signaling in the left thalamus in healthy controls, a higher pH was associated with higher lipid signaling in the CRPS patients. Thus, as pH seems to differently be associated with lipid signaling in healthy

controls and CRPS patients, the mechanisms underlying such signaling in these two groups need to be investigated. Healthy controls seem to maintain pH homeostasis and the regulation of acid-base balance in brain which shows a stability of cerebral pH,¹⁵ decreasing Lip09 signals in left thalamus. Intracellular pH regulation in the brain is important in both physiological and physiopathological conditions because pH changes result in altered neuronal excitability¹⁶ and may have a key role in the regulation of glial-neuronal metabolism of Gln/Glu metabolism.¹⁷ Maintaining physiological pH is required for survival, and exposure to alkaline chemicals such as ammonia elicits severe pain and inflammation through unknown mechanisms.¹⁸ Thus, the elevated pH seems to be associated with the increase of neuroinflammation, affecting higher lipid signals in thalamus of CRPS patients. Considering that oxygen resulted in pain relief and a reduction in blood alkalosis in headache pain,¹⁹ the increase of RBC, principal means of delivering oxygen, seems to affect lower Lip13a signals in the right thalamus of healthy controls. However, as CRPS patients did not show this association between RBC and

lipid signals, imbalance and dysfunctional interactions between oxygen and CO₂ may be associated with neuroinflammation and lipid signals in CRPS.

For healthy controls, the increased lipid signaling in the thalamus was associated with higher levels of NAA and mI in the thalamus. These positive correlations between lipid signaling and NAA and mI in the thalamus seem to be associated with repair and recovery mechanisms. In other words, as lipid signals indicative of neuronal cell death⁴ were associated with increases in NAA as a neuronal marker²⁰ and mI as a marker of glial activation²¹ in the thalamus, neuronal cell death in healthy controls may promote new cell proliferation, neurogenesis, and recovery, thus maintaining functional homeostasis in the brain. Higher levels of NAA accompanied by higher lipid signaling seem to be associated with an increase in neurogenesis, which protects against lipid-related neuronal cell death in healthy controls. However, given that the brains of adult rats exposed to traumatic brain injury showed long-term upregulation of inflammation and suppression of cell proliferation,²² trauma-mediated neuroinflammation in CRPS may suppress normal neurogenesis and promote pathophysiological symptoms. As a result, normal and healthy interactions between peripheral measures and central metabolites, which act against lipid-related neuronal cell death and improve functional homeostasis in the brain, seem to be impaired in CRPS patients, resulting in severe chronic pain and complex pathophysiological symptoms. NAA is present predominantly in neuronal cell bodies, where it acts as a neuronal marker,²³ and levels of NAA were lower in the dorsolateral prefrontal cortex of CRPS 1 patients in MRS study.²⁴ Although elevated mI may mediate neuropathic pain as a marker of glial activation,^{25,26} neuroglia may show normal beneficial functions that maintain homeostasis, form myelin, and provide support and protection for neurons in healthy brain.²⁷ Thus, the associations of higher levels of mI with higher lipid signaling are indicative of an increase in glial cell production, as a beneficial mechanism against lipid-related neuroinflammation, evidenced by the production of neurogenesis-related glial cells in healthy controls. However, given that CRPS patients did not show any relationship between NAA or mI levels and lipid signaling, they may lose any benefits of these mechanisms due to chronic pain-related pathology. Thus, impairments of repair systems from neuron to glia which may be associated with neurogenesis need to be investigated in CRPS patients in future studies.

Considering that higher levels of WBCs, CO₂, basophils, uric acid, total protein, and albumin in peripheral blood decrease lipid signaling in the right thalamus in CRPS patients, the beneficial actions of these peripheral measures should be investigated in relation to lipid and neuronal cell death, depending on the right thalamus-

dominance mechanisms of CRPS. Higher levels of Na, PCT, MPV, PDW, and lymphocytes in peripheral blood showed an association with lower lipid signaling in the left thalamus of the CRPS patients. However, higher levels of calcium, MCH, and neutrophils were associated with higher lipid signaling in the left thalamus of the CRPS patients. Thus, the increased or decreased levels of peripheral measures are associated with lipid signaling in the left thalamus of CRPS patients. In addition, different peripheral measures were associated with right versus left thalamus lipid signaling in the CRPS patients. Thus, the mechanisms underlying such signaling should be investigated according to the right or left thalamus, presence of Lip13a or Lip09 signaling, and different types of peripheral measures in CRPS patients.

This is a pilot study with a small sample size and a preliminary study to be progressed in the following research. Multiple correlations can be accounted for with Bonferroni and other corrections, or by the approach of controlling the false discover rate, but these approaches are not always needed, given that reducing the type I error for null associations increases the type II error for those associations that are not null.^{28,29} So, we used a rather liberal threshold for significance in correlation analysis. Some people may insist that values with a CRLB < 20% were viewed as being reliable, but metabolites were considered reliably quantified if the CRLB were < 50%.³⁰ Also, more liberal and challengeable approach can lead new important findings and make a new way to go breakthrough, given that considering as being reliable values with a CRLB < 30% can reduce loss of sample numbers which may happen when a CRLB < 20% viewed as being reliable. Thus, values with a CRLB < 30% in our study can be viewed as being important to lead progress in human neuroscience and more active and dynamic researches related to MRS.

In conclusion, abnormal interactions between Lip13a and Lip09 in the thalamus and peripheral measures and central metabolites may mediate the complex pathophysiological mechanisms underlying CRPS, including involvement of the central nervous system and peripheral nervous system. Lip13a-dependent right thalamus and Lip09-dependent left thalamus may be important to elucidate abnormal interactions between lipids in the central thalamus and peripheral measures in CRPS patients, suggestive of unique mechanisms underlying lipid-associated pathophysiology in CRPS, which may be used to develop personalized therapies according to the dominant side of the thalamus (right vs. left) and the presence and interactions of specific metabolites. In addition, impairments of repair systems from neuron to glia associated with NAA and mI may contribute to chronic pathological symptoms in CRPS patients.

Author contributions

Jung YH contributed to the statistical analysis and interpretation of data, writing manuscript for content including introduction, results and discussion, and acquisition of data. **Kim HJ** contributed to the MRS data processing and writing manuscript for the content of MRS data processing. **Jeon SY** contributed to the study concept and design, writing manuscript for methods, and acquisition of data. **Kwon JM** contributed to the MRS data processing. **DS Lee** contributed to the acquisition of data and discussion. **SH Choi** contributed to the correcting of manuscript, comments for discussion, responsibility for conduct of research, and obtaining funding. **Kang DH** contributed to the study supervision, responsibility for conduct of research and final approval, and obtaining funding.

Acknowledgments

The authors wish to thank all participants for their valuable time engaging with this research. Also, the authors would like to thank Prof. Hyeonjin Kim for MRS data processing. The English in this article has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: <http://www.textcheck.com/certificate/LR4oIQ>

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Grant No. R-20150902-002176 from the IT R&D program of MSIP (Ministry of Science, Information and Communication Technology and Future Planning).

References

- Bussa M, Mascaro A, Cuffaro L, et al. Adult complex regional pain syndrome type I: a narrative review. *PM R* 2017; 9: 707–719.
- Marinus J, Moseley GL, Birklein F, et al. Clinical features and pathophysiology of complex regional pain syndrome. *Lancet Neurol* 2011; 10: 637–648.
- Jeon SY, Seo S, Lee JS, et al. [11C]-(R)-PK11195 positron emission tomography in patients with complex regional pain syndrome: a pilot study. *Medicine (Baltimore)* 2017; 96: e5735.
- Zoula S, Hérigault G, Ziegler A, et al. Correlation between the occurrence of 1H-MRS lipid signal, necrosis and lipid droplets during C6 rat glioma development. *NMR Biomed* 2003; 16: 199–212.
- Majno G and Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* 1995; 146: 3–15.
- Bussa M, Guttilla D, Lucia M, et al. Complex regional pain syndrome type I: a comprehensive review. *Acta Anaesthesiol Scand* 2015; 59: 685–697.
- Hauser J, Hsu B and Nader ND. Inflammatory processes in complex regional pain syndromes. *Immunol Invest* 2013; 42: 263–272.
- Weyrich AS and Zimmerman GA. Platelets: signaling cells in the immune continuum. *Trends Immunol* 2004; 25: 489–495.
- Schroeder JT. Basophils beyond effector cells of allergic inflammation. *Adv Immunol* 2009; 101: 123–161.
- Ferreira CR, Silber MH, Chang T, et al. Cerebral lipid accumulation detected by mrs in a child with carnitine palmitoyltransferase 2 deficiency: a case report and review of the literature on genetic etiologies of lipid peaks on MRS. *JIMD Rep* 2016; 28: 69–74.
- Apkarian AV, Bushnell MC, Treede R-D, et al. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain* 2005; 9: 463–484.
- Kimura T, Sako K, Tanaka K, et al. Evaluation of the response of metastatic brain tumors to stereotactic radiosurgery by proton magnetic resonance spectroscopy, 201TlCl single-photon emission computerized tomography, and gadolinium-enhanced magnetic resonance imaging. *J Neurosurg* 2004; 100: 835–841.
- Wolinsky JS, Narayana PA and Fenstermacher MJ. Proton magnetic resonance spectroscopy in multiple sclerosis. *Neurology* 1990; 40: 1764–1769.
- Garraud O and Cognasse F. Platelet Toll-like receptor expression: the link between “danger” ligands and inflammation. *Inflamm Iadt* 2010; 9: 322–333.
- Rabary O, Bousofara M and Grimaud D. Acid-base equilibrium and the brain. *Ann Fr Anesth Reanim* 1994; 13: 111–122.
- Ruffin VA, Salameh AI, Boron WF, et al. Intracellular pH regulation by acid-base transporters in mammalian neurons. *Front Physiol* 2014; 5: 43.
- Nissim I. Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am J Physiol* 1999; 277: F493–F497.
- Dhaka A, Uzzell V, Dubin AE, et al. TRPV1 is activated by both acidic and basic pH. *J Neurosci* 2009; 29: 153–158.
- Benedetti F and Dogue S. Different placebos, different mechanisms, different outcomes: lessons for clinical trials. *PLoS One* 2015; 10: e0140967.
- Soher BJ, Wu WE, Tal A, et al. Automated whole-brain N-acetylaspartate proton MRS quantification. *NMR Biomed* 2014; 27: 1275–1284.
- Plitman E, de la Fuente-Sandoval C, Reyes-Madrigal F, et al. Elevated myo-inositol, choline, and glutamate levels in the associative striatum of antipsychotic-naive patients with first-episode psychosis: a proton magnetic resonance spectroscopy study with implications for glial dysfunction. *Schizophr Bull* 2016; 42: 415–424.
- Acosta SA, Tajiri N, Shinozuka K, et al. Long-term upregulation of inflammation and suppression of cell proliferation in the brain of adult rats exposed to traumatic brain injury using the controlled cortical impact model. *PLoS One* 2013; 8: e53376.
- Chatham JC and Blackband SJ. Nuclear magnetic resonance spectroscopy and imaging in animal research. *Ilar J* 2001; 42: 189–208.

24. Grachev ID, Thomas PS and Ramachandran TS. Decreased levels of N-acetylaspartate in dorsolateral prefrontal cortex in a case of intractable severe sympathetically mediated chronic pain (complex regional pain syndrome, type I). *Brain Cogn* 2002; 49: 102–113.
25. Pattany PM, Yeziarski RP, Widerström-Noga EG, et al. Proton magnetic resonance spectroscopy of the thalamus in patients with chronic neuropathic pain after spinal cord injury. *AJNR Am J Neuroradiol* 2002; 23: 901–905.
26. Chang L, Munsaka SM, Kraft-Terry S, et al. Magnetic resonance spectroscopy to assess neuroinflammation and neuropathic pain. *J Neuroimmune Pharmacol* 2013; 8: 576–593.
27. Jessen KR and Mirsky R. Glial cells in the enteric nervous system contain glial fibrillary acidic protein. *Nature* 1980; 286: 736–737.
28. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990; 1: 43–46.
29. Saville DJ. Multiple comparison procedures: the practical solution. *Am Stat* 1990; 44: 174–180.
30. Bednařík P, Moheet A, Deelchand DK, et al. Feasibility and reproducibility of neurochemical profile quantification in the human hippocampus at 3 T. *NMR Biomed* 2015; 28: 685–693.