Review

Lysophosphatidic acids in cerebrospinal fluids: Potential biomarkers and therapeutic targets for neuropathic pain

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Abstract Recent advances in lipidomics have allowed for the measurement of precise levels of biologically active lipids in clinical human samples, which can aid us in understanding the involvement of bioactive lipids in the pathogenesis of human diseases. Among the various human diseases, a variety of membrane lipid-derived mediators have been demonstrated to possess important roles in the initiation, maintenance, and modulation of neuropathic pain (NP). Lysophosphatidic acid (LPA) has been identified as being the main initiator of NP based on experimental animal models, as well as clinical studies using a lipidomics approach. Currently, there is no specific medical treatment and no objective laboratory testing for NP. Based on these conditions, we have described the possible implementation of inhibitors of autotaxin, a producing enzyme for LPA, and LPA measurements in the cerebrospinal fluid (CSF) as a therapeutic target and a potential diagnostic marker.

Key words: NP: neuropathic pain, CSF: cerebrospinal fluid, LPC: lysophosphatidylcholine, LPA: lysophosphatidic acid, ATX: autotaxin

Introduction

Many elegant basic studies have identified the important physiological roles of functional lipids, such as eicosanoids, lysophospholipids (LPLs), and ceramides[1−8]. To introduce the findings of these basic studies into clinical medicine, it is very important to investigate the modulation of these lipid mediators in human samples. In addition, the measurement of lipid mediators can sometimes help researchers in understanding their unexpected roles in the pathogenesis of human diseases.

In these translational research and reverse translational research studies, the lipidomics approach via the use of mass spectrometry functions well. Mass spectrometry-based lipidomics approaches can enable researchers to detect and quantify many types of lipids; in the past decade, research studies in lipidomics have tremendously increased due to improved instrumentation, such as the shift from low resolution to high mass resolution procedures. In this study, we used reversed-phase liquid chromatography−mass spectrometry, which separates lipid species from the same lipid by their cumulative carbon number-double bond index or the composition of their fatty acyl chains[9]. Additionally,

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we used imaging lipidomics, among other high mass resolution instrumental approaches, such as shotgun lipidomics and matrix-assisted laser desorption ionization-time of light.

When we previously investigated the modulation of LPLs in plasma samples taken from subjects with acute coronary syndrome, we first observed the possible significance of lysophosphatidic acid (LPA) with a w3 acyl chain and other LPLs, such as lysophosphatidylserine (LPS) and lysophosphatidylinositol (LPI), in the pathogenesis of acute coronary syndrome[10,11]. This observation prompted us to investigate their roles in pathological conditions, such as inflammation and platelet activation, which are involved in the pathogenesis of acute coronary syndrome[12–15].

In this review, we explain the roles of LPA in the pathogenesis of neuropathic pain (NP), which have been proposed from basic studies and clinical research that has primarily been conducted by our team via a lipidomics approach; additionally, we discuss possible future medical applications.

1. LPA-LPC-ATX Axis

Lysophosphatidic acid (LPA) is one of the biologically active lipid mediators that acts through its defined G protein-coupled receptors LPA_{1-6} and is mainly produced by autotaxin (ATX) from lysophosphatidylcholine (LPC) extracellularly, and from phosphatidic acid (PA) via diacylation of phospholipase A_2 (PLA_2) intracellularly[5]. LPA is involved in many cellular and biological processes, including cell proliferation, migration, anti-apoptotic actions, the enhancement of secretion of cytokines and chemokines, the induction of arteriosclerosis[16], cancer cell invasion[17], and neuropathic pain (NP)[18].

In the last two decades, many studies have been performed by different groups on the physiological and pathological roles of LPA and its related factors, including LPC, ATX, and LPA receptors. Although the receptors have not been identified, LPC is also a biologically active lipid known to act as a pro-inflammatory[19], oxidative stress, and apoptosis inducing factor[20], in addition to being a substrate of ATX for the production of LPA. LPC is produced from phosphatidylcholine (PC) which is the major component of the cell membrane, via the cleavage of PLA_2. Moreover, ATX is one of the well-studied enzymes associated with LPA signaling and exhibits widespread tissue expression with high levels in the brain, which results in higher levels of it in the cerebrospinal fluid (CSF) than in the blood[21]. ATX activity in human melanoma cells was initially reported to be a cell motility stimulating factor[22]. Currently, it is now clear that the activity of ATX is a result of LPA signaling. Therefore, the quantitative determination of ATX in serum samples is used as a surrogate marker of LPA due to the stringent conditions required for the precise measurement of LPA in plasma samples[23]. Serum ATX has been established as a novel and reliable marker of liver fibrosis[24,25]. In addition, almost all of the actions of LPA are mediated by its receptors (LPA_{1-6}), and all of the LPA receptors are expressed in the nervous system and involved in neurodevelopmental processes[26], as well as in the initiation of NP.

2. LPA in Neuropathic Pain

NP is a chronic pain with refractive characteristics, and it occurs due to damage to the somatosensory neuronal axes. The clinical phenotypes of NP are quite different from those of acute pain, which is effectively treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids in a consistent manner, even though the initial cause of the pain has been resolved. NP exhibits abnormal pain symptoms, such as hyperalgesia and allodynia, and it is divided into two broad categories known as peripheral and central NP, depending on the location of the damage. Additionally, it occurs as a secondary symptom in lumbar spinal canal stenosis (LSS), diabetes, herpes zoster infections, the effects of chemotherapy, and after a stroke[27].

Multiple mechanisms, as well as the key molecules in these mechanisms, including cytokines (Il-6, TNFa), chemokines, brain-derived neurotrophic factor (BDNF), neurotransmitters (GABA and glutamate), neurotrophins (nerve growth factor), VGF-derived neuropeptides, ATP, lipid mediators (prostanoids and lysophospholipids), and their receptors, have been demonstrated to be involved in the manifestation of NP symptoms[28–30].

A variety of biologically active lipids are produced during NP, wherein some of the lipids are induced, whereas others prevent its progression. Among these proposed molecules, LPA is promising for NP initiation and maintenance. In 2004, Ueda et al. first demonstrated that the LPC-LPA-LPA1 axis is involved in the pathogenesis of pain via basic experiments. A source of LPA can be explained by its local production or conversion from LPC extracellularly via the action of ATX. LPC itself was also reported to be directly involved in NP, mainly explained by its conversion to

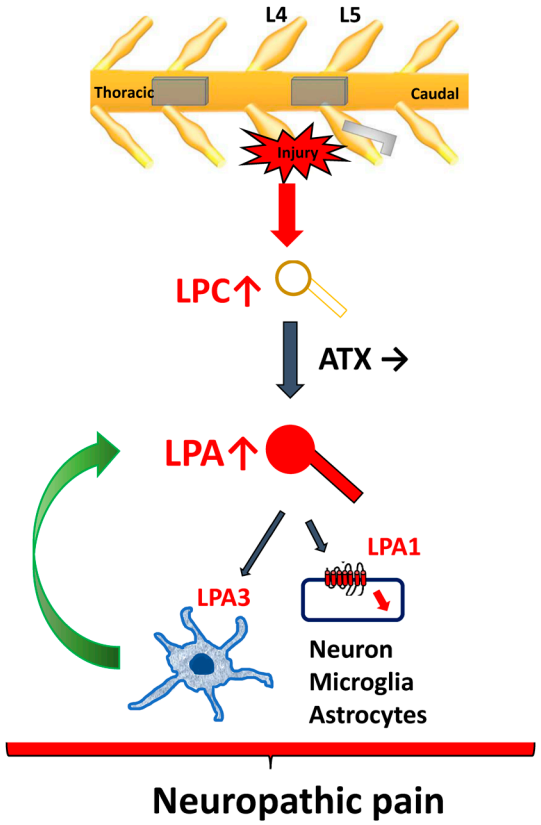

Fig. 1. Hypothetical scheme of the involvement of lysophosphatidic acid and its receptors 1 and 3 in the mechanism of neuropathic pain.
In two different NP models, under the compression of the spinal cord or DRG, LPC, which is a substrate of ATX, increases in the CSF. The level and activity of the ATX enzyme that produces LPA from LPC are not changed. LPA levels were also increased in CSF and acted on neurons, microglia, and astrocytes through its receptors LPA_1 and LPA_3, which play important roles in the feed-forward system underlying NP.

LPA[18]. The direct plantar administration of LPA initiated nociceptive responses that were abolished by a genetic deficiency of LPA_1 or pretreatment with the LPA_1 expression inhibitor Rho-kinase[31], and the intrathecal application of LPA resulted in NP behaviors in animal experiments. Many studies have suggested that there are direct and indirect involvements of LPA and LPA_1 in demyelination, allodynia, demyelination-associated sprouting, and abnormal pain synapses by using NP model animals and procedures including peripheral sciatic nerve ligation (pSNL) and ex vivo preparations[32–36]. The stimulation of LPA_1 was shown to lead to the upregulation of $Ca_v\alpha 2\delta 1$, which enhances pain transmission and becomes a reason for hyperalgesia[37]. When regarding other LPA receptors, LPA_3 was reported to amplify LPA production through the activation and accumulation of microglia [38,39].

Concordant with previous reports[31–36], we have also demonstrated increased levels of LPA and LPC in both the spinal cord or dorsal root ganglion (DRG) tissue samples and in the CSF, wherein we used lipidomics approaches in two different animal models for NP. One model involved a preclinical model of the narrowing of the spinal canal or cauda equina compression (CEC) without the direct injury of the nerve, while still resulting in NP with motor dysfunction[40]. The other is LSS model or mechanical compression of the dorsal root ganglion (CD) with a decreased threshold of pain[41]. In both studies, LPC and LPA measurements were performed in the plasma, CSF (Fig. 1), and spinal cord or DRG tissue samples, in conjunction with the characterization of the behavior. The chromatograms of various LPC and LPA species in CSF shown in Fig. 2. In the CEC model study, we confirmed the involvement of LPA in NP initiation and maintenance by measuring LPC, LPA, and the expression levels of LPA receptors in a time-course manner, and the increased levels of LPC species were also confirmed via the MALDI-MSI analysis[40]. The levels of LPA and LPC that were measured in the CSF of patients with NP demonstrated associations with the degree of pain[42] and the severity of symptoms[43]. All of these animal models and clinical studies demonstrated crucial roles of the LPA-LPC-ATX axis in NP, among other factors involved in the pain mechanism (Fig. 1).

3. CSF Is an Ideal Sample to Measure LPA

To introduce the physiological roles of LPA that have been demonstrated by basic studies into clinical settings, we and other researchers have measured LPA levels by using liquid chromatography-tandem mass spectrometry (LC–MS/MS) in the blood[44], CSF[45], synovial fluid[46], ascites[47], and aqueous humor[48]. Blood samples contain high amounts of both substrate (LPC) and enzyme (ATX), thus resulting in the synthesis of LPA even after sample collection, which requires stringent conditions by suppressing the activity of platelets or by inhibiting ATX[49]. In healthy people, CSF has larger quantities of ATX than in blood samples, whereas CSF has almost no measurable levels of LPC[45]. Considering the low endogenous concentration of the lipids around 500 times lower than in serum[50] and less environmental influences by blood cells on lipid concentrations[51, 52], we believe that CSF is one of the most suitable specimens among above mentioned biological fluids,

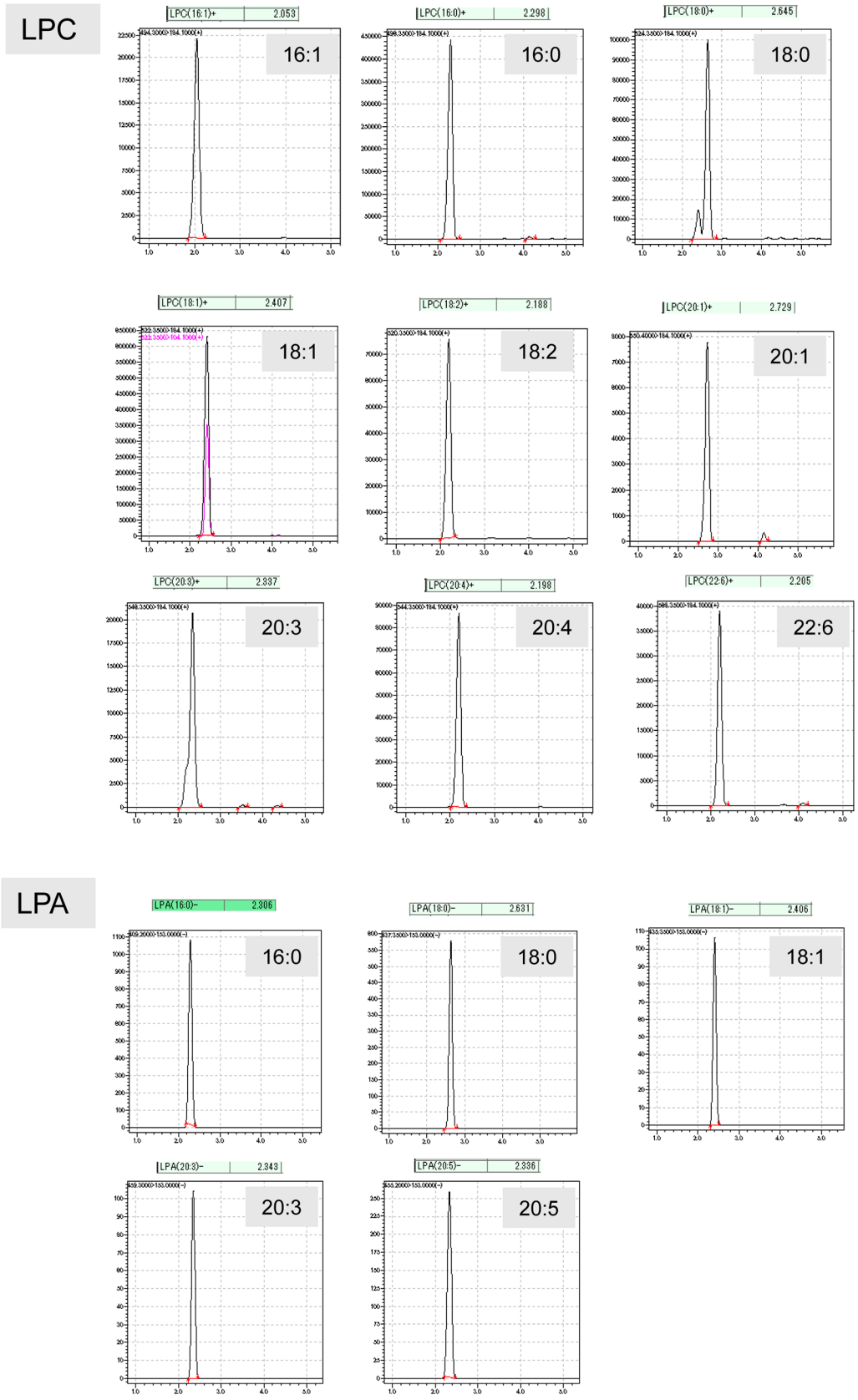

Fig. 2. Chromatograms for LPC and LPA species in CSF samples.

The representative chromatograms of various molecular species of the LPC and LPA in CSF samples are shown.

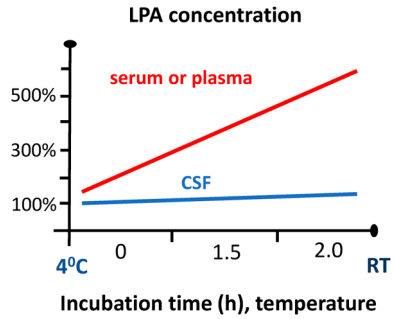

Fig. 3. Influence of incubation time and temperature on the concentration of the LPA in the serum or CSF samples.
Incubation time and temperature-dependent changes were subtle in the CSF. LPA levels were not altered in comparison to the plasma/serum LPA levels.

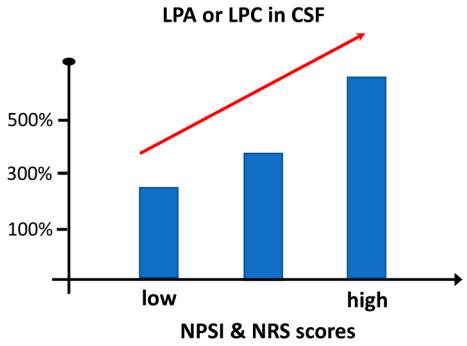

Fig. 4. LPC and LPA levels were higher in the patients' CSF with severe NPSI group.
LPC and LPA species were higher in severe than mild NP patient CSF samples as classified by the Japanese version of the neuropathic pain symptom inventory (NPSI) and Zurich Claudication Questionnaire (ZCQ) scores.

including blood samples, for the evaluation of lysophospholipids (LPLs), such as LPA. We evaluated the matrix effects together with validation of the within-run and between-day precisions by using CSF with low or high LPLs levels and a standard mixture[45]. In CSF samples, we observed much smaller changes in LPL levels dependent on incubation time at room temperature than in serum samples, as shown in Fig. 3, which suggested usefulness of the LPLs measurements in CSF in clinical laboratory settings. Invasiveness in sampling CSF, compared with other samples, was the only disadvantage of using LC-MS/MS, however the merit should overcome this invasiveness, in the situation where it is difficult to judge the suitability for operation.

4. LPA in CSF in Human Neuropathic Pain

There have been two clinical studies conducted by our group wherein we used CSF obtained from NP patients with different etiologies for the measurements of the LPC and LPA to investigate their association with NP[42,43]. Based on accurate selection, the participating patients' pain symptoms were assessed by using the Japanese version of the neuropathic pain symptom inventory (NPSI) and the numeric rating scale (NRS) for further assessment. A total of 12 molecular species of LPC and LPA were measured by using LC-MS/MS in 18 patients[42]. Total LPA was significantly correlated with total NPSI (including pressing pain and paroxysmal pain) scores and average NRS scores (Fig. 4). Total NPSI scores were correlated with 18:1 and 20:4 LPA, whereas average NRS scores correlated with 16:0, 16:1, and 18:1 LPA. All of the molecular species of LPA were clearly and positively correlated with the corresponding LPC species[42]. This study indicated an association of NP symptoms with LPA.

As mentioned in the *Introduction*, lumbar spinal stenosis (LSS) is one of the diseases representing NP in elderly patients, with the following pain symptoms being observed: low back pain, radiating lower leg pain (with or without foot numbness), and dysesthesia. For further study, a total of 28 patients with LSS and 15 control patients were enrolled in our study[43], and CSF was also used as the main measurement tool for this study for the measurement of LPLs. In addition to NPSI, the Zurich Claudication Questionnaire (ZCQ) was completed by all of the participants. Based on these scoring systems, LSS patients were grouped into mild and severe groups. All of the measured LPLs, including LPC, LPA, and lysophosphatidylinositol (LPI), were significantly higher in the CSF of LSS patients than in the control group[43]. In addition to these findings, the levels of some species of LPC and LPA were significantly higher in the severe group than in the mild group of LSS patients. Total LPA or all of the molecular species of LPA were shown to be strongly correlated in severe group patients with total LPC or corresponding species of LPC, according to the NPSI scoring[43]. These clinical studies indicated an association of LPA levels that were measured in the CSF with NP symptoms and disease severity.

In addition, NP induced an effect of chemotherapy, especially with taxane, which was associated with genetic polymorphisms (two single nucleotide polymorphisms) of LPA_1 but not with LPA_{2-6}[53]. The screening of the genetic polymorphisms in LPA_1 could predict the possible onset of chemotherapy-induced NP, which will be associated with effective therapy.

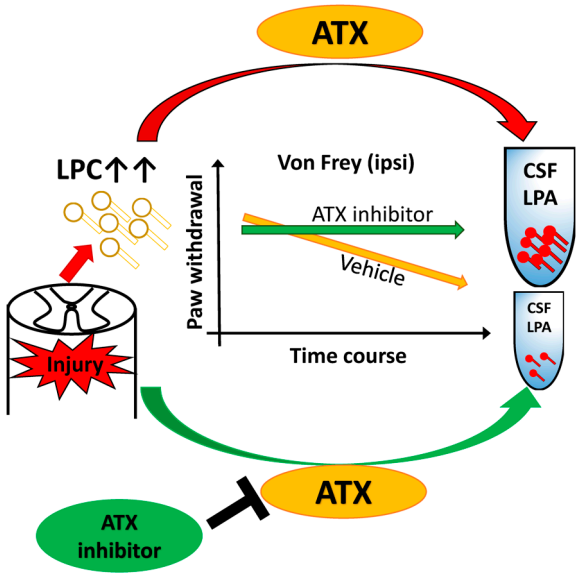

Fig. 5. Increased LPC and LPA levels were decreased due to inhibition of ATX with amelioration of pain symptoms.

Schematic model of the effect of ATX activity in the CD model. When ATX is fully active, the levels of LPC (yellow symbol) and LPA (red symbol) increase in the CSF. The ATX inhibitor suppressed ATX activity and predicted beyond the production of LPA. The threshold of the pain was recovered from mechanical pain by administration of an ATX inhibitor (green arrow).

5. The LPA-LPC-ATX Axis Is a Potential Therapeutic Target for Neuropathic Pain

The involvement of LPA in human pain diseases, as described above, prompted us to investigate the possibility that LPA is a target for the treatment of NP. As mentioned above, LPA is produced from LPC by ATX activity and induces NP via its $LPA_{1,3}$ receptors (Fig. 1). In the CD model, an aligned study using an ATX inhibitor (ONO-8430506) was performed to prove the possibility of the LPA-LPC-ATX axis as a potential therapeutic target in NP (Figs. 1, 5). Obviously, in the CSF, the levels of LPC and LPA were increased from the 1st day after surgery with a time course-dependent decrease in CEC but were sustained at high levels in the CD model (Figs. 1, 5). These increased levels of LPA returned to levels similar to those of the control when the ATX inhibitor was orally administered (Fig. 5). The therapeutic effects were also observed in the mechanical threshold of the pain, which was significantly decreased on the ipsilateral side, as shown in Fig. 5. The involvement of microglia and astrocytes was also inhibited in a time-dependent manner[41]. The expression level of ATX, which is a limiting enzyme for producing LPA from LPC, was not changed during the study, except for its activity, which was totally inhibited in the plasma and partially inhibited in the CSF[40,41] when the ATX inhibitor was orally administered (Figs. 1, 5). These studies demonstrated the importance of the LPA-LPC-ATX axis in NP and suggested the possible use of the ATX inhibitor for the treatment of NP (at least the type of NP caused by LSS).

6. Perspective for the Clinical Usefulness of Lipidomics for Neuropathic Pain

Our clinical and basic research with lipidomics methods, as described above, confirmed the involvement of LPA in human NP. These results have demonstrated the possible medical application of these findings as biomarkers and therapeutic targets. When regarding applications for clinical laboratory testing, the measurements of LPA and/or LPC in the CSF can be an objective marker for quantifying the degree of pain or for the diagnosis of the underlying diseases causing pain. At present, no quantitative clinical laboratory testing exists for the evaluation of pain, and physicians evaluate the degree of pain by using complicated and subjective questionnaire forms. Moreover, the elevation of LPA and/or LPC will exclude patients with pain in which psychogenic factors are largely involved. Our recent study suggested that the measurements of LPA and/or LPC will also be useful to discriminate the neuropathic pain caused by LSS from that caused by non-LSS diseases, such as diabetic neuropathy. Therefore, the development of LPA and LPC measurement systems in CSF will help physicians in evaluating the degree of pain and in judging the application of surgical treatment.

Similar to our study in the area of NP, as reviewed in the present article, the lipidomics approach will largely contribute to the understanding of human diseases and can facilitate translational and reverse translational research studies in various diseases.

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